Recent Advances on Natural Products, covering all the latest and outstanding developments in the area

Tribute to Prof. Otto R. Gottlieb

XXVII Annual Meeting on Micromolecular Evolution, Systematics and Ecology

Reflections on the Current Status of Chemosystematics

November 4th to 7th, 2007, Hotel Colina Verde, São Pedro, São Paulo State, Brazil.

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WELCOME FROM THE ORGANIZING COMMITTEE

On behalf of the Organizing Committee, we are glad to welcome all participants in the 1st Brazilian Conference on Natural Products (1st BCNP) and the XXVII Annual Meeting on Micromolecular Evolution, Systematics and Ecology (RESEM), which have been held from November 4th to 7th, 2007 at Hotel Colina Verde in São Pedro, São Paulo State, Brazil.

The 1st BCNP will be organized by the Natural Product (PN) Division of the Brazilian Chemical Society (SBQ) at intervals of two years. The first one is a Tribute to Prof. Otto R. Gottlieb for his outstanding contributions to Natural Product. Prof. Gottlieb was born in Brno, Czechoslovakia, on August 31st, 1920, and immigrated to Brazil in 1939. Fortunately, he opted for his Brazilian nationality. Professor Gottlieb ranks as one of the most influential professionals in Organic Chemistry and the Chemistry of Natural Products in Brazil. Having developed an intense interest in the molecular diversity of the rich Brazilian flora at an early stage of his career, he went on to pioneer the introduction of Phytochemistry as a major discipline in Brazil, and he has settled several graduate courses and research programs in many Brazilian institutions. He has supervised over 120 graduate students, many of whom now hold influential positions in Brazilian universities and research organizations. He pioneered the study of the Brazilian plant biodiversity searching for chemotaxonomic markers. The establishment of an interdisciplinary program of chemobiology pursuing the rationalization of evolution, systematics and ecology of plants as a guide to the search for plant-derived bioactive substances, represented a further facet of his broad phytochemical interests. Gottlieb at an early stage of his activities on chemobiology initiated the present series of Annual Meetings on Micromolecular Evolution, Systematics and Ecology (RESEM), which now has been happening for 27 years. He continues to sponsor RESEM and now the Natural Product Division of the Brazilian Chemical Society became an important instrument for maintaining it at intervals of two years into the international BCNP.

The XXVII RESEM program is focused on “Reflections on the Current Status of Chemosystematics”. A most distinguished roll of speakers will dedicate special attention on the future directions for research on chemosystematics in Brazil.

The purpose of the 1st BCNP was to bring together renowned specialists, researchers and students from all over the world, and from all area of the Natural Products sciences, to meet, discuss, and exchange their views and experiences on topics related to “Recent Advances on Natural Products, covering all the latest and outstanding developments in the area”

The Plenary and Short Lectures have been arranged to foster studies on: Biodiversity & Natural Products; Biological and Pharmacological Activity of Natural Products; Manufacturing and Quality Control of Herbal Drugs and Essential Oils; Biosynthesis and Molecular Biology of Natural Products; Recent Advances on Isolation and Structure Elucidation of Secondary Metabolites.

The Organizing Committee has labored to provide a truly prominent slate of lecturers and excellent posters, world-class accommodations, and hospitality second to none. All they have endeavored to maximize the interaction of all the participants and speakers by having a single venue and no parallel sessions. We are happy to announce that the program has attracted over 300 participants from 5 countries.

We would like to thank CNPq, CAPES and FAPESP for their willingness to underwrite the financial obligations of this symposium, to the students who became involved in the organization of this Meeting, particularly to Márcio Santos Soares by intense dedication in the administrative activities during the several preparative phases of this event. We are also very grateful to all members of the Organizing Committee for what they have done to make this Meeting the success that we hope it will be.

Finally, we hope to all of you a good stay in Brazil, and a successful and enjoyable Meeting.

Prof. Dr. Raimundo Braz Filho
Chair, Organizing Committee

Prof. Dr. M. Fátima das G. F. da Silva
Secretary General, Organizing Committee
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Natural compounds are evolutionary selected and pre-validated by Nature, displaying a unique chemical diversity and a corresponding diversity of biological activities. These features make them highly interesting for studies of chemical biology, and in the pharmaceutical industry for development of new leads. Of utmost importance, for a rational discovery and exploration of new biologically active compounds, are two aspects. One of these would be the identification and charting of the biologically relevant chemical space, the other a similar charting of the corresponding evolutionary space (e.g. Bohlin et al., 2007 pro parte).

The first key to this is the coverage of the natural products’ chemical space. For this purpose we introduced ChemGPS-NP (Larsson et al., 2005; 2007). This new tool is tuned for handling the chemical diversity encountered in natural products research, in contrast to previous tools focused on the much more restricted drug-like chemical space. The aim is to provide a framework for making compound classification and comparison more efficient and stringent, to identify volumes of chemical space related to particular biological activities, and to track changes in chemical properties due to, for example, evolutionary traits and modifications in biosynthesis. Physical-chemical properties not directly discernible from structural data can be discovered, making selection more efficient and increasing the probability of hit generation when screening natural compounds and analogues.

The second key would consequently be to explore evolutionary space, or essentially to elucidate robust phylogenies for the relevant groups of organisms under study. From this basis, reflecting the evolutionary history and hence biosynthesis development, further conclusions can be drawn (e.g. el-Seedi et al., 2005, Backlund et al., 2006). From these initial attempts, brief discussions on the intersection of chemical and evolutionary space of iridoids, betalains, and sesquiterpenes will be expanded.

Bohlin, L., Göransson, U., Backlund, A. Modern pharmacognosy: Connecting biology and chemistry.
SHORT LECTURES
ABSTRACTS
Secondary metabolites played an important role along de period 1960-1980 as systematic characters for delimitation of angiosperm groups above family level and for the establishment of relationships among them. They were also highly relevant for the establishment of kinships among genera, species and infraspecific categories. Chemical characters were object of much debate among taxonomists, because chemical evidences often conflicted with comparative morphology. Nonetheless, the systematic relevance of several classes of secondary metabolites (tyrosine derived alkaloids, betalaines, glycosinolates, iridoids, sesquiterpene lactones, polyacetylenes) gained recognition by proponents of the main angiosperm systems of classification along the second half of the last century, although with much disagreement as to the relative importance of chemical evidence over morphology. For example, while iridoids distribution indicated affinities among a broad range of dicotyledon families, some authors preferred to recognize as taxonomically coherent only a restricted group of iridoid bearing families, while assigning a condition of convergence to the occurrence of iridoids in other taxa.

The advent of molecular (macromolecular) systematics, starting in the early 80’s, led to a decline of the prestige of micromolecular characters as systematic evidence. For phylogenetic purposes, molecular evidence has turned out the most reliable tool, all other types of evidence playing a subsidiary role. Like most phenotypic characters, secondary metabolites provide limited numbers of characters for phylogenetic reconstructions, as opposed to the numerous molecular characters that can be sampled from several nuclear, plastidial and mitochondrial DNA regions. In addition, many phenotypic characters (the chemical ones included) are subject to environmental influences, which pose much caution as to their use in systematics and phylogeny.

In most cases of disagreements formerly observed and derived from incongruence between chemical and morphological evidences, angiosperm molecular phylogenies have agreed with kinships suggested by micromolecule distributions.

Chemical systematics remains a highly important shortcut for prospecting economically important chemicals, in particular for medicinal purposes.

Quite often molecular phylogenies are used for the establishment of evolutionary trends of phenotypic characters. Hence, chemical evolution is currently determined upon a topology based on molecular evidences, rather than being an independent basis for the establishment of phylogenies.

On the other hand, chemical characters are taxonomically useful towards three main objectives: 1) establishing affinities among taxa, for which no molecular evidence is available; 2) adding phenotypic sinapomorphies to branches corresponding to important clades in molecular phylogenies; 3) by means of combination with molecular markers, bringing additional support to phylogenies, especially in cases of low tree resolution or low clade support.
Além de constituintes de óleos essenciais, os terpenóides e os compostos fenólicos são as classes de metabólitos secundários mais investigadas de Asteraceae brasileiras. Dentre os terpenóides, destacam-se os sesqui, di e triterpenos, enquanto que os flavonóides são os mais comuns dentre os polifenóis. O interesse nestes grupos substâncias é devido à elevada diversidade estrutural que apresentam, à sua importância para estudos quimiotaxonômicos e ao seu potencial biológico. Salienta-se que no Brasil várias Asteraceae são utilizadas como plantas medicinais, seja em produtos comerciais ou em preparados tradicionais.

Nos últimos anos, foram investigadas quimicamente várias espécies de gêneros pertencentes a grupos brasileiros e sul americanos. Foram isoladas e identificadas lactonas sesquiterpênicas, diterpenóides derivados do caurano e pimarano, além de flavonóides. O perfil químico de tricomas glandulares de algumas espécies foi estabelecido. Merecem destaque os estudos filogenético e etnofarmacológico de algumas espécies de cerrado.

Com base nos resultados destes estudos, constatou-se a elevada quimiodiversidade das espécies investigadas, tendo sido identificadas várias substâncias novas. As relações entre os perfis químicos e a classificação taxonômica de algumas espécies foi avaliada, sendo possível efetuar interessantes discussões. Através de estudos multidisciplinares, o potencial biológico de extratos brutos e de diversas substâncias foi revelado, em especial de terpenóides. Por exemplo, descobriu-se lactonas sesquiterpênicas com potente ação citotóxica, leishmanicida e antinfiamatória e diterpenos com pronunciado efeito antiespasmódico. Foram encontrados extratos brutos com atividade antiinflamatória e com efeito fagoinibidor.

Estudos computacionais realizados com diversas moléculas de Asteraceae levaram à elaboração de modelos direcionados para a coleta racional de espécies vegetais e para a análise quimiotaxonômica de tribos e de subtribos, para a atribuição automática de deslocamentos químicos de RMN $^1$H e predição de tempos de retenção para cromatografia líquida de fase reversa. Com base na quimiotaxonomia e na utilização de bancos de dados de estruturas, pretende-se estudar o metabolismo secundário e efetuar triagem virtual de moléculas frente a alvos biológicos.

Conclui-se que a investigação das Asteraceae brasileiras é relevante para a área de química de produtos naturais do país e uma fonte promissora de moléculas de interesse, seja para a química, biologia ou informática. Logo, estimula-se a formação de grupos de pesquisa multidisciplinares para diversificar a pesquisa das potencialidades de espécies e de moléculas desta família.
Plants grown under elevated [CO$_2$] exhibit increase of their biomass due to a higher rate of photosynthesis. Although a number of results have been published with temperate tree species, relatively little is known about the physiological responses of Neotropical trees. We have recently demonstrated that seedlings of Neotropical legume trees indeed grow faster, accumulating more carbon in their bodies. Furthermore, the level of incorporation of carbon for each species was proportional to their respective life span, indicating that the rates of carbon sequestration is higher in fast growing species and lower in slow-growing species. In other words, ecological succession is probably the more efficient way to maintain constant carbon sequestration. We also performed experiments with sugarcane (a C4 plant) growing them under elevated [CO$_2$]. We found that as photosynthesis is stimulated, near 60% more biomass is produced in elevated CO$_2$ with slightly higher sugar concentration. The reason for obtaining higher photosynthesis is likely to be related to an increase in the electron transport genes in sugarcane leaves. This poses a dilemma: shall we plant sugarcane everywhere or regenerate/preserve forests? Before deciding to go ahead with either of the decisions, one has to bear in mind that the amount of carbon in the entire Brazilian sugarcane crop is about 8 million tons, whereas in the forests only in South America the stocks are of about 70 billion tons. Thus, regeneration or preservation alone are quantitatively much more efficient in avoiding carbon emissions to the atmosphere. It is important to remember that we have already burnt out 3 billion tons of carbon of the Amazon, which means that we could not recover the carbon lost even if we doubled sugarcane production and continue to produce it for 100 years. On the other hand, ethanol produced from sugarcane has some potential to mitigate carbon emissions. Probably the best solution would be the “game-theory” one, which is to increase cane biotechnology at the maximum possible, therefore maximizing profit, but at the same time contemplate forest preservation and regeneration, even if this means having forests strategically planted in sugarcane fields, which could limit production to some extent. This would probably turn a product that could be called Brazilian Environmentally Friendly Ethanol (BREFE) more competitive in the world market. Increasing the production of BREFE is probably one of the best ways to go for a country who flirts with the idea of being one of the environmental superpowers in the future. MCT, FAPESP, CNPq.
OVERVIEW OF GLOBAL ENVIRONMENTAL CHANGE

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This overview will cover some of the key developments of GEC science over the last 5 years since the Amsterdam Conference in 2001. It will focus mostly on GEC at the regional scale, that is, how changes occurring at hot spots of the Planet can influence the global environment and vice-versa. The influence of the rapid rates of land use change in Amazonia on regional climate and on global biogeochemical and the impact of GEC on the sustainability of that region will be used as illustrations of scientific advancements in regional GEC science. Examples of GEC for Africa and Asia will also be presented.

The overview will also dwell on the emergence of applications of Earth System Science, which challenge the scientific community and the GEC Programmes to translate dense and reliable information on global and regional environmental changes into actions toward a sustainable future for the Planet which can make a difference in the debate on the environmental, social and economic aspects of sustainability. This is particularly important for developing countries in order to resolve the apparent dilemma of sustainable management of the environment in the face of urgently needed economic growth. Special attention will be given to the requirements of Earth observations from space for the development of Earth System Science.
The known organic compounds before the XVIII century were from natural origin with the exception of ether and acetic acid, both obtained from ethanol. Scheele (1842-1886) was the first chemist to develop a methodology to extract natural compounds from live organisms, mostly plants. He succeeded in obtaining acids from plants and animals, by an acid/base extraction. He also obtained sugars and glycerin. Lowitz e Kirchhoff isolated glucose and fructose and observed that they were different from sucrose. Lavoisier, working in the oxidation of sugars, found lactic acid as a product of this reaction. He also found that the relation between oxygen and hydrogen in sugars is the same that in water.

In the beginning of XIX century, in France, natural product chemistry had a remarkable advance. Fixed oils, alkaloids, colorful compounds were isolated and analyzed regarding the percentage of carbon and hydrogen. In 1817, after the achievement of crystallized morphine by Seturner, the alkaloid class was recognized. It was at that time that sugars, amino acids, terpenes, flavonoids and other aromatic compounds were discovered. In 1835 thirty-five alkaloids were known. The use of alkaloids and other natural compounds in medicine as well as pigments made chemistry vary attractive. Chevreul, working with coloring material from plants isolated brasilein from *Caesalpinia echinata* Lam (Pau Brasil) and indigo from *Indigosfera tinctoria*. At that time about one hundred essential oils had been used for different purposes.

In the middle of XIX century, chemistry began to move to German. After the chemical structural ideas of Kekule, van’t Hoff and other chemists the structural determination of natural products became one of the main fields in chemistry research. The way to achieve this goal was by synthesis and degradation of natural products. The work of Bredt with camphor, Willstätter with tropanic alkaloids and Wallach with monoterpenes were remarkable, but there is no doubt that Fischer was the one who developed the greatest work in this area. He elucidated the structure of the hexoses, isolated and elucidated the structure of 12 natural purins, receiving the 1902 Nobel Prize. This prize was given to 12 natural products chemists during the first half of XX century; among them, Ruzicka received the Prize in 1922 for his extensive work with terpenes and other natural products. In my opinion, the work of Semmler dealing with the structure of santalene, a tricycle sesquiterpene, in the beginning of 1900 was one of the most important in the field.

In the middle of XX century, biosyntheses was one of the important challenges of natural product chemistry. At that time, structural determination had the aid of NMR and mass spectroscopy measurements. Names such as Djerassi and Nakanish, both still alive, make great contribution in the field.

Professor Otto R.Gottlieb started with the study of essential oils when he perceived the importance of the knew techniques (NMR, MS) in the structural determination of natural products. He decided to search that knowledge and when came back to Brazil in 1964 he began to teach modern natural products chemistry all over the country. By giving lectures he taught hundreds of students about the organic chemistry of natural products. In the beginning, Gottlieb phytochemistry research was mainly in the structural determination of compounds from Amazon plants. Then, the study about the systematic, evolution and ecology of plants became more important to him. He published around 700 papers and oriented more than one hundred graduate students. He won more than 40 awards and was indicated by the Brazilian scientific society to compete to the Nobel Prize in 1999.
ORAL SESSION ABSTRACTS
SELF ORGANIZING MAPS AS TOOL FOR TAXONOMIC CLASSIFICATIONS AT LOWER HIERARCHICAL LEVELS

Vicente P. Emerenciano\textsuperscript{1}, Marcus T. Scotti\textsuperscript{1}, Marcelo J. P. Ferreira\textsuperscript{1}, Mauro V. Correia\textsuperscript{1}, Sandra A. V. Alvarenga\textsuperscript{2}, Gilberto V. Rodrigues\textsuperscript{3}

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The use of chemical data as auxiliary data in the classification of vegetable taxa has been employed since some decades. In these works several methods and techniques were utilized in order to use the chemical data as variables for the classification of the most varied vegetable groups. The objective of this work is to show a novel application of Self-Organizing Maps (SOM) as an alternative and efficient technique which, making use of chemical data, can also be utilized in classification of botanical taxa, i.e. chemosystematics.

The SOM has cluster analysis characteristics similar to those of more classical unsupervised algorithms. One of the most important advantages of a Kohonen network\textsuperscript{1} is visualize relationships of objects, into 2-dimensional space, preserving the essential topological features of data. Another advantage, as others artificial neural networks procedures, is associated to the fact that SOM can “learn” and connect information which is extremely important in chemistry where the relationship between cause and effect has not known yet. In contrast to the traditional statistical methods, SOM are not only restricted to linear correlations or linear spaces. Consequently, they can be applied in problems involving classification, prediction and visualization of chemical data. As opposed to the multilayer neural networks, the SOM has relatively little been used in chemistry and mainly little been used in the chemistry of natural products.

The botanical group selected for this study was the Asteraceae family which constitutes a group of plants spread widely across the world, comprising about 23000 species. According to Bremer\textsuperscript{2} the family is divided into four subfamilies and 17 tribes. Over 5000 species of the family had already been chemically studied, from which ca. 7000 micromolecular compounds had been isolated at that time. In this study, the number of occurrences of each chemical class at the hierarquical level analyzed was utilized, comprising monoterpenes, sesquiterpenes, sesquiterpene lactones, diterpenes, triterpenes, flavonoids, coumarins, polyacetylenes and benzofurans isolated from 650 genera grouped according Bremer\textsuperscript{2}. The choice of such metabolites is owing to a vast record of them in this family.

The presence of the most representative 144 skeletal types were extracted from a database containing 38000 occurrences of compounds isolated from Asteraceae. These skeletal types were inserted in the Kohonen network as input data. The SOM was able to separate the four subfamilies of Asteraceae with a performance of 86%. Among the most studied tribes, pertaining to the Asteroideae subfamily, the unsupervisoned network classifies four tribes with high degree of recognition (80% and 70% for training and test series, respectively).

Through the use of SOM phylogenetic relationships among the subfamilies and tribes of Asteraceae could be established. Thus, the method can be applied as a powerful and complementary tool in plant classification.

References
The Amazonian rain forest holds the highest diversity of plant in the world and among these plants *Minquartia guianensis* Aubl. (Olacaceae) can be found in Acre, Amazonas, Roraima, Pará and Amapá States in Brazil, where its trunk is widely used as a light post on account of all the types of bad whether it can withstand. Popularly it is known as acariquara, acariquara-roxa, acari among others. A chemical study with the stem bark of *M. guianensis* collected in Ecuador yielded the minquartynoic acid, a cytotoxic polyacetylene and it also showed moderate *in vitro* activity against *Plasmodium falciparum* and *Leishmania major*. Another chemical study with barks of *M. guianensis* from Equador showed the presence of the triterpenes eritrodiol and betulin and also the acetylene minquartynoic acid and the lichexanthone. The present study was performed with leaves of *M. guianensis* which were extracted with dichloromethane, methanol and water three times each using ultrasound for 20 minutes. The dichloromethanic extract fractionation was made using several chromatographic techniques which allowed isolating the triterpenes: squalene (1), lupen-3-ona (2), taraxerol (3) and lupeol (4). The chemical structure identification was made based on spectral $^1$H and $^{13}$C Nuclear Magnetic Resonance (NMR) data and as compared to literature.

In the literature available so far, triterpenes were only previously reported in two species belonging to Olacaceae family: *Heisteria nitida* (lupeol) and *Minquartia guianensis* (eritrodiol – a β-amirin derivative and betulin – a lupeol derivative) both collected in Equador, in the Amazonian rain forest. Our work corroborates that triterpenes could be used as chemical and geographic markers of these genus.

Acknowledgements: FAPEAM and PPBio/MCT.

Refs.
INHIBITION OF TOPOISOMERASE I BY PHYSALINS FROM *Physalis angulata* L. IN VITRO

Cavalcanti, B.C.¹, Sombra, C.M.L.¹, Bezerra, D.P.¹, Veras, M.L.², Pessoa, O.D.L.², Moraes, M.O.¹, Costa-Lotufo, L.V.¹, Pessoa, C.¹*

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Crude extracts of *Physalis angulata* L. (Solanaceae) and several isolated secondary metabolites exhibit a wide variety of biological activities such as antimicrobial, antiinflammatory, trypanocidal, and cytotoxic. The cytotoxicity of this plant has been attributed to the physalins, compounds characterized as withasteroids, a series of C28-steroidal lactones structurally based on the ergostane skeleton which are commonly produced by Solanaceae plants (Cárdenas et al., 1994). There are some evidences that physalins might be able to affect DNA synthesis (Magalhães et al., 2006), so these compounds might be a suitable candidate to cause inhibition of topoisomerase. To test whether cytotoxic properties were related to topoisomerase inhibition, this protein was evaluated in a cell-free system. Purified human topoisomerase I was incubated with physalins B, D and F (5 or 10 µg/mL) in the presence of supercoiled plasmid DNA, and the products were subjected to electrophoresis to separate closed and open circular DNA. In this case, the relaxation of DNA was inhibited in both tested concentrations for only physalins B and D. Further studies in the structure-activity relationship of physalins and their derivatives are still in progress. Support: CNPq, FUNCAP and Institute Claude Bernard.

Refs.


POSTER SESSION
ABSTRACTS
The Atlantic forest from São Paulo is characterized by a great diversity of Myrtaceae species. These species are considered as a source for essential oils with recognized pharmacological activities such as eugenol, main component from clove oil (*Syzygium aromaticum* (L.) Merr. & L.M.Perry). The production and the composition of essential oils may be affected by several factors such as phenology, climate, geographic localization, genetic variability and endogenous rhythms. However, studies relating the volatile oil variation in Myrtaceae with these factors are scarce. In this way, we proposed an analytical method to study the seasonal variation of the volatile oil using as model *Myrcia macrocarpa* DC which is native from the Atlantic Rain forest.

**Materials and Methods**

The leaves of three specimens were collected at the State Park Fontes do Ipiranga (São Paulo, SP) in the period of January 2005 to January 2006. The essential oils were obtained by hydro-distillation in a Clevenger-type apparatus for 4 h and further analyzed by gas chromatography coupled with mass spectrometer (GC/MS). The raw ion-chromatogram data, more precisely, abundance of different fragments (m/z) in function of the retention time for 21 samples, were exported in ASCII file format. The data sets were inserted into MATLAB 6.5 program to generate contour plots of the chromatograms. Mass fragments (m/z) ranges most frequently found in the different samples were selected, after visual analysis of the contour plots, submitted to “area normalization” and tested to Principal Components Analysis (PCA) using the Uncrambler v. 9.5 program.

**Results and Discussion**

The mass fragments (m/z) ranges selected and submitted to PCA were: 55, 67, 77-79, 80-84, 91-97, 105-110, 120-123, 133-135, 139, 147-160, 133-135, 139, 147-150, 160-163, 204-207. The first principal component explains to 83% of whole variance and the second only 9%, totalizing 91%. Analyzing the scorer plot, it can be noticed that there were no significant differences among individuals collected in the same month and year. However, a significant difference was verified among samples of different months and for same samples collected in the same month of different years.

**Conclusion**

PCA was an effective tool to verify seasonal and individual variations using only raw CG/MS data of the volatile oils from *M. macrocarpa* DC.

Ref.

S. Wold et al., *Chemometrics and Intelligent Laboratory Systems*, 2, 37 (1987)
USE OF BACKPROPAGATION ARTIFICIAL NEURAL NETWORKS TO PREDICT
THE OCCURRENCES OF CHEMICAL CLASSES IN ASTERACEAE

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The Asteraceae family comprises about 23000 species, whose economical and medicinal importance has been widely described. Botanically, the family has been divided into subfamilies and tribes by several authors and, chemically this group has been extremely studied from which an enormous variety of chemical classes have been isolated: terpenoids, coumarins, flavonoids, polyacetylenes and benzofurans. Until the 90’s, about 7000 species of the family already had some type of chemical study. The chemical constituents isolated from this species (38000 occurrences) were stored in our database and the number of species a priori is considered representative for an approach by artificial neural networks (ANN)1. The research on phytochemistry and the search for new biologically active substances from plants has been a constant scientific activity for several decades. Usually the factors that direct this search type are ethnobotanical information coming from the popular use of plants or obtained from chemotaxonomic studies. In spite of a great number of studied species, the information on them stored so far still is scarce, due to the phytochemical study of each species is rather incomplete. Certain genera have many interesting species and are extremely studied, what generates plenty of data. A typical example in our database is the genus Artemisia, whose number of studied species/existent species is 250/390, that is 64%. However, for other genera also present in our database, this ratio may be very small, 8/120 for the genus Kaonosphyllon taken as an example, that is, only 7%. Techniques of multivariate statistics have been used to approach the existence of correlations among occurrences of chemical classes in the family but the results of this methodology still are insufficient to determine species to be analyzed accurately. As the data are somewhat imprecise, an approach using ANN may be more precise for the analysis of this type of phytochemical information.

The aim of this work is to predict the presence or absence and the occurrence number of the chemical classes of natural products in genera of the Asteraceae through ANN.

A backpropagation neural network was training with nine variables representing the chemical classes of compounds (inputs data) in 400 genera. The outputs were heuristic variables scaled from 0 to 1 and representing the rare, frequent or very frequent probability to found a specific chemical class in genera of family. The ANN was able to predict the presence or absence, as well as the heuristic degree of probability of all chemical classes with 84.3%. The external set containing 100 genera was submitted to the ANN analysis.

From this procedure, the ANN was able to predict with 79.1% the presence of chemical class and classify correctly the heuristic probability of occurrence.

The search for potentially active compounds has been the objective of several research groups all around the world. A single heuristic information coming from the researcher that knows the database of the Asteraceae has shown itself here quite interesting as a new approach to be introduced in ANN. The results showed here demonstrated this application and also encourage us to continue the research based on data from specific families of plants using this methodology to indicate promising plants that should be studied.

References

Chemotaxonomic Relationships in Celastraceae

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Celastraceae sensu lato, including Hippocrateaceae genera, comprises a group of woody lianas, shrubs and trees with a pantropical distribution. Some species are used for medicinal purposes, as ornamentals plants and used socially as a stimulant. Various authors have considered Hippocrateaceae and Celastraceae as two distinct families but other taxonomists have been argued for the unified family. The production of micromolecules in Celastraceae sensu lato has been characterized mainly by the occurrence of terpenoids, especially dihydroagarofuran sesquiterpene polyol ester, alkaloids, friedelane triterpenoids and some unusual substances as dimmers and trimmers of sesquiterpenes, diterpenes and triterpenes. The most known biogenetic feature is the production of quinonemethides, a kind of nortriterpenoid. In order to deal with a great number of compounds and botanical informations in a chemotaxonomic study, the use of multivariate statistical methods as principal component analyses (PCA) has been implemented. This powerful tool enables to reduce the dimensionality of the data set without a significant loss of information. In this paper the PCA was employed in a chemical database containing 664 occurrences of triterpenes and used to investigate some intergeneric relationships in Celastraceae.

This study involved the tribes Cassineae, Celastraeae, Euonymeae, Lophopetaleae, Hippocrateeae and Salaciae since they have a more representative chemical profile (96% of the triterpene occurrences). The triterpene’s occurrences of Celastraceae s.l. allowed making a model to explain the genera relationships. Celastraceae phylogeny inferred from 26 S nuclear ribosomal DNA genes¹ was used as a base to pointed out correlations with the chemical data. In this analysis two main groups of genera could be recognized. The first group was chemically characterized by no production of the 26(14→15)-abeo-24-D:A-friedo-nor-oleanane and low production of the lupane skeleton. The second group showed the production of all celastraceous skeletal types of triterpenes.

Comparison of PCA and cladistic analysis showed Cassine s.s distinct from Elaeodendron. Such distinction has also been supported by differences in pollen, bark and wood anatomy². Cassine s.s. produces quinonemethide triterpenoids derived from the 24,29–D:A–friedo–dinor–oleanane while Elaeodendron does not. Maytenus s.l. (including Gymnosporia and Tricera) is not resolved as a natural group¹. Our results support the distinction of Gymnosporia from Maytenus once the later produces high diversity of substances and skeleton types while Gymnosporia appear to have a tenuous concentration in the oleanane skeleton production. PCA analysis for tribes shows that all celastraceous tribes are very similar on the chemical point of view. This fact corroborates with the idea of a unified family including Hippocrateaceae genera within Celastraceae¹.

References
The Asteraceae family has been intensely studied by researchers of the diverse areas, such as chemists, pharmaceutics, botanicals, etc due its large chemical and morphologic diversity. Several classifications systems intend to cluster the genera in tribe according to morphological, macromolecular and micromolecular criteria. From the point of view of the tribes, since Reading’s Symposium, several authors attribute different interpretations for groups which classification is still not clear, for example the tribes Mutiseae, Helenieae, Heliantheae and others. The use of chemical data as auxiliary data in the classification of Asteraceae taxa has been employed in the last decades. In these papers several methods and techniques were used in order to utilize the chemical data as variables for the classification of the most varied sufamilies, tribes and subtribes of the family. The aim of this paper is to use chemical data related to isolated secondary metabolites of Asteraceae, which are available in our database containing all chemical information about this family, to compare them with the Wagenitz and Bremer’s classification employing principal component analysis (PCA).

The power of PCA analysis for discriminate groups in phenetic studies were already published. The principal advantage of this method is the possibility of to work with several variables when they are highly correlated. This methodology avoids the redundance of the multivariate analysis and reduces the dimensionality of the data without of information prejudice. In some very advanced taxons the diversity of chemical classes and / or skeletal types makes impossible the inspection of the data in graphics without the dimensionality reduction. Thus, the PCA was applied to Asteraceae database containing 38000 occurrences of compounds isolated from their species. The chemical classes used in this study comprising monoterpenes, sesquiterpenes, sesquiterpene lactones, diterpenes, triterpenes, flavonoids, coumarins, polyacetylenes and benzofurans. The results were analyzed according to the Bremer and Wagnetiz classification and show a great similarity with both classification systems. The separation of the Cichorioideae and Asteroideae subfamilies could be evidencied from chemical data once the production of secondary metabolites is less expressive in the Cichorioideae. This observation is in agreement with the botanical classification that ranks this group as the more basal in Asteraceae. The very small number of isolated compounds of Barnadesieae tribe was the responsible for its position as an outlier in the PCA analysis. Therefore, the primitive position within the family based on DNA data appears to be confirmed by the chemical data. In the other hand, the separation of the cluster formed by the Inuleae s.l. is not well confirmed by the chemical data. The use of PCA in the taxonomic studies could be compared with the two types of classification obtained to Asteraceae family and some correlations between the chemical and botanical data were obtained. However, some doubts remain about the evolution of this family and we considered that the introduction of the one or more chemical descriptors could be useful to improve the discussion about the evolution of the groups within the family.

References
Magnolia and Talauma are the most important genera in Magnoliaceae. Several phytochemical studies have been carried on Magnolia while Talauma has received little attention. The present work describes the results of phytochemical study of leaves of T. ovata. Chromatographic fractionation of the dichloromethane extract yielded three known benzofuranoid neolignans. The compounds were identified by NMR 1D and 2D as well as comparison with reported data as acuminatin, licarin A and 7-(4-hydroxy-3-methoxyphenyl)-1’-trans-propenal-3’-methoxy-8-methyldihydrobenzofuran. The last one has been previously obtained only by synthesis\(^1\). Neolignans of this type are been reported for the first time in Talauma, however are common in Magnolia. These results support the proposal of reducing Talauma to a sub-genus of Magnolia.

Refs.
The taxonomic classification in Myrtaceae is difficult and chemical data could be useful to investigate the delimitation of botanical groups. With this aim the composition of essential oils of Brazilian *Eugenia* and *Myrcia* was analyzed statistically. Chemical data was getting through review in the literature. These data was analyzed by the Principal Component Analysis (PCA) and Cluster Analysis. Twelve variables, corresponding to monoterpenes, acyclic sesquiterpenes and ten cyclic skeletons of sesquiterpenes were used. In *Eugenia*, the first component was positively determined by the skeletons germacrane, bicyclogermacrane and elemene and the second component by content of monoterpenes and skeleton caryophyllane. The PCA did not result in a clear grouping of species. In *Myrcia*, the first component was determined by the skeletons humulane and bisabolane and the second component by eudesmane and aromadendrane. The PCA resulted in two near groups, showing that *Myrcia* is a more homogenous group than *Eugenia*. The PCA of *Myrcia* and *Eugenia* together showed *Myrcia* as a group immerse in *Eugenia*. 
EVOLUTIONARY TENDENCIES IN SWARTZIA GENUS: BIOGENETIC PROPOSAL FOR SWARTZIARBOREOLS

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This work gives continuation to the chemiosystematic study of plants from the Swartzia genus regarding evolutionary tendencies [1], by taking into account the disparity and the diversity of specific metabolites isolated and identified in them [2].

The biogenetic substitution of the compounds originated from the shikimate route by the compounds originated from the acetate route can be taken as an evolutionary tendency since the later ones are found in more recent angiosperms [2].

This evolution can be observed in the plants of Swartzia genus since S. polyphylla, S. ulei, S. leiocalycina and S. laevicarpa produce isoflavonoids and S. arborescens and S. langsdorffii produce abieta-8,11,13-triene diterpenoid derivatives named swartziarboreols [3].

In order to explain the biodiversity of isolated swartziarboreols, it is reported the proposal of a biogenetic pathway based in that reported for ferruginol [4], including a pair of epimers (1, 1’, 2 and 2’), which were recently detected [5].

References:

Propolis is a complex resinous substance used by bees to seal their hives. It is gathered from plants modified by the bees to produce the finished material. A range of medicinal properties have been attributed to propolis, antimicrobial, anti-hepatotoxic, anti-inflammatory, immunostimulant, anti-oxidative, anti-cancer properties, among others. Propolis may consist of flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, sesquiterpenes, quinones, coumarins, steroids, amino acids, sugars, proteins and inorganic compounds. The exact composition of propolis varies according to its variety and geographical source. The most often commercialized propolis type in Brazil is green propolis, which has plant material collected by bees from *Baccharis dracunculifolia*. A new type of propolis from Maceió (AL), namely red propolis, has been shown to derive from *Dalbergia ecastophyllum* and to have composition distinct from other known propolis types. The purpose of this research was to determine the total contents of phenolic compounds and total flavonoids and identify individual constituents of a sample of Maceió red propolis, and to evaluate its antimicrobial and anti-oxidative activities. Total phenols were determined by the Folin–Ciocalteu method and total flavonoids were determined by the AlCl₃ method. Identification of constituents was based on HPLC/ESIMS and GC/EIMS analyses. Antimicrobial activities determined using the macrodilution method in broth with the following microorganisms *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Escherichia coli*, *Proteus mirabilis* and *Streptococcus pyogenes*. Antioxidant activities were evaluated by DPPH and β-caroten/linoleic acid methods. Methanolic extracts exhibited 24.13% of total phenols and 3.55% of total flavonoids. Mains constituents identified were the isoflavonoids 2’-hydroxy-4’,7-dimethoxyisoflavan, 7,2’-dihydroxy-4’-methoxyisoflavan (vestitol) and 2’,4’,7-trihydroxyisoflavonone. Other constituents found were the phenylpropanoids *trans*-anethol, methyleugenol, elimicin, methoxyleugenol and cis-asarone, and the triterpenic alcohols α- and β-amyrins and lupeol. Methanolic extracts (2.0 mg/mL) exhibited 84.5% of antioxidant activity relative rutin by the β-caroten/linoleic acid method, while 25 µg/mL methanolic extracts had 39.1% of antioxidant activity relative rutin by the DPPH method. Methanolic extracts inhibited growth of all microorganisms tested.

FAPESP, CNPq
Coumarins from *Euxylophora paraensis* and their Chemosystematics Significance

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*Euxylophora paraensis* Hub. (Rutaceae) is a South American tree which is sometimes used in the United States as 'Brazilian Satinwood' for the manufacture of fine furniture [1]. It is known to contain a series of rare indolopyridoquinazoline alkaloids related to 1-hydroxyrutaecarpine, 2-quinolone N-methylflindersine, 4-quinolones spectabiline, furoquinoline skimmianine and two 4-hydroxy-2,2,6-trimethyl-3,4,5,6-tetrahydro-2H-pyran[3,2-c]quinolin-5-one and 4-(2,3-dihydroxy-3-methylbutoxy)-1-methylquinolin-2(1H)-one, dimeric quinolinone alkaloids, as (6α,7α,14α)-6,6,9,16-tetramethyl-7-(2-methylprop-1-enyl)-6,6a,7,9,14a,l6-hexahydro-H,15H -quino[3″,4″:5′,6′]pyrano [2′,3′:4,5] pyrano-[3,2-c]quinoline-8,15-dione [2].

Our taxonomic interest in the Rutaceae, stimulated an investigation of the leaves and stem of *E. paraensis*. Stem afforded N-methylflindersine, limonoid limonin and two coumarins marmesin (1) and 8-methoxymarmesin (2) [3]. The extracts from leaves afforded three coumarins pimpinellin (3), 8-methoxypsoralen (4), lanatin (5) and the furoquinolin alkaloid γ-fagarin. Coumarin 5 appears to be new as natural product, it was found at literature as synthetic derivative [x]. This is the first record of the five latter coumarins from *Euxylophora*. The results provide firm support for including this genus in the Rutaceae.

ANTHRANILATE ALKALOIDS, COUMARIN AND LIMONOIDS FROM 
NYCTICALANTHUS SPECIOSUS AND THEIR CHEMOSYSTEMATICS 
SIGNIFICANCE

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Nycticalanthus speciosus Ducke is a tree found only in north of Brazil, Manaus, 
Amazonas [1]. No previous phytochemical work has been reported on Nycticalanthus. 
Recently, we have described the isolation and identification of six known alkaloids dictamine, 
γ-fagarine, skimianine, 1-methyl-4-methoxyquinolin-2-one, N-methylflindersin and 
rutaecarpin, the new alkaloid 8,8a-seco-8-carboxy-14-N-methylrutaecarpin, the coumarin 
 seselin, and three new limonoids methyl 2-hydroxy-epiisoobacunoate (1), methyl 2,21-
dihydroxy-23-oxoepiisoobacunoate (2) and 7-acetoxy-21-hydroxy-23-oxoteleanine (3) [2]. 
Thus, our taxonomic interest in the Rutaceae stimulated an investigation of other plant organ 
of N. speciosus. The extracts from the stem bark afforded the limonoids 1, methyl 2-acetoxy-
21-hydroxy-23-oxoepiisoobacunoate (4), two alkaloids 4-methoxyquinolin-2-one (5), 
dictamine (6) and the coumarin 5-methoxyseselin (7).

Seção 1.01   The results provide firm support for including the genus Nycticalanthu 
in the Rutaceae.

espécie Nycticalanthus speciosus (Rutaceae) e biossíntese de cumarinas preniladas. Tese de 
Saint-Hilaire (1823, 1824, 1825) cited Cusparia, Angostura, and Conchocarpus among the synonyms of Galipea Aubl., and described several new species in Galipea and two in Ticorea Aubl. Those he described in Galipea belong in Conchocarpus, and those he described in Ticorea belong in Galipea. His erroneous circumscriptions of these genera were perpetuated by de Candolle (1824), Jussieu (1825), and Bentham & Hooker (1862). Engler, in his treatments of the Rutaceae in Martius’s “Flora Brasiliensis” (1874) and in “Die Natürlichen Pflanzenfamilien” (1986, 1931), defined these genera somewhat more clearly. He limited Ticorea to species with five fertile stamens by transferring to Galipea the two species (with two fertile stamens) described by Saint-Hilaire. He limited Galipea to species with connate carpels by excluding from it the apocarpus species, i.e., the species described by Saint-Hilaire (which, as noted above, belong in Conchocarpus) and the type of Angostura, which he called Cusparia trifoliate (Willd.) Engl. He recognized these as one genus, Cusparia, still confounding Angostura and Conchocarpus. He also failed to realize that his Galipea bracteata (Nees & Mart.) Engl. was congeneric with his C. trifoliate. These errors were rectified by J. A. Kallunki & J. R. Pirani (1998) recently. Angostura Roem. & Schult. as understood by Engler was defined more narrowly by J. A. Kallunki & J. R. Pirani. The species excluded from Angostura were recognized as species of Conchocarpus J. C. Mikan. Three new species of Angostura (A. alipes Kallunki, A. quinquefolia Kallunki, A. simplex Kallunki) were described, and three new combinations in this genus were made [A. bracteata (Nees ξ Mart.) Kallunki, A. granulosa (Kallunki) Kallunki, A. longiflora (K. Krause) Kallunki]. They recognized seven species of Angostura, the six latter and its type A. trifoliata (Willd.) T. S. Elias. The major source of both 2-alkyl and 2-arylquinolines are these genera. The co-occurrence of different structural types of quinoline and acridone alkaloids suggests affinity between these genera and supported J. A. Kallunki & J. R. Pirani taxonomic conclusions (1998).

The increasing interest in the use of medicinal herbs requires consistent and fast methods for the identification of the phytochemical constituents and for the quality control of phytotherapics. In the case of non-polar extracts, the biological and pharmacological activities were often believed to be due to a number of triterpenoid and steroid compounds, such as α- and β-amyrins, lupeol, betulin and betulinic acid\(^1,2\). As part of our ongoing research on bioactive compounds from Brazilian plants for the treatment of tropical diseases, we have investigated species of *Casearia*, a genus belonging to the Flacourtiaceae family, with approximately 1300 known species\(^3\). In this work, we have employed gas chromatography (GC-FID) to investigate the hexane extracts composition of four *Casearia* species (*C. gossypiosperma*, *C. obliquoa*, *C. decandra*, and *C. rupestris*).

The analysis were carried out using the Crevelin *et al.*\(^4\) modified method, which is rapid and simple, and does not require pre-derivatization of the crude botanical extract. The extracts (10 mg – leaves and twig) were dissolved in chloroform (3 mL) and submitted to solid phase extraction on silica gel (200 mg), eluted with chloroform (10 mL). The fraction obtained was dried, dissolved again in chloroform (1 mg/mL) and analyzed by GC-FID in duplicate. All the hexane extracts were analyzed by GC-FID on a Varian model CP-3800 gas chromatograph, with temperature of injector and of flame ionization detector adjusted to 260 °C and 290 °C, respectively. The injected volume was 2.0 \(\mu\)L. SPB-50 (cross-linked 50% phenyl-methyl-silicone, 30 m × 0.25 mm × 0.25 \(\mu\)m) capillary column was employed and the column temperature was 280 °C (isotherm). Triterpenes and sterols were identified by comparison of the relative retention (RR) of the samples with the RR of the standard sterols and triterpenes. Cholesterol was used as the internal standard.

The results show that the steroid β-sitosterol is present in all the analyzed extracts. Stigmasterol and campesterol were detected only in the leaves and twigs extracts of *C. gossypiosperma*. The triterpenes α-amyrin and β-amyrin were only detected in *C. gossypiosperma* (twigs) and *C. rupestris* (leaves), respectively, while the lupeol acetate was detected in twigs of *C. gossypiosperma*. These results clearly demonstrate that the chemical profiles of the four *Casearia* species concerning sterols are very similar, just the species *C. gossypiosperma* and *C. rupestris* presented small differences in the steroidal and triterpenic composition. In summary, we have used a simple, rapid, and relatively cheap method to identify triterpenes and sterols from hexane extracts of *Casearia* species using GC-FID.

Refs.
The Neem tree is well adapted to hot, dry climates where shade temperatures often reach 50 degrees Celsius and annual rainfall ranges from 400 to 1,200 millimeters. It has successfully been introduced in northern, northeastern and western regions of Brazil. Chinaberry tree is native to Asia and was widely introduced in Brazil as an ornamental plant. These trees have a better potential to survive low temperatures than Neem trees. The latter has been grafted to stems of *M. azedarach* and the tree has been adapted well in cool climates. These grafts growing in southern regions of Brazil are part of breeding and selection program of Agronomic Institute of Paraná (Brazil) aimed at development vigorous Neem trees to be established in more cool regions. This is the first time that the graft of *A. indica* on *M. azedarach* rootstock has been investigated. Thus, roots, stems, leaves and seeds of this graft were examined in order to determine if secondary metabolites present in the rootstock could be translocated into the *A. indica*. Extraction of Azadirachtin A from all parts of the Neem tree has been described in the inaccessible Japanese patent literature (Kanaki, 2007), but it has been frequently reported to be present at highest concentration in the mature seeds (Akhila et al, 1999). However, the concentrations of azadirachtin A may vary due to fluctuating contents in the natural sources, or susceptibility of the compounds to environmental influences as heat, light, etc., or when the tree budded on a second one as *Melia azedarach* as a rootstock. High-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) was used to develop a rapid and sensitive method for detecting azadirachtin A on all aerial parts of this graft. As expected, azadirachtin A was nearly in equal proportion in fresh juvenile, matured and fully matured seed kernels, dry fully matured seed kernels. It was present in smaller proportion in fresh immature fruits, in general the content of azadirachtin A increase with growth of the fruit. On the other hand, peak area obtained in the SRM chromatogram of dry seed coat cannot be directly compared, since large differences (13.0<<20.0 mg.mL$^{-1}$) in concentration make comparisons invalid. The analysis of dry stem without bark indicated a notable concentration of azadirachtin A, but it appeared with a significant decrease in dry stem bark and flowers. The limit of detection of azadirachtin A was 25 ng.mL$^{-1}$. It means that the proposed method should have the ability to detect azadirachtin A if it had been present in leaves. The absence of azadirachtin A in leaves could be attributed to the influence of graft on the biosyntheses of this limonoid in this organ. Leaves of *A. indica* have been the subject of a number of investigations that have yielded many limonoids, but seldom have been cited the presence of azadirachtin A. Method for extracting azadirachtin A from leaves has been described in the inaccessible Japanese patent literature (Kanaki, 2007), by alkaline ionized water with a stable pH for a long period. Thus, it is premature to draw any conclusions about the role of graft on the absence of azadirachtin A, until the leaves of *A. indica* on *M. azedarach* can be evaluated by Kanaki method.

References
The family Myrsinaceae consists of nearly 1000 species of trees and shrubs spread in 33 genera and is characterized by the presence of benzoquinones, anthraquinones, flavonol glycosides and a number of triterpenoids based on oleanane and/or ursane skeleton. In Brazil, the genus Myrsine (family Myrsinaceae) is represented by at least 34 species and is widely used in traditional medicine as anthelmintics and antibacterials. Myrsine rubra M.F. Freitas & L.S. Kinoshita is a Brazilian native high sized tree, distributed in Atlantic Coast from Espirito Santo to Paraná state, occurring in sand dunes (restinga).

A chemical and antibacterial investigation of the AcOEt extract of the leaves and stems of M. rubra has led to the isolation of two active flavonol glycosides new within this specie.

Both extracts showed broad spectrum antibacterial activity against five ATCC standard bacterial strains Staphylococcus aureus (ATCC 25923), S. aureus (ATCC 29213), S. epidermidis (ATCC 12223), Enterococcus faecalis (ATCC 29212) and Escherichia coli (ATCC 25922) determined by the disk diffusion method.

These data indicate that in addition to the potential employment of many Myrsine species as anthelmintic, M. rubra can be considered a potential source of promising antibacterial agents for treatment of intestinal or respiratory tract infections in man.

References
2. L.O.M. Arot et al., Phytochemistry, 45, 1107 (1996)
CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF THE ESSENTIAL OIL FROM OCOTEA NOTATA (NEES) MEZ. (LAURACEAE).

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Plants of the genus Ocotea (Lauraceae), commonly known in Brazil as “cinnamons” (canelas), are traditionally used in folk medicine for treating several diseases which manifest themselves by symptoms such as pain, neuralgia, dyspepsia and anorexia.1 Many Ocotea species have caught the attention of phytochemists and pharmacologists for the presence of unusual lignans, neolignans and alkaloids, and some of them are reputed traditional remedies or possess a proven pharmacotherapeutic action.2 Due to the importance of this genus, we report in this work the chemical study and biological activity evaluation of the essential oil from Ocotea notata (Nees) Mez, which is distributed over the Brazilian Coast from Sergipe to Paraná State, mainly occurring in sand dunes regions (restinga).

The leaves were hydrodistilled according to Brazilian Pharmacopoeia methods, and 1,0 mL of an yellow-green oil was obtained. Essential oil yield was determined on a volume to dry weight basis as 0.12%. GC-MS analysis of the oil led to the identification of 12 constituents (83,08% of the total) by their quantitative data and Kovat’s indices. The major constituents were mainly sesquiterpenes (54,69%): germacrene A (22,67%) and β-caryophyllene (22,88%) were the most abundant. The concentration of monoterpenes was 28,39%, with important amounts of α-pinene (8,65%); β-pinene (6,65%) and terpinolene (5,5%).

In vitro general toxicity of the essential oil, obtained by means of Brine Shrimp Lethality Test (BSL), was also evaluated. The oil exerted a relatively high toxic activity with LCso 2.37 μg/mL.

Antibacterial activity of the essential oil was also checked by employing the standard disks diffusion technique against gram positive (Enterococcus foecalis ATCC 29212, Staphylococcus aureus ATCC 25923, S. aureus ATCC 29213, S. epidermidis ATCC 12223) and gram negative strain (Escherichia coli ATCC 25922).3

The essential oil of O. notata leaves proved to possess interesting properties, emerging from both its chemical composition and from the evaluation of its in vitro biological activities. Moreover, the general toxicity exerted by the oil and its specific action against gram positive bacteria (E. foecalis, S. aureus, S. epidermidis), may suggest its use as a functional fragrance.

References
With the richest primate fauna of the world, Brazil shelters 17 genera and 70 species of monkeys and tamarins, of which approximately 50% are endemic. Although Amazônia concentrates the highest diversity of primates, some of the most threatened species are found in the Atlantic Forest, exactly for being endemic of an almost destroyed ecosystem, with all its remainders already degraded. The golden-lion-tamarin Leontopithecus rosalia, endemic of the State of Rio de Janeiro, became the symbol of a well succeeded program of reproduction in captivity, as a result of the national and international effort for its survival. Usually, the food selection by primates is related with its palatability or digestibility, caloric or nutritional value, or the degree of toxicity. Thus, \( L. \) rosalia prefers leaves with high protein value and low levels of fibers and secondary metabolites (digestive inhibitors and toxins). The present study aimed to analyze some aspects of the chemoecological relation between \( L. \) rosalia and vegetable species. A careful bibliographical survey of the plant species used in the feeding of the golden-lion-tamarin was carried out, and was followed by field observations on the behavior of groups of golden-lion-tamarins in relation to the vegetal species inside of the Conservation Unit REBIO-Poço das Antas (RJ). The vegetal species were harvested from Fazenda do Estreito, inside the limits of the EPA Bacia do Rio São João (RJ). Leaves of Cecropia hololeuca, Henriettea saldanhaei, Miconia cinnamomifolia and Miconia latecrenata and fruits of \( C. \) hololeuca and \( Euterpe edulis \) were collected, extracted with MeOH:H\(_2\)O (1:1) and submitted to total phenol analysis by the method of Folin-Denis, using tannic acid as standard. The results of the total phenol analyses revealed a high concentration in leaves of \( M. \) cinnamomifolia, \( C. \) hololeuca and \( M. \) latecrenata. In turn, a low total phenols concentration was observed in fruits of \( C. \) hololeuca and a greater one in pulp and rinds of \( E. \) edulis. It was also observed that the tamarins eat the pulp of \( E. \) edulis fruits, discarding the rind and, moreover, that they consume the fruits still green. The relation between the plants chemistry and the alimentary habit of \( L. \) rosalia seems to indicate that these primates probably possess physiological mechanisms able to diminish the activity of plant secondary metabolites.
The Instituto de Química Agrícola (IQA), so named in 1934, replaced The Instituto de Química, created in 1918, by Mário Saraiva (1885-1950), a physician from Bahia. In 1962, the IQA was closed down, and its most important researchers, among which were, Walter B. Mors, Benjamin Gilbert, and Otto Gottlieb, were dispersed throughout the country, creating new research institutes, most of them in the area of Natural Products Chemistry.

The Instituto de Química focused on agricultural, industrial and livestock research, and on contributions to the teaching of chemistry. In 1934, when it was renamed as Instituto de Química Agrícola, its attributions were modified.

Walter B. Mors joined the IQA in 1947 to research into plants toxic to livestock. At the same time, Otto Gottlieb was invited to join the IQA group of researchers; one of his activities was to extract the essential oil of pau-rosa (Brazilian rosewood). In 1958, invited by Walter Mors, Benjamin Gilbert came to Brazil to work with infrared spectroscopy.

After the IQA was closed down, in 1962, Walter B. Mors and Benjamin Gilbert helped to create the Natural Products Research Center at the Pharmacy School in the then called Universidade do Brasil, today, The Núcleo de Pesquisas de Produtos Naturais of The Universidade Federal do Rio de Janeiro (NPPN/UFRJ). Although retired in 1991, Walter B. Mors, is still heads the research at NPPN.

After working at many Brazilian institutions, Benjamin Gilbert became in 1987 a researcher of Fundação Oswaldo Cruz (FIOCRUZ), where he is still active.

Otto Gottlieb, after a long peregrination for almost the entire territory of Brazil, structuring research groups in natural products, finally stopped at The Instituto de Química of The Universidade de São Paulo (USP), in which he stayed till his compulsory retirement. He was the Brazilian researcher who got closer to winning a Nobel Prize. Since 2003, he has been a Visiting Professor at The Universidade Federal Fluminense, UFF.

The IQA was essential to the implantation and structuring of the research groups in natural products now in existence in Brazil. After its extinction, its ideas were disseminated throughout the national territory, and these scientists we’ve mentioned deserve an important place in the history of Brazilian Science.

REFS.

1. Rheinboldt, H. Em. As Ciências no Brasil v.2;Azevedo F., org.; ed. UFRJ, 98 (1994).
PLENARY LECTURES
ABSTRACTS
Brazil, known worldwide as the country of soccer and samba, also has a fascinating history on the Chemistry of Natural Products, since the colonization times, when several European scientists came to our country aiming to discover, and to study plants of our huge biodiversity. From this ancient time, there are several important ethnographies, among these, Historia Naturalis Brasilae, written by W. Piso and G. Marcgraf in 1648, and Systema Materiae Medica e Vegetabilis Brasiliensis from Carl Friedrich Philip Martius, 1843 are considered the most important documents, which described the potential of our biodiversity, further scientifically demonstrated in the several studies published by Pelletier and Caventou, with plants of Brazilian flora containing alkaloids. Nevertheless, modern Phytochemistry’s in Brazil had its acme during the last century due to the expertise of several researchers, who contributed for the advance on Natural Product Chemistry in Brazil. Obviously, three researchers established the basis for the development and consolidation of this area. In Sao Paulo, Prof. Gottlieb studying plants from Amazonian Region, especially Lauraceae and Myristicaceae gave deep contribution on structure elucidation and biosynthesis of lignans and neolignans, and so far established important connections between secondary metabolites and plant phylogeny of various taxonomic groups. In Rio de Janeiro, Profs. Walter B. Mors and Benjamin Gilbert dedicated their efforts to secondary metabolites of medicinal plants, increasing the chemical profile of several Brazilian plant species.

In 1996, the number of lectures given at the 20th IUPAC Symposium on the Chemistry of Natural Products held in Chicago demonstrated that the main focus of developed countries was dedicated to macromolecules. Maybe inspired in the first world’s trend, the major Brazilian drug discovery was not derived from a micromolecule from plant, but was resulted from biochemical research accomplished by Prof. Sergio Ferreira. It involved bradykinin and a potentiatior derivative, which after exploited by Squibb resulted in the development of captopril, a drug used until nowadays in the treatment of high blood pressure.

Recently, the ripening of researchers who work on Natural Products, and the new trends of this area have contributed for the establishment of new groups, which subject of research are focused on the search for bioactive micro- and macromolecules, chemical ecology, marine derivative compounds, associated microorganisms, biosynthesis, and metabolomics. These new trends also require, of course, a multidisciplinary approach involving botanists, chemists, biologists, pharmacists, and pharmacologists, which is an important initiative to study our huge biodiversity.

Recently, the Biota Program, supported by FAPESP was considered a valuable tool to map and catalogue the biodiversity of the main biomes of the State, including flora, fauna, microorganisms, and insects aiming their preservation and sustainable uses. This new conception of program toward biodiversity would fit nicely into the expertise of natural product chemists: For example, molecular biology techniques would be important tools not only for biotechnology, but also in the characterization of medicinal plants. Proteomics and metabolomics open exciting opportunities for the knowledge of tropical and equatorial plant species, and for studying the effects of medicinal plants. Taking in mind these considerations, we can give tremendous contribution for the future on Natural Products Chemistry in Brazil.
The discipline of organic chemistry, with its objective of understanding the chemistry of living organisms, had its origins two centuries ago. During the 19th century, the important theoretical concepts of molecular structure and stereochemistry were developed, and great progress in recognizing the relationship between structure and reactivity was made, along with the development of synthetic methods. In the 20th century, these areas continued to make enormous progress, and many reaction mechanisms were elucidated. In addition, separation techniques improved dramatically, and physical methods of structure determination became incredibly powerful. It has long been recognized that certain compounds, such as the alpha amino acids, sugars, fats, and nucleic acid bases (“primary metabolites”) are common to just about all organisms, while others, such as alkaloids, antibiotics, terpenes, etc. (“secondary metabolites”) occur much more sporadically, perhaps only in a single species.

In the early days of studying secondary metabolites, or “natural products,” the emphasis was on characterizing the structures of compounds of particular interest, which often meant compounds with important biological activity. Interest in devising laboratory syntheses and in understanding biosynthetic mechanisms quickly followed. During the last half century, chemists have become more aware of the value of studying the roles of secondary metabolites in the lives of the organisms that produce them, and these studies have given rise to the discipline of chemical ecology. In this lecture, selected examples of elucidating the chemistry underlying signaling mechanisms among insects, spiders, snakes, and plants will be presented. Interestingly, the majority of currently used pharmaceutical compounds trace their origins to such signaling molecules, which have always been central to the drug discovery process. Newly improved strategies for the characterization of naturally-occurring small molecules hold the promise of rapid progress in the discovery of new drugs and new drug targets.

Because of the current worldwide high rate of species extinction, it is urgent for chemists and biologists to devote increasing attention to the chemical riches provided to us by the world’s biodiversity. Nowhere in the world is this opportunity, as well as the challenge of taking full advantage of it, greater than in Brazil!
Biodiversity is our only renewable natural resource. Long time ago our ancestors found all kind of uses for plants, microorganisms, etc. But as we are so used to our daily life, most people do not realize that it is based on these natural resources. We all know that milk comes from a factory! But in fact we use plants and other organisms for the production of e.g. food, medicines, dyes, flavors, fragrances, agrochemicals, clothing, paper, as well as for construction, fuel and many other applications. Much of the economic activities in the world are based on this. So we should be very grateful with the heritage of our ancestors. Still there is a lot of this traditional knowledge that has not yet been really explored, e.g. there is an estimated number of medicinal plants of 40,000-70,000 species, most of which have never been studied in detail. Using this knowledge in combination with our powerful scientific tools there are many possibilities for developing novel products and concepts from biodiversity. Biodiversity as source for developing novel medicines will be discussed in some more detail.
Pharmacogenomics of Artesunate in Cancer

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Secondary metabolites from plants serve as defence against herbivores, microbes, viruses, or competing plants. Many medicinal plants have pharmacological activities and may, thus, be a source for novel treatment strategies. We have systematically analyzed medicinal plants used in traditional Chinese medicine during the past decade and focused our interest on Artemisia annua L. (qinhao, sweet wormwood). We found that the active principle of Artemisia annua L., artemisinin, exerts not only anti-malarial activity but also profound cytotoxicity against tumor cells. The inhibitory activity of artemisinin and its derivatives towards cancer cells is in the nano- to micromolar range. Candidate genes that may contribute to the sensitivity and resistance of tumor cells to artemisinins were identified by pharmacogenomic and molecular pharmacological approaches. Target validation was performed using cell lines transfected with candidate genes or corresponding knockout cells. These genes are from classes with different biological function; for example, regulation of proliferation (BUB3, cyclins, CDC25A), angiogenesis (vascular endothelial growth factor and its receptor, matrix metalloproteinase-9, angiostatin, thrombospondin-1) or apoptosis (BCL-2, BAX). Artesunate triggers apoptosis both by p53-dependent and -independent pathways. Anti-oxidant stress genes (thioredoxin, catalase, \( \gamma \)-glutamyl-cysteine synthetase, glutathione S-transferases) as well as the epidermal growth factor receptor confer resistance to artesunate. Cell lines over-expressing genes that confer resistance to established anti-tumor drugs (MDR1, MRP1, BCRP, dihydrofolate reductase, ribonucleotide reductase) were not cross-resistant to artesunate, indicating that artesunate is not involved in multidrug resistance. The anticancer activity of artesunate has also been shown in human xenograft tumors in mice. First encouraging experiences in the clinical treatment of patients suffering from uveal melanoma suggest larger clinical trials with artesunate for cancer treatment in the near future.

Active compounds from plants used traditionally to treat cancer

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Drugs based on natural products from plants and fungi comprise a high percentage of chemotherapeutic agents in clinical use. In addition, many plants are considered to give protection against the development of cancer because of their antimutagenic, antioxidant and estrogenic properties.

Over the last 15 years our laboratory has studied plants from a variety of cultures which have the reputation of being useful in cancer. Bioassays using crude extracts have been used to test for cytotoxic effects and for activities that might upregulate the mammalian cell’s own defence mechanisms. If activity is established, bioassay-guided fractionation is carried out to isolate active compounds, whose structure is then determined using advanced spectroscopic methods. The Sulforhodamine B assay for cytotoxicity has been our standard technique and a range of cancer and non-cancer cell lines has been used in order to determine specificity. Tests for antioxidant and estrogen receptor binding effects have also been used.

Work on upregulation of glutathione-S-transferase has been investigated for some plants from Malaysia and species of *Alpinia* have shown both cytotoxic and GST-upregulation effects, thus they display a dual activity which might explain traditional usage.

As well as testing activities of the crude extracts, the possibility of their containing ‘pro-drugs’ has been investigated by comparing cytotoxicities pre- and post-incubation with enzyme systems analogous to those occurring within the body for plants used traditionally against cancer in Thailand and China. Recent studies on the mode of action of active compounds from Nigerian and Chinese species will be discussed.

References


Plant Secondary Metabolites: Their Role in the Human Diet
There is increasing evidence that plant secondary metabolites such as flavonoids and phenolic compounds have a key role in the reduced incidences of cardiovascular disease and cancer that are associated with the long-term consumption of a fruit and vegetable-rich diet. Originally these effects were thought to be due the antioxidant properties of these compounds but it is becoming evident that the diverse protective effects are not due solely to \textit{in vivo} increases in antioxidant levels and that other mechanisms are also involved. Qualitative and quantitative variations in the amounts of flavonoids and phenolic compounds in different fruits and vegetables as well as beverages such as red wine, fruit juices, tea and coffee, will be discussed. The bioavailability of these compounds is an important issue with some components being absorbed in the small intestine and others in the large intestine where they are also subjected to catabolism by colonic bacteria. This topic will be discussed with reference to feeding studies with flavonols (onions and tomato juice), anthocyanins (strawberries) and green tea (flavan-3-ols) with healthy human subjects and volunteers with an ileostomy.
The future of natural products research

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In the past, natural products made many important contributions to chemistry, biology, and medicine. Today those contributions are either forgotten or dismissed as historical relics of a bygone era, and the role of natural products is diminished. What can be done to incorporate natural products into scientific discovery, especially into modern drug discovery, in a time when high-throughput screening and genome sequencing are the major discovery paradigms? There are a number of answers, some requiring relatively minor technical changes and some requiring much deeper changes.

Since modern drug discovery is dominated by high-throughput screening, natural products simply have to be compatible with this technology. Approaches such as partial purification in advance of screening must be adopted. An example of how natural products can be adopted for use in a novel high-throughput screen called the small molecule microarray will serve as an example.

The emphasis on genome sequencing argues that natural products should make use of sequencing information and more generally DNA-based discovery methods to find new natural products. It also suggests that researchers focus on more genetically tractable organisms that can be cultured – and genetically manipulated – in the laboratory. Two examples will be used to illustrate this part of the lecture: 1) The first begins with the observation that sequenced bacterial (and fungal) genomes show many biosynthetic gene clusters for which no corresponding small molecule is known. In the case of the very well studied producer of ivermectin, *Streptomyces avermitilis*, we can associate small molecules with approximately 10% of the identified clusters, meaning that 90% of the natural products made by *S. avermitilis* remain to be discovered. Members of this 90% are called cryptic metabolites, and bacillaene and other molecules will illustrate approaches to studying them systematically. 2) One way to try to break the repression that prevents cryptic metabolites from being studies is heterologous expression of cryptic (or simply unknown) genes in a host organism. Pantocin A will show how one small molecule discovered by heterologous expression was studied, and large scale heterologous expression studies both on *Pantoea agglomerans* and *Photorhabdus luminescens* will illustrate how new molecules can be discovered.

Finally, there is the old approach of using chemical ecology to discover new molecules, but with the new twist that it should focus only genetically tractable organisms. Two examples of this approach will be given: the first involves a developmental switch in the model organism *Caenorhabditis elegans*, and the second involves the interaction of the Southern Pine Beetle, two of its symbionts, and the parasite of one of the symbionts.
Biosynthesis of Lignins and Lignans: A Tribute to Otto R. Gottlieb

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This contribution reflects upon the influence of Professor Otto on our work, on science in Brazil and throughout the world. He has left a remarkable legacy.

In this lecture, I emphasize the importance of the evolution and diversity of biochemical pathways in vascular plants. These have led to formation of a remarkable array of plant polyphenols, including allyl/proplyenyl phenols, lignans, structural lignins, (iso)flavonoids and, etc. that are present in the 350,000 or so extant species – all areas that Prof. Otto gave leadership to from a scientific standpoint. It is those phenolics that were largely responsible for the successful colonization of plants to land. Our world thus depends on their quite wonderful (biochemical) world. I discuss the progress in various selected areas, and the exciting progress that has been made.

**Allylphenol/proplyenyl phenols:** These include monomeric, highly valued, flavor and fragrance molecules such as chavicol and eugenol. Their popular use by the western civilization began with imports along the spice route several centuries ago. Yet their biosynthetic pathways were only delineated last year with discovery of chavicol/eugenol synthases (CS/ES). These proteins belong to the same family as pinoresinol-lariciresinol reductases, isoflavone reductases and phenylcoumaran benzylic ether reductases which we had characterized earlier. Their discoveries are described, together with the proposed catalytic mechanism, and the future potential of these phenols.

**Lignans:** In an analogous manner, lignans have a long, remarkable, history of medicinal usage, in protecting against various diseases, as well as in plant defenses. For example, podophyllotoxin (an antiviral) can be converted into etoposide, tenoposide and Etophos®, which are widely used in cancer treatment. Others such as tetramethyl nordihydroguaiaretic acid show excellent promise in treatment of recalcitrant head and neck malignancies in NIH-supported trials.

The progress made in defining the biochemical processes in lignan formation are described: this includes mechanistic aspects involved in dirigent-mediated stereoselective coupling of E-coniferyl alcohol to afford (+)-pinoresinol, as indicated by site-directed mutagenesis, assays, and X-ray structure. In addition, the basis for enantiospecificity of pinoresinol-lariciresinol reductases which can lead to various health-promoting and/or medicinal lignans is discussed, together with the current knowledge of how the creosote bush lignans (nordihydroguaiaretic acid) and related lignans are formed.

**Cell wall biosynthetic lignins:** Currently a rather controversial topic involves that of lignin macromolecular assembly and configuration. These biopolymers are very highly conserved, in terms of strong evolutionary pressure to form them from monolignols (as well as partially from monolignol esters in grasses.) The trials and tribulations that the lignin field has undergone for nearly five decades are described, this resulting in large part because no experiments had ever been designed to distinguish between a presumed random versus that of non-random assembly. Detailed discussion is given of the monolignol forming biochemical machinery, and modulation of its biosynthesis (e.g. mutants/down-regulated plant lines and the phenotypes so obtained). In particular, attention is given to beginning to resolve the severe limitations that currently exist in macromolecular lignin analyses. Discussed are trends obtained consistent with formation of a small but finite number of lignin macromolecular structures that are monolignol-derived.
Advances on the Assignment of Absolute Configuration by $^1$H-NMR

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Abstract:
The assignment of the absolute configuration by NMR is particularly useful in cases where the amount of sample is limited, no monocrystals are available or a fast and inexpensive method is needed. In its classical approach, the substrate is separately derivatized with the two enantiomers of a chiral auxiliary reagent and the absolute configuration is obtained by comparison of the $^1$H-NMR spectra of the two resulting diastereomers (1). Alternatively, two spectra of only one derivative taken at two different temperatures or before/after addition of a chelating agent can be employed (2). Also, modifications that reduce or eliminate the manipulation of the sample, making the assignment an almost automatic procedure, have been described (1).

In this presentation, I will show first the foundations of the procedure and its application to a variety of monofunctional chiral compounds (alcohols, amines carboxylic acids, cyanohydrins…). Next, application of this methodology to the assignment of some polyfunctional compounds such as diols, aminoalcohols and triols (3) will be presented and illustrated with their actual $^1$H-NMR spectra.

References:
SHORT LECTURES
ABSTRACTS
The discovery of a new chemical substance frequently triggers a breakthrough in basic science.\textsuperscript{1} Various natural products with extraordinary structures and significant biological activities have been isolated and characterized from marine organisms. These bioactive compounds are promising candidates for drugs or biological probes for physiological studies. It is important to search continually for unexpected and unforeseen compounds from nature. To overcome the difficulty of targeted pursuit for such bioactive key compounds, careful observation in the field is extremely important.

Recently, we isolated two bioactive compounds from the symbiotic marine dinoflagellate \textit{Symbiodinium} sp., and named symbioimine\textsuperscript{2} and symbiodinolide.\textsuperscript{3} Symbioimine significantly inhibited the differentiation of RAW264 cells into osteoclasts. Thus, it is an antiresorptive drug candidate for the prevention and treatment of osteoporosis in postmenopausal women. Symbiodinolide, a novel 62-membered polyol macrolide, exhibited a potent voltage-dependent \textsuperscript{2+} channel-opening activity at 7 nM. The isolation, structure, biological activities, and synthetic studies of these two compounds will be described.

References
\textsuperscript{1} Uemura, D. \textit{Chem. Rec.} \textbf{2006}, 6, 235.
Solanaceae is an economically important botanical family either as source of foods or pharmacological active principles. A wide array of bioactive secondary metabolites have been isolated from solanaceae species, like alkaloids, whitanolides, sesquiterpenes, sugar esters and many other chemical classes of natural products. Despite their usefulness for different human activities, the great majority of these compounds are components of either the preformed or the induced defensive system of the plant. To understand properly their role within this system, it must be kept in mind that, from an ecological point of view, interactions between plants and other organisms are essentially physico-chemical in nature. The border line for these interactions is at the surface of the leaf. An insect is either attracted or repelled towards a plant, following complex and specific physicochemical cues, a neighbouring plant can be inhibited through the release of allelochemicals from the surface of the plant and many parasitic microorganisms like fungi and bacteria must germinate there prior invasion of the vegetal. A very interesting way of combining these defensive and communication tools often found in a great number of solanaceae species is through epidermal hairs. Almost twenty different types of hairs have been described for solanaceae and one third of them are glandular. Trichomes are glandular hairs where the plant stores bioactive compounds and freed them into the environment due to an external stimulus. Sugar esters are wide spectrum bioactive natural products secreted by type IV trichomes in Nicotiana, Datura and Solanum genera, which have found an application as bioinsecticides in the US market. Of the different bioactivities described for these compounds the allelopathic, anti-insect, antibacterial and antifungal ones can be highlighted. Structurally, they are poliesters of common sugars like sucrose and glucose and short chain ramified fatty acids. Looking for new germoplasms sources of these compounds, we broaden the study of the trichome bearing solanaceae, looking for new sugaresters structures and selective bio-activities. SEM investigation of the leaf surface in some native Solanaceae species allowed the recognition of Type IV trichomes and the chemical investigations confirmed the presence of acylsugars. Normal sugaresters will be reported for the first time in inflorescence trichomes of Nicotiana glauca, a glabrous native shrub where the presence of such compounds is ecologically relevant and in Salpichroa, a Solanaceae genus where sugar esters will be reported for the first time. New type of structures of acyl sugars from weeds that are non tuberous Solanum species will be presented. These new structures are glycosides of different sugar (arabinose, arabinoxylans, rhamnogluicosil) molecules and medium to long chain R-β-hydroxiacids, n-esterified with lauric, miristic, palmitic and stearic acids. The biological activity of these compounds have been tested in antiinsect, allelopathic, antifungal and antibacterial bioassays and the results obtained will be discussed in terms of the ecological significance of these findings. These compounds have shown a very broad spectrum in the different bioassays. Acyl sugars from non tuberous Solanum species showed a level of bioactivity which in some cases is similar to that of commercial agrochemicals.
In Brazil, five different native vegetation types can be found: forest (floresta amazônica and floresta atlântica), savannah (cerrado), prairie (caatinga), and mangroves and sandunes (dunas e manguezais). From them, the “caatinga” (in the native language “clear forest”), characteristic of the northeastern Brazil, is the most neglected and unexplored relative to its importance as a source of plants with chemical constituents showing potential use.

The Ceará State, one of the poorest Brazilian states, in the middle part of the region inside the “caatinga dominium”, besides its wonderful beaches and sand dunes is privileged with at least two other vegetation niches of Atlantic forest and savannah, thus becoming a prolific source of plants with potential use. Its people, either peasant or urban, make use of several plants with ethnobotanic value making them excellent subjects for research purposes and we have, as part of our research endeavor, studied several native plants with reputed popular medicinal uses.

In the “caatinga”, where just a dry and a rainy season occur, is incredible high the incidence of plants presenting pleasant smell what makes it a source of economically exploitable essential oil producer plants such as *Lippia sidoides*, *Ocimum gratissimum* and several others from the Euphorbiaceae, Lamiaceae, Mirtaceae and Verbenaceae, for instance. *Myracrodruon urundeuva* (Anacardiaceae), a well disperse tree through the caatinga, and popularly used as anti-inflammatory, has shown at least five chemotypes based on the major volatile component of its essential oil: $\gamma$-ocimene and $\gamma$-3-carene, as the major ones, followed by limonene, $\delta$-pinene and myrcene.

Related to the non-volatile compounds we have focused on the Asteraceae (*Egletes viscosa*), Boraginaceae (*Cordia piauiensis*), Leguminosae (*Amburana cearensis*, *Copaefera langsdorfi and Harpalyce brasiliana*), and the Solanaceae (*Acnistus arborescens* and *Physalis angulata*). Through a multidisciplinary program (Chemistry, Botany, Agronomy and Pharmacology) we have studied those plant species to characterize a vast class of secondary metabolites such as flavonoids (particularly the pterocarpan), steroids (including the whitasteroids), phenylpropanoids, quinone and hydroquinone terpenoids, mono, sesqui, di and triterpenoids, and saponins. The pharmacological activities range from antimicrobial to anti-inflammatory, muscle relaxant, anti-ophidian and, particularly, cytotoxic. For *Egletes viscosa* and *Amburana cearensis* seed propagation experiments were successfully performed making them available for further studies.

The project has received grants from the funding Brazilian governmental agencies: **CNPq, PRONEX, CAPES, FINEP, FUNCAP** and **BNB**
The state of Mato Grosso do Sul, located in the central-western region of Brazil, is home of three major biomes, namely “cerrado”, “pantanal” and tropical forest. The “cerrado” landscape, which is characterized by extensive savanna-like formations, with different vegetation types, including grassland with scattered shrubs and trees with peculiar leathery leaves and twisted trunks covered by a thick bark, consists of 61% of the state vegetation. The “pantanal”, the world’s largest wetland area, which was recognized by UNESCO as one of the most exuberant and diversified natural Earth resources, is found in Brazil in only two states: Mato Grosso and Mato Grosso do Sul, the latter comprising 2/3 of the total area of the Brazilian “pantanal”, which is 150,355 km$^2$. The vast range of biodiversity of the two ecosystems has led to an increasing interest in the chemical investigation of plants endemic to these regions, as rich sources of biologically active compounds.

One of the studies developed by our research group in the Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil, is the search for potential anticancer constituents in plants collected in the “cerrado” and “pantanal” of Mato Grosso do Sul, by evaluating the mutagenicity, antimutagenicity and cytotoxicity of their crude extracts and isolated compounds. The methods employed for assessing these bioactivities comprise the following assays: the micronucleus test (evaluation of micronuclei frequency in the peripheral blood of fish), analysis of DNA strand breaks using the comet assay (single-cell gel electrophoresis) on mammalian cells, the wing somatic mutation and recombination test (SMART) in Drosophila melanogaster and cytotoxicity against human cancer cell lines (MTT colorimetric assay). In this lecture, some of the significant results obtained by our group in this study will be presented.
Bioactivity-guided chemical studies of natural products isolated from plants that interact and inhibit photosynthesis.

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In the photosynthetic machinery of chloroplasts occur the energetic metabolism of plants. This is a preferred target for allelochemicals that inhibit PSII at different level of the electron transport redox chain of the chloroplast. Interruption of the electron transport in the electron transport chain of PSII or PSI by natural product can be detected by polarography or by transient of chlorophyll $a$ fluorescence induction curve (Kautsky curve), known as JIP test. Secondary metabolites that transform the regular OJIP sequence into an OJ curve have a similar site of interaction and inhibition of the phenylureas and triazines herbicides, or the natural product sorgoleone, which act as inhibitors of the D1 protein of photosynthetic electron transport chain located at the reducing side of PSII, that displaces $Q_b$. Secondary metabolites as tricolorin A isolated from Ipomoea tricolor (Achnine et al., Physiol Plant., 1999) and trachyloban-19-oic acid, isolated from Iostephanne heterophylla (Asteraceae) (Hernández-T. et al., Pestic Biochem Physiol 2003) behaved like DCMU herbicide. Other natural products affect the J – I phase of fluorescence in a similar way as Tris does, which is an indication that the site of interaction and inhibition is the water-splitting enzyme of chloroplasts. Odoratol is a natural protolimonoid, isolated from Cedrela odorata (Meliaceae). Comparative analysis of odoratol and some of its derivatives indicate that the diol moiety at position 23 and 24 of the side chain of this compound is an important structural requirement for its inhibitory activity (Achnine et al., J. Agric Food Chem 1998). Annonaceous acetogenins as squamocin, bullatacin and motrillin, bis-tetrahydrofuran isolated from Annona purpurea (Annonaceae) inhibited ATP synthesis and uncoupled electron transport, indicating that act as Hill reaction inhibitors. Their target site was located at the oxygen-evolving complex (OEC). These acetogenins also present uncoupler activity as they enhanced light-activated Mg$^{2+}$-ATPase, and basal electron flow (Chàvez et al., Physiol Plant 2001). Bioactivity-guided fractionation and isolation. - The air-dried leaves of Croton ciliatoglanduliferus were ground and extracted at room temperature with $n$-hexane. The solvent was evaporated in vacuo to give a crude extract that inhibited ATP synthesis. The $I_{50}$ value was 42 ppm. To know the compound responsible for this inhibitory activity, the n-hexane extract was fractionated by column chromatography then by thin-layer chromatography: two compounds were isolated and characterized as the flavonoids retusin (5-hydroxy-3,7,3¢,4¢-tetramethoxyflavone) (1) and pachypodol (5,4¢-dihydroxy-3,7,3¢-trimethoxyflavone) (2). Both compounds were separated by repeated runs in preparative thin-layer chromatography (hexane-ethyl acetate 80:20 v/v), and their structures were confirmed by comparison of their physical (melting points) and spectroscopical properties (UV, 1HNMR, 13CNMR, HMQC, HMBC, and NOESY data). Compound 2 was the most active on ATP synthesis inhibition. The $I_{50}$ value was 51 $\mu$M. 2 Behaves as a Hill reaction inhibitor. It inhibited the uncoupled electron flow on photosystem II partial reaction from water to dichlorophenol indophenol (DCPIP) and from water to sodium silicomolybdate. However, the uncoupled partial reaction from diphenylcarbazide to DCPIP and the uncoupled photosystem I from DCPIP$\text{red}$ to MV were not inhibited by 2. Therefore, pachypodol inhibits the water-splitting enzyme activity (González-V. et al., J. Agric Food Chem 2006). Bioactivity-guided fractionation of the dried stems of Croton ciliatoglanduliferus led to the isolation of labdanes as labdane 8α,15 diol (1) and its acetyl derivative (2). Both labdanes show inhibitory activity of ATP synthesis and electron flow. The site of inhibition on thylakoids electron transport chain was located for 1 at the oxygen-evolving complex (OEC), and for 2 at the oxygen-evolving complex (OEC) and at $P_{680}$ in PSII and at the span of P700 to FX in PSI. This is the first time that we found an allelochemical that inhibits PSI, the labdane 8α,15 diol (Morales-F. et al., Photosynth Res, 2007).
THE ACTIVATION OF PLANT RESISTANCE AND THE RELATIONSHIP BETWEEN PLANT PHYTOPHAGOUS INSECTS AND PARASITOIDS.

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Plant resistance to insect relies on the ability of individual cells to perceive herbivores, to transmit this information systematically, and them to trigger defenses that impair herbivore performance. Plants have evolved a variety of mechanisms to withstand the damage and stresses caused by pathogens and herbivorous animals, as well as by many abiotic factors. One such mechanism involves the emission of volatile compounds, either constitutively or as a result of biotic infestation or physical damage, which can affect pathogen development and the behaviour of insect herbivores searching for a food source. Plant volatiles that are induced on damage to repel insect attack also can act as an indirect plant defense mechanism by attracting other insects that prey on or parasite the herbivores. Such compounds may act as signals between plants, whereby defense mechanisms are induced in undamaged plants in response to volatiles produced by neighbouring infested plant. We will discuss the role of volatiles produced by plant defense mechanism during herbibory act by indirect effect by attracting predators and parasitoids of pest insect.
Natural products and analogues with antifungal properties

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The \textit{in vitro} antifungal properties and studies of mode of action of natural compounds and synthetic analogues are reported here.

Bioautography and dilution assays were used as a first order screen to determine the Minima Inhibitory and Fungicidal Concentrations (MIC and MFC) following the guidelines of NCCLS (now CLSI). Then, higher order tests such as the determination of MIC\textsubscript{80} and MIC\textsubscript{50} % inhibition at different concentrations, time to-kill studies against standardized as well as clinical isolates of fungal species, and others, allowed us to have a wide view on the antifungal properties of the compounds under study. Targeted assays including the detection of fungal cell wall inhibitors, the binding to ergosterol, the observation of curling, shortening and other type of hypha malformations were performed to the most active compounds. The synthesis and evaluation of series of analogues of active compounds allowed us to perform SAR studies and to design better structures for further development.

Extracts from fruits of \textit{Phytolacca tetramera} (P.t.), aerial parts of \textit{Polygonum ferrugineum} (P.f.), \textit{Polygonum acuminatum} (P.a.) and \textit{Zuccagnia punctata} (Z.p.), showed the best activities against a panel of standardized opportunistic and pathogenic fungi for human beings. The bioassay-guided fractionation led to the isolation of Phytolaccoside B from P.t., three chalcones and for the first time an homoisoflavanone from P.f., polygodial from P.a., flavonoids, chalcones and a cinnamic ester from Z.p. In addition, the isolation and characterization of antifungal soybean infecting fungi allowed us to have a panel of phytopathogenic fungi against which new compounds from \textit{Zuccagnia punctata} were detected.

Synthetic series of chalcones, homoallylamines, and cyclic imides analogues of those isolated from natural sources, were tested and SAR studies were performed with them. A thorough study on the stability of cyclic imides into the growth media allowed us to determine the structures responsible for the activity.

Acknowledgements: ANPCyT PICT R 260, RIBIOFAR (CYTED), UNR.

References:

1-Escalante et al., \textit{J. Ethnopharm.} 82, 29, 2002;
2-López et al., \textit{Phytochem.}, 67, 2152, 2006;
3-Derita et al., Biochem. Syst. Ecol. 2007, in press;
4-Svetaz et al., Planta Med. in press, 2007; 4Svetaz et al.,
5-J. Agric. Food Chem. 52, 3297, 2004;
8- Suvire et al., Bioorg. Med. Chem. 14,1851, 2006;
11-Sortino et al., manuscript to be submitted to Bioorg. Med. Chem. 2007.
Natural products, especially those derived from higher plants and microorganisms; have over the centuries, contributed greatly for the development of modern therapeutic drugs. Some important classes of drugs currently used in the therapeutic, namely, anti-cancer, analgesics; antibacterials were developed directly or indirectly from natural sources. Natural products can be used in several ways to develop new drugs: a) they are used as source of direct therapeutic agents, (both as pure drugs and phytomedicines);b) they serve as raw material for elaboration of complex semi-synthetic drugs; c) they are used as prototypes for design of lead and complexes molecules and d) they can be used as taxonomic markers for discovery of new drugs. Currently, about one-third of the best-selling drugs in the word are natural products or their derivatives.

In the second part of my presentation, I will discuss some recent pre-clinical studies carried out with the Brazilian medicinal plant *Cordia verbenacea*. Since 1998, our own group and several other Brazilian research groups have studied this plant with the aim of developing a phytomedicine with scientific proof of safety, efficacy and quality according to the Brazilian guidelines established by ANVISA. The essential oil obtained from the leaf of *C. verbenacea* was studied phytochemically every month for a year and the active markers - the sesquiterpenes α humulene and trans-caryophyllene - were analyzed both phytochemically and pharmacologically to investigate their seasonal fluctuation. Preclinical topical and systemic anti-inflammatory and antinociceptive studies were carried out with the oil and active compounds from *C. verbenacea* in several pharmacological in-vivo models of pain and inflammation. The oil and active compounds α-humulene and trans caryophyllene revealed a strong and long-lasting anti-inflammatory and antinociceptive actions with potency and efficacy comparable with the anti-inflammatory drugs currently available on the market. Their mechanisms of action are quite complex and are related mainly with their ability to suppress the expression of the key enzymes involved in the inflammatory processes such as, COX2 and iNOS and also the synthesis and expression of the pro-inflammatory cytokine, namely TNFα and IL1-β, due to their ability to interfere with the activation of nuclear factor ©§B. Preclinical acute and repeated toxicological studies carried out on rodents and dogs revealed that the essential oil of *C. verbenacea* standardized as 0.5% in α humulene was well tolerated, presenting no evidence of important side effects. After several multicenter clinical studies (phase I, II and III) the topical anti-inflammatory product Acheflan® produced from *C. verbenacea* was approved by the Brazilian FDA-like agency NVISA and is currently in the market to treat trauma, tendinitis and myofascial pain.
Natural products constitute small molecules produced by plants, microorganisms, marine invertebrates, and insects. Many natural products have evolved for the purpose of enhancing the survival of the producing organism and therefore should have some biological and/or ecological relevance. Over the past few decades natural products research has undergone a number of significant changes and a variety of natural products have been isolated, characterized, and synthesized, providing treatments for many otherwise incurable human and animal diseases, and agrochemicals for improved food production. Growing body of evidence suggests that plant-associated microorganisms, especially endophytic and rhizosphere microorganisms, represent a huge and largely untapped resource of natural products with chemical structures that have been optimized by evolution for biological and/or ecological relevance (Gunatilaka, 2006). Contrary to common assumptions concerning limited biodiversity in arid regions, we have encountered a rich and diverse range of microorganisms living in association with plant communities of the U.S. Southwestern desert. In our search for novel small molecule bioactive agents from this niche, we have constructed a microbial library consisting of numerous endophytic and rhizosphere fungal strains. Extracts derived from cultures of some selected strains have been screened in assays for inhibition of cancer cell proliferation and migration (Zhan et al., 2007), and heat shock modulation (Turbyville et al., 2006). Organisms producing metabolites active in these assays were identified, cultured on large-scale and the derived extracts have been subjected to bioactivity-guided fractionation to obtain a variety of natural products with diverse structures, and potential applications in agriculture and cancer chemotherapeutics. In this presentation our approach to search for bioactive and/or novel natural products from a hitherto under-explored niche and manipulation of some plant-associated and other microorganisms to obtain “unnatural” natural products will be illustrated.

Financial support for this work was generously provided by the U.S. National Institutes of Health through the National Cancer Institute (Grant R01 CA90265), and the Arizona Biomedical Research Commission.

References


A survey by the National Cancer Institute (J. Nat. Prod., 66, 1022 (2003)) showed that 61% of the 877 small-molecule new chemical entities introduced as drugs worldwide during 1981–2002 could be traced to or were inspired by natural products. These include natural products (6%), natural product derivatives (27%), synthetic compounds with natural-product-derived pharmacophores (5%), and synthetic compounds designed on the basis of knowledge gained from a natural product (that is, a natural product mimic; 23%).

The influence of natural products is therefore significant even in therapeutic areas for which they might not seem relevant, such as cholesterol management, diabetes, arthritis, and depression. The increasing importance of countries such as India and China, where the healthcare systems have for centuries and continue today to rely on their traditional plant-based medicines has helped to foster a growing interest in the West in traditional medicines and their relevance in treating many of the diseases that afflict the world today. Scientists and companies are now therefore using some of these traditional medicines and their modern counterparts as a ‘discovery engine’ for developing new licensed pharmaceutical drugs. This contrasts with last century where the advent of synthetic chemistry and the discovery and use of New Chemical Entities (NCEs) in drug development dominated the pharmaceutical industry and resulted in fewer and fewer drugs being developed from plant sources in the West.

In recent years the FDA, the EMEA and the MHRA in the UK have reviewed the regulatory frameworks governing the development and use of drugs based on natural products. The FDA Guidance states, "because of the unique nature of botanicals, the FDA finds it appropriate to apply regulatory policies that differ from those applied to synthetic, semi-synthetic, or otherwise highly purified or chemically modified drugs (including antibiotics)". Under these guidelines, botanical drugs can be developed more quickly and more cheaply than conventional synthetic NCE pharmaceuticals. In the UK it is now possible to register herbal medicines that have an established traditional use. In order to do so they need to meet required standards of safety and quality. The alternative is to obtain a product licence which requires evaluation of safety, quality and efficacy (or effectiveness). As there are so many products on the market that need to be registered, current products will not have to comply with the legislation until April 2011.

Thus there is increasing recognition that natural products for medicinal use need to be registered. Randomised controlled clinical trials are the gold standard for evaluating safety and efficacy. Design of such studies is key to getting appropriate data. Clinical trials for natural products should differ somewhat from those of pharmaceutical trials using NCEs, as most of these products have already shown a history of use but many of the basic principles are the same. These issues will be discussed in the presentation.
The texture and particularly the flavour of foods are major determinants of food preferences. Where they have a choice, few consumers are willing to substantially compromise such sensory qualities for other benefits. Reduced energy “diet” products have formed a well-established market sector, but in general there has been resistance to the adoption of diets with reduced levels of salt, fat and sugar. Some products have been successful, but only when product performance substantially matches that of the traditional product. Where functional ingredients or nutraceuticals are incorporated into foods it may be necessary to make other changes to the formulation, or to develop different products, in order to overcome such resistance. There is some evidence that male and female consumers perceive “healthiness” in different ways, and that European and North American female consumers may be more willing to select foods perceived as healthy.
A breakthrough in isoprenoid biochemistry: the discovery of the methylerythritol phosphate pathway for the formation of isoprene units in bacteria and plants

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Incorporation of tritium labelled precursors (acetate and glucose isotopomers) into the bacterial triterpenoids of the hopane series and isoprenoids from plant plastids disclosed a novel biosynthetic pathway towards isoprene units, corresponding to an alternative to the classical mevalonate pathway. Complete elucidation required molecular biology techniques, including gene identification and characterization of the enzymes.

Starting from pyruvate 1 and glyceraldehyde phosphate 2, isopentenyl diphosphate 9 and dimethylallyl diphosphate 10, the universal isoprenoid precursors are synthesized via deoxyxylulose phosphate 4, four methylerythritol derivatives 5-7 and 2-methylbutene-1,4-diol diphosphate 8. Incorporation of deuterium labelled precursors pointed out unprecedented features and threw light on the stereochemical course of the last two reactions catalysed by Fe/S cluster enzymes. This pathway occurs in most eubacteria, in the Plasmodium spp. responsible for malaria and in the chloroplasts of all higher plants, where it responsible for the formation of hemi-, mono- and diterpenes, as well as the carotenoids and the prenyl chain of plastoquinone. The most striking aspects of discovery and elucidation of this long overlooked biosynthetic route will be discussed.

M. Rohmer, Pure Appl. Chem. 79, 739-751 (2007), and references cited therein.
Phytochemical and biological investigation carried out on Piperaceae species have led to the identification of 4-nerolidylcatecol (antioxidant), amides (isobutil, piperidine, and piperitone) (insecticide, antifungal, molluscide)\textsuperscript{1-4}, chromenes and benzoic acid derivatives (antifungal, antitumoral)\textsuperscript{5,6}, tetrahydrofuran lignans (trypanocidal)\textsuperscript{7}. Based on such potential regarding bioprospecting, the investigation of their biosynthesis and regulations are of paramount importance. A number of enzymes involved in the biosynthesis of terpenes (IPP synthase and isomerases, prenyltransferases), phenylpropanoids, amides and meroterpenes (PAL, SCoA ligases, PKS III and amide synthases) are involved in the formation of a number of bioactive compounds in \textit{Piper tuberculatum}, \textit{P. aduncum}, \textit{P. arboreum}, \textit{P. crassinervium}, \textit{Piper gaudichaudianum} and \textit{Potomorphe umbellata} (Piperaceae)\textsuperscript{8-12}. The biosynthetic investigation of specific compounds and associated pathways have been carried out by means of feeding experiments with labeled precursors which have identified the major enzymes and their intracellular localization (microsome, cytosol, plastid and membrane)\textsuperscript{8-12}. The purification, analysis of proteins have been carried out by chromatographic steps, 2D-PAGE and MALDI-ToF, respectively.

References
Mimicry and Deception used in chemical communication

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The channels of communication among living organisms can be summarized as tactile, chemical, visual and acoustic. Insects seem to rely more heavily on chemical signals than on any other form of communication however more recent research reveals that even unicellular organisms like bacteria do depend on chemical communication to survive. These signals are named as semiochemicals or infochemicals and serve as a form of “language” that mediate interactions between living organisms and are divided into interspecific (pheromones) and intraspecific signals (allochemicals). Surviving means being able to fight, eat and reproduce, therefore sex attractants, trail marking and defense substances do have an enormous impact on all living organisms and these are extensively employed either “correctly” or mimicking other species pheromones in order to get a survival advantage. Well documented examples are the Orchidaceae mimicking bee virgin queen sexual pheromones, fatty acids, thus pollination is rather effective in these orchid species that offer little rewards\(^1\). Fatty acids are also the communication basis of the first-instar larvae of the blister beetle *Meloe franciscanis* which mimick female sexual pheromones of the solitary bee *Habropoda pallida*\(^2\) thus ensuring the beetles transportation and survival. Among the unicellular organisms homoserine lactones are used as chemical communication and these are fatty acid derivatives. Consequently the role of chemical substances in communication will be central focus of this presentation.

THE USE OF COUNTERCURRENT CHROMATOGRAPHY IN THE SEPARATION OF NATURAL PRODUCTS

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The separation of bioactive secondary metabolites from crude plant extracts has always been a challenge to natural products researchers and CCC offers many advantages compared to traditional phytochemical techniques of purification, especially upon those where chromatography with solid supports is used.¹ The main advantage of CCC is that it is a form of liquid-liquid chromatography, which does not use a solid support; there can be no loss of compounds or bioactivity due to interactions between the solid phase and the target compounds. Here we show some examples of the purification of plant-derived compounds using isocratic and gradient elution modes in CCC. The first example shows the isolation of dammarane triterpenos from the dichloromethane extract of leaves of Cabralea canjerana (Meliaceae), with anti-micobacterial activity. Eachlerianic acid was isolated as the major compound in this extract with solvent system hexane-ethyl acetate-methanol-water 1:1.5:2.5:1 v:v:v:v, aqueous phase as mobile. The second example shows the isolation of simple phenolic acids (protocatecuric and vanillic acids) and rhamnosyl derivatives of quercetin from the ethyl acetate extract of leaves of Bathysa australis (Rubiaceae). The solvent system was composed of hexane-ethyl acetate-methanol-water 1:2:0.8:1, organic phase as mobile. The phenolic acids and the flavonoids are excellent anti-oxidant compounds. Two examples of separation by gradient elution are the isolation of phenylpropanoids and iridoid glycosides from Stachytarpheta cayennensis² (Verbenaceae) and flavonoid glycosides from Siparuna guianensis¹ (Siparunaceae). In the case of Stachytarpheta, the ethyl acetate extract of the roots was submitted to a four-step gradient elution with the solvent system ethyl acetate-butanol-water 1:x:1, x = 0.05; 0.2; 0.5 and 1. The gradual raise of BuOH in the organic mobile phase led to the isolation of three phenylpropanoids (martynoside, isoverbascoside and verbascoside) and three iridoids, one of which ipolamiide. For the ethyl acetate extract of S. guianensis, a two-step gradient composed of hexane-ethyl acetate-methanol-water 0.6:4:x:1, x = 0.05 and 0.7 was used, followed by isocratic elution, leading to the isolation of quercetin-3-O-rutinoside and quercetin-7-O-rutinoside. All separations were done with PC INC. equipment, 80ml coil, at a flow rate of 2ml/min. and 850rpm. The separations lasted about 3-4h (except for the gradient elutions) and consumed not more than 2l of solvent. The samples varied from 200mg to 1g. These examples show the versatility of the technique.

Refs.
Natural Products encompass a very broad research area which includes several complementary activities and expertise. Due to its large complexity, this area nowadays has been subdivided in several niches according to the final interest, each one covering different aspects of the broader area. Independently of the selected sub-area, the Separation Sciences has being playing a very important role on the development of this important scientific area, being widely used for extraction, isolation, separation and identification of most Natural Products constituents in either analytical, preparative and industrial scales.

In this presentation, recent advances in the area of Separation Sciences and either its actual or potential applications to the Natural Products area will be presented and its major advantages and limitations discussed. Emphasis will be done on:

- Modern Sample Preparation Techniques (in special those environmentally friend such as SPME, SBSE,HS-SME, SFE on both analytical and Prep scale ) according to a “Green Chemistry” approach;

- High Resolution and small scale chromatographic techniques (HRGC, c-LC, UHP-LC, c-SFC, High Temperature GC, Temperature Programmed-LC, Multidimensional and Comprehensive GCxCG and LCxLC, and so on);

- Hyphenated Techniques involving separation and identification techniques (LC/LC/MS/MS), SPME/GC/MS, SBSE/LC/MS, among others.

In each case either an actual or a potential application in the area of Natural Products will be shown and discussed, emphasizing the “Green Chemistry” approach (significant reduction in the use of solvents; avoiding the use of organic solvents; dramatic reduction in waste production; use of reduced sample amounts).
Identification of quantitative and/or qualitative protein expression differences and characterization of specific proteomes (e.g., cancer cell proteome) will advance clinical diagnostics and drug discovery. Up and down-regulation and the appearance or disappearance of hundreds and sometimes even thousands of proteins can be encountered in cellular systems subjected to changes in physiological conditions, such as cancer. This lecture aims to discuss novel proteomics tools with the main emphasis on mapping diseased cell proteomes and discover new surrogate biological markers (biomarkers). Orthogonal and distinctly unrelated separation techniques such as multidimensional electrophoresis and chromatographic methods provide excellent resolving power, while their miniaturization enables rapid analysis times. Advanced bioinformatics tools are used for quantification and comparison of individual proteins and up and down-regulation domains between samples, e.g., cancer cells vs. normal cells, anticancer drug treated cells vs. non-treated cells, etc. Sophisticated data mining approaches (bioinformatics) are applied to find biomarkers of clinical interest.
ORAL SESSION A: Biodiversity & Natural Products
A Novel NMDA Receptor Antagonist Isolated from the Venom of the Social Wasp *Polybia paulista*

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Keywords: insect venom, NMDA antagonists, anticonvulsivants, social wasps.

The venomous insects use low molecular mass neurotoxins to cause injuries to mammal’s nervous systems; however, some times these toxins may present a neuroprotective action under specific physiological conditions. Wasps have been bioprospected for the search of such types of neuroprotective agents. The venom of the social wasp *Polybia paulista* was extracted and fractionated under reversed phase HPLC, resulting in the separation of 13 different fractions, which were screened for anticonvulsivant effect in male Wistar rats, by using in vitro preparation of brain slices in an electrophysiology system. The fraction presenting neuroprotective effect was submitted to structural elucidation by using NMR analysis combining 1-D ($^1$H and $^{13}$C) and 2-D (gCOSY, gHSBC and gHMBC contour map), in addition of mass spectrometry (ESI-MS, MS/MS, HRMS). In the present work, we found out a novel compound which was named as histaminyl-carboxy pyranose. This novel compound blocks strongly the glutamatergic NMDA-dependent receptors. The synthesis and further studies of this molecule can lead to new potential antiepileptic drugs in the future.

Financial Support: FAPESP/ BIOprospecTA (FAPESP) / CNPq
Inflammation-inducing Substance from Marine Animals

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The amphinomid polychaetes are popularly known as “fire worms” or “bristle worms”, and they are included among marine dangerous animals. They are frequently found at a sand-muddy beach or shallow water in the Temperate and Sub-tropical zones. They possess large satae capable of penetrating human skin, causing allergic reactions. The search of inflammation-inducing substances has been studied\(^1\); however, the bioactive substances are not yet elucidated.

In this study, we attempted to isolate the active substance guided by a direct induction of inflammation to a mouse footpad. Specimens of fire worms *Erythoe complanata* were collected on a tidal wetland of Awase beach of Okinawa island, Japan. And, we found the methanolic extract of the animals exhibited an inflammation-inducing activity to a mouse footpad by subcutaneous administration. The water-soluble bioactive substance, eurythotoxin, was purified by repeating chromatography (polystyrene beads and silica gel), and its structure was characterized by a trimethyl ammonium group and an olefinic carbon chain.

The detailed structure elucidation and biological properties of this compound will be discussed.

![Erythoe complanata](image1)

*Erythoe complanata* body length: 7 cm

![structure of eurythotoxin](image2)

structure of eurythotoxin

Ref.
NEW ALKALOIDS OF THE SEA SPONGE Aplysina fistularis

Narlize S. Lira, Ricardo C. Montes, José G. de Sena Filho, Micheline de A. Lima, Daniel Esdras de A. Uchoa, Emídio V. L. da Cunha, José M. Barbosa Filho, Raimundo Braz Filho, Celidarque da Silva Dias.

Aplysina fistularis is a Verongida sea sponge of the family Aplysinidae, presents as a tubular form, concise and elastic, yellow and frequently found in the Brazilian coast. This genre is known for their isoxazolines alkaloids with residues of bromated tirosines presenting anti-tumor, antimicrobial and toxic pharmacological activities which display a large range of main reasons to study this specie. Aiming to obtain more information about this sponge studies were carried out towards its chemical structure. The sponge was collected in João Pessoa-PB with geographic reference the localisation: 07°02’S 34°34’W. The material was identified by the zoologist Ulisses Pinheiro from the Laboratório de Invertebrados Marinhos da Universidade Estadual do Sudoeste da Bahia – UESB, and then dried out (45°C) and powdered in minced until 1.7 Kg, submitted to macerated with ethanol 95% obtaining an ethanolic extract (EE). The phytochemical screening revealed alkaloids to which the research was directed resulting in quaternaries alkaloids FAQ (2,276 Kg). The FAQ was submitted to chromatographic analysis in Sephadex LH-20 in ethyl acetate and methanol 1:1, obtaining 21 fractions organized by their Rf’s after CCDA analysis under ultraviolet light. The sample 1-3 yielded the following yellow amorphous solid and identified though 1D and 2D NMR (500 MHz) in methanol-d4 as being 2-(3-methoxy-4-(4-methoxy-5-(trimethylammonium) ethyl) furan-3-yl) furan-2-yl)-N,N,N-trimethylethanaminium.

This study showed that Aplysina fistularis holds a quaternary alkaloid still not reported in the literature and has a high importance in the specie.

Keywords: Aplysinidae, Aplysina fistularis, Quaternaries Alkaloids

Figure 1. 2-(3-methoxy-4-(3’-methoxy-2’-(trimethylammonium) ethyl) furan-2’-yl) furan-2-yl)-N,N,N- trimethylethanaminium.

Refs
Flavonoids are a widespread class of natural compounds showing wide range of biological activities. One of their main reported activities is the capacity to act as antioxidant by scavenging free radicals. The genus *Tephrosia* has been known as important source of flavonoid compounds but there are very few papers involving chemical studies of this plant. This work describes the chemical study of hexane and ethyl acetate extracts by chromatographic techniques such as column (CC), preparative thin layer (PTLC), HPLC and GC-MS, that resulted in the isolation of flavones, flavanones, chalcones, rotenoids, pterocapanes and biflavonoids. Furthermore, three new compounds, two biflavonoids and one chalcone, have been isolated. Their structures were unambiguously determinated by NMR and MS spectra.

The biflavonoid, named toxicarine B, was isolated by CC and PTLC on the hexane extract of the plant. The structure of this compound presented two equivalent flavane units linked by C-4/4a and C-5/5a, forming an extra ring. This purpose is in agreement with $^1$H and $^{13}$CNMR and NOESY spectra. Another biflavonoid toxicarine A, isolated from both hexane and ethyl acetate extracts, was identified by NMR and MS spectra. This compound is constituted by one unit of 5,7-dimethoxyflavane and one unit of the flavanone glabranine. The third compound, toxicariachalcone, presented the substituted groups at ring A with one hydroxyl, two methoxy and one prenyl groups. This pattern of oxigenation at C-5, C-7 and C-9 and C-prenilation at C-8 was observated in all of the flavonoids isolated from this plant. The chalcone structure was attributed by NMR and MS spectra.

The bioactivity of the extracts of this plant had been tested and showed, preliminarily, mortality against *Artemia salina*, antifungal properties in a TLC bioautography using *Cladosporium herbarum* and antioxidant activities evaluated by hydrogen-donating ability (DPPH$^*$ assay) and inhibition of lipid peroxidation.

CHARACTERIZATION AND DETECTION OF MAJOR MICROMOLECULAR CONSTITUENTS FROM SPECIES OF CERRADO AND ATLANTIC FOREST USING HPLC/HRMS/DAD COMBINED WITH IN SILICO TECHNIQUES


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The Brazilian biodiversity is a rich source to search for biological active compounds and its preservation is an important goal both for the intrinsic value of this enormous biological resource as well as for its potential as new sources of pharmaceuticals, cosmetics, and agrochemicals. Recently we are incorporating the development of dereplication methodologies using hyphenated chromatographic techniques such as HPLC/HRMS/DAD to accelerate the screening of crude extracts from plant species that exhibited prior in vitro antioxidant and antimalarial properties. Quite recently we are combining in silico techniques, using well-known established data-bases, to construct a micromolecular profile of the crude matrixes helping on the selection of valuable plant species. In this work we will discuss how the combination of those techniques was useful for the study of endophytic fungi and plant extracts of species from Cerrado and Atlantic Forest, specially the case of Strychnos brasiliensis (Loganiaceae) a genus bearing as chemomarkers monoterpene indole alkaloids. The crude extract from S. brasiliensis (1.0 mg) was initially analyzed by HPLC/HRMS/DAD. The resulting TIC (Total Ion Chromatogram) was analyzed, processed and interpreted, generating for each major extracted peak, a high resolution molecular formulae. Combining this data with a major natural product database, we were able to detect some indolic monoterpene alkaloids such as brucine and icajinine, previously reported in other Strychnos species. The detection of quinolinic alkaloids such as cusparine, chimaicine and mainly quinine, called our attention since S. brasiliensis is commonly confused with S. pseudoquina, a nearby species popularly used against malaria. Some triterpenes such as fridelin, β-amirin, β-sitosterol and stigmasterol were also generated for the detected mass fragments. The co-occurrence of fridelin and β-amyrin which showed an identical high resolution mass (m/z: 426.38617) was solved after an MS-MS analysis of this ion. A fragment at m/z 208.40 originated by a retro Diels-Alder rearrangement only possible for β-amyrin confirmed the presence of this triterpene in the series.
ISOLATION AND ANTIRADICAL EVALUATION OF CINNAMIC ACID DERIVATIVES FROM BACCHARIS REGNELLI (ASTERACEAE)

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Baccharis genus is widespread in tropical areas of South America, and comprises about 500 species, of which 120 occur in Brazil¹. Many of them are used in folk medicine to treat gastrointestinal and liver disorders, diabetes, anti-inflammatory processes etc. The main compounds identified in this genus are diterpenoids, triterpenoids and flavonoids. In the present work we report the isolation of two cinnamic acid derivatives from Baccharis regnellii leaves collected in Campos do Jordão, São Paulo. Moreover, the extracts and pure substances were submitted to the luminol/hemin/hydrogen peroxide chemiluminescence assay to evaluate their antiradical activity². This technique allows the determination of the number of radicals scavenged (n) of pure compounds as well as complex mixtures, in relation to Trolox®. The active MeOH extract was partitioned with hexane, CH₂Cl₂, EtOAc and n-butanol. The EtOAc phase (2.3 g) showed anti-radical potential and was subjected to chromatographic separation in Sephadex LH-20 (MeOH) to afford 14 groups. After purifications steps based on silica gel chromatography (step gradient of CHCl₃ and MeOH), compound 1 (R = CH₃, 25.9 mg) was obtained from group 8 (300 mg) and compound 2 (R = H, 9.8 mg) from group 9 (130 mg). The ¹H NMR spectra (pyridine-d₅) of these substances showed signals at δ 6.94 – 7.35 or δ 6.88 – 7.62, characteristic for aromatic hydrogens and at δ 8.03 (1H, d, J = 15.8 Hz) and δ 6.76/6.68 (1H, d, J = 15.8 Hz) attributed to trans olefinic hydrogens. The sugar moiety was identified as glucosil by the signals at δ 5.85/5.84 (1H, d, J = 6.3 Hz) and δ 4.37/4.34 (m). The presence of three methoxy groups in 1 was proposed by the singlets appearing at δ 3.77 (6H, s) and δ 3.80 (3H, s), whereas substance 2 possess only two methoxy groups as indicated by the signal at δ 3.72 (6H, s). Analysis of the ¹³C NMR, HMQC and HMBC spectra allowed the characterization of the new compound 1 as 4'-O-β-D-glucopyranosyl-3',5'-dimethoxybenzyl-ferulate and the known compound 2 as 4'-O-β-D-glucopyranosyl-3',5'-dimethoxybenzyl-caffeate, previously isolated from B. articulata³. Compound 2 presented a higher antiradical capacity (n = 0.7) than 1 (n = 0.4), but both were less active than Trolox (n = 2).

Cyclic peptides are widely accumulated in animals and microorganisms, and it is relatively rare in higher plants, being distributed mainly in Euphorbiaceae, Rubiaceae and Violaceae. This class of peptide has recently received special attention due to its large range of biological activity. Also these compounds have often been used as models for studies of structural features of proteins, which conformational determination of such cyclic peptides is an important step to exhibit local interactions which could initiate the folding of native proteins process\(^1\). Here, we report the isolation, purification, and characterization of the cyclic peptides accumulated in three Jatropha species occurring in the Northeast of Brazil. The latex of three species: *Jatropha curcas* L., *J. multifida* L. and *J. gossypifolia* L. were partitioned with ethyl acetate and fractionated on Sephadex\(^\text{G15}\). The crude peptidic fractions obtained from each plant species were analysed by \(^{18}\text{C}\) reverse phase HPLC to reveal several peaks. Each peak was resolved into pure peptides, the novel jatrophidin I \((1)\) and the pohlianin A \((2)\) (*J. curcas*); labaditin \((3)\) and biobollein \((4)\) (*J. multifida*) and cyclogossine A \((5)\) and cyclogossine B \((6)\) (*J. gossypifolia*) by multi-step preparative HPLC. The structure of all cyclic peptides, including the new one were elucidated by a combination of chemical degradation, amino acid analysis, mass spectrometry and two-dimensional \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectroscopy. The NMR studies in conjunction with molecular modeling using distance geometry calculations revealed that the new peptide \(1\) exists as two conformers of a cyclic structure. The isolates were also examined against four fungi strains; however \(1\) and \(2\) showed week effect against these strains. Compound \(2\), \(5\) and \(6\) was also submitted to *in vitro* heme interaction assay, which showed antimalarial activity. These results can be useful to map the peptidic composition of other Brazilian plant species, which represent another potential source of bioactive compounds. (FAPESP, CNPq, CAPES).

The anti-insectan activity of ethanolic extracts of different plant parts was evaluated for nine species belonging to five families: Bignoniaceae: Clytostoma callistegioides, Dolichandra cynanchoides, Macfadyena unguis-cati; Sapindaceae: Dodonaea viscosa, Allophylus aedulis, Serjania meridionalis; Lamiaceae: Salvia procurrens; Solanaceae: Lycium cestroides; and Phytolaccaceae: Phytolacca dioica. Aphid settling inhibition assays were done with a feeding generalist, Myzus persicae, and a specialist that feeds on grasses, Rhopalosiphum padi (Hemiptera: Aphididae). Antifeedant assays were performed with adults of the specialist Epilachna paenulata (Coleoptera: Coccinellidae) and larvae of the generalist Spodoptera littoralis (Lepidoptera: Noctuidae). Settling inhibition was calculated as percent SI = 1 - (% of aphids on treated surfaces / % of aphids on a control surface) / 100.\(^1\) Feeding reduction was expressed as % FR = [1 - (treatment consumption/ control consumption)] / 100.\(^2\) The results showed that only the twig extracts from A. aedulis (Sapindaceae) showed a significant settling inhibition effect against M. persicae (% SI = 77.6 ± 1.8), although it was inactive against R. padi (% SI = 24.5 ± 7.0). Several leaf extracts showed significant feeding reduction against E. paenulata: the three Bignoniaceae species [C. callistegioides, (% FR = 100 ± 0), D. cynanchoides (% FR = 85 ± 8), M. unguis-cati (% FR = 82 ± 8)], the three Sapindaceae (D. viscosa, % FR = 91 ± 4; A. aedulis % FR = 90 ± 6 and S. meridionalis % FR = 93 ± 6) and L. cestroides (Solanaceae, % FR = 100 ± 0). However, none of these extracts were active against S. littoralis.

The relatively negative results in the aphid settling assay may indicate that these plant species are not-resistant against aphids, or that aphid settling inhibition activity may be found in lipophilic compounds located at the leaf surface. The results in the feeding reduction assays may be explained by the different feeding strategies of the two herbivores tested. As a generalist, S. littoralis is able to ingest a variety of plant foods, and is therefore expected to exhibit greater resistance toward plant chemical defenses. This trait, however, would not be expected for specialists such as E. paenulata, which has only been selected to withstand secondary metabolites from its food plants (Cucurbitaceae).

ORAL SESSION B: Biological and Pharmacological Activity of Natural Products
Evaluation of Glyceraldehyde-3-Phosphate Dehydrogenase Natural Product Inhibitors by Isothermal Titration Calorimetry

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Abstract

Isothermal titration calorimetry (ITC) has been employed in kinetic parameters determination of enzymes belonging to every enzyme classification. The general principle is to measure the heat-flow, which is proportional to the rate of the enzymatic catalysis. In this work, we report virtual and docking screenings followed by structure-activity relationships (SAR) that were successfully employed in the search for new Trypanosoma cruzi glyceraldehyde-3-phosphate dehydrogenase (GAPDH) inhibitors. Calorimetric assay for T. cruzi GAPDH (EC 1.2.1.12) was used to determine the interactions between selected natural products and the target enzyme. Some exhibited lower IC₅₀ values against T. cruzi GAPDH as compared to early-published GAPDH natural product inhibitors. Michaelis-Menten kinetic parameters were obtained from a pseudo-first-order method yielding for catalysed G3P conversion to 1,3-DPG

\[ k_{cat} = 88.3 \pm 1.7, \ K_M = 30.7 \pm 1.1 \ \mu M \] and \[ k_{cat}/K_M = 2.88 \pm 0.05 \times 10^6 \ M^{-1} s^{-1} \]. This assay allows the search and development of new compounds for Chagas’ disease chemotherapy. It is also envisaged that ITC has a great potential for new applications in the field of affinity kinetics. (CNPq, FAPESP)
**NATURAL PRODUCTS AS INHIBITORS OF CATHEPSIN V: IDENTIFICATION, BIOLOGICAL ACTIVITY AND MOLECULAR MODELING**

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**Key words:** cathepsin V; natural products; docking.

**Introduction:** Coronary arterial disease is one of the major causes of human death. In this context, atherosclerosis, which is characterized by a thickening and loss of elasticity of the arterial wall, is the leading cause of cardiac illness. Cathepsins are papain-like cysteine proteases with elastolytic activities that have been identified in macrophages present in plaque areas of diseased blood vessels. Nearly 60% of the total elastolytic activity of macrophages is related to cysteine proteases with cathepsins V, K, and S contributing equally. However, cathepsin V (cath-V) is the isoform that exhibits the most potent elastase activity. Therefore, cath-V enzyme is a potential molecular target for the treatment of atherosclerosis.$^{1,2}$

In the present work, a series of 170 compounds isolated from Brazilian plants were tested and some of them identified as potent reversible inhibitors of cath-V. Moreover, aiming at gaining further insights into the inhibitors’ binding mode, a molecular modeling study was carried out.

**Experimental Procedures:** The biological activity of the isolated compounds was assessed for their ability to inhibit cath-V by fluorescence assays using the fluorogenic substrate 7-amino-4-methyl coumarin (MCA) in black 96-well microplate. The IC$_{50}$ values (concentration required for 50% inhibition of cath-V) of each inhibitor were calculated by non linear curve-fitting regression analysis of the experimental data. Subsequently, kinetics studies were performed to evaluate the inhibition mechanism, the $K_i$ (inhibitory constant) values of the inhibitors and the selectivity toward cath-V. Molecular modeling tools were applied in order to investigate the inhibitors binding mode within receptor. Accordingly, the docking programs FlexX and GOLD were employed to sampling the inhibitors conformers within the cath-V binding site and a structure-based pharmacophore model was used for analyzing the proposed binding mode.

**Conclusions:** The kinetic studies revealed that this novel identified series of natural products are potent inhibitors of cath-V. Furthermore, the assays also showed significant differences on the selective activity towards cathepsins K, L and S. Finally, the molecular modeling studies suggested a suitable binding mode that is in good agreement with the inhibitors’ mechanism of action and can be used for designing selective compounds with improved affininity toward cath-V enzyme.

**Refs:**
The Cytotoxic, Trypanocidal and Antileishmanial Activity of Alkaloids Isolated from *Duguetia furfuracea* - Annonaceae

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*Duguetia furfuracea* (Annonaceae) is a shrub distributed throughout the Brazilian state of Mato Grosso do Sul. It is known as “araticum-seco” and in folk medicine its powdered seeds are mixed with water for use against pediculosis, whereas an infusion of the twigs and leaves are used to treat rheumatism

From alkaloid extract of the subterranean stem bark of *Duguetia furfuracea* were obtained five alkaloids. These compounds were evaluated in vitro against *Trypanosoma cruzi*, *Leishmania amazonensis* and three human cancer cell lines (Colon-HCT-8, glioblastoma-SF925 and breast-MDAMB435 human cancer cells). The new alkaloid, duguetine β-N-oxide, was found to have potent antileishmanial (Table 1) and cytotoxic (Table 2) activities. This substance and duguetine were much potent against the human cell lines, although they are aporphinic alkaloids and have N-methyl substituent making their structure very non-planar and would seem to make them poor candidates for DNA binding by intercalation. The oxoaporphine alkaloid, dicentrinone, had not considerable cytotoxic activity, although of its very planar, but it showed the highest antileishmanial activity. Only duguetine and dicentrinone were active against trypomastigote forms and presented the lowest IC₅₀ values.

![Duguetine β-N-oxide](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Trypanocidal IC₅₀ (µg/mL)</th>
<th>Antileishmanial IC₅₀ (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duguetine</td>
<td>9.32</td>
<td>4.32</td>
</tr>
<tr>
<td>Duguetine β-N-oxide</td>
<td>30.79</td>
<td>0.11</td>
</tr>
<tr>
<td>Dicentrinone</td>
<td>18.83</td>
<td>0.01</td>
</tr>
<tr>
<td>N-methyltetrahydropalmatine</td>
<td>9072</td>
<td>17.03</td>
</tr>
<tr>
<td>N-methylglaucine</td>
<td>4957</td>
<td>4.88</td>
</tr>
</tbody>
</table>

Table 2 – Cytotoxic activity of compounds isolated from *D. furfuracea* for Tumor Cell Lines

<table>
<thead>
<tr>
<th>Cell line (% cytotoxic)</th>
<th>HCT-8 (IC₅₀)</th>
<th>SF295 (IC₅₀)</th>
<th>MDAMB35 (IC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duguetine</td>
<td>91.1 ± 0.8 (0.5)</td>
<td>86.0 ± 1.1 (0.5)</td>
<td>98.1 ± 0.1 (4.4)</td>
</tr>
<tr>
<td>Duguetine β-N-oxide</td>
<td>92.0 ± 1.0 (0.7)</td>
<td>87.1 ± 0.7 (0.6)</td>
<td>84.5 ± 1.6 (2.7)</td>
</tr>
<tr>
<td>Dicentrinone</td>
<td>68.0 ± 0.7</td>
<td>50.3 ± 1.5</td>
<td>37.5 ± 4.0</td>
</tr>
<tr>
<td>N-methyltetrahydropalmatine</td>
<td>57.6 ± 0.7</td>
<td>39.8 ± 0.7</td>
<td>31.1 ± 0.8</td>
</tr>
<tr>
<td>N-methylglaucine</td>
<td>45.2 ± 2.1</td>
<td>25.1 ± 2.3</td>
<td>15.1 ± 6.4</td>
</tr>
</tbody>
</table>

Colon (HCT-8); glioblastoma (SF925) and breast (MDAMB435) human cancer cells. Doxorubicin was used as positive control. All compounds were tested concentration of 25 µg/mL and only active compounds were calculate IC₅₀ (µg/mL) values and 95% confidence interval obtained by nonlinear regression.

Therefore, the results obtained demonstrate that *D. furfuracea* contain potential compounds, mainly duguetine, duguetine β-N-oxide and dicentrinone, for use as agents against *Trypanosoma cruzi*, *Leishmania amazonensis* and the human cell lines.

Refs.
Many authors have already emphasized that phytochemicals from spices, such as peppers, have biological applications [1, 2, 3, 4]. Piperlonguminine is a known alkaloid amide from peppers, including *Piper divaricatum*. The aim of this study was to investigate the *in vitro* and *in vivo* antitumor effect of piperlonguminine in experimental models. Hematological, biochemical, and histopathological analyses were performed in order to evaluate the toxicological aspects. The cytotoxicity of piperlonguminine was tested against HL-60 (leukaemias), MDA-MB-435 (breast), SF-295 (brain), and HCT-8 (colon) human cancer cell lines using MTT assay. For the *in vivo* antitumor activity, Sarcoma 180 cells were injected (2×10^6 cells/animal/s.c.) in mice left hind limbs. One day after, the animals were treated intraperitoneally with piperlonguminine (25 or 50 mg/kg/day) for 7 days. Negative control was treated with the vehicle (4% DMSO) used for diluting the tested substance. The 5-Fluorouracil was used as positive control. On day 8, the mice were sacrificed and tumors and organs were excised, weighed and submitted to histopathology analysis. Piperlonguminine did not show significant *in vitro* cytotoxic effect at the experimental exposure levels, but showed *in vivo* antitumor effect. The inhibition rates were 38.71 % and 40.68 %, after 7 days of treatment, at the doses of 25 mg/kg and 50 mg/kg, respectively. As described in previous studies, one important structural requirement to direct cytotoxic effects in tumor cells is the presence of two α,ß-unsaturated carbonyl moieties as observed for plipilatine [1, 2, 3, 4]. On the other hand, when we considered *in vivo* antitumor activity, the presence of the methylenedioxyphenyl and the alicyclic amide group are important structural requirements to be considered [2, 4]. The histopathological analysis suggests that the liver and the kidney were only weakly affected by piperlonguminine treatment. Neither the enzymatic activity of transaminases (aspartate aminotransferase-AST and alanine aminotransferase-ALT) nor urea levels were significantly altered. In the hematological analysis, all parameters analyzed remained constant after piperlonguminine treatment. In conclusion, these data reinforce the anticancer potential of spices components. Supported by: CNPq, CAPES, BNB, FUNCAP, FINEP, Claude Bernard Institute.

Key words: *Piper divaricatum*, Piperlonguminine, Antitumor activity, Toxicity

Refs.
The chemical study of *Psychotria umbellata* Vell. (Rubiaceae) leaves led to isolation of psychollatine (1), a glycoside monoterpene indole alkaloid\(^1\) that exhibits an interesting psychopharmacological profile, including anxiolytic, antidepressive and amnesic effects in mice models\(^2\). Molecular modeling has been used as an auxiliary tool to evaluate the conformations adopted by new molecules, including alkaloids, together with coupling constants obtained by NMR analysis and correlations observed in 2D NMR techniques\(^3\). In the present work, a conformational study of psychollatine (1) was carried out, in order to elucidate the results obtained by NMR techniques and to establish the conformational features of 1. The molecular structure of compound 1 was submitted to semiempirical calculations using RM1 Hamiltonian of the MOPAC 7.0 program and all obtained conformers were minimized until the achievement of gradient norm values below 0.001. Theoretical \(J_{HH}\) coupling constants were calculated employing the Karplus equation (parameterized by Hasnoot-Altona) and compared with the experimental values. From the conformational analyses by RM1, four minimum energy conformations were evaluated for psychollatine (1), being two half-chair due to the inversion of the dihydropiran ring (ring E) of the iridoid moiety (defined as conformational substates \(E_a\) and \(E_b\)) and two half-chairs on ring C from the tetrahydro-\(\beta\)-carboline system (defined as conformational substates \(C_c\) and \(C_d\)). These conformations were named \(1E_aC_c\), \(1E_aC_d\), \(1E_bC_c\), \(1E_bC_d\). The theoretical coupling constants calculated by RM1 for the conformations \(1E_aC_c\) and \(1E_aC_d\) presented good agreement with the experimental data. However, for the conformers \(1E_bC_c\) and \(1E_bC_d\), a significant difference between theoretical and experimental data was observed for the coupling constant between H-20 and H-21, suggesting that in the analytical conditions the substate \(E_a\) of the dihydropiran ring could be predominant. For the ring C, the theoretical \(J_{HH}\) coupling constants calculated for all conformers evaluated presented values closely related to experimental data. Thus, a detailed analysis of 1D and 2D NMR spectral data together with the conformational study could reinforces the hypothesis of a dihedral angle near 180° between H-20 and H-21. Moreover, the combined results of spectroscopic and molecular modeling data suggests that psychollatine (1) is a structure formed by the combination of a geniposide derivative and triptamine.

Refs.
ORAL SESSION C: Manufacturing and Quality Control of Herbal Drugs and Essential Oils
The aim of this study was to evaluate the differences in the HPLC chromatographic profile between two varieties of transgenic sugarcane (“Bowman-Birk” and “Kunitz”, furnished by Prof. Marcio de Castro Silva-ESALQ/USP) and, consequently, to identify the secondary metabolites which are responsible for these differences. For this purpose, two pattern recognition methods, partial least squares discriminant analysis (PLS-DA) and principal component analysis (PCA) were performed [1-2]. High-performance liquid chromatography (HPLC) method with photo-diode array (DAD) detection, previously described [3], was used in order to obtain the HPLC fingerprints of flavonoids of sugarcane leaves for chemometrics analysis. Preprocessing (baseline correction and autoscaling) and pretreatments (correlation optimized warping- COW) of fingerprints chromatograms were required to reduce drifts in the retention times as well as alignment correction in the chromatographic data, due to its peak shape and area [4]. PLS-DA (RMSECV = 0.0662, RCV = 0.991 using two latent variables) successfully classified the leaves of two sugarcane varieties. PC1 versus PC2 scores plot (describing 84.2% of total variance) effectively distinguished the Kunitz and Bowman-Birk samples groups. Through PLS-DA it was possible to decrease the number of variables from 2701 to 70 and thus improve the PCA results. From the loadings (PC1 and PC2), it could be conclude that mainly 7 peaks were able to discriminate the two sets of transgenic sugarcane leaves (tR1 = 6.74, tR2 = 11.8, tR3 = 17.8, tR4 = 19.9, tR5 = 23.2, tR6 = 27.2, tR7 = 45.1 min). Some of the compounds corresponding to those peaks were elucidated by comparison with previous LC-MS data [6-7]. These results demonstrated that our approach is capable of identify the metabolites which are significant for the discrimination of these two transgenic sugarcane leaves.

Acknowledgments: CAPES, CNPq, FAPESP

Refs.
1. S. Wold et al., Chemometrics and Intelligent Laboratory Systems, 2, 37 (1987)
Brazilian Aristolochia genus (Aristolochiaceae) consists of approximately 100 species. In traditional medicine, these plants are known as “one thousand men”, and have been mainly used as abortifacients, stomachics, antiphidians, antiasthmatics, expectorants, and, recently, in slimming therapies. Despite the significant number of Brazilian Aristolochia species, the volatile compounds they contain are known for only a few species. The goals of the present study were to investigate the nature of essential oils from roots of 10 Aristolochia species, and correlate their oil compositions to morphological groups for the identification of these species. The variation in the chemical constitutions of the essential oils was examined by taking into account the various species and duration of extraction. The essential oils from the roots of each individual were obtained by hydrodistillation in a modified Clevenger apparatus and were analyzed by GC-MS. More than 50 essential oils were obtained from the roots of the plants under diverse durations of extraction (0.5, 2, and 4 h). A total of 75 compounds were identified in the analyzed oils by comparison of their mass spectra with those reported in the literature.

The highest concentration of monoterpene hydrocarbons was observed in the oils of A. arcuata, A. galeata, A. gigantea, A. malmeana, and A. melastoma, whereas the oil of A. pubescens showed a predominance of oxygenated monoterpenes. That of A. esperanzae showed a predominance of oxygenated sesquiterpenes hydrocarbons, whereas those of A. chamissonis, A. elegans, and A. lagesiana showed a predominance of oxygenated sesquiterpenes. The principal component analysis (PCA) was individually applied on datasets of normalized chromatograms from roots oils. These analyses showed that 2 h of hydrodistillation is the best duration when the oils are used for discriminate species. Analysis of the PCs (PC1 and PC3) enabled classification of the species into four morphological groups. A group consisted of A. arcuata, A. chamissonis, A. lagesiana, A. melastoma, and A. pubescens. A. gigantea and A. elegans were separate in two different groups. A. esperanzae, A. galeata, and A. malmeana may be in a fourth group that was more similar to the first group. These results are consistent to presented by Hoehne (1942). The GC-MS and exploratory multivariate analyses of the chemical constituents of the essential oils from roots of 10 Aristolochia species, which were obtained after 2 h of hydrodistillation, are good tools for helping to identify and also classify Aristolochia species into morphological groups. These analyses could aid in the identification of further specimen belonging to Aristolochia genus, including those available in the popular markets.

Refs.
1. L.M.X. Lopes et al., Research Advances in Phytochemistry, 2, 19 (2001)
3. F.C. Hoehne, Flora Brasílica: Aristolochiaceae, 15t.2 (1942)
TAXONOMIC DIFFERENTIATION BETWEEN *PHYLLANTHUS* SPECIES IN THE INQUIRY OF THE AUTHENTICITY OF COMMERCIAL SAMPLES OF “QUEBRA-PEDRA”

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*Phyllanthus niruri* and *P. tenellus* species, known in the Brazilian folk as “quebra-pedra”, are described in the fourth edition of the Brazilian Pharmacopeia and its use is mainly related to treatment of patients with urolithiasis\(^1\). One of the problems faced in the market of this herbal drug is that most of the times, different species of *Phyllanthus* end up consumed without any criterion. In this work are described a methodology for taxonomic differentiation of five different *Phyllanthus* species (*P. niruri, P. tenellus, P. carolininesis, P. amarus e P. urinaria*), all of then cultivated in a controlled conditions in the herbarium of CPQBA-UNICAMP, as well as, four commercial samples founded in the local commercial market. The technique used for this proposed was \(^1^H\) High Resolution Magic Angle Spinning (HR-MAS) NMR, which gotten useful information directly from vegetable material without any pré-treatment or extraction process, allied to chemometric analysis from the data set obtained.

All \(^1^H\) HR-MAS NMR measurements were carried through on a Bruker Avance DRX 400 instrument (operating at 400.23 MHz for Hydrogen) equipped with a 4mm HR-MAS probe head and zirconium rotor. A few drops of D\(_2\)O were added in the samples for field homogeneity adjustment and the spectra are referenced using TMSP-2,2,3,3-D\(_4\) (sodium-3-trimethylsilylpropionate) like an internal reference. The spectra were collected using 5 kHz spinning speed without temperature regulation using the Carr–Purcell–Meiboom–Gill\(^2\) (CPMG) spin-echo pulse sequence. Water suppression was included in the CPMG sequence. Data analyses on generated data matrices from \(^1^H\) HR-MAS NMR were performed by the Pirouette\(^\circledR\) software (v.2.02, Infometrix, USA) and Principal Component Analysis (PCA) method were used for data analysis.

From the PCA scores plot it is possible to distinguish all the species *P. niruri, P. tenellus, P. carolininesis, P. amarus e P. urinaria* and also to observe that only one of commercial samples which is closed with the *P. niruri*, one of the species recommended for the Brazilian Pharmacopeia and used for the treatment of urolithiasis. An other commercial sample is closed with the *P. urinaria*. However other two samples are not related with the genero *Phyllanthus*, most probably, because this samples including other vegetal species. It is important to mention that without the use of chemometrics tools will be impossible to distinguish the \(^1^H\) NMR spectra and certainly to classify the commercial samples in on of the pattern species.

Refs.
GC-MS, PCA and HCA were applied to evaluate the variability of the essential oils of Lippia graveolens HBK (Family: Verbenaceae). L. graveolens, a shrub to 2 m tall known as Mexican oregano in Mexico and Central America, grows wild in arid regions in the east of Guatemala. The leaves of L. graveolens, are used for treatment of colds, bronchitis, asthma and as seasoning for food preparations [1]. The essential oil of L. graveolens has showed activity against Gram-positive and Gram-negative bacteria and fungi [2]. L. graveolens has showed essential oil yields higher than 3 % (w/dw) by hydrodistillation. Previous works have described three chemotypes (thymol, carvacrol and mixed chemotypes) for L. graveolens. Fischer et al. [3], found significant differences in yield and composition in the essential oil from experimental cultivation of L. graveolens from five different populations. They found yields by hydrodistillation up to 3.6 % (w/dw) and thymol (6.8-80.6%), carvacrol (1.1-44.2%), β-caryophyllene (2.8-8.7%) and p-cymene (2.7-6.9%) as major components.

The aim of the study was to apply the Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) multivariate techniques to differentiate the chemotypes of L. graveolens populations based in their essential oils composition.

Aerial parts of 14 individuals of L. graveolens HBK were collected in January 2006, from seven populations located in three eastern departments of Guatemala. Essential oils were extracted by hydrodistillation for 3h using a Clevenger type apparatus and analyzed by Gas Chromatography/Mass spectrometry (GC-MS) using a HP5 (5% phenylmethylsilicone) column (25m x 0.2 mm, 0.25 µm film thickness). Temperature program was 60°C-240°C (7min) at 3°C.min⁻¹. The compounds were identified by their mass spectra, external standards as reference and retention indices correlated with those published by specialized literature. Multivariate Analysis was performed with the Program XLSTAT version 2007. There was coincidence between the results obtained by PCA and HCA with the major compounds in the essential oils. Thus, eight essential oil samples from populations of the provinces of Zacapa and El Progreso were classified in the first component corresponding to thymol chemotype, with thymol in 45.7-78.7%. Three samples classified in the second component corresponded to carvacrol chemotype, with carvacrol in 43.8-47.4% (populations from the province of Chiquimula) and three corresponded to the mixed chemotype (populations from the provinces of Zacapa and El Progreso).

INFLUENCE OF SOLAR RADIATION IN PHENOLIC COMPOUNDS PRODUCTION IN MIKANIA GLOMERATA.

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In the last years, with the increase in ozone hole, plants are more susceptible to the effects of solar radiation. Once several authors mention the influence of solar radiation in the composition of secondary metabolites¹, this work seeks to evaluate the variation in composition of phenolic compounds (phenylpropanoids and flavonoids) of M. glomerata grown under four levels of radiation: full sun, 25%, 50% and 75% of radiation interference. The analysis of secondary metabolites was made by HPLC-DAD-MS. The majority compounds were isolated in HPLC in preparative scale and identified through their UV spectra and ¹H, ¹³C, DEPT, HMBC and HMQC NMR data, as the chlorogenic, fertaric and 3,5-dicaffeoyl quinic acids and the mikanin-3-O-sulfate flavonoid. To the experiments 30mg of the dry and powdered leaves were used, as methodology previously described². The analyses were accomplished in HPLC-DAD, equipped with two monolithic (Onyx™ 100 x 4,6 mm - C-18 Phenomenex) columns in line and a pre-column of the same material. As mobile phase was used H₂O:acetic acid 1% (phase A) and MeCN:acetic acid 1% (phase B), 3% of B during 5 min., 3-20% B (5-30 min.), 20-35% B (30-35 min.), 35% B (35-36 min.), 35-100% B (36-41 min.), and more 4 min. to return to the initial conditions and re-equilibrate the column, flow rates were 3ml/min. Quantification and detection limits were evaluated. The quantified compound exhibited coefficients of linear regression higher than 0.999 and a wide linearity strip. The recovery of the method was in interval of 85 to 93% for phenylpropanoid derivatives and around 100% for the flavonoid, with a variation coefficient smaller than 5% calculated at different fortification levels. The analyses showed that phenylpropanoid derivatives exhibited an increase in concentration of up to 20 times with the increase of light incidence, while the flavonoid did not suffer great influence. On the attempt to relate these results with the location of these compounds in the leaf, photomicrographies were obtained in a laser scanning confocal microscopy (TCS SP2 - Leica Microsystems), as described methodology³, where the presence of glandular trichome was observed in the abaxial surface of the leaves. After revelation with NP-PEG, flavonoid presence was visualized inside the trichome, while phenylpropanoid derivatives were distributed in all leaf. With these results we can justify the increase of only a class of substances, once compounds stored in glandular trichome of Asteraceae usually do not suffer increase after the adult phase of the leaves⁴.

Refs.

BOSC-5
ORAL SESSION D: Biosynthesis and Molecular Biology of Natural Products
Many 9,9'-deoxygenated lignans present beneficial medicinal properties, including nordihydroguaiaretic acid (NDGA) from the creosote bush (*Larrea tridentata*), whose tetramethyl derivative (M4N) is currently undergoing Phase II trials for treatment of brain and CNS tumors; others include the insecticidal/antimicrobial conocarpan from the tropical plant *Piper regnellii*.

Through biosynthetic studies in basil (*Ocimum basilicum*), we have established that the allyl/propenyl phenol monomers (*i.e.* putative precursors in the biosynthesis of 9,9'-deoxygenated lignans [1]) are formed by regiospecific reduction of monolignol esters (or quinone methide derivatives thereof) such as *p*-coumaryl acetate and *p*-coumaryl coumarate [2, 3]. We found these reactions to be performed by homologues of PIP reductases (Pinoresinol-lariciresinol, Isoflavone and Phenylcoumaran benzyl ether reductases), and we have now characterized such homologues in other plant systems [4].

The presumed metabolism leading to 9,9'-deoxygenated lignans was investigated in cell-free extracts from young leaves of *P. regnellii*; the latter can promote the coupling of *p*-anol (but not chavicol) in the presence of H$_2$O$_2$, to afford mainly conocarpan. The conocarpan thus formed though is apparently racemic; non-specific oxidations catalyzed by horseradish peroxidase, in the presence of H$_2$O$_2$, yield nearly identical product mixtures. However, a subsequent enzymatic dehydrogenation of conocarpan in the presence of H$_2$O$_2$ was observed, forming the benzofuran neolignan eupomatenoiid-6. The enzyme responsible for this reaction is being purified and appears to preferentially utilize the (−) antipode of racemic conocarpan. Future experiments will further characterize the processes described above, especially regarding substrate coupling, and examine their participation in 9,9'-deoxygenated lignan metabolism in the two species.

References:
Lentinus species (basidiomycete) are important sources of secondary metabolites and have provided some biologically active compounds, such as the antibiotic cortinellin and antimicrobial sesquiterpene hirsutane\(^1\). This work reports the first chemical investigation of *L. striguellus*. This fungus was cultivated in 14 L of peptone-yeast extract medium for 28 days under static condition. Mycelium was separated from the liquid medium, dried until constant weigh and subjected to extraction with hexane, hexane/EtOAc 50%, EtOAc and MeOH. Column chromatography of Hex/AcOEt 50% extract (388.1 mg) allowed the isolation of the indol alkaloid echinuline\(^2\) (1, 16.0 mg) and anthraquinone physcion\(^3\) (2, 5.0 mg). *L. striguellus* was also grown in a shaker (150 rpm) using the same procedure previously described. Partition of the liquid medium (7 L) with AcOEt afforded 1.4 g of extract, which was chromatographed and provided 2,2-dimethyl-6-methoxy-4-chromanone (3, 35.0 mg), 2,2-dimethyl-3-hydroxy-6-methoxycroman (4, 12.8 mg) and 2,2-dimethyl-3,4-dihydroxy-6-methoxycroman\(^4\) (5, 7.5 mg). Structures were elucidated by MS and \(^1\)H and \(^13\)C NMR analysis. Compounds 3 and 4 were previously isolated from *L. Conatus*\(^1\) and all other substances are being described for the first time in *Lentinus* genus.

**Ref**.s.
PARTIAL PURIFICATION AND CHARACTERIZATION OF EUPOMATENOI-6 SYNTHASE FROM PIPER REGNELLII

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\textit{P. regnellii} Miq. is popularly known as “pariparoba” and can be found in lowlands of neotropical regions. Its major secondary metabolites described so far include several phenylpropanoids and dihydrobenzofuran neolignans with antibacterial, antifungal and antichagasic activities (Benevides et al., 1999).

The biosynthesis of neolignan (+)-conocarpan requires \( p \)-hydroxypropenylbenzene as precursor which can be dimerized by enantioselective oxidative coupling (Sartorelli et al., 2001). The benzofuran neolignan eupomatenoid-6 is apparently formed by dehydrogenation of conocarpan, but the mechanism of such conversion remains to be clarified.

The enzyme that catalyzes the conversion of (+)-conocarpan in its dehydrogenated analogue, with hydrogen peroxide as co-factor, has been partially purified from the leaves of \textit{P. regnellii} and characterized in terms of its pH and temperature optima, substrate specificity and kinetic parameters.

Enzymes that perform similar reactions have been described for sterols and fatty acids and the reaction mechanism usually involves \( \text{NAD}^+ \) or \( \text{NADP}^+ \) in some cases (Atawong et al., 2003) or \( \text{NADH} \) or \( \text{NADPH} \) and molecular oxygen in others (Taton and Rahier, 1996 Behrouzian and Buist, 2003), but none has been described to perform dehydrogenation with hydrogen peroxide as co-factor and thus the mechanism of this reaction still needs to be investigated.

Refs:
Microorganisms like fungi are multi-enzyme systems that undergo chemical transformations in the presence of different types of starting materials. Therefore these organisms are an efficient and cost-effective alternative to obtain interesting compounds that are difficult to be synthesized and that show lower toxicity and/or enhanced activity.

The sesquiterpene lactone budlein A (1) is the main constituent of the sunflower-like plant *Viguiera robusta* (Asteraceae). It has important biological effects including potent cytotoxic activity against human tumor cell lines as well as *in vitro* and *in vivo* anti-inflammatory effect. Nevertheless, sometimes compounds of this type present toxic properties due to the α,β-unsaturated system in the lactone ring. However, the obtention of analogues of 1 by traditional approaches, i.e. derivatization, is quite complex. Thus, we decided to perform the biotransformation of 1 using the fungus *Aspergillus terreus*.

Budlein A was incubated on Czapeck liquid medium and after 120 h at 30 ºC and 120 rpm the culture filtrates were extracted with CHCl₃. The organic layer was chromatographed over silica gel and the biotransformed products (2 and 3) were purified by HPLC. The structures were elucidated by NMR spectrometry and HRESIMS.

The biotransformation of 1 afforded two new and unusual terpenoid products which are difficult to be obtained by traditional synthetic reactions. The transformation carried out by *A. terreus* involved a double bond isomerization of the side chain ester at C-8 (3), the reduction of the double bond at C-2 and the complete removal of the lactone moiety followed by the formation of a new bond between C-7 and C-3 (2 and 3). The 3D structure of 2 is also shown. So far, the enzymes involved in these reactions were not identified, but they were responsible for the elimination of the putative toxic moiety of the molecule.

This is a good example of the versatility of a microbial-catalyzed biotransformation leading to unusual derivatives with potential pharmacological interest. The next step is to evaluate the biological effects of the products 2 and 3.

Refs
THE EFFECT OF PHENYLALANINE ADDITION ON THE BIOSYNTHESIS OF BIS-PHENYLPROPANOIDS AMIDES IN THE FUNGUS *PENICILLIUM SP.* ISOLATED AS ENDOPHYTE FROM *MELIA AZEDARACH*.

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The associations between plants and microorganisms are being considered an important and emergent research area and many important active substances were discovered in the past few years. In this context, *Penicillium* sp., a fungus isolated as endophyte from *Melia azedarach* (Meliaceae), produces many interesting secondary metabolites such as the bis-phenylpropanoid amides, Brasiliamides A (1) and E (2). These Brasiliamides seem to cause convulsion in silkworms (*Bombix mori*)¹, and researchers even consider these compounds as a possible new group of tremorgenic substances¹. These amides also showed activity against gram-positive bacteria *Bacillus subtilis* in bioassays recently conducted at LaBioMMi (DQ/UFSCar). Because of their fast and easy development in artificial mediums, the microorganisms provide many manipulation possibilities to increase the production of secondary metabolites. During early experimental works, it were started studies to find the best conditions for the production of bis-phenylpropanoid amides, which are probably made through condensation of two amino acid unities. In this sense, the aim of this work was to develop and apply analytical methodologies to study the influence of L-phenylalanine on the *Penicillium* sp. metabolism and Brasiliamides production, and to verify the best production period, in order to increase the yield of these compounds, once these substances shows interesting parasitic and bactericide activities. The experimental procedures started with the inoculum preparation which was obtained by suspending spores from 14-day-old cultures in water to a density of (1–2) x10⁷ spores/mL. The microorganism was cultivated at 25° in 125 mL Erlenmeyer flasks containing 30g of rice. To study the effect of the amino acid on amide production, the medium was supplemented with 25 mg of L-phenylalanine during inoculation. Secondary metabolites were extracted with organic solvents, and samples for analysis were taken at 2, 3, 5, and 10 days intervals. Qualitative analyses were made using a triple quadrupole mass spectrometer in previously optimized conditions. It was verified that the addition of L-phenylalanine in the culture medium increases the production of Brasiliamide A and the maximum production of this metabolite occurs at 28 days. An interesting fact is that the Brasiliamide production is verified in 2-days culture. In conclusion, the best medium and period of cultivation were optimized and the Brasiliamide A production maximized, indicating that L-phenylalanine could be the precursor on its biosynthesis, which will be further proved by adding labeled L-phenylalanine in the culture medium.

POSTER SESSION ABSTRACTS
Inhibition of ascitic Ehrlich tumor growth by intraperitoneal injection of *Pfaffia paniculata* butanolic residue (Brazilian ginseng).

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Antineoplastic effects of butanolic residue of *Pfaffia paniculata* in Ehrlich tumor-bearing mice by oral treatment was reported previously. The aim of this study was to investigate the effects of this residue by intraperitoneal treatment. A toxicity study was performed in which doses of 12.5 mg/Kg; 25mg/Kg e 50mg/Kg of butanolic residue of *P. paniculata* were administered by intraperitoneal injection for seven days in Swiss mice do not bearing Ehrlich ascitic tumor. The mice did not lose weight during the treatment and any pathologic alteration was observed by histologic assessment of the collected organs. The effect of this residue on the ascitic Ehrlich tumor in Swiss male mice was then investigated. Male mice received, by intraperitoneal injection, once a day, 12.5mg/Kg; 25mg/Kg or 50mg/Kg of butanolic residue of PP or saline solution (PBS), as control, for seven days. This protocol started in the same day of tumor inoculation with 5x10^6 cells i.p. the concentration of 50mg/Kg demonstrated a lower number of tumoral cells than control, thus the protocol was repeated with this concentration. The ascitic tumor was evaluated by the quantification of the volume of the ascitic fluid, relative number of tumor cells, number of dead tumoral cells and total number of tumor cells, morphology of tumor cells and histopathologic alteration in representative slices of the organs. A decrease in tumoral cells number per ml was observed in *P. paniculata* treated mice, that was followed by a numerical decrease in the total numbers of tumor cells in the ascitic fluid colected. These results may indicate the presence of tumor–cell inhibitory effects by *P. paniculata* residue.

Refs.
In Vivo ANTICÂNCER ACTIVITY EVALUATION OF BY-PRODUCTS FROM THE PRODUCTION OF ARTEMISININ FROM ARTEMISIA annua L. (ASTERACEAE)

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Malaria is one of the major causes of mortality in the Brazil and worldwide. At CPQBA-UNICAMP the artemisinin production from Artemisia annua L (Asteraceae) in large scale was patented. The production process generates a sesquiterpene-enriched residue. Therefore we started investigation on the biological potential of this material. The objective of this work was to evaluate the "in vivo" anticancer activity of Fraction (F2S) obtained by filter column chromatography from the residue generated in the artemisinin pilot scale production that previously demonstrated activity when tested on "in vitro" anticancer assay on nine human cell lines. The Ehrlich “in vivo” Tumor Model was selected as the standard assay. Ehrlich tumor cells were maintained in male Swiss mice through serial i.p. inoculation in 7-day intervals in an ascitis form. After preparing the cells, the total number was determined by Newbauer chamber using trypan blue dye exclusion method (Dagli, 1992), with tumor cell viability always higher than 90%. The cells were then diluted in saline (0,9%) for final inoculation density (1 x 10⁵ cells/mL). Fraction (F2S) was previously submitted to acute toxicity (LD₅₀) study in order to determine which concentrations could give the potential therapeutic activity but not kill the animals, as described by Litchfield & Wilcoxon (1953). In the EAT assay, five groups of eight animals received 1 * 10⁵ cells/mL, via i.p. The drug administration (via i.p.) began on the fourth day after inoculation, and the groups received the following treatments: Grupo 1 - saline (vehicle) negative control, group 2 - doxorubicina (5 mg/kg) positive control and group 3 - fraction (F2S), doses (200 mg/Kg), group 4 – fraction (F2S) dose (300 mg/Kg) and group 5 - fraction (F2S), doses (400 mg/Kg). The animals were treated again in the same doses in the 14th and 21st days after inoculation, and every day all animals were observed and scored for behavior and clinical conditions (Ullman-Cullere, 1999). When one animal got the highest punctuation, this animal was sacrificed and this day considered the time of the animal’s death. With EAT model, Fraction’s F2S antitumoral action was confirmed by the prolongation of the life-span of treated groups. The expectation is that an active compound can act in tumor cells curing some percentage of them and/or prolonging the life span of others. These results are very important, considering that EAT is a very aggressive tumor, having killed the controlled group after 20 days of the tumor cells’ inoculation. With 200, 300 and 400mg/Kg doses tested in this same date (20º post-treatment), all animals survived. On the 40th day post-treatment all three sample animal groups continued alive. On the 90 th day post-treatment, when assay was ended, the three sample groups treated with F2S had 50% animal survival. The doses of 200, 300 and 400mg/Kg had no adverse reactions demonstrating a potential "in vivo" anticancer activity for fraction F2S, prompting further studies in Hollow fiber models with human tumor cell lines.

IN VITRO ANTICANCER ACTIVITY OF Calea pinnatifida (R.Br.) Less. LEAVES

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Calea pinnatifida (R.Br.) Less., Asteraceae is popularly known as aruca or erva-de-lagarto, used in folk medicine to treat amoebiasis and giardiasis. Twelve constituents of C. pinnatifida (R.Br.) Less. were identified by Kato et al. (1994) in the essential oil, extracted from aerial parts.

The scope of this study was to evaluate “in vitro” antiproliferative activity of Calea pinnatifida (R.Br.) Less. crude extract and to isolate the actives fractions by activity guided fractionation.

The aerial parts of Calea pinnatifida (R.Br.) Less. were collected in the experimental field of CPQBA – Unicamp. Grinded dry leaves were extracted on Soxhlet with methylene chloride. The extract was dried under vacuum, yielding crude methylene chloride extract (ED) (9.9%).

The crude methylene chloride extract was purified by column filter chromatography. The fraction that presented the best antiproliferative activity in the “in vitro” assay was submitted to an acid-base extraction. Three fractions were obtained, a neutral non-polar fraction (NNP); a basic fraction (B) and an acid fraction (A) that were submitted to the anticancer “in vitro” assay. The samples were evaluated for anticancer activity in UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, non-small cells), OVCAR03 (ovarian), PC03 (prostate), HT-29 (colon), 786-0 (renal) and NCI-ADR (ovarian expressing phenotype multiple drugs resistance) cancer cell lines “in vitro”. A 48 h SRB cell viability assay was performed to determine growth inhibition and cytotoxic properties of the compounds. Cells were treated with at least four different concentrations levels ranging from 0.25 to 250 µm/mL with determination of total growth inhibition (TGI) parameter.

The crude methylene chloride extract (ED) presented selectivity concentration-dependent to cancer cell line 786-0 (TGI: 32.05). This result is important because there is no specific treatment for renal cancer.

The neutral non-polar fraction (NNP) presented selectivity concentration-dependent for cancer cell lines NCI-460 (TGI: 5.42), MCF-7 (TGI: 4.08), UACC-62 (TGI: 5.59) and 786-0 (TGI: 2.51). The basic fraction (B) presented selectivity concentration-dependent for cancer cell lines NCI-ADR (TGI: 4.47) and acid fraction (A) presented selectivity concentration-dependent for lines UACC-62 (TGI: 8.91) and 786-0 (TGI: 8.19). The clean up process of the crude methylene chloride extract permitted the identification of fractions with potential selectivity anticancer activity that will be further studied in “in vivo” experimental cancer models.

Introduction. Trypanosomiasis and leishmaniasis have reemerged over the last few decades as important threats to human health and economical development. Flavonoids have been known to exert diverse biological effects, particularly, acting as antioxidants and prophylactic agents against several diseases, including parasitic diseases. Computer-aided molecular design methods are considered a rational tool to find new drugs for the treatment of the mentioned diseases. Objective. Application of Principal Component (PC) and Consensus PC (CPC) analysis to a set of 106 flavonoid derivatives (aglycones and glycosides, and other compounds of phenolic and phenylpropanoid nature) having antiprotozoal activity against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovani*, and cytotoxicity for mammalian L6 cells. Methodology. Three-dimensional molecular structures from Protein Data Bank (PDB) were used as starting geometries of ligands. The flavonoids and derivatives models were minimized employing the AM1 semiempirical method (HyperChem 6.0). PCA and CPCA were applied using VolSurf+ program. Those chemometric methods were used to generate findings for elucidating the structure-activity relationships of the investigated compounds. Results. In a preliminary investigation, a classification between the 3D models and antiprotozoal and cytotoxicity activities, considering a dataset of 105 compounds and 114 descriptors, was searched employing PCA. Two significant principal components (PCs), explaining about 70% of the total variance of the matrix, were found by a cross-validation technique. The loadings plot indicated the variables log p c-hex, logd-10, G, R, CW1-8, W1-8, which were mixed and hydrophilic descriptors, as having greater influence in antiprotozoal activity. CPCA was performed, and PC1 and PC2 explained about 75% of original information, but 85% of the total explained variance was computed with H$_2$Oprobe. Conclusion. Those findings can be used to understand the investigated system and establish the relevant descriptors to the biological activity and cytotoxicity. The chemometric tools used here were the beginning of a search for designing new flavonoid agents having antiprotozoal activity.

Refs.
USE OF SELF-ORGANIZING MAPS OF THE FLAVONOIDS AND ANALOGUES WITH ANTIPROTOZOAL ACTIVITIES

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**Introduction**

Trypanosomiasis and leishmaniasis have reemerged over the last few decades as important threats to human health and economical development. Flavonoids have been known to act as antioxidants and preventive agents against several diseases. There is the need for the development of new, cheap, safe, and easy-to-administer molecules for the treatment of these infectious diseases.

**Objective**

We investigated 105 flavonoid glycosides and other phenolic compounds with antiprotozoal activities against *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovani* and cytotoxicities for mammalian L6 cells.

**Methodology**

106 flavonoids and derivates were submitted geometry optimization using the AM1 semiempirical quantum chemical method by the Hyperchem v. 6.0 software. The compounds were divided in approximately 50% active and 50% inactive, observing pIC\(_{50}\) against *T. cruzi*, *T. brucei*, *L. donovani* and pED\(_{50}\) for cytotoxicity. The molecules were saved as MolFiles for computing 1664 molecular descriptors using DRAGON Professional v. 5.4. Redundant and noisy information were excluded. The 658 remaining descriptors were used for performing a Kohonen Neural Network. The Multiple Linear Regression (MLR) models by using genetic algorithms (GA) to perform variable selection using MobyDigs v. 1.0 software. Kohonen maps were performed by SOM (Self-Organizing Map) Toolbox 2.0 for Matlab 6.5, using all (658) descriptors. SOM results obtained using only the selected descriptors (about 8) by MLR using GA were compared with the previous ones.

**Conclusion**

For all descriptors, training sets with activities against *T. cruzi*, *T. brucei*, *L. donovani* and cytotoxicity shows a match, respectively, 72.7%, 72.5%, 77.3%, 72.9% (active group) and 89.3%, 71.4%, 73.3%, 80.0% (inactive group). The test set calculated with *T. cruzi*, *T. brucei*, *L. donovani* and cytotoxicity showed the matches, respectively, 75.0%, 68.8%, 37.5% and 62.5%. Thus is possible to use SOM as a filter for a virtual screening. For selected descriptors, training sets shows a match, respectively, 75.8%, 87.5%, 72.7%, 82.7% (active group) and 91.1%, 89.8%, 80.0%, 81.1% (inactive group). The test set calculated with *T. cruzi*, *T. brucei*, *L. donovani* and cytotoxicity showed the matches, respectively, 75.0%, 68.8%, 62.5% and 56.3%. The selected variables were capable to divide the compounds mainly with activities against the *T. cruzi*.

**Refs.**

ANTIBACTERIAL AND ANTIMICROBIAL ACTIVITY OF EXTRACTS FROM

*Typha latifolia*

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*Typha latifolia* inhabits marshes, shallow, ditches and wet wastes along river. It is a perennial herb from a creeping rhizome and a dark brown pistil. The cattails are folk remedy for several diseases¹. In connection with our study of antimicrobial activity of plants from Northern São Paulo State, it has been presented the preliminary results of antimicrobial activity from extracts of *T. latifolia*.

The plant material was collected from “varzea” near of Assis, São Paulo, Brazil. From dry and powdered stalk has gotten the following extracts: hexane (EHTa), chloroform (ECTa), ethyl acetate (EAcTa) and methanol (EMEOHTa). All these extracts were prepared from by dynamic maceration of *T. latifolia* for 2h. Dried material (30g) was extracted with hexane (30 mL) and repeated for twice with 30 mL of the same solvent. After this, the extracts were filtered off and plant residue was treated with ethyl acetate. The same procedure was applied for methanol and final residue discarded. From of these extracts were carried out the antimicrobial activity by MIC against the bacteria *Pseudomonas aeruginosa* (ATCC 13388), *Escherichia coli* (ATCC 11775), *Rhodococcus equi* (ATCC 25729), *Micrococcus luteus* (ATCC 4698), *Staphylococcus epidermes* (ATCC 12228), *Salmonella choleraesuis* (ATCC 10708), *Enterococcus faecium* (ATCC 5079), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 6538), *Enterococcus hirae* (ATCC 10541) and against the yeast *Candida albicans* (ATCC 10231). EHTa showed activity to *Rhodococcus equi* (0,25 mg/mL), EAcTa showed inhibition (0,50 mg/mL) to *Bacillus subtilis* (0,50 mg/mL) and *Candida Albicans* (0,50 mg/mL).

All the rest the microorganisms tested presented no activity (> 1,0 mg/mL).²

Refs.


BPS-6
ANTIMICROBIAL ACTIVITY OF EXTRACTS OBTAINED FROM THE WASTE RESIDUE OF Agave sisalana

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Agave sisalana is a plant that has an attractive commercial value because of its applications like rope industry, tapestry, drugs, animal food, etc. By the other side, the waste aqueous residue, rich in steroidal saponins, has been discarded. The aim of this research was to study the antimicrobial activity of MIC (Minimal Inhibitory Concentration) from the extracts obtained from this residue.

Firstly, it was done a reduction of ten times of the water volume of the waste residue by heating, followed by evaporation of residual water at r.t. From this crude material, it was done a ressuspension in water and it prepared the following extracts by liquid-liquid extraction: butanolic (EBAS) and ethyl acetate (EAAS). Some crystals was formed in the EBAS extract, (called EBASc), which were separated and used on antimicrobial activity testing without any further purifying treatment. From the same crude and evaporated material it was prepared the hidroalcoholic extracts: ethanol:water 70:30 (EAqAS70) and ethanol:water 50:50 (EAqAS50).

Second, the waste residue was diluted with ethanol in a proportion residue:ethanol 50:50 v/v. There was a formation of a precipitate, which was filtered and discarded. The liquid portion evaporated (r.t.) and the extract obtained was called EVAS extract.

The antimicrobial activity of these extracts was tested against the yeast Candida albicans (ATCC 10231) and following the bacteria: Pseudomonas aeruginosa (ATCC 13388), Escherichia coli (ATCC 11775), Rhodococcus equi (ATCC 25729), Micrococcus luteus (ATCC 4698), Staphylococcus epidermes (ATCC 12228), Salmonella choleraesuis (ATCC 10708), Enterococcus faecium (CCT 5079), Bacillus subtilis (ATCC 6051), Staphylococcus aureus (ATCC 6538), Enterococcus hirae (ATCC 10541).

EBASc showed strong activity against Candida albicans (0,3mg/ml) and Rhodococcus equi (0,075 mg/ml), moderate inhibition against Enterococcus hirae (0,7 mg/ml) and no activity to others microorganisms used. EBAS showed stronger activity than EBASc against the Candida albicans (0,15 mg/ml) and Rhodococcus equi (0,05 mg/ml), but a discrete inhibition against Enterococcus hirae (0,9 mg/ml), Staphylococcus aureus (1.0 mg/ml) and no activity to others microorganisms. EAqAS70 showed only one strong activity against Rhodococcus equi (0,3 mg/ml). All the rest of tested extracts showed no activity. It was considered “no activity” when the microorganisms tested presented inhibition > 1,0 mg/mL. As expected, the antimicrobial activities were concentrated in saponin fraction.

Refs.
Pyrostegia venusta is a climber with delicate stems of the family Bignoniaceae that is represented by more than 100 genera and about 800 species. In this plant are found flavonoids, rutin and phenolics compounds. In connection with our study of antimicrobial activity of plants from Northern São Paulo State, we are now reporting our preliminary results concerning Minimal Inhibitory Concentration (MIC) of hexanic, ethyl acetate and metanolic extracts from P. venusta.

Leaves of P. venusta were collected in an area of "cerrado" of Assis, São Paulo, Brazil. Dried leaves (30g) were macerated with 30 mL of hexane with agitation for 2h. Subsequently, the extract was filtered and plant residue has re-extracted with two portions of 30mL hexane each and evaporated. This extracted was the hexanic extract (EHPV). The same preparation was used with ethyl acetate (EAPV), methanol (EMPV) and chloroform (ECPV).

The antimicrobial activity of EHPV and EAPV was tested against the yeast Candida albicans (ATCC 10231) and following bacteria: Pseudomonas aeroginosa (ATCC 13388), Escherichia coli (ATCC 11775), Rhodococcus equi (ATCC 25729), Micrococcus luteus (ATCC 4698), Staphylococcus epidermes (ATCC 12228), Salmonella choleraesuis (ATCC 10708), Enterococcus faecium (CCT 5079), Bacillus subtilis (ATCC 6051), Staphylococcus aureus (ATCC 6538), Enterococcus hirae (ATCC 10541). EAPV showed strong activity against Candida albicans (0,3mg/ml), discrete inhibition against Staphylococcus aureus (1,0 mg/ml) and no activity to others microorganisms used. EHPV showed moderate inhibition against Staphylococcus aureus (0,9mg/ml), good inhibition against Enterococcus hirae (0,5mg/ml) and no activity to others microorganisms used. It was considered “no activity” when the microorganisms tested presented inhibition > 1,0 mg/mL. These results indicate EAPV and EHPV extracts, obtained from Pyrostegia venusta, have the better potential for an antimicrobial activity.

(a) Refs
SELF-ORGANIZING MAPS TO PREDICT THE ANTI-VIRAL ACTIVITY OF SESQUITERPENE LACTONES IN THE SUBGENOMIC HCV REPLICON SYSTEM

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2 - Instituto de Ciências Exatas e Biológicas, Universidade Federal de Ouro Preto

Introduction
Some sesquiterpene lactones (SLs) are the active compounds of a great number of traditionally medicinal plants, mainly from the Asteraceae family, and possess considerable anti-microbiologic activity. Studies in vitro have shown the anti-viral activity in the subgenomic Hepatitis C virus (HCV) replicon system; proving that screening of natural products is a viable and fast way for identifying novel molecular diversity as potential drug leads.\textsuperscript{1}

In this study we investigated a set of 19 different molecules, represented by 5 skeletons of SLs (11 germacranolides, 4 eudesmanolides, 1 guaianolide and 1 pseudoguaianolide) and 2 additional lactones, in what it says respect of their anti HCV properties.

Materials and Methods
These molecules were classified as actives (12 molecules with EC\textsubscript{50} < 10\textmu M) and as inactives (7 molecules with EC\textsubscript{50} > 10\textmu M), and were drawn into SISTEMAT X and had their activity data inserted. After that, they were codified in 3D using .mol (mdl) files. Molecular modelling computations were performed on Hyperchem 7.0 for Windows. The molecules were subjected to geometry optimization and conformational analysis. Semi-empirical quantum chemical method used was AM1 and the root mean square gradient value of 0.001 kcal/mol as termination condition. The molecules were saved as MolFiles for computing various molecular descriptors using DRAGON Professional v. 5.4. These descriptors were used for performing a Kohonen Neural Network. All descriptors provided by DRAGON were calculated, totaling 1664 descriptors. For each block of descriptors, the constant variables were excluded, as well as those that presented only a different value of the series. Kohonen maps were trained using Matlab 6.5 and SOM (Self-Organizing Map) Toolbox 2.0. We used MobyDigs program for the calculation of Multiple Linear Regression (MLR) models by using genetic algorithms, with pEC\textsubscript{50} = –log EC\textsubscript{50} (EC\textsubscript{50} provided by the anti-viral activity of the 12 molecules with specific activity in the subgenomic HCV replicon system) as dependent variable, and all DRAGON descriptors as independent variables. The selected descriptors were reanalyzed at SOM Toolbox.

Results and Conclusions
The 3D-MoRSE descriptor showed the best results, with 100% of match for the inactive compounds, 83.3% for the active ones and 89.5% for the entire block. The less satisfactory values were shown by Randic descriptor block that reached 57.1% of correctly prediction for inactive molecules, 100% for active ones and 84.2% for the total test. After the MLR analysis, the descriptors (MOR11\textmu, G1\textmu and HATS3\textmu) were selected by the most significant equation ($n=12, r^2=0.94, Q_{cv}^2=0.88, s=0.05, F=-39.6$). The SOM obtained for these three descriptors showed best results (85.7% of match for the inactive compounds, 91.7% for the active ones and 89.5% for all). SOM is a possible tool to be used as a filter for a virtual screening for SLs.

Reference
ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF CRUDE EXTRACT AND ISOLATED COMPOUNDS FROM Nectandra megapotamica (Lauraceae)

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The bark of Nectandra megapotamica (Spreng) Chodat et Hassler (Lauraceae) is used in Brazilian folk medicine as an anti-rheumatic and to relieve pain¹. The chromatographic fractionation of the crude hydroalcoholic extract of the barks of N. megapotamica (ENM) afforded the isolation of α-asarone (1) and eleven lignans, including veraguensin (2) and galgravin (3). Regarding the analgesic and anti-inflammatory activities, the oral administration of ENM (500mg/kg) produced a significant inhibition of the constrictions induced by acetic acid by 82.2%, while α-asarone (1), veraguensin (2), and galgravin (3), at the dose of 20mg/kg (p.o.) showed 60.5%, 71.3%, and 70.6% of inhibition, respectively. In the carrageenan-induced paw edema, veraguensin (2) and galgravin (3), at the dose of 20mg/kg (p.o.), showed a significant anti-edematous effect of 41.2% and 71.4%, respectively. The compound 1 and ENM were inactive in this assay at the tested doses. In the hot plate test, the oral administration of α-asarone (20 mg/kg) and ENM (300 mg/kg) produced a significant analgesic effect, while 2 and 3 were inactive. In the in vitro COX-2 and NFkB assays, all tested samples were inactive. The results taken together suggested that 2 and 3 possessed peripheral analgesic activity, while the analgesic properties of ENM may be due mainly to the presence of α-asarone².

Refs
POTENTIAL HYPOGLYCEMIC EFFECTS OF *LEANDRA LACUNOSA* EXTRACT IN ALLOXAN-INDUCED DIABETIC RATS

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*Diabetes mellitus* is a major endocrine disorder affecting nearly 10% of the population all over the world. Despite of the introduction of hypoglycemic agents, diabetes and related complications continue to be a serious medical problem. Medicinal plants have been used as an alternative treatment of diabetes. *Leandra lacunosa* (Melastomataceae) has been used in folkloric Brazilian medicine for treatment of this disease.

The aim of the present work was to evaluate the antidiabetic activity of the hydroalcoholic extract of aerial parts of *L. lacunosa* in the alloxan-induced diabetic rats. The extract of the plant were obtained by maceration with ethanol/water (8:2 v/v). Diabetes was induced in rats by i.v. administration of 150 mg/kg of alloxan monohydrate. After 2 weeks, animals with level of blood glucose higher than 200 mg/dL were selected. In this experiment were used 3 groups of 5 rats each: group I (negative control - treated with saline), group II (positive control – chlorpropamide 40 mg/kg) and group III (diabetic rats treated with of hydroalcoholic extract 500 mg/kg). Blood glucose level was measured just prior to and 1, 2, and 4 h after drug administration. Treatment with hydroalcoholic extract of *L. lacunosa* indicates a significant reduction in blood glucose levels at 4h samples (Table 1).

The present study confirms the folkloric use of this specie for treatment of diabetes. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of these hypoglycemic effects. [Financial support: FAPESP]

**Table 1:** Effect of hydroalcoholic extract of *L. lacunosa* on blood glucose level in Alloxan-induced diabetic rats

<table>
<thead>
<tr>
<th>Samples</th>
<th>0 h</th>
<th>1 h</th>
<th>2 h</th>
<th>4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>hydroalcoholic extract&lt;sup&gt;a&lt;/sup&gt;</td>
<td>319.4 ± 12.9</td>
<td>255.8 ± 45.4</td>
<td>240.0 ± 46.6</td>
<td>166.6 ± 31.5</td>
</tr>
<tr>
<td>Positive control&lt;sup&gt;b&lt;/sup&gt;</td>
<td>252.4 ± 21.8</td>
<td>154.4 ± 21.0</td>
<td>140.4 ± 35.4</td>
<td>116.4 ± 49.3</td>
</tr>
<tr>
<td>Negative control&lt;sup&gt;c&lt;/sup&gt;</td>
<td>218.8 ± 25.0</td>
<td>249.8 ± 22.0</td>
<td>293.6 ± 37.7</td>
<td>246.6 ± 22.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> 500 mg/kg  <sup>b</sup> chlorpropamide 40 mg/kg  <sup>c</sup> Saline.

The values (mg/dL) are expressed as means ± S.E.M. (n=5) determined at different time (h) after treatment. The percentage of reduction is shown in brackets.
This paper reports the bioassay guided isolation and structure elucidation of compounds isolated from *Pterodon pubescens* Benth. methylene chloride-soluble fraction evaluated for anticancer activity in UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, non-small cells), OVCAR03 (ovarian), PC03 (prostate), HT-29 (colon), 786-0 (renal) and NCI-ADR (ovarian expressing phenotype multiple drugs resistance) cancer cell lines *in vitro*. A 48 h SRB cell viability assay was performed to determine growth inhibition and cytotoxic properties of the compounds. Cells were treated with at least four different concentrations ranging from 0.25 to 250 µm/mL with determination of total growth inhibition (TGI) parameter. Compounds, 7β–diacetoxyvouacapane (1), 6α–7β–diacetoxyvouacapane (2), methyl 6α–7β–dihydroxyvouacapan–17β-oate (4) were identified based on comparison of experimental 1H and 13C – NMR with reported spectral by Fascio et. al (1976), Campos et. al. (1994) and Ortalo-Magne et.al. (2005). Novel Compound 3 was deduced as having an elemental formula C\(_{22}\)H\(_{32}\)O\(_4\), by HREI-MS (observed M\(^+\) = 360.23556), which indicated seven insaturation sites. Infrared absorptions at 3449 (OH), 1713 (C=O) cm\(^{-1}\) provided evidences for hydroxyl and carbonyl functionalities. When acetylated with excess acetic anhydride/pyridine, this compound showed identical 1H and 13C – NMR spectral data to compound 6α–7β–dianetoxyvouacapane (2) (HREI-MS 402,2630), suggesting that the difference in compound 3 was an acetyl group either in C-6 or C-7. Compound 3 1H– NMR spectra data lacked a signal at δ 5.24 pm due to H-7α and the appearance of a signal at δ 3.4 pm characteristic of hydroxyl group. Therefore these data suggested compound 3 to have the same relative configuration to 6α–7β–dianetoxyvouacapane (2) with the hydroxy group attached β to C-7 whereas the acetyl group positioned α at C-6. Novel compound 3 (TGI 4.48 µg/mL) proved to be potent for ovarian cancer cells that express multidrug resistance phenotype (NCI-ADR) (TGI 4.48 µg/mL).

Plants have long been an invaluable source of medication in human communities worldwide. The Brazilian flora is vast and varied and each of its biomas is rich in species, many with medicinal attributes. The Endopleura uchi (Huber) Cuatrec. is a member of the family Humiriaceae and mollis is common in the Brazilian states of Pará and Amazonas, in the north of Brazil. According Sant’ana et al. (2000) and Ferreira et al. (2005), the stem of this plant has two major compounds: bergenine and 8,10 – dimetoxiberginine. The E. uchi is largely used by the population to combat miomas, although there are just a few biological studies with its extract. This study objectified evaluates the pharmacology activities of this plant. In the first part of this study, the extract of the stem of E. uchi was obtained by decoction with water. This extract was dried and used to the studies of acute oral toxicology (doses of 500, 1000 and 2000 mg/kg), according the OECD Guideline (2002). The extract was also used in the pharmacological study of intestinal motility (Wong et al., 1981) in dose of 200 mg/kg. The result showed that E. uchi aqueous extract offers a wide margin of safety and cannot be considered toxic. In the study of intestinal motility effect, the group treated with plant extract, the motility was decreased by 10,8% in the dose of 200 mg/kg of extract when compared with the control group, that received just water. It can be conclude that the extract of E. uchi presents an inhibition effect on the intestinal motility.

Refs.
Dimorphandra mollis is a Brazilian plant largely used in folk medicine with antioxidant potential for containing flavonoids. Its fruit pericarp is used in Brazil to treat ulcers, to reduce inflammation and to heal wounds (Lorenzi & Matos, 2002). From the fruits it can be extract flavonóides, mainly rutin and quercetin (Sousa et al., 1991). The study was performed on fruits from a Dimorphandra mollis tree growing in the Araraquara district of São Paulo State. The dried and comminuted plant material was extracted in ethanol. We have evaluated the crude ethanolic extract of D. mollis fruits as a potential antioxidant source using an assay based on the bleaching of the radical monocation 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS●+) and by DPPH scavenging capacity (Oliveira et al, 2007). Cysteine was used as a positive antioxidant control evaluated at different concentrations. Crude ethanolic extract showed marked action over DPPH (IC₅₀= 79,5 ± 3,5 µg/ml) and radical (IC₅₀= 3,45 ± 0,071 µg/ml). Both extracts and cysteine (DPPH: IC₅₀= 21 ± 1,4 µg/ml; ABTS●+: IC₅₀= 1,3 ± 0,14 µg/ml) were able to scavenge radicals at low levels. From the results, we can conclude that the crude ethanolic extract from Dimorphandra mollis fruits has a powerful antioxidant potential.

Refs.
Introduction

Tissue damage due to oxidative stress is directly linked to development of many, if not all, human morbidity factors and chronic diseases. In this context, the search for dietary natural occurring molecules with antioxidant activity, such flavonoids, became essential. The DPPH system is a stable radical-generating procedure that simulates the biological free radical producing mechanism and because of this, the system can be used to measure the biological antioxidant activities of electron accepting compounds like flavonoids. The $^{13}$C NMR, as molecular descriptors, can be used to describe the molecular structure. These data give rich information about the molecular structure and they are sensitive to detect differences on the molecule.

Objective

In this study we investigated a set of 41 flavonoids (23 flavones and 18 flavonols) analyzing their structures and biological antioxidant activity$^1$. Molecular information given by the $^{13}$C NMR data were used to predict the antioxidant activity of the flavonoids, sorting them in active or inactive compounds from the ANNs (artificial neural networks).

Materials and Methods

$^{13}$C NMR data were used to perform a Kohonen self-organizing map study, analyzing the influence and weight that each carbon has in the activity. Thus, the compounds were classified as active if they exhibited $IC_{50}<200\mu M$ and inactive if they exhibited values of $IC_{50}>200\mu M$. The $^{13}$C NMR chemical shifts of flavonoids were used as the input data and the separation between active and inactive compounds were obtained. The unsupervised training were performed using the SOM Toolbox version 2.0 for Matlab version 6.5 computing environment by MathWorks, Inc.

Results and Conclusion

The results from Kohonen map show a highly significant match with high percentage for both groups (80%). Analyzing the groups separately the major mach occurred among the active compounds (84%). This could be explained by the common chemical environment exhibited by the active molecules once these compounds have a pattern of substitution that influences the electromagnetic surroundings in the same way. The importance of these carbons was confirmed in the $^{13}$C NMR analysis. Through the $^{13}$C NMR chemical shifts the ANN achieve a significant result, sorting the active and inactive molecules with minimal errors (20%). This supports the idea that $^{13}$C NMR data can be used to perform classification of flavonoids taking into account the biological activity, making clear that radical scavenging activity is highly correlated with the chemical environment.

Reference

The seminal contribution of Gottlieb and co-workers to chemotaxonomy resulted in several postulates about the evolution of secondary metabolites in plants. One of them suggests that “The evolution of micromolecules proceeds by oxidation. The relatively highly oxidized compounds characterize new chemical lines”. Recently, it was suggested that the oxidative pathways in plants occur parallel to protective mechanisms against oxidative degradation. The same skeletal types, of a chemical class, can be found in different tribes, subtribes or even genera. In such cases, a method to distinguish one tribe from the rest is through comparison of sets of shared skeletons or substitutional diversification of such skeletons. The base of this procedure is: different broken metabolic are characterized by sets of compound, and their structures 3D should be used in the differentiation process.

Objectives
In this study we verify and quantify the existent relationship of the oxidation number (NO\textsubscript{X}) of diterpenes, in the 13 tribes (Anthemideae, Arctoteae, Astereae, Calenduleae, Cardueae, Eupatorieae, Gnaphalieae, Helienieae, Heliantheae, Inuleae, Mutisieae, Senecioneae, Vernonieae) of the Asteraceae family, with the RDF descriptors from 3D structures.

Material and Methods
The coordinates 3D of 2150 diterpenes were generated through the program SISTEMAT X, starting from 2D representation. Molecular modeling computations were performed on SPARTAN for Windows v. 4.0 software. The molecules were subjected to geometry optimization and conformational analysis. The semi-empirical quantum chemical method used was AM1. Respective botanical occurrences for each compound and its oxidation number were extracted of the program SISTEMAT X. Molecules with their energies minimized were saved as MDL MolFiles for computing RDF descriptors using the program DRAGON Professional v. 5.4.

The program BuildQSAR was used to select the variables for the generation of a linear model (MLR - multiple linear regression) with up to 4 variables.

Results and Conclusions
The most statistically regression equation, largest squared cross-validation correlation coefficient (Q\textsubscript{cv}^2), is:

\[
\text{NO}_X = +1,168(\pm0,277) \text{ RDF070u} -2,754(\pm0,749) \text{ RDF095u}
\]
\[+6,776(\pm1,995) \text{ RDF135u} -2,083(\pm0,701) \text{ RDF150u} -35,588(\pm2,998)
\]

\(n=013; r^2=0,931; s=0,670; F=27,266; Q_{cv}^2=0,856; \text{SPRESS}=0,972\)

Although there is a high correlation between the NO\textsubscript{X} and the RDF descriptors, the tribes was not distributed by the evolution order proposed by Bremer. Tribes considered less "developed", in other words, basal tribes of the Bremer’s diagram, as Arctoteae present high value of the average of oxidation number of the present diterpenes, while tribes more "developed", as Anthemideae, present low values.

Refs.
USE OF SELF-ORGANIZING MAPS AND $^{13}$C NMR SPECTRAL DATA TO PREDICT ALDOSE REDUCTASE ACTIVITY OF FLAVONOIDES

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The aldose reductase (AR) enzyme is responsible to reduce glucose to sorbitol using as a cofactor the nicotinamide-adenine dinucleotide phosphate (NADPH). Simultaneously, another enzyme, sorbitol dehydrogenase, oxidizes sorbitol to fructose. However, in diabetes conditions, glucose level in this pathway is increased and sorbitol is produced faster than oxidized to fructose. The accumulation of sorbitol in lens leads to cataract formation. Flavonoids are phenolic compounds isolated from a wide range of vascular plants, with over 8000 individual compounds known. They possess a high variety of biological activities. Structure-activity relationships of flavonoids are important due to the facility of the analysis once they are natural active substances that show small plane molecules with few functional groups. These compounds contain a basic structure constituted of 15 carbon atoms arranged in three rings (C$_6$-C$_3$-C$_6$) and showed the molecular structural requirements for a remarkable AR inhibition effect. To describe the molecular structure the $^{13}$C NMR can be used. These data give rich information about the molecular structure and they are sensitive to detect small differences on the molecule. The most used ANN architecture for pattern recognition is the Kohonen network also named of Self-Organizing Map (SOM). A SOM can map multivariate data onto a two dimensional grid, grouping similar patterns near each other. In this study, we correlate $^{13}$C NMR data with a set of 71 flavonoids with AR inhibition effect, obtained from literature. For some structures, the $^{13}$C NMR data were obtained using ACDlabs software. Since flavonoids are plane aromatic compounds, the aditivity model can be applied to predict $^{13}$C NMR data.

The unsupervised training was performed using the SOM Toolbox version 2.0 for Matlab version 6.5 computing environment by MathWorks, Inc. The correlation between the $^{13}$C NMR data to the biological activity was performed using SOM. For that, the compounds were classified as active if they exhibited $pIC_{50}=\log_{10}IC_{50}>5.69\mu M$ and inactive if they exhibited values of $pIC_{50}<5.35\mu M$. The compounds were first divided into two subsets: one training set composed of 54 molecules and one external test set composed of 17 compounds (around 20% of the whole set). The test set contains representative samples of trained group and includes the range of activity values of the training group.

The training set shows a highly significant match (85%) for active and inactive groups. Analyzing the groups separately the major match occurred among the active compounds (89%). The test group confirms the analysis success showing the highly significant match with high percentage for both groups (88%). A remarkable 100% match for the active group and a good match of 75% for the inactive compounds support the validation of the study. This difference was observed once the active compounds exhibited a common chemical environment i.e., a same pattern of substitution, thus these groups influence the electromagnetic surroundings in the same way.

Refs.
Sesquiterpene lactones are a large group of natural products from which more than 4000 structures are known mostly isolated from the Asteraceae family. These compounds are described as the active substances of various medicinal plants used in traditional medicine and possess a wide variety of biological and pharmacological activities, such as antimicrobial, cytotoxic and anti-inflammatory activities. There are strong indications that the activities are mediated by a general mechanism chemically dependent of the presence of α,β-unsaturated carbonyl structures, such as α-methylene-γ-lactones or α,β-unsaturated cyclopentenones or conjugated esters. These functional groups react with nucleophiles, especially sulfhydryl group of cysteine, by a Michael-type addition.

Although the number of sesquiterpene lactone skeletons is limited, a variety of substituents and their position and configuration isomerism, leads to an enormous number of possible structures. Complete structure determination (constitution, configuration and conformation) often represents a hard task. Thus, the $^1$H and $^{13}$C NMR spectroscopy play a very important role in the structural determination process as well as a valuable source of data for correlation between the structure features, NMR parameters and biological activities.

The aim of this work is to predict the anti-inflammatory activity of sesquiterpene lactones using the $^{13}$C NMR data and self-organizing maps (SOM).

To generate a SOM for the prediction of the anti-inflammatory activity, the data set of 58 sesquiterpene lactones containing their inhibitory concentrations ($\mu$M) in the NF-κB DNA binding assay and their $^{13}$C NMR data was employed. The NF-κB is a central mediator of the human immune system promoting the expression of over 400 target genes in response to inflammatory stimulators. To get better clustering, it was necessary to reduce the number of activity classes, which are divided in nine groups because of the experimental procedure, and to combine compounds with similar activity. Therefore, two classes of sesquiterpene lactones are generated: Class A – shows the compounds with high activities ($IC_{100}= 5 – 50\mu$M); Class I – contains the compounds with low activities ($IC_{100}= 100 – 300\mu$M).

The internal validation revealed that a correct prediction of NF-κB inhibitory activity was possible to 81.03%. The validation with an external data set that was not used during selection and training was carried out. External validation with 10 compounds, divided equally in the two classes, resulted in a correct prediction of 80%. The model can be used to search and develop lead structures with a strong inhibitory activity on NF-κB DNA binding; a good prediction is especially interesting for the higher activity class where our model, using only $^{13}$C NMR data, allows the correct prediction of 31 from 38 sesquiterpene lactones (81.58%) belonging to the class A containing the higher active compounds. Therefore, from these results one can be concluded that our model is a valuable tool for the prediction of the NF-κB inhibitory activity and for the screening of lead structures possessing this activity.

References
Piper species have also been described to contain several prenylated benzoic acids, which showed antimicrobial, moluscicidal, antifungal and anti-radicalar activities. Based on such information, the MeOH extract from leaves of *P. caldense* was evaluated to its ability to scavenge 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH) *in vitro* assay. Thus, aiming to isolate the active compound, the crude extract (542 mg) was subjected to chromatographic separation in Sephadex LH-20 (MeOH) affording four groups (I – IV) in which the anti-radicalar potential was detected in two of them (II and III). The \(^1\)H NMR spectrum of group II (147 mg) showed a 1,3,4,5-tetrasubstituted aromatic ring due the signals at δ 7.34 (d, \(J = 2.0\) Hz) and 7.42 (d, \(J = 2.0\) Hz) and a prenylated chain due the four singlets at δ 1.59, 1.62, 1.67 and 1.73, assigned to methyl groups and broad multiplets at δ 5.11 – 5.17 and at δ 2.07 – 2.27, assigned to hydrogens linked to sp\(^2\) and sp\(^3\) carbons, respectively. The triplet at δ 6.78 (\(J = 7.4\) Hz, 1H), suggested the presence of and carboxyl group in the side chain. The \(^13\)C NMR spectra (BBD and DEPT 135°) showed 27 signals, two of them assigned to carboxyl groups, six to aromatic ring and 19 to side chain carbons. Analysis of DQ-COSY, HMQC, HMBC and NOESY spectra allowed the characterization of active compound as 3-(11'-carboxyl-3',7',15'-trimethyl-hexadeca-2'E,6'E,10'E,14'E-tetraenyl)-4,5-dihydroxy-benzoic acid (1), named caldensinic acid. In order to evaluate and compare the anti-radicalar activities, 1 was acetylated to \(1a\) and both compounds were subjected to the DPPH-scavenging assay, using Trolox® as positive control. Compound 1 had the highest hydrogen-donating capacity in comparison to \(1a\), but it was twice less active than Trolox®. Thus, this evaluation suggested that the presence of catechol unit is important to anti-radicalar activity of these prenylated benzoic acid derivatives, which have been found in several *Piper* species.

![Chemical Structure](image)

\(1\) caldensinic acid \(\text{R} = \text{H}\)  
\(1a\) diacetoxy caldensinic acid \(\text{R} = \text{Ac}\)

POLAR COMPOUNDS ISOLATED FROM *Maytenus truncata* Reissek (*Celastraceae*)

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*Maytenus truncata* [“Caminha de anjo” (Angel’s little bed) or “Arvore de Natal” (Christmas tree)] is generally used in traditional medicine of the peoples who live around “Rio das Contas” region, Jequié, Bahia, Brazil, in the form of infusion or decoct for treatment of gastrointestinal tract diseases, hemorrhages and “women’s diseases”. For these reasons there are great interest to know the chemical structure of polar compounds of this plant. From *M. truncata* dried fragmented leaves or branches using phytochemical processes, were possible to obtain the following polar constituents: sulphate in organic salt (1), the polyol dulcitol or galactitol (2), the polyphenols pro-anthocyanidin (3) and 4’-O-methylepigallocatechin (4), 3β-stigmast-5-enyl β-D-glucopyranoside of (5) and the alkaloid maitein (6). These compounds were identified by spectrometric methods (IV, \(^1\)H and \(^13\)C NMR) and thin layer chromatography using in this case comparison with authentic samples. The results obtained are not contrary to popular use of *M. truncata* decoct, once polyphenols compounds, as the flavonoids 3 and 4, present antioxidant activity and protector factor\(^1\).

References

Baccharis dracunculifolia D.C. (Asteraceae) is the most important plant source of the Brazilian green propolis, which is popularly known for its antimicrobial activity\textsuperscript{1,2}. B. dracunculifolia is used in folk medicine as anti-inflammatory\textsuperscript{3}. The aim of this work was to evaluate the analgesic and anti-inflammatory activities of the leaves hydroalcoholic extract of B. dracunculifolia (BdE). The crude extract (BdE), at different doses, was evaluated by using the acetic acid-induced writhing in mice, paw edema induced by both carrageenan and histamine in rats. In addition, the in vitro effect of the BdE on the activity of cyclooxygenase-2 (COX-2) enzyme was also evaluated. The results showed that BdE reduced the number of abdominal contortions by 45.4\% (at 200 mg/kg, p.o.) and 76.2\% (at 500 mg/kg, p.o.) in the acetic acid-induced writhing in mice. Regarding the anti-inflammatory assay, BdE displayed a significant anti-inflammatory activity in the paw oedema assay in rats induced by carrageenan (48.9\% of edema inhibition at 400 mg/kg, p.o.), but it did not provide a significant reduction (p > 0.05 Student “t” test) in the paw edema assay induced by histamine in comparison with the positive control (Hydroxyzine, at 10 mg/kg, p.o.). In the in vitro COX-2 assay, the COX-2 enzyme activity was inhibited by BdE, which showed an IC\textsubscript{50} value of 45 \(\mu\)g/mL. The results obtained in this work confirmed the traditional indications of B. dracunculifolia as anti-inflammatory. Also, both the reduction in the paw edema induced by carrageenan and the acetic acid-induced writhes result from the anti-inflammatory activity of BdE, which may be related to the inhibition of the COX-2 enzyme activity.

Refs
ANTILEISHMANIAL AND ANTIMALARIAL ACTIVITIES OF TETRAHYDROFURAN LIGNANS FROM Nectandra megapotamica (LAURACEAE)

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The bark of Nectandra megapotamica (Spreng) Chodat et Hassler (Lauraceae) is used in Brazilian folk medicine as an anti-rheumatic and to relieve pain¹,². Seven tetrahydrofuran lignans, isolated from the crude hydroalcoholic extract of the barks of N. megapotamica (Lauraceae), were evaluated for their in vitro antileishmanial and antimalarial activities. Among the evaluated compounds, machilin-G (1a) and veraguensin (2a) showed the highest antileishmanial activities, displaying, for both compounds, an IC₅₀ value of 18 µg/mL and IC₉₀ value of 36 µg/mL, while galgravin (1b), nectandrin-A (1c), nectandrin-B (1d), calopeptin (2b), and ganshisandrine (3) were inactive against Leishmania donovani. In the antimalarial assay against Plasmodium falciparum, it was observed that calopeptin (2b) displayed moderate activity, with IC₅₀ values of 3800 ng/mL (against D6 clone) and 3900 ng/mL (against W2 clone), while the lignans 1a-1d, 2a, and 3 were inactive. In order to compare the effect on the parasites with the toxicity to mammalian cells, the cytotoxic activity of the isolated compounds were evaluated against the VERO cells, showing that all evaluated tetrahydrofuran lignans exhibited no cytotoxicity in the maximum dose tested.

Refs
PHYTOCHEMICAL STUDY AND COMPARATIVE EVALUATION OF THE IN VITRO ANTIMICROBIAL ACTIVITY OF Baccharis dracunculifolia (ASTERACEAE) AND BRAZILIAN GREEN PROPOLIS


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Baccharis dracunculifolia D.C. (Asteraceae) is the most important plant source of the Brazilian green propolis (BGP), which is popularly known for its antimicrobial activity1. B. dracunculifolia and BGP display similar anticariogenic2, anti-ulcer3, and immunomodulatory4 activities. However, it is still unknown whether B. dracunculifolia displays the same antimicrobial activities reported for BGP. The aim of this work was to evaluate the in vitro antimicrobial activity of B. dracunculifolia and some of its isolated compounds. The results showed that the leaves extract of B. dracunculifolia (BdE) presents antimicrobial activity, mainly against Candida krusei (IC50: 65 µg mL−1) and Cryptococcus neoformans (IC50: 40 µg mL−1). In comparison to the BdE, green propolis hydroalcoholic extract (GPE) showed better antimicrobial activity, especially against Candida krusei (IC50: 9 µg mL−1). The chromatographic fractionation of the BdE was carried out, affording the isolation of several compounds, including ursolic acid (1), 2α-hydroxy-ursolic acid (2), isosakuranetin (3), aromadendrin-4′-methylether (4), baccharin (5), viscidone (6), and hautriwaic acid lactone (7). Compounds 1 (IC50: 5 µg mL−1) and 2 (IC50: 3 µg mL−1) showed antibacterial activity against methicillin-resistant Staphylococcus aureus, while compound 3 was active against C. neoformans (IC50: 15 µg mL−1). All evaluated samples exhibited no cytotoxicity against the VERO cells in the maximum dose tested. The results showed that the BdE, similar to the GPE, displays in vitro antimicrobial activity, which may be related to the effect of several compounds present in the crude extracts3.

Refs
Several aporphine alkaloids possess significant in vitro cytotoxic properties against a number of human cancer cell lines and this class of secondary metabolites is of wide occurrence in members of the family Lauraceae. In a continuation of our search for potential antitumor agents from plants endemic to Mato Grosso do Sul, Brazil, the composition of the alkaloid extract of the leaves from Ocotea acutifolia (Nees) Mez. (Lauraceae) was studied. This work led to the isolation of two new, ocoteine N-oxide (1) and nor-ocoxylonine (2) and eight known aporphinoid alkaloids, ocoxylonine (3), ocoteine (4), O-methylcassyfiline (5), dicentrine (6), nor-dicentrine (7), leucoxine (8), isodomesticine (9) and neolitsine (10). Compound 10 is reported for the first time in the genus Ocotea. Air-dried and powdered leaves (500 g) of O. acutifolia were extracted at room temperature with EtOH. After concentration in vacuo, the EtOH extract was treated with 10% acetic acid and then partitioned with EtOAc to yield the EtOAc layer and the acid layer. The latter was adjusted to pH 8 with NH₄OH and extracted with EtOAc to yield the crude alkaloid extract. This was repeatedly subjected to column and flash chromatography on silica gel, gel filtration on Sephadex LH-20 and reversed phase HPLC separations to afford alkaloids 1-10. The structural elucidation of these isolates was mainly based on 1D- and 2D-NMR spectroscopic techniques. Alkaloids 1, 2, 4, 8, and a mixture of 4 and 6 and of 5 and 7 were tested for their in vitro cytotoxic properties against the Hep2 (larynx carcinoma) human cancer cell line (Table 1). Ocoteine (4) and a mixture of 4 and dicentrine (6) displayed significant cytotoxic activity; compounds 1, 8 and a mixture of 5 and 7 exhibited weak cytotoxicity, while 2 was inactive in this assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀(µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32.75</td>
</tr>
<tr>
<td>2</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>4</td>
<td>8.14</td>
</tr>
<tr>
<td>8</td>
<td>37.23</td>
</tr>
<tr>
<td>mixture of 4 and 6</td>
<td>10.08</td>
</tr>
<tr>
<td>mixture of 5 and 7</td>
<td>43.78</td>
</tr>
<tr>
<td>Cisplatin (positive control)</td>
<td>1.64</td>
</tr>
</tbody>
</table>

GASTROPROTECTION OF *Plinia edulis* (VELL.) SOBRAL (MYRTACEAE)

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Introduction: *Plinia edulis*, medicinal plant known as “cambucá”, is native in Brazilian Atlantic Rain Forest. However, no reports are available on the relationship between the activity of its extract and gastric ulceration. The objective of this work was to investigate the gastroprotective effect of the aqueous ethanol leaf extract and its fractions at the HCl/ethanol induced ulcers model. In addition, the gastroprotective effect of ursolic acid was accessed.

Materials and Methods: An oral dose of 100 mg/kg body wt. was initially used to establish a general profile of the antiulcerogenic activity of the extract and its fractions at HCl/ethanol model in rats. Lansoprazole at 30 mg/kg was used as the reference drug. Ursolic acid was administered at 50 mg/kg. The stomachs were excised and the ulceration area (mm²) determined. The extract was submitted to partition with organic solvents of increasing polarity and bio-guided chromatographic procedures and spectrometric analyses were performed in order to obtain purified fractions for detection of the plant metabolites involved in the gastroprotective activity.

Results and Discussion: The extract showed extremely significant antiulcer activity at the used doses being more active than lansoprazole. Among the fractions obtained by partition, the hexane fraction was the most effective, but has shown lower activity than the crude extract. β-amyrin, β-sitosterol, ursolic acid, maslinic acid and corosolic acid were identified in the hexane fraction. Although others titerpenes are well known as gastroprotectors, ursolic acid reduced the lesion area, but has not shown significant activity at this model. These results suggest that the extremely high activity observed in the aqueous ethanol extract of leaves of *P. edulis* is probably due to synergism among chemical constituents and the titerpenes are partially involved in the gastroprotective mechanisms.

Acknowledgements: To CNPq, CAPES and FAPEAM for fellowships and to CNPq, CAPES, FAPESP and FAPEAM for financial support.
SCREENING OF ACTIVE SUBSTANCES FROM Serjania erecta RADLK AS MEMORY STIMULANT

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Plants such as Ginkgo biloba, Ptychopetalum olacoides, Pseudocalymma elegans, Salvia officinalis, Physostigma venenosum, Galanthus nivalis L., Huperzia serrata and Leucojum vernum L. have been evaluated in neurological disturbances and the Alzheimer disease. The neotropical class Serjania Mill. have about 203 species known at the moment, being the most numerous of this tribe, in which it is find the Sapindaceaes family, which is rich in saponins, an active compound of adaptogen plants, such as Paullinia cupana and Cardiospermum halicacabum, with known depurative, tonic-stimulant and immunomodulator actions. Serjania erecta Radlk, popularly known as “retrato-de-téiu” and “cinco-folhas”, belongs to this family. It is endemic to sensu stricto cerrado, and it is an arbustive plant (Silva, 1998). The leaves are used as menstrual regulator, healing and antiseptic. According to Guarim Neto and his collaborators (2000), from the leaves it is prepared some teas that help in the ulcers treatment, and the root is used for the high blood pressure treatment. The objective of this project is to find out a potential drug that could prevent or delay neuronal degenerations, with an improvement of the cognitive and comportamental losses, mainly in the Alzheimer disease, that reaches an incidence of more than one million people in Brazil, through the pharmacological characterization in vivo and in vitro of the vegetal extract of Serjania erecta Radlk and its purified fractions, with an approach to the following aspects: purification, isolation and chemistry characterization, pharmacological study directed to the enzymatic properties (AChE and BuChE) and biochemistry (lipoperoxidation assay) of fractions and purified substances, pharmacological and comportamental study through the Passive Avoidance and the Elevated Plus Maze methods, induced by scopolamine, and by the fractions and purified substances. The preliminary results obtained with the aqueous extract of Serjania erecta were: presence of pharmacologically active substances: steroidal saponins, glucosilated flavonoides and condensed tannins, and the comportamental assay with rodents showed cognitive improvement (memory and attention), with antioxidant and enzymatic protection, favorable results in the hematological and biochemistry analyses, suggesting adaptogen action, without toxicity, that, therefore, stimulates the continuity of the research about Serjania erecta Radlk, evaluating the physiological actions directly in the central nervous system, action mechanism, elucidation of the chemistry structures present in the active compounds, and a second pre-clinic phase in non-rodents.

Refs.
IN VITRO ANTICANCER ACTIVITY OF EXTRACTS OF PLANT SPECIES FROM SÃO PAULO STATE

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Ethnomedicine has contributed for the development of many important plant-derived drugs. The study of natural products represents an important source of knowledge in the discovery of new anticancer drugs and infectious diseases. About 67% of effective drugs used in chemotherapy are derived from natural products.

This study is part of the Bioprospecta (Biota-Fapesp) program that has as major aim the identification and characterization of São Paulo state biodiversity, and definition of mechanisms for conservation and sustainable use.

The scope of this study was to evaluate “in vitro” antiproliferative activity of crude ethanol extracts of 25 plant species collected among the Piperaceae, Apocynaceae, Verbenaceae, Melastomataceae, Asteraceae, Marantaceae, Commelinaceae, Myrtaceae, Malpighiaceae, Solanaceae, Ericaceae, Fabaceae, Apocaceae families from São Paulo state.

The plant species were collected in Serra da Mantiqueira – SP, by Dr. Ming and identified by Dra. Torres. Grinded dry plant species were extracted on Soxhlet with ethanol. The extracts obtained were cleaned with active charcoal for chlorophyll removal, dried under vacuum, freeze dried and submitted to anticancer “in vitro” assay. The samples were evaluated for anticancer activity in UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, non-small cells), OVCAR03 (ovarian), PC0 3 (prostate), HT-29 (colon), 786-0 (renal) and NCI-ADR (ovarian expressing phenotype multiple drugs resistance) cancer cell lines. A 48 h SRB cell viability assay was performed to determine growth inhibition and cytotoxic properties of the crude extracts. Cells were treated with at least four different concentrations levels ranging from 0.25 to 250 µM/mL with determination of growth inhibition of 50% of the cells (GI50); total growth inhibition (TGI) and concentration need to kill 50% of the cells (LC50) parameters.

Among the extracts under study we observed a concentration-dependent selectivity for cell line 786-0 for samples from Apoaceae, Solanaceae, Myrtaceae, Commelinaceae, Asteraceae, Verbenaceae, Apocynaceae and Piperaceae families, compared to the positive control (doxorubicina). Whereas plant extracts from Ericaceae, Malpighiaceae and Melastomataceae demonstrated a concentration-dependent selectivity for NCI-ADR cell lines.

**IN VITRO ANTIPROLIFERATIVE ACTIVITY OF CAMPOMANESIA guazumifolia (CAMBESS.) O. BERG FRACTIONS**

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Key words: *Campomanesia guazumifolia*, antiproliferative activity

*Campomanesia guazumifolia* (Cambess.) O. Berg is popularly known as Sete-Capotés, pertaining to the Myrtaceae family. In the south of Brazil the population uses this plant to treat gastric disorders. Markman et al (2004) studied the antiulcerogenic activity of *Campomanesia xanthocarpa*. Fernandes et al (2003) and Limberger et al (2001) studied the essential oil composition of some *Campomanesia* species. This study reports the bioassay guided fractionation of crude *Campomanesia* dichloromethane and ethanol soluble fractions evaluated for *in vitro* anticancer activity in UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, non-small cells), OVCAR03 (ovarian), PC0 3 (prostate), HT-29 (colon), 786-0 (renal) and NCI-ADR (ovarian expressing phenotype multiple drugs resistance) cancer cell lines. A 48 h SRB cell viability assay was performed to determine growth inhibition and cytotoxic properties of the fractions. Cells were treated with at least four different concentrations levels ranging from 0.25 to 250 µm/mL with determination of total growth inhibition expressed by three concentration-dependent parameter; concentration that produces 50% growth inhibition (GI50), concentration which totally inhibits cell growth (TGI) and concentration that produces 50% lethal cell killing (LC50). The aerial parts of the *Campomanesia guazumifolia* (Cambess.) O. Berg were collected in CPQBA-Unicamp experimental field on March 2007. Leaves *in natura* were grinded with dry ice, and extracted with dichloromethane. After filtration, the solvent was evaporated under vacuum 40ºC, supplying the diclorometanic crude extract (DRE). The plant residue was retaken in ethanol, extracted with producing the ethanolic crude extract (ERE). This extract (ERE) presented antiproliferative activity superior to the dichloromethane crude extract (DRE). Therefore ERE was further extracted with variation of pH producing four fractions denominated: fraction A (acid fractions), fr B (basic fractions), fr NA (neutral apolar fraction) and fr NP (neutral polar fraction). Fr NA showed selectivity in a concentration-dependent way for HT-29 (colon) cell line with the most potent GI50, TGI and LC50 values. Fraction B showed inhibition in a concentration-dependent way for MCF-7 (breast), NCI 460 (lung), UACC62 (melanome) and OVCAR-3 (ovary) cell lines, whereas fraction NP inhibited all cell lines, and fraction A was inactive.


CNPQ, FAPESP
Antioxidant activity of plant extract is often associated with the presence of polyphenolic compounds, which have an important role in stabilizing lipid oxidation and have inhibitory effects on mutagenesis and carcinogenesis in human when is daily ingested from a diet rich in fruits and vegetables\(^1,2\). It is well known that this activity is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals. These reactive species are formed by the normal metabolism of aerobic cells starting from the oxygen molecular reduction. When these radicals had an excessive formation induce oxidative damage to biomolecules, which eventually causes numerous neurodegenerative disease\(^3\). This work describes the total phenolic (TP) content by Folin-Ciocalteu\(^4\), DPPH free radical scavenging (FRS)\(^5\) and total antioxidant (TAA) activities by FTC method\(^6\) of extracts and isolated flavonols from leaves of *Tournefortia bicolor*. The air-dried leaves were extracted at room temp. with acetone and EtOH 90%. These extracts were suspended in MeOH-H\(_2\)O (3:2), extracted successively with C\(_6\)H\(_6\), CHCl\(_3\) and EtOAc and were evaluated for their TP content, FRS and TAA activities. The EtOAc fractions from both crude extracts showed a high phenolic content (acetone: 365.18 mg/g D.W and EtOH: 691.78 mg/g D.W). Thus, these fractions were evaluated for FRS and TAA activities, where EtOAc fraction (EtOH extract) showed a significant FRS (IC\(_{50}\) 30.0 ± 3.2 µg/mL) and TAA (85.44% at 100 µg/mL) activities. This fraction was filtered on silica gel column and the most active CH\(_2\)Cl\(_2\)-EtOAc 1:1 sub-fraction \(\geq 1000\) mg/g D.W, FRS (IC\(_{50}\) 12.8±5.6 µg/mL) and TAA inhibition (85.17% at 25 µg/mL), after successive chromatographic fractionations (silica gel and Sephadex LH-20) afforded rutin (1, 25 mg) and tiliroside (2, 15 mg). On the other hand, EtOAc fraction (acetone extract) with a good FRS (101.0 ± 3.5 µg/mL) and TAA (78.37% at 50 µg/mL) activities, after the same experimental procedure afforded quercetin (3, 25 mg). These flavonols (1-3) were also evaluated and showed a significant FRS and a good TAA inhibition at 25 µg/mL when compared with standard used (AA, α-TC and BHT) (Table 1). These results suggest that this species contain phenolic compounds that may act as antioxidant.

<table>
<thead>
<tr>
<th>Seção 1.02 Isolated Substances and Standards</th>
<th>DPPH FRS* IC(_{50}) (µg/mL)</th>
<th>TAA Inhibition (%) at 25 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin (1)</td>
<td>21.82 ± 3.3</td>
<td>84.01</td>
</tr>
<tr>
<td>Tiliroside (2)</td>
<td>50.4 ± 4.2</td>
<td>80.26</td>
</tr>
<tr>
<td>Quercetin (3)</td>
<td>7.67±3.6</td>
<td>90.0</td>
</tr>
<tr>
<td>Ascorbic acid (AA)</td>
<td>37.37 ± 3.18</td>
<td>91.0</td>
</tr>
<tr>
<td>α-Tocopherol (α-TC)</td>
<td>92.51 ± 3.17</td>
<td>91.0</td>
</tr>
<tr>
<td>BHT</td>
<td>73.78 ± 13.73</td>
<td>91.0</td>
</tr>
</tbody>
</table>

* The average values of three assays \((n = 3)\) are presented as mean±S.D. *at 25 µg/mL.


Support: CNPq, FAPEAL, IMSEAR and BNB-RENORBIO.
RADICAL SCAVENGING AND ANTIOXIDANT ACTIVITIES OF PHENOLIC COMPOUNDS AND EXTRACTS FROM FRUITS OF *Triplaris americana* L.

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Damage from free radicals has been implicated in the some degenerative disorders, cancer, heart disease, diabetes, and atherosclerosis. Antioxidants can protect the human body these radicals and retard the process of many chronic diseases. In recent years, several methods such as DPPH and ferric thiocyanate (FTC), have been used to determine the anti-radical and antioxidant capacities of the biologic samples, plant extracts and isolated compounds. Thus, this work describes the total phenolics (TP) content, free radical scavenging (FRS) and total antioxidant activities (TAA) by Folin-Ciocalteu, DPPH and FTC methods, respectively, of isolated compounds and crude extracts from fruits of *Triplaris americana* L. The fresh fruits were extracted at room temp. with C₆H₁₄ and MeOH. The MeOH extract was suspended in MeOH-H₂O (3:2) and extracted successively with C₆H₁₄, CHCl₃ and EtOAc. The obtained fractions were evaluated and the EtOAc and MeOH-H₂O extracts showed significant TP content, FRS and TAA activities (Table 1), which the relationship between these activities for many of crude extracts was positive and significant (p<0.05). These results suggest that this species contain a variety of phenolic compounds that may act as antioxidant. So, these fractions were filtrated on silica gel column with solvent of different polarities and the most active EtOAc sub-fractions (C and E), after successive chromatographic fractionations and TLC preparative, afforded 1 (13 mg) and 2 (24 mg) from C and 1 (14 mg) and 3 (7 mg) from E. These compounds were also evaluated and showed significant FRS and good antioxidant activities (at 25 µg/mL) when compared with standards used (AC, α-TC and BHT) (Table 1). Compound 2 that has an arabinofuranosyl unity at C-3 was lightly more active as antioxidant than 3. This fact can probably be associated to the degree purity this last compound.

Table 1. Total phenolics content, free radical scavenging and total antioxidant activities of crude extracts and isolated substances (1-3).

<table>
<thead>
<tr>
<th>Crude extracts/Isolated compounds/ Standards used</th>
<th>TP Content * (mg/g D.W GAE)</th>
<th>DPPH* (IC₅₀ (µg/mL))</th>
<th>TAA Inhibition (%)* at 25 µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>EtOAc</td>
<td>997.3 ± 3.55</td>
<td>52.58 ± 3.13</td>
<td>88.3</td>
</tr>
<tr>
<td>CH₃Cl₂ (A)</td>
<td>NT</td>
<td>&gt; 200</td>
<td>NT</td>
</tr>
<tr>
<td>CH₃Cl₂-EtOAc 1:1 (B)</td>
<td>&gt; 1000</td>
<td>24.32 ± 3.04</td>
<td>82.3</td>
</tr>
<tr>
<td>EtOAc (C)</td>
<td>&gt; 1000</td>
<td>37.93 ± 6.12</td>
<td>93.0</td>
</tr>
<tr>
<td>EtOAc-MeOH 1:1 (D)</td>
<td>1000 ± 33.45</td>
<td>51.92 ± 1.83</td>
<td>94.0</td>
</tr>
<tr>
<td>MeOH-H₂O → EtOAc (E)</td>
<td>601.8 ± 15.02</td>
<td>71.99 ± 7.19</td>
<td>90.8</td>
</tr>
<tr>
<td>Gallic acid (1)</td>
<td>14.10 ± 6.10</td>
<td>90.53</td>
<td></td>
</tr>
<tr>
<td>3-O-α-Arabinofuranosylquercetin (2)</td>
<td>30.64 ± 1.75</td>
<td>90.71</td>
<td></td>
</tr>
<tr>
<td>Quercetin (3)</td>
<td>27.98 ± 5.46</td>
<td>82.58</td>
<td></td>
</tr>
<tr>
<td>Ascorbic acid (AC)</td>
<td>37.37 ± 3.18</td>
<td>91.0</td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (α-TC)</td>
<td>92.51 ± 3.17</td>
<td>91.0</td>
<td></td>
</tr>
<tr>
<td>BHT</td>
<td>73.78 ± 13.73</td>
<td>NT</td>
<td></td>
</tr>
</tbody>
</table>

NT = Not tested. * The average values of three assays (n = 3) are presented as mean ± S.D.


Support: CNPq, FAPEAL, IMSEAR and BNB-RENNOR BIO.
Statistical design to development of *Lychnophora ericoides* leaves extract with high vicenin-2 content by chemical and biological monitoring.

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**Introduction.** The vicenin-2, antinflammatory flavone, was present in *L. ericoides* leaves, therefore sesquiterpenes lactones can attribute toxic properties for the extract\(^a\). **Objective.** The aim was develop a standard liquid extract from *L. ericoides* leaves with high vicenin-2 containing using a factorial design and genotoxic monitoring. **Methodology.** Extract development. The factorial design \(^2\) was used to development of *L. ericoides* leaves extract with high vicenin-2 content. The variables studied were: extraction time; plant: solvent ratio and alcoholic content. The vicenin-2 levels were analyzed using validated HPLC method. The statistical analyses of experimental design were generated with Statistica 5.0\(^b\). **Genotoxicity monitoring by Comet Assay.** Around \(0.5 \times 10^5\) HTC cells (rat hepatoma cells (*Rattus norvegicus*)), were incubated in culture tubes containing culture medium. Cells were treated with ethanolic extract and their alcoholic vehicle. MMS (alkylating agent) was used as positive control and PBS as negative control. The cells were prepared for the comet assay according to the protocol\(^c\) and they were then viewed with a fluorescence microscope\(^c\). The data was carried out using ANOVA for all the results of each test and Dunnet’s test to compare the number of cells with DNA damage in the comet assay, with the mean values in PBS or MMS controls (p < 0.05). **Results.** The best level of vicenin-2 was found to ethanol 20\(^{\circ}\)GL (EET 20). Although the vicenin-2 chemical monitoring had showed the best concentration in EET 20, the folk medicine extraction fabrication practices ethanol with major alcoholic graduation (around 80\(^{\circ}\) GL) (EET 80). Because this fact ours studies included the genotoxicity monitoring of both extracts (EET 20 and EET 80). The damaging score show that the solvents ethanol 80 \(^{\circ}\)GL and 20 \(^{\circ}\)GL alone do not induce hepatomes DNA damage. The EET 20 presented lower damaging score than negative control (score = 8) in both concentration tested 25 \(\mu\)L (score = 5.3) and 50 \(\mu\)L (score = 7.7). This result suggests a genoprotective effect for EET 20. However, the EET 80 presented damaging score near than positive control, so the EET 80 damaging score was 26.7 for 25 \(\mu\)L concentration and 140.7 for 50 \(\mu\)L. The positive control score was 201. These data show the high genotoxic effect to extract prepared with ethanol 80 \(^{\circ}\)GL. **Conclusion.** The EET 20 had the best extractive efficiency for vicenin-2 and the extract with high vicenin-2 level showed no genotoxicity effect. The combined of chemical and biological results can be more effective to obtain better results using few experiments. **Financial support.** Fapesp and Fundação Araucária.

**References**


CYTOTOXIC ACTIVITY OF COMPOUNDS ISOLATED FROM ENDOPHYTIC FUNGI OF Smallanthus sonchifolius (ASTERACEAE)

Margareth B. C. Gallo¹; Andrew S. Nunes²; Bruno C. Cavalcanti²; Manoel O. Moraes²; Letícia V. Costa-Lotufo²; Cláudia Pessoa²; Jairo K. Bastos¹; Mônica T. Pupo¹

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²Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Universidade Federal do Ceará.

Yacon (S. sonchifolius) originally cultivated in the Andean highlands, was introduced in Brazil in 1989. Its tubers are reported to contain a high concentration of oligofructans, polysacharides not metabolized by human beings thus employed as diet food. Besides, leaves are sources of kaurene-type diterpenoids presenting antifungal activity.¹ Endophytes are microorganisms regarded as mutualists when living inside plants without causing diseases and are innovative fonts of bioactive secondary metabolites. From the leaves and roots, subsequent to surface-sterilizing procedures, was isolated Papulospora immersa (PI) and from the stem Phoma betae Frank (PB). They were cultivated on rice medium at 30°C for 20 and 60 days, respectively. Both media were extracted with ethanol and partitioned in ethyl acetate yielding the correspondent fractions (EAF). These fractions were effective in the cytotoxic bioassay, that was assessed using the MTT assay at a single sample concentration (100 µg/mL for extracts) in four human tumor cell lines, HCT-8 (colon), SF295 (glioblastoma), LH-60 (promyelocitic leukemia) and MDA-MB435 (breast), for 72 h. Doxorubicin was used as positive control. PI-EAF produced ergost-4-en-3-one (1), ergost-4,6,8(14),22-tetraen-3-one (2) and an additional substance not yet characterized (3), whereas compounds 1 and dimethylterephtalate (4) were isolated from PB-EAF. Compounds 1, 3 and 4 displayed an IC₅₀ > 25 µg/mL in all biossayed tumor cells whilst substance 2 was primarily active against colon and leukemia cell lines (Table1). Until now, from both PI and PB was isolated compound 2 whose cytotoxic effect was considered moderate. So it has not been viewed as the mainly active compound responsible for the initial extract activities and a more detailed chromatographic screening of the extracts is been carried on in order to isolate further cytotoxic compounds. In addition, PB is a notable species for its ability of biosynthesizing aphidicolan-16β-ol synthase, a key enzyme in the biosynthesis of an unusual tetracyclic diterpene and important anticancer agent named aphidicolin.² Moreover chemical study of PI is pioneer and can contribute to the knowledge of the chemical profile of the genus.

Table 1. IC₅₀ (µg/mL) of ergost-4,6,8(14),22-tetraen-3-one

<table>
<thead>
<tr>
<th>Compound</th>
<th>HCT-8</th>
<th>SF295</th>
<th>LH-60</th>
<th>MDA-MB435</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>6.2</td>
<td>17.0</td>
<td>5.3</td>
<td>14.1</td>
</tr>
</tbody>
</table>

References
LARVICIDAL ACTIVITY OF GRANDISIN AGAINST Aedes aegypti L.

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²Departamento de Ciências Biológicas/CECETEN, Universidade Severino Sombra/USS, Vassouras, RJ, Brazil.
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INTRODUCTION:
Dengue is a disease caused by an arborivus transmitted by Aedes aegypti (Diptera: Culicidae) and with predominantly occurrence in tropical and subtropical countries. Since no effective vaccine is available, the strategy for disease prevention has been addressed to vector control using natural insecticides from plants. The aim of this study was to evaluate the larvicidal activity of lignan grandisin isolated from Piper solmsianum (Piperaceae).

EXPERIMENTAL:
P. solmsianum was collected in Picinguaba, Ubatuba, SP and the identification of the botanical material has been reported previously (Martins et al., 2000). The purification of the lignan grandisin from aerial parts has been carried out as described (Martins et al., 2003).

Ae. aegypti eggs were collected in Seropédica and Nova Iguaçu, RJ, from ovitraps methods (Alencar et al., 2004) and has been maintained in the Laboratory of Diptera/IOC/RJ. The grandisin solution (30µL/mL) was applied on Petri dish with dechlorinated water (30mL) at final concentrations of 1µg/mL⁻¹, 10µg/mL⁻¹ and 100µg/mL⁻¹ containing fish food suspension (1g/larvae). The L1 larvae (25 larvae/groups) of Ae. aegypti in triplicate were evaluated for grandisin effect and the controls groups included acetone solution (without grandisin) and untreated solution. The mosquitoes were maintained at 27±1°C and 70±10% RH during all the experiments and the toxicity and growth development of Ae. aegypti were evaluated during 15 days.

RESULTS AND CONCLUSION:
Ae. aegypti treated with grandisin solutions showed viability of 32% (1 µg/mL⁻¹) and 12% (10 µg/mL⁻¹) in the larval stage, and only 4% (1 µg/mL⁻¹) and 8% (10 µg/mL⁻¹) of emergence have been observed. The grandisin treatment showed 100% larvae toxicity at 100 µg/mL⁻¹ and a LD₅₀ of 2.37 µg/mL⁻¹. The activity of the lignans from Piper species suggests their potential in developing new useful types of mosquito’s control. (FAPERJ; FAPESP; CAPES; IOC).

REFERENCES
CHROMOBACTERIUM CELL LINES AS SOURCE OF NEW ANTICANCER CHEMOTHERAPEUTICS AGENTS COLLECTED AT AMAZON REGION

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\textsuperscript{1} Centro Pluridisciplinar de Pesquisas Químicas Biológicas e Agrícolas, Universidade Estadual de Campinas, \textsuperscript{2} IB/Unesp Botucatu, \textsuperscript{3} ICB-USP/São Paulo

With more than 10 million new cases yearly, cancer has become one of the most devastating diseases worldwide. Despite all the known treatments, the results are still unsatisfactory and cancer has become a leading cause of death. In 2005, cancer was responsible for 13\% of all deaths worldwide, showing the need for development of new anti-cancer drugs\textsuperscript{1}.

Microorganisms have become an important source of natural products for research and of great interest to pharmaceutical industries. Many known antibiotics, such as, Penicillin are secondary metabolites originated from microorganisms\textsuperscript{2}. Violacein, a purple pigment extracted from \textit{Chromobacterium violaceum}, has been reported to have antibiotic, trypanocide and antiviral properties. Several studies have shown that violacein is also capable of inducing apoptosis in a variety of cancer cell lines\textsuperscript{3, 4}. The aim of this work was to study \textit{Chromobacterium sp.} strains crude extracts to identify compounds with growth-inhibitory or toxic effects on particular human tumor cells. The extracts were prepared by soxhlet using chloroform, ethyl ether and ethanol sequently. The antitumor activities were evaluated on eight different human tumor cell lines: lung (NCI460), colon (HT29), breast (MCF7), prostate (PCO3), ovarian (OVCAR03), melanoma (UACC62), renal (7860) and ovarian expressing phenotype multiple drugs resistance (NCIADR). The results showed that the crude extracts inhibited cancer cell line growth in a concentration-dependent way. The parameters GI\textsubscript{50} (50\% growth inhibition), TGI (total growth inhibition) and LC\textsubscript{50} (50\% lethal concentration) values were determined. A mean graph according to Holbeck\textsuperscript{5} for CBMAI 305 and CBMAI 310 strains of \textit{Chromobacterium sp.} crude chloroform extracts corroborated the selectivity for NCI.ADR cell lines in a concentration-dependent way, whereas the positive control, doxorubicin, was unable to inhibit this cell line’s growth.

\textit{Chromobacterium} sp. 305 and 310 strains presented another interesting characteristic: phylogenetic analysis of RNA ribosomal 16S gene show that CBMAI 305 strain grouped with \textit{Chromobacterium violaceum} type strain, however, CBMAI 310 show phylogenetically related distant of the two described species type, \textit{C. violaceum} and \textit{C. suttsugae}, suggesting another species for the genus.

Therefore the results reported herein indicate that \textit{Chromobacterium} extracts produce potent anticancer metabolites that after further phytochemical & pharmacological studies might lead to interesting new pharmacophores capable of providing new anticancer chemotherapeutics agents.

\begin{enumerate}
\item Stewart, B.W., World Cancer Report (2003)
\item Diggins, F.W.E., British Journal of Biomedical Science, 56 (2), 83-93 (1999)
\item Carvalho, D.D \textit{et al}, Toxicology in Vitro, 20, 1514-1521 (2006)
\end{enumerate}
The family Meliaceae is a source of numerous secondary metabolites that exhibit significant insecticidal activity. Screening programs of Meliaceae have identified the genus *Trichilia* as a source for potential development. This work describes the results of the search for insecticidal compounds from the Meliaceae family. The samples assayed against fall armyworm *Spodoptera frugiperda* were the hexane, methanol and hydromethanol extracts of twigs, leaves, seeds, aril and exocarp of *T. catigua*; twigs, leaves, fruits, seeds, aril and exocarp of *T. claussenii* and twigs, leaves and fruits of *T. elegans*. The hexane and methanol extracts of leaves, hexane of twigs and methanol of fruits from *T. claussenii* showed to possess the higher insecticidal activity against *S. frugiperda*. Nevertheless, the hexane and methanol extracts of fruits from *T. elegans* showed the highest rate of larval mortality (100%). The hydromethanol extract of *T. elegans* showed moderate insecticidal activity and also showed growth inhibition and antifeedant activities. The active extracts were fractionated leading to the isolation of 20 compounds, including limonoids, triterpenes, coumarins, steroids and one sesquiterpene. From the hexane extract of leaves from *T. claussenii* were isolated the steroids β-sitosterol, stigmasterol, campesterol and sitostenone; the triterpenoids α-amyrin, β-amyrin, lupeol, lupenone, in addition to the cycloartane triterpene 24-methylen-26-hydroxycicloartan-3-on and sesquiterpene criptomeridiol. From the methanol extract of leaves of *T. claussenii* were isolated the steroids 24-methylen-3β,4β,22α-trihydroxy-colesterol and 3-β-O-β-D-glucopyranosilsitosterol. From the methanol extract of fruits of *T. elegans* were isolated the coumarins 6,7-dimethoxycoumarin, 6-methoxy-7-hydroxycoumarin and 7-hydroxycoumarin. The same coumarins and the steroids were also isolated from the hexane extract of fruits from *T. elegans*. The limonoids cedrelone and 11β-methoxycedrelone were isolated from the hexane extract of aril from *T. catigua*. Limonoids methylangolensate together with the photogedunin epimeric mixture were isolated from the methanol extract of aril from *T. catigua*. This is the first report of the isolation of limonoids from *T. catigua*.

References

Antinociceptive effect of crude metanolic extract of *Condalia buxifolia*.

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*Condalia buxifolia* is a tree that grows in the south of Brazil, Uruguay and Argentina, where it is used by the local people as antitermic and in the treatment of dysenteries. The aim of the present work was to evaluate the potential antinociceptive activity of the crude metanolic extract (CME) of *Condalia buxifolia*. The antinociceptive activity was investigated in male mice, weight 20-25g, n=7-8, in the experimental models of acetic acid-induced abdominal constriction and hot-plate. The results showed that in the acetic acid-induced abdominal constriction the doses of 100 and 1000 mg/kg of the CME given orally significantly inhibited the constriction in 42.8% and 43%, respectively, comparing to the control group. When the CME of *Condalia buxifolia* was given by intraperitoneal route, the doses of 10, 30 and 100 mg/kg inhibited the constrictions in 67.8%, 58.6% and 68%, respectively, comparing to the control group. The hot-plate test demonstrated that the CME of *Condalia buxifolia* given orally significantly increased the average time of latency for the withdrawal of the hind paw, showing the peak of the antinociceptive activity in the fourth hour after administration. The tested doses of 100, 300 and 1000 mg/kg respectively increased the latency for the withdrawal of the hind paw in 103, 104 and 73%, comparing to the initial measure. When given by intraperitoneal route (10, 30 and 100 mg/kg), only the dose of 100 mg/kg significantly changed the latency response in the hot-plate. There was no significant difference when compared to the group morphine (positive control). The attained results describe for the first time the antinociceptive properties of *Condalia buxifolia* in the acetic acid-induced abdominal constrictions and hot-plate tests, representing a scientific contribution for the rational use of medicinal plants.
Plants have long been an available source of medicines in human communities worldwide. The Brazilian flora is vast, varied and each one of its biomas are rich in species, most of them with medicinal attributes. The *Endopleura uchi* (Huber) Cuatrec. is a member of the family Humiriaceae and is common in the Brazilian states of Pará and Amazonas, in the north of Brazil. According Sant’ana *et al.* (2000) and Ferreira *et al.* (2005), the stem of this plant has two major compounds: bergenin and 8,10 – dimethoxibergenin. *Endopleura uchi* is largely used by the people to combat miomas, although there are just a few biological studies with its extract. The aim of this study was to evaluate the pharmacologic activities of this plant. In the first part of this study, the extract of the bark of *E. uchi* was obtained by decoction with water. This extract was dried and used in the pharmacological study of anti-inflammatory activity with rat paw edema model (Winter *et al.*, 1962) in a dose of 100 and 200 mg/Kg. The result showed that in the rat paw model, the inflammation response was significantly reduced in the oral treatment of animals with plant extract with a maximum decrease in paw volume by 18.7% in the dose of 100 mg/Kg and 26.9% with the dose of 200 mg/Kg. It can be concluded that the extract of *E. uchi* has anti-inflammatory activity.

Refs.
PRELIMINARY STUDIES OF ANTIMICROBIAL ACTIVITY OF *Endopleura uchi* (Huber) Cuatrec. (HUMIRIACEAE) BARKS


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The great diversity of plant species available in Brazil raise interest from pharmaceutical industries and some research groups directionated at development of new phytoterapic drugs. *Endopleura uchi* (Huber) Cuatrec. belongs to Humiriaceae family and is common in the Brazilian states of Pará and Amazonas, in the north of Brazil. The stem of this plant has two major compounds, according to Sant’ana et al. (2000): bergenin and 8,10 – dimethoxiberginin. The barks are largely used by the population to combat miomas, although there are just a few biological studies with its extract. This study aimed evaluates the antimicrobial activity of dry aqueous extract and the dry ethanolic extract 50% in front of *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Bacillus subtilis* (ATCC 9372), *Proteus mirabilis* (ATCC 25933), *Pseudomonas aeruginosa* (CC), *Shigella somnei* (CC), *Enterococcus faecalis* (CC). The aqueous extract was obtained by infusion at 10% concentration and posterior lyophilization. The ethanolic extract 50% was obtained by turboextraction and concentrated under reduced pressure in rotatory evaporator until dryness. The extracts were resuspended in dimethylsulfoxide (DMSO) to obtain the stock solution 1:1 (v/v). One colony of each strain was inoculated in brain and heart infusion and incubated at 37 °C during 24 h. After the inocula were prepared by adjusting the turbidity of the suspension to 0,5 McFarland standard with saline solution 0,9%. To Petri dish were added Muller-Hinton Agar, in which 150µL of bacterial suspension in adequate concentration were added. Petri dish prepared and homogeneized, after solidification were used to evaluate antimicrobial activity through *templates technique*. Each well received 50µL of ampicillin solution (50µg/mL); 100µL of each *E. uchi* extract and 50µL of DMSO as negative control. The plates were incubated at 37 °C for 24 h. Bacterial growth inhibition was determined by the inhibition haloes around the wells. Our preliminary results showed no inhibition of bacterial growth for all strains tested with this extract concentration. However, the antimicrobial activity must be best studied using different extract concentration and other techniques to explore this biological activity.
The Agave sisalana (sisal) presents fungicidal substances that inhibits Saccharomyces cerevisiae and other fungus as Cryptococcus neoformans and Crebrothecium ashbyi. Saccharomyces cerevisiae is the responsible microorganism in the process of the industrial alcoholic fermentation. It is presented as deteriorant in the production of soft drinks. Although the Lactobacillus fermentum is an important contaminant in the process of the industrial alcoholic fermentation. This work aimed at evaluation the capacity of growth inhibition Agave sisalana extracts in five different concentrations (from 3750 ppm to 234.37 ppm) against two distinct lineages of Saccharomyces cerevisiae (CCT 4370 and FCLA M26), and Lactobacillus fermentum (CCT 1396 and CCT 0559).

The Minimal Inhibitory Concentration (MIC) was determined by the method of macrodilution with following adaptations: 6 mL of medium MRS (Man Rogosa Sharpe) (Difco), in pH 6.0, for L. fermentum and formulated medium 2% of sucrose, in pH 5.0 for S. cerevisiae. Both sterilized 121°C per 15 minutes. Inoculate was standardized in accordance with the Macfarland 0.5 in aseptic conditions. The cultures have been incubated 30°C. The cellular growth of the bacteria and the yeast was measured in spectrophotometer at 600nm. The MIC was carried through in triplicate and it was done a mean of percentage of inhibition results. It had an inhibition against S. cerevisiae (CCT 4370) in 94% on the concentration of 468.75 ppm. For the FCLA M26 lineage there was an inhibition of 100% on the 937.5 ppm. However, for both the lineage of L. fermentum, there was not inhibition in the growth (MIC >3750 ppm). The gotten results are excellent and suggest that new assays must be carried through objectifying its application in the microbiology control in the nutritious industry.

Refs.
PASSIFLORA EDULIS FORMA FLAVICARPA DEGENER (MARACUJÁ) INHIBIT INFLAMMATORY RESPONSE IN A MURINE MODEL


Introduction: In Brazil there are several native plants of the genus Passiflora, known as maracujás. Some of these species are cultivated because their fruits are used in the juice preparation. Additionally, the tea of their leaves are used in the popular medicine as sedative, tranquilizer, against cutaneous inflammatory diseases, and intermittent fever. Objectives: The aim of this study was to evaluate the anti-inflammatory activity of fractions and compounds isolated from leaves of Passiflora edulis forma flavicarpa Degener (P. edulis) in the mouse model of pleurisy induced by carrageenan. Material and methods: The leaves of P. edulis were collected in October of 2003 in Antônio Carlos, SC, Brazil (FLOR 33886). The powdered leaves were extracted under infusion for 10 min and partitioned with ethyl acetate (EtOAc) and n-butanol (BuOH). The BuOH fraction (BF) was chromatographed on a flash column, in silica gel, resulting in four sub-fractions named A-D. Further, from the C sub-fraction (CSF), three compounds were isolated by chromatographic procedures. In the in vivo study non-fasted adult Swiss mice of both sexes (18–22 g) were used throughout the experiments. The mouse model of pleurisy was induced by carrageenan (Cg, 1%). Different groups of animals were pretreated (0.5 h, i.p.) with BF, A-D sub-fractions or isolated compounds 1, 2 and 3. The total and differential leukocytes and myeloperoxidase (MPO) levels in the pleural fluid were evaluated 4 h after Cg injection. Statistical differences between groups were determined by analysis of variance (ANOVA) and complemented with either Dunnett’s or Student t tests. Values of P < 0.05 were considered as indicative of significance.

Results: BF fraction (50 and 100 mg/kg) inhibited leukocytes from 31±4.0 to 57±5.4 (P < 0.05), neutrophils from 38±4.8 to 67±6.0 (P < 0.05) and MPO levels from 63±44 to 62±37 (P < 0.01). Amongst the A-D sub-fractions, the CSF showed best result. This fraction at doses of 50 mg/Kg was capable to inhibit leukocytes by 50±71, neutrophils by 72±0.60 (P < 0.01) and MPO levels by 60±36 (P < 0.05). Compounds 1 and 2 (25 mg/kg), isolated from CSF also inhibit leukocytes by 57±10 and by 43±8.6 (P < 0.01), and neutrophils by 53±13 and by 41±8.0 (P < 0.01), respectively. Only the compound 1 decreased MPO levels by 43±27 (P < 0.05). Compound 3 did not change the studied inflammatory parameters. Compound 1, 2 and 3 were identified as flavonoids on the basis of UV and are now being elucidated by NMR spectroscopy (results not shown). Conclusion: The P. edulis leaves showed promising results as anti-inflammatory agent by inhibiting activated leukocytes due to neutrophils to the site of inflammation by carrageenan in mice. The present study provides evidences that flavonoids, can be, in part, responsible for this activity.

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EVALUATION OF THE ANTIOXIDIZING ACTIVITY OF ISOLATE D*IMENIA AMERICANA L. EXTRACTS AND COMPOUNDS

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Irreversible oxidative damage to biomolecules caused by free radicals is one of the factors associated to the development of several degenerative diseases such as atherosclerosis, Parkinson’s disease, Alzheimer’s disease, cardiovascular diseases, cancer, diabetes, etc.¹ To evaluate the antioxidizing activity of vegetal extracts and their pure compounds, the assay with radical DPPH is frequently used as it is a comparatively easy and fast method.² In this work, the antioxidizing activity of ethanol extracts of the bark, stem wood, and leaves of Ximenia americana was evaluated. The hexane, chloroform, ethyl acetate, and hydromethanol fractions obtained by liquid-liquid partition of extracts and of pure compounds derived from the ethyl acetate fractions of leaves and bark were submitted to quantitative antioxidant assays. Activity was evaluated according to the methodology described in literature with small modifications and the consumption of free radical DPPH in samples was monitored by measuring the decrease in absorbance of solutions with different concentrations in a UV-Vis spectrophotometer at 516 nm wavelength with rutin and gallic acid as positive controls. The intensity of the capacity of the samples to arrest free radicals was quantitatively evaluated by the percent inhibition value. At 100 µg/mL, EtOH extracts of stem bark, leaves, and stem wood presented a percent inhibition of radical DPPH of 94.75% ± 0.27, 57.27% ± 4.20, and 42.83% ± 2.43, respectively. Therefore, stem bark presented antioxidizing activity comparable to that of the positive control, gallic acid, (94.84% ± 0.44), and larger than that of rutin (89.25% ± 0.25). The hexane fractions of the three EtOH extracts presented low percent inhibition of radical DPPH, showing it to be poor in antioxidizing compounds. At 100 µg/mL, ethyl acetate extracts of stem bark, leaves, and stem wood reduced DPPH by 93.49% ± 2.85, 92.33% ± 3.12, and 46.65% ± 2.83, respectively. The ethyl acetate stem wood fraction was more active at concentrations of 250 µg/mL (82.34% ± 5.39), and 200 µg/mL (72.87% ± 6.94). Flavonoids epi-cathequin, from stem bark, and quercetin, from leaves, were isolated from the ethyl acetate fractions. At 50 µg/mL, the isolated compounds, quercetin and epi-catequin and the positive controls, gallic acid and rutin, presented DPPH percent inhibition of 93.84% ± 0.13, 62.62% ± 2.5, and 83.59% ± 3.77, and 64.34% ± 3.73, respectively. The IC₅₀ values of extracts, fractions, and compounds were calculated and the results show that the ethyl acetate fractions of X. americana stem bark (29.62 ± 3.00) and leaves (33.03 ± 2.66) are the most active. The high antioxidizing activity of these fractions is justified by the presence of epi-cathequin (34.64 ± 3.00 µg/mL) and quercetin (16.40 ± 1.30 µg/mL).

Malaria remains one of the major public health problems in most tropical countries. According to the WHO, about 300-500 million new cases are recorded every year. In Brazil, it was recorded 600 thousand cases in 2005. There are around 152 thousand people living in high-risk areas concentrated in the Legal Amazon - a 5.1 million square km administrative unit that encompasses the Brazilian states of Acre, Amazonas, Roraima, Amapá, Pará, Rondônia, and portions of the states of Maranhão, Tocantins and Mato Grosso. In search of new antimalarial drugs based on ethnopharmacological study, we singled species *Cecropia pachystachya* for study. We evaluated its schizonticide activity in blood and carried out its chemical study. Rats infected with *P. berghei* were treated orally with different doses in four consecutive days. *In vitro* tests for *P. falciparum* were carried out by the tritiated hypoxanthine incorporation method according to methodology described in literature. Amounts of 125, 250, and 500 mg/kg of the root raw ethanolic extract reduced parasitemia by 48 to 66% on days 5 and 7 after inoculation in relation to untreated control rats. *In vitro* tests with 50 and 25 µg/ml doses inhibited *P. falciparum* parasitemia by up to 90%. The liquid-liquid partition of this extract resulted in hexane, chloroform, ethyl acetate, and hydromethanolic fractions, which were also submitted to antimalarial tests. Only the hexane fraction was active, reducing parasitemia by 42% *in vivo* at 500 mg/kg and by 60% *in vitro* at 50 µg/ml. After silica gel filtration, the seven subfractions obtained from this fraction were submitted to bioassay and four reduced parasitemia by 32 to 62% *in vivo* at 500 mg/kg. Subfractions MRP1.2 (3.45g) and MRP1.7 (3.92g) were submitted to silica gel column chromatography and two compounds were obtained, CPMR-1, inodorous white flakes identified as β-sitosteral (steroid), and CPMR-6, an inodorous amorphous white solid identified as tormentic acid (triterpenes). Both were submitted to antimalarial tests. In *in vitro* tests, tormentic acid was active with IC₅₀ of 12.14 µg/ml, while β-sitosteral was inactive. In *in vivo* tests, tormentic acid and β-sitosteral were partially active at 5 mg/ml. Higher concentrations must be tested. In summary, it is observed a higher activity for tormentic acid in *in vitro* tests than in *in vivo* tests. A new molecule extracted from a medicinal plant confirmed the importance of this approach in the search for new antimalarial drugs.
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Serjania lethalis A.St.- Hil., a woody climbing vine known colloquially as Timbó, is popularly used for fishing as an attractant. The crude ethanol extract obtained from the trunk of the plant was subject to a liquid-liquid partition after solubilization in methanol:water (2:3; 1.5L) with the following solvents: n-hexane (5x400 mL), chloroform (5x400 mL) and ethyl acetate (5x400mL). After having solvent removed at low pressure by rotavapor, the fractions were subject to bioassay. The ethyl acetate extract was shown to have more inhibitory activity on two resistant strains of Staphylococcus aureus. The results show that the stem and leaf extracts of Serjania lethalis may be sources of antibiotic activity against susceptible and resistant Staphylococcus aureus (strain ATCC25922). The minimum inhibitory concentration (MIC) values for the fractions derived were less than 100 mg/ml.

Dialysis was the method of purification selected, using a synthetic membrane in which was placed 2g of fraction (ACOEt), then immersed in 300mL of deionized water. The fractionate water was changed every 24 hours, obtaining a total of six fractions which were then freeze dried. Fraction 1 (originating from the dialysis) was qualitatively analyzed by Thin Layer Chromatography (TLC) with the mobile phase Pyridine:Butanol:Water (100:35:25) and cerium (IV) sulfate as the developer, which revealed a single spot Rf=0.5. HPLC was used to analyze the efficiency of separation, with a silica column of 0.46x15 cm with the mobile phase dichloromethane:hexane (60:20, v/v). The chromatogram revealed several bands, with at least two of good resolution (Fig. 1). The results obtained suggest that compounds 1 and 2 are heterosides.

Figure 1 - Chromatographic profile of fraction 1 (dialysis) and isolation of compound 1.

Ref.:
CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACTS OF *Lippia sidoides* CHAM. OBTAINED BY DIFFERENT EXTRACTIVE METHODS IN *Artemia salina* LEACH

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BACKGROUND: *Lippia sidoides* Cham. (Verbenaceae) is a Brazilian plant, which is popularly known as “alecrim-pimenta”. Its aerial parts are used in the traditional medicine as an anti-infective and antiseptic agent (Matos, 1994). Previous studies have reported that the essential oil of this species shows different values of systemic toxicity and little cutaneous toxicity (Fontenelle, et al. 2007, Albuquerque, 2006, Couto, et al, 2000, Mendonça, et al, 1990). However there is no information about the comparative toxicity *in vivo* of crude extracts obtained from its aerial parts.

OBJECTIVE: The aim of this study was to compare the cytotoxicity of two different ethanolic extracts obtained from the leaves of *L. sidoides* by soxhlet extraction and maceration.

METODOLOGY: *Lippia sidoides* Cham. was cultivated in Ribeirão Preto, SP; the leaves were collected in March 2007. The dried plant material was powdered (600 µm) and extracted with EtOH in a Soxhlet apparatus for about 29-34 h. For the ethanolic macerate, dried leaves were extracted at room temperature in 95% EtOH for 21 days (three times, 7 days) under frequent shaking. The extraction experiments were made in triplicate. The dried ethanolic extracts were screened for toxicity with larvae of *A. salina*. The test was performed in triplicate in wells, with extract concentrations of 4, 40 and 400 µg/mL.

RESULTS AND CONCLUSION: The soxhlet extract showed a LC 50 of 50.91 µg/mL and for the macerate, the LC 50 was 45.39 µg/mL. According to Costa et al. 2001, the ethanolic extract of *L. sidoides* contains constituents with cytotoxic potential against tumoral cells. In this work the cytotoxicity of the macerate and the soxhlet extract does not differ statistically. These results suggest that the fraction responsible for the cytotoxicity does not contain unstable or thermolable substances.

Refs:
IN VITRO ANTIOXIDANT ACTIVITY OF Senecio crassiflorus (Poir.) DC. var. crassiflorus

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BACKGROUND: In recent years, much attention has been directed to the involvement of free radicals in pathogenesis of many diseases. At the same time, a great scientific advance has been verified involving the chemical and pharmacological analysis of medicinal plants in order to identify new compounds with therapeutical properties as an important source of natural biological active products1. Senecio species are rich in secondary metabolites which exhibit various pharmacological activities, such as antioxidant2, antibacterial, antiinflammatory, cytotoxic, antimalarial, antymycotic and antihelmintic activities3. S. crassiflorus var. crassiflorus (Asteraceae) is an herb, native of the Brazilian south coast, known as margarida-das-dunas among others. The antibacterial activity of its essential oils has been evidenced recently5. OBJECTIVE: Evaluation the antioxidant properties of the ethyl acetate fraction, obtained by the sequential fractionation of the ethanolic macerate from the fresh aerial parts of Senecio crassiflorus var. crassiflorus.

METODOLOGY: The fractions were obtained by liquid-liquid extraction of the ethanolic extract with hexane, dichloromethane, ethyl acetate and butanol. Thin Layer Chromatography (TLC) of the fractions was performed in order to detect the presence of constituents with potential antioxidant activity. Lipid peroxidation in Rat’s brain was induced by Fe2+ and the influences of the ethyl acetate fraction on the thiobarbituric acid reactive species (TBARS) levels were measured. The tested concentrations ranged from 2 µg/mL up to 160 µg/mL and three samples of each concentration were assayed. The results were analyzed by ANOVA. RESULTS: TLC analysis indicates the presence of phenolic compounds in the ethyl acetate fraction. This fraction caused a significant decrease (p<0,001) in the iron-induced TBARS production at all tested concentrations.

CONCLUSION: The ethyl acetate fraction of S. crassiflorus var. crassiflorus exhibits a great antioxidant activity. The active constituents are phenolic compounds, probably flavonoids. This group of substances is often found in herbal ethyl acetate fractions. As polyphenoles, flavonoids have the ideal structure to bind free radicals and thus act as scavengers and might be responsible for the antioxidant properties of the tested plant material. Further studies are necessary to determine the possible utilization of S. crassiflorus extracts for the treatment of human diseases related to oxidative stress.

FINANCIAL SUPPORT: CNPq.

KEY-WORDS: Senecio crassiflorus var. crassiflorus; antioxidant activity; TBARS

Refs.
3. L. N. Francrscato; Tese (Mestrado em Ciências Farmacêuticas – Controle e avaliação de insumos e produtos farmacêuticos), Universidade Federal de Santa Maria, RS, Brasil, 154 (2007).
TOTAL PHENOLIC AND FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITIES OF BRAZILIAN PROPOLIS SAMPLES

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Propolis is a resinous substance constituted mainly by beeswax and plant material. Its colour and texture are highly variable, and its chemical composition also differs according to the hive geographic location. Bees use propolis to seal opens in the hive, to cover the internal surface in order to keep the internal temperature at 35ºC and to maintain an antiseptic environment, among another purposes. Propolis pharmacological properties are known since remote times. Among well-known properties, emphasis has been given to antiinflamatory, antimicrobian, citotoxic and antioxidant activities. Propolis samples from different places have distinct compounds and properties, therefore chemical studies are important, since pharmacologically active new substances might be uncovered. The present study aims to analyze the antioxidant properties of six Brazilian green propolis samples and test a possible relationship among phenolic and flavonoid contents and antioxidant activities. Analyses of antioxidant activity followed two methods: reduction of the free radical DPPH (2,2-diphenyl-1-picrylhidrazyl) and β-carotene discoloring method. Total phenolic compounds were determined by the Folin-Ciocaulteau and total content of flavones and flavonols (flavonoid classes with higher antioxidant properties) by the AlCl₃ methods. Possible relationships among antioxidant activities and phenolic and flavonoid contents were tested by the minimum square method, assuming as significant values of $R^2 > 0.5$. With DPPH method, antioxidant activities of 30 µg/mL methanolic extracts ranged from 14.6 to 27.2%. Results of β-carotene discoloring method with 1 mg/mL methanolic extracts ranged from 71.2 to 84.1%. Total phenols ranged from 10.9% and 16.5% and total flavonoid contents ranged from 2.2% to 4.3%. Statistical tests showed that a significant relationship exists between antioxidant activity towards DPPH and flavonoid content ($R^2 = 0.5939$), but not towards β-carotene/linoleic acid ($R^2 = 0.357$). Total phenolic contents have no correlation with either antioxidant method ($R^2 = 0.396$ and 0.012). Since propolis fractions with lower polarity than methanolic extracts (hexan, chloroform, ethyl acetate) also exhibited antioxidant activities, compounds other then phenylpropanoids and flavonoids are likely to act as antioxidants, mainly by the β-carotene discoloring method. This would account for the lack of correlation above mentioned. (FAPESP, CNPq)

Refs.
Chagas' disease is a widespread illness which still does not have an efficient treatment or a prophylactic drug for its control. Thus, the search for new compounds actives on *Trypanosoma cruzi*, the etiological agent, is extremely needed and higher plants are promising sources of them.¹

In the present work, we evaluate the trypanocidal activity of 15 crude extracts and 14 compounds isolated from *Cedrela fissilis* (Meliaceae). The trypanocidal activity was assessed *in vitro* against trypromastigote forms of *T. cruzi*.² Extracts from fruits, branches, stem, roots and leaves were assayed, and 11 of them were significantly actives (lysis % ≥ 50); the dichloromethane extract of roots was the most active one, reducing 97.4 % of the parasite number. Such results suggested that *C. fissilis* is a promising source of trypanocidal compounds. In fact, the chemical investigation of this specie allowed the isolation of several compounds,³⁴⁵ among them 4 limonoids (3β-acetoxycarapin, 7-deacetoxy-7-oxogedunin, gedunin, and photogedunin) and 10 triterpenes (hispidol A, piscidinol A, pentaol, nilocetin, odoratol, iso-odoratol,odoratone, 11-ooxoolanonic acid, oleanonic acid, and oleanolic acid), which were assayed on *T. cruzi*. The results showed that the triterpenes were more actives than the limonoids, and the odoratol (1) was the most active one, with an IC₅₀ value of 23.4 µM. This is a promising result since gentian violet, the currently prophylactic drug, has a higher IC₅₀ (83 µM).⁶ Moreover, in the present investigation, a new triterpene, 11-oxooleanonic acid (2), was identified through spectral data.

Therefore, *Cedrela fissilis* is a suitable source on the search of new compounds to control Chagas' disease, such as the triterpene odoratol (1), which has higher trypanocidal action than gentian violet.

References
Evaluation of mutagenic activity of amentoflavone and methanol extract of *Byrsonima crassa* measured by mutation reverse assay*

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*Byrsonima crassa* Niedenzu is a plant popularly used for the treatment of gastric dysfunctions and pertaining to the Brazilian central vegetation. The methanol extract, obtained by maceration, contains catechin, tannins, terpenes and flavonoids; both mutagenic potential and antioxidant properties have been ascribed to flavonoids. The mutagenicity of some flavonoids is believed to be associated with the formation of reactive oxygen species and seems to depend on the number and position of hydroxyl groups. In the present study the mutagenic activity of the methanol, chloroform and 80% aqueous methanol extracts, as well as ethyl acetate and aqueous sub-fractions of this medicinal plant were evaluated by *Salmonella typhimurium* assay, using strains TA100, TA98, TA102 and TA97a. The reverse mutation assay is used to evaluation of different mutagenic mechanisms. These assays are performed with and without S9 mix. The results showed mutagenic activity of the methanolic extract in the TA98 strain without S9. The acetate fraction showed strong signs of mutagenicity without S9, suggesting that in this enriched fraction were concentrated the compounds that induced mutagenic activity. The aqueous fraction showed no mutagenic activity. The TLC and HSCCC analyses of the acetate fraction with some standard compounds permitted the isolation of the quercetin-3-O-β-D-galactopyranoside, quercetin-3-O-α-L-arabinopyranoside, amentoflavone, methyl gallate and (+)-catechin, of which only the amentoflavone exhibited positive mutagenicity to TA98 (+S9, -S9).

Financial support: FAPESP-BIOTA
ANTIOXIDANT POTENTIAL OF METHANOLIC ROOT EXTRACT OF
PYLOCARPUS SPICATUS IN A CELL OXIDATIVE STRESS INDUCING MODEL

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There is a great biodiversity in Brazil that was not completely studied, yet. Therefore, it
is important to know the biological (and possible pharmacological) effects of plant extracts,
like antioxidant activity, in different biological systems. In this context, we searched the
antioxidant properties of methanolic root extract of Pylocarpus spicatus, a plant found in the
Northeast of Brazil, using cell oxidative stress promoted by catechol (1,2-
dihydroxybenzene) 1,2 as an experimental model.

The extract was donated by LAPEMM and dissolved in DMSO (to obtain a final
growth of this diluent in the medium of 0.5 %). Catechol was dissolved in HCl
(0,01M). The cells used in tests were of the GL-15 line (human glioblastoma); they were
cultivated and treated during 48 hours with increasing concentrations of both catechol and the
extract to determine the IC50 for each one. After that, cells in culture were pretreated with
increasing concentrations of extract (below toxic concentrations) and catechol at 1 mM to
demonstrate protective effect. The cell viability, measured by MTT method 3 was compared
between the groups.

The catechol presented an IC50 of 912 µM (Fig.1). The IC50 could not be calculated for
the extract because it did not kill 50% of cells even at the maximal concentration (3000
µg/mL) (Fig.2). The methanolic root extract of Pylocarpus spicatus presented protective
activity at three tested concentrations (6 .µg/mL, 30 .µg/mL e
100 µg/mL) against the effects os catechol at 1mM during 64h (Fig.3).

The development of antioxidant therapies could give support to treatments of several
diseases. Moreover, the use of natural products to promote health is an important perspective.
Thus, these are relevant data to know relevant activities of Pylocarpus spicatus.

Refs.
ACTION OF *Pothomorphe umbellata* AND *Struthanthus* sp EXTRACTS TO A CLINICAL ISOLATE *Trichophyton rubrum*

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*Pothomorphe umbellata* is a characteristic plant of the Brazilian flora, known popularly as “caapeba” or “pariparoba” that presents anti-inflammatory, analgesic, anti-PAF and antioxidant actions. *Struthanthus* sp is a hemi-parasite Brazilian plant of which there are few biological studies. In two past decades the incidence of infections caused by dermatophytes and other fungi has increased, due to the inefficiency of some drugs to these agents. Works in literature indicate pharmacological action for several plant species, including its use in the popular medicine. The objective of this work was to evaluate the antifungal activity of *Pothomorphe umbellata* and *Struthanthus* sp extracts to a clinical isolate of *Trichophyton rubrum* with resistance antifungal history. The tests were performed according the NCCLS M38-P (NCCLS, 2002), using plates of microtitulation for the determination of minimal inhibitory concentration (MIC). Aerial parts of plants were dried and converted to powder and submitted to extraction in ambient temperature during approximately 10 days. Ethanolic and methanolic extracts, obtained by maceration with ethanol 70% and methanol, respectively, were lyophilized. The extracts were dissolved in DMSO to obtain stock solution concentration of 50mg/mL. The growth inhibition was observed after incubation of 7 days at 28°C to determine MIC. The Ethanolic and methanolic extracts were tested and presented good antifungal action in vitro, with MIC values of 312.5 and 156.25 µg/mL respectively. Our results suggest that the extracts may be explored to the discovery of substances that posses the antifungal activity.

Refs.
Introduction: The red alga *Chondrophyccus flagelliferus* belongs to the *Laurencia* complex\(^1\), and its preliminary chromatographic analysis showed the presence of steroids, sesquiterpenes and glycolipids. Marine glycolipids, as well as those from terrestrial organisms, are amphiphilic compounds which are currently classified into two groups: glycoglycerolipids (GGLs) and glycosphingolipids (GSLs). These substances have demonstrated immunostimulant, antitumor and antiviral activities\(^2\). Herpes Simplex Virus types 1 and 2 (HSV-1 and 2) infect a great number of people all over the world and their resistance to the available treatment makes the herpetic infections a serious problem of public health.

Objective: The aim of the present study was to evaluate the potential anti-HSV-1 and anti-HSV-2 activities the aqueous extract of the red seaweed *Chondrophyccus flagelliferus*.

Methodology: This alga was collected at Bombinhas Beach (Santa Catarina, Brazil) and extracted with ethanol 70%. This extract was partitioned with ethyl acetate, yielding an organic and an aqueous extracts. The aqueous extract cytotoxicity was evaluated by MTT assay on VERO and GMK AH1 cells. The anti-HSV-1 (strains 29R and KOS) and anti-HSV-2 (strain 333) activities were evaluated by plaque number reduction assay on VERO and GMK AH1 cells, respectively.

Results: The tested extract showed no cytotoxic effects on these cells, at the maximum evaluated concentration of 0.5 mg/mL, and it significantly inhibited the replication of the tested viruses: HSV-2 strain 333 (77 %), HSV-1 strain 29R (92%) and HSV-1 strain KOS (97%). Conclusions: The presence of glycolipids in the aqueous extract of *Chondrophyccus flagelliferus* could explain the detected activity, and additional studies will be carried out with the isolated substances from this extract to confirm this hypothesis.

Ref.
Financial support: CNPq, FAPESC, CAPES
ALKYL PHENOLS, BIFLAVONOIDS, DIPHENYL ETHER AND A NEW DEPSIDE FROM SCHINOPSIS BRASILIENSIS ENGL.

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Species of the Anacardiaceae family has spread around the world and some of them have economical and commercial importance. The presence of alkyl and alkenyl phenols, catechols, resorcionols, hydroquinones is the cause of the contact dermatitis that characterize the poison species of this family. Schinopsis brasiliensis Engl. is popularly known in Brazil as “barauna”. It is a large tree well known in the Brazilian northeastern and it is employed by the local population in the cattle verminosis and, the wood is also employed in constructions. For this reason, today it is considerate by the brazilian authorities as an endangered species.

This work describes the chemical study of the methanol extract of wood of S. brasiliensis The CHCl3 and EtOAc fractions obtained from the partition of MeOH extract were submitted to different chromatographic procedures over silica gel and Sephadex LH-20. These procedures permitted to isolate vanillin, vanillic acid, 2,4-dihydroxybenzaldeid, methyl 2,4-dihydroxybenzoate, 3,4,5-trimethoxybenzil alcohol, methyl gallate, gallic acid, siringaresinol, β-sitosterol, estigmast-4-en-3-one, methyl 3,5-dicloro-6-(6-hydroxy-4-methoxy-3-methoxycarbonil-2-methyl-phenoxy)-2-hydroxy-4-methyl-benzoate (1), luxenchalcone (2), besides the new compounds methyl 2,4-dihydroxy-3,6-dimethyl-benzoate, methyl 2-hydroxy-3,6-dimethyl-4-(3,4-dimethoxy-benzyloxy)-benzoate (3), 4′-methoxy-7-hydroxy-flavanone-(3→3′)4′′-methoxy-3′′,7′′-dihydroxy-flavanone (4) and a mixture of seven methyl n-alkyl-2-hydroxy-4-methoxybenzoate. All the structures were elucidated by empiric data calculation and different techniques of NMR and MS of the pure compounds or their acetyl derivatives. Compound 1 is an unusual plant compound which was previously isolated from Byrsonima microphylla (Malpighiaceae)1. The biflavonoid (2) was only obtained from Luxemburgia octandra (Ochnaceae)2.

(CNPq, FAPESB, CAPES, PRONEX).

Refs.
1. J. H. C. Rocha et al., Bioscience, Biotechnology, and Biochemistry, 70, 2759 (2006)
ANTIMICROBIAL ACTIVITIES OF THE EXTRACTS OF FUNGAL SPECIES: Xylaria spp., Crinipellis perniciosa, Trichoderma stromaticum AND Phytophthora palmivora.

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The purpose of this work was screened for antimicrobial activities the extracts of five fungi species, namely, Crinipellis perniciosa, Trichoderma stromaticum, Phytophthora palmivora and two unidentified species Xylaria sp\textsuperscript{1} and Xylaria sp\textsuperscript{2}. The fungi were inoculated in solid (rice) and liquid (malt, PD and Czapek) media for period of 20 to 40 days. The solid medium of Xylaria sp\textsuperscript{1}, Xylaria sp\textsuperscript{2} and C. perniciosa, were extracted with methanol. The liquid medium were extracted with methanol and methanol/dichloromethane (mycelium) and the broth with ethyl acetate at pH=5 and pH=9. The extract of the solid media obtained for Xylaria sp\textsuperscript{1} was sequentially extracted with hexane, chloroform and ethyl acetate at pH=5 and pH=9. The methanol and methanol/dichloromethane extracts of the mycelium were sequentially extracted with chloroform and ethyl acetate at pH=5 and pH=9. The broth of T. stromaticum and P. palmivora species were extracted with ethyl acetate and the solid medium were divided in two portions, the first was extracted with methanol and the other with acetone. In total 38 extracts were screened. Antimicrobial tests were carried out using the technique of Minimal Inhibitory Concentration (MIC) against different strains of Gram-positive bacteria (Bacillus subtilis, Staphylococcus aureus, Streptococcus mutans and Micrococcus luteus), Gram-negative bacteria (Salmonella typhimurium, Pseudomonas aeruginosa and Escherichia coli) and fungi (Candida albicans, Aspergillus niger, Cladosporium cladosporioides and Crinipellis perniciosa). Most of the extracts of the species tested were active against Gram-positive bacteria strain. The species, T. stromaticum, was found to be the most active with values of the MIC equal, or next to the inhibitory concentration of the control (chloranfenicol). From Gram-negative bacteria strain, Salmonella typhimurium was most susceptible to extracts assayed and P. palmivora extracts were the most active. For the E. coli bacteria, only the T. stromaticum specie was active and for P. aeruginosa the C. perniciosa and T. stromaticum species were actives. Concerning the fungi, the T. stromaticum specie was found the most active against C. cladosporioides strain and only one specie of the genus Xylaria was active against A. niger. The results indicate the potential of these species to produces drugs to the treatment of microbial infections. The active extracts will be fractioned seeking the isolation actives metabolites.
ANTIMALARIAL CHROMENES FROM PIPER SPECIES AND PYRONES DERIVATIVES

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Almost one-half of the world’s population lives under constant exposure to several tropical diseases including malaria, which is responsible for about 2 million deaths each year (World Health Organization, 1997). Malaria is caused by protozoan parasites of Plasmodium species, the most deadly of this being P. falciparum. In spite of several natural alkaloids are well established as antimalarial drugs, such as quinine from Cinchona tree and artemisin from Artemisia annua, the development of resistance requires the discovery of new antimalarial compounds.

Several classes of phytochemicals have been isolated from Piperaceae species, such as amides, chromenes, phenylpropanoids, lignans, neolignans and others compounds of mixed-biosynthetic origin[1]. The biological properties displayed included the antifungal[2], antioxidant and trypanocidal activities [3].

Quinolines-containing antimalarial drugs interfere in heme detoxification pathways in the blood stages of malaria parasite’s life cycle. The parasites degrade hemoglobin in the acidic vacuole producing free heme and reactive oxygen species as toxic by-products. A heme polymerization reaction serves to detoxify this molecule by connecting heme monomers together to form the insoluble hemozoin, commonly referred as “malaria pigment”. In this work we evaluated the interaction of chromenes isolated from Piperaceae species with heme. Additional analogs included pyrones and coumarins isolated from other plant sources. Compounds 1-17 were spectrophotometrically analyzed in a microplate reader using 6 µM heme bovine suspended in 40 % DMSO, and 0.02 M HEPES pH 7.5 [4]. Compounds 1, 3-5, 9, and 12-15 affected the UV spectral characteristics of heme by showing intense absorbance decrease at λ 401 nm, which evidenced the ability to complex with heme group. Additionally, they showed similar effect to chloroquine, used as positive control. Therefore, these compounds require further specific assays in order to explore their interaction with heme, using NMR, and in vitro assays on Plasmodium cell culture.

References
Antinociceptive and toxicological activities of *Zeyheria montana* (Bignoniaceae) ethanolic extract.


Unidade de Biotecnologia-UNAERP.

Pain and inflammatory processes, particularly those chronic ones, are often linked to several diseases and to continuous public health problems. In recent decades, the search for new analgesic drugs has been motivated by actual drugs costs or effectiveness distortions. In this work, we evaluated the antinociceptive potential and some few toxicological properties of the ethanolic extract from *Z. montana* leaves (75; 150; 300 mg/Kg, ip). Acetic acid-induced writhes in mice were significantly inhibited by extract doses of 75 mg/Kg (67.27%), 150 mg/Kg (49.38%) and 300 mg/Kg (82.87%). Besides peripheral analgesic activity, *Z. montana* extract induced, apparently, central antinociceptive effect at the dose of 300 mg/Kg (ip) in rats. In this case, the latency time until the appearance of the writhing/stretching response of the rats was significantly increased by 46.5%. Some few sign of toxicity, including the ulcerogenic ones, was absent to mice treated with those extract on an extensive range of doses. The LD$_{50}$ was > 2000 mg/Kg. These results suggest that the *Z. montana* extract exhibits both central and peripheral antinociceptive activities with little toxic effects. Finally, the search for new compounds from different biological sources, chemical natures or different mode of actions could be an attractive alternative to development of new drugs more effective or cheaper than the actual ones.

**Keywords:** Antinociception; Plant extract; *Z. Montana*; Hargreaves test; acetic acid-induced writhes.

**Financial support:** UNAERP
Evaluation of antinociceptive and toxicological activities of *Serjania erecta* (Sapindaceae) leaves extract


Unidade de Biotecnologia-UNAERP.

Antinociceptive and toxicological activities of the ethanolic extract from *S. erecta* leaves were studied. Antinociceptive evaluation was carried out against chemical and thermal stimuli in mice and rats, respectively, and toxicological studies (LD50), including the ulcerogenic test, were conducted in mice. Acetic acid-induced writhes were significantly inhibited by extract doses of 75 mg/Kg, (60.40%), 150 mg/Kg (64.37%) and 300 mg/Kg, ip (61.77%). Apparently, no central antinociception was observed for all extract doses tested. Some a few signs of toxicity, including the ulcerogenic effect, were not observed to rats treated with those extract on an extensive range of doses. The LD50 was > 2,000 mg/Kg. These results suggest that the *S. erecta* extract exhibits a peripheral antinociceptive activity with little toxic effects. Finally, the search for new compounds from different biological sources, chemical natures or different mode of actions could be an attractive alternative to development of new drugs more effective or cheaper than the actual ones.

**Keywords:** Antinociception; Plant extract; *S. erecta*; Hargreaves test; acetic acid-induced writhes.

**Financial support:** UNAERP.
Nematocidal effects of medicinal plants against Pratylenchus zeae and Pratylenchus jaehni.

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Synthetic nematicides have been broadly used in the past decades for the control of plant parasitic nematodes. Despite their relevant effectiveness, these compounds can cause great damage to the environment, and to the human or the animal health. The growing searches for new nematicides, particularly of natural ones, may conduce to higher safety and efficiency to the nematode control. In this work it was evaluated the in vitro nematocidal effect of ethanolic extracts obtained from selected plant species: Tithonia diversifolia, Eclipta alba and Mikania glomerata (Asteraceae); Tabernaemontana catharinensis and Mandevilla velutina (Apocynaceae); Casearia sylvestris (Salicaceae); Zeyheria montana (Bignoniaceae); Lippia alba (Verbenaceae); Croton antisiphiliticus (Euphorbiaceae) and Serjania erecta (Sapindaceae) against the plant parasitic nematodes Pratylenchus zeae (Nematoda: Pratylenchidae) and Pratylenchus jaehni (Nematoda: Pratylenchidae). Moreover, a preliminary phytochemical characterization of these plant extracts was performed in order to associate these data to those observed in nematocidal assays. Our results indicated a significant nematocidal activity of the analyzed extracts, in special those demonstrated by E. alba (DL50 (ppm) = 304.08; 55.32 – P. zeae and DL50 (ppm) = >1000; 212.82 – P. jaehni; 12 and 24 h, respectively), T. catharinensis (DL50 (ppm) = 215.26; 60.04 – P. zeae and DL50 (ppm) = 825.44; 376.60 – P. jaehni; 12 and 24 h, respectively), C. sylvestris (DL50 (ppm) = 198.05; 56.94 – P. zeae and DL50 (ppm) = 747.98; 322.98 – P. jaehni; 12 and 24 h, respectively), Z. montana (DL50 (ppm) = 166.43; 34.08 – P. zeae and DL50 (ppm) = >1000; 427.34 – P. jaehni; 12 and 24 h, respectively) and S. erecta (DL50 (ppm) = 178.74; 74.12 – P. zeae e DL50 (ppm) = 689.24; 249.50 – P. jaehni; 12 and 24 h, respectively). Thus, these data show that the evaluated plants presented significant nematocidal effects, which are of high economic or environmental interest and useful towards the growth of agricultural activities worldwide.

**Keywords:** Plant extracts; Plant parasitic nematodes; Pratylenchus jaehni; Pratylenchus zeae.  
**Financial support:** Capes and UNAERP.
Ocimum gratissimum, originating in the Orient, is widespread throughout tropical countries including Brazil, where it is popularly known as "alfavacão, alfavaca and alfavaca-cravo". This species is used in traditional medicines in South America and Africa for a variety of therapeutic purposes which includes its use in treating bacterial infections, diarrhoea, diabetes\(^1\). Recently we have examined the antifungal activity of the essential oil obtained by steam-distillation (1.1% w/w) of the aerial parts of \textit{O. gratissimum}. The results revealed that the essential oil inhibited the growth of \textit{Botryosphaeria rhodina}, \textit{Rhizoctonia} sp., two strains of \textit{Alternaria} sp., \textit{Aspergillus niger}, and \textit{Penicillium chrysogenum}. The compound that showed antifungal activity was isolated and identified as eugenol by TLC bioautography technique. GC/MS analysis showed that eugenol was the main constituent of the essential oil studied\(^2\). In continuation of the studies on \textit{O. gratissimum} we have examined the aerial parts of the plant. Differently of the essential oil, there are few studies reported on the macerated extract of this species. Powdered aerial parts were continuously extracted with hexane-dichloromethane-ethanol mixture (1:3:1) at room temperature. The obtained extract (25.0g) was chromatographed on silica gel column eluted with hexane, dichloromethane and ethanol to yield aliphatic hydrocarbon mixture (\(C_{31}, C_{33}, C_{34}, C_{35}\)), eugenol, caryophyllene oxide, a mixture of stigmasterol/sitosterol and large quantity of ursolic acid (3.5g). Ursolic acid has attracted considerable interest because of its range of biological activities such as cytotoxic, anti-mutagenic, antiviral, anti-invasive, trypanocidal and other activities that are of interest to modern pharmacology\(^3,4\). This is the first report of ursolic acid in \textit{Ocimum gratissimum} species.

\begin{figure}[h]
\centering
\includegraphics[width=0.2\textwidth]{ursolic_acid.png}
\caption{Ursolic acid}
\end{figure}

2. T. J. de Faria \textit{et al.}, \textit{Brazilian Archives of Biology and Technology}, 49, 867 (2006).
The species of the Myrtaceae family practically occur in the whole world. Some species of this family are used in Brazil in the treatment of diabetics, rheumatism, as astringents, anti-inflammatory and in the treatment of chronic ulcers, hemorrhages, fever, cystitis and urethritis. The plants of this family are characterized by the production of tannins, flavonoids, mono-, sesqui- and triterpenes, derivatives of the fluorgluconol, chromenes and stilbenes. The aim of this work is the phytochemistry study of the non-volatile extracts of Myrcia hiemalis leaves, as well as the establishment of its biological properties. The leaves were harvested in the sand dunes of Salvador, Bahia, northeastern region of Brazil in 2005. The dried leaves were ground and extracted with ethanol. Them, the extract was partitioned with hexane, dichloromethane and ethyl acetate. Antimicrobial tests were carried out with the extract using the technique of Minimal Inhibitory Concentration (MIC) with the following microorganisms: Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella, choleraesuis, Aspergillus niger, Cladosporium cladosporioides, Candida albicans and Crinipellis perniciosa. The hexane and ethyl acetate extracts were inactive. The dichloromethane extract presented activity against Gram-positive bacteria as well as the fungus C. perniciosa. Seven components had been isolated and identified from the dichloromethane extract: myricitrin, daucosterol, 2',4'-dihydroxy-3',5'-dimethyl-6'-methoxychalcone, 2',6'-dihydroxy-3'-methyl-4' methoxychalcone, 2',3',4'-trihydroxy-5'-methyl-6'-methoxychalcone, 7-hydroxy-6,8-dimethyl 5-methoxyflavanone and 5,7-dihydroxy-6,8-dimethylflavanone. The structures of the substances were determined using 1H and 13C NMR techniques. The isolated substances were assayed against above microorganisms and the 2',4'-dihydroxy-3',5'-dimethyl-6'- methoxychalcone presented antimicrobial activity against M. luteus and the substance 7- hydroxy-6,8-dimethyl-5-methoxyflavanone acted against B. subtilis and M. luteus. Anti- Trypanosoma cruzi and anti-Leishmania amazonensis tests were done with isolated substances using a technique developed in the FIOCRUZ-BA. The 2',4'-dihydroxy-3',5'- dimethyl-6'-methoxychalcone was active against both parasites (anti-T. cruzi: IC50 13,12 µM; anti-L. amazonensis: 100% inhibition in 50 µM) and 2',6'-dihydroxy-3'-methyl-4' methoxychalcone presented activity against T. cruzi. (IC50 35,95 µM). Supported by CNPq, FAPESB, FINEP and CAPES.
INSECTICIDAL ACTIVITY OF Trichilia sp. (MELIACEAE) EXTRACTS AGAINST Atta sexdens rubropilosa

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The family Meliaceae includes many plants that are sources of valuable timber and many of them have wide ranging of uses in ethnomedicine. Numerous secondary metabolites that exhibit significant insecticidal activity were already found in Trichilia genus¹. The leaf-cutting ants of the genera Atta and Acromyrmex use mostly fresh plant fragments to raise their symbiotic fungi² and are the cause of considerable economic damage, due to defoliation that they cause³. Control of this pest is still problematic, presenting only temporary effects and is sometimes, harmful to the environment, to man and other animals. Consequently, an extensive search for alternate methods to control these insects has been made in an attempt of substitute traditional agrochemicals with high specificity and, therefore, causing less damage to the environment. In this context, this work evaluated the toxic effect of dichloromethane and methanol extracts of leaves, stem, bark, branches and thin branches of Trichilia sp against leaf-cutting ants Atta sexdens rubropilosa as source for less toxic compounds that could be used as a soft control method. The extracts were incorporated in artificial diet for the ingestion toxicity test for the ants. For each treatment were used 50 ants distributed in 10 Petri plates, lined with filter paper. The Petri plates were maintained in B.O.D, at 24 °C and R. U.H. above 70 %. The ants mortality were accomplished daily, during 25 days and the obtained data of the bioassay were analyzed through the comparison of the survival curves by the test "log rank" (Prism 3.0). The dichloromethane and methanol extracts of bark, branches, thin branches and methanol extracts leaves and stem of Trichilia sp showed high activity against leaf-cutting ants Atta sexdens rubropilosa, while leaves and stem dichloromethane extracts showed lower rates of activities. The dichloromethane extract of stem was fractionated with the objective of testing the pure compounds and led to isolation of steroids, triterpene, acid cinamic derived and coumarin characteristics of this genus. These compounds were identified by comparison of their NMR, MS, UV and IR data.


FAPESP, CNPq, CAPES
Plinia cauliflora LEAVES AGAINST Candida albicans

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In the last few years, resistance of Candida albicans to antifungal drugs has become a fact of concern. So, plant extracts and compounds activity evaluation has been focused in order to obtain new agents against Candida. The objective of this work was to determine the activity of Plinia cauliflora leaves against C. albicans. The leaves extract was obtained with 70% ethanol by percolation and dried under reduced pressure. Liquid-liquid partition of the extract (15.0 g) was done with 500 mL of water and 500 mL of ethylacetate and after, with 500 mL of n-butanol. Crude extract and the fractions of ethylacetate (EtOAcFr), butanol (BuOHFr) and water (WFr) were totally dried and 25 mg/mL of each one were separately dissolved in 1 mL of DMSO with subsequent dilution in RPMI-1640 medium (1:2; v/v). Determination of Minimum Inhibitory Concentration (MIC) was done according NCCLS M27-A2 at 96-wells plates. Final samples concentration was 1.56 mg/mL and final C. albicans (ATCC 64548) inoculum was 2.5-5.0x10³ cells/mL. Controls of 32 µg/mL of amphotericin B, DMSO:RPMI (1:2; v/v), medium and growth were also done. It was proceeded serial dilutions in the plates that were incubated for 48 h at 25 °C. After that time, subcultures of the plates were done in agar-Sabouraud plates and incubated for 48 h at 25 °C to determine Minimum Fungicidal Concentration (MFC). MIC measurement was done by addition of 2% TTC. The test was done in duplicate. WFr showed the best values of MIC and MFC. BuOHFr showed the smaller value of MIC but MFC was higher than start concentration. EtOAcFr had intermediated MIC values and MFC was higher than start concentration. Crude extract showed the highest MIC value. Based on those results, it was possible to observe better activities against C. albicans for WFr and BuOHFr, therefore, it is necessary to continue the studies of this promissory extract by evaluation of its constituents.

Support: PADC-UNESP

Refs.
Pharmacological and Phytochemical characterization of Tabernaemontana catharinensis (Apocynaceae) stem bark extract.

R.C., Gomesa; Guenka, L.C.a; A., Coutinho-Netoa; V.L., Meloa; V.C., Fernandesa; G., Dagravaa; W.S., Santosb; P.S., Pereiraa; L.B., Coutoa; R.O., Belebonia

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Analgesic, anti-inflammatory and toxic activities, as well as the phytochemical profile of the ethanolic extract from T. catharinensis (Apocynaceae) stem bark were studied in this work. Analgesic evaluation was carried out against chemical and thermal stimuli. Anti-inflammatory activity was investigated on carrageenin-induced edema in rats and toxicological studies (LD50) were conducted in mice. Phytochemical analyses were performed according standardized methodology. Acetic acid-induced writhes were significantly inhibited by extract doses of 37.5 mg/Kg (40.97%), 75 mg/Kg (77.70%) and 150 mg/Kg (88.98%). Besides peripheral analgesic activity, the T. catharinensis extract induced central analgesia at all doses tested, particularly noticed 60 and 90 min following administration. The extract significantly reduced edema formation by 30.35% (37.5 mg/Kg), 34.46% (75 mg/Kg) and 56.42% (150 mg/Kg) when assessed 180 min following carrageenan intraplantar injection, demonstrating anti-inflammatory action. The calculated LD50 value was 2200 mg/Kg on toxicological assay. Phytochemical analyses of ethanolic extract from T. catharinensis stem bark showed the presence of alkaloids and terpenoids, which might be responsible for the observed pharmacological activities.

Keywords: Anti-inflammatory; Antinociceptive; Plant extract; Tabernaemontana catharinensis.

Financial support: UNAERP.
Oxidative processes are an important factor in the pathogenesis of several disorders, including neurodegenerative diseases like Parkinson’s Disease (PD) and Alzheimer’s disease (AD). Therefore, the search for new compounds with antioxidant activity is currently the focus of many studies. Recent works have demonstrated that the toxicity of 1,2-dihydroxybenzene (catechol), a metabolite of benzene, involves inhibition of the respiratory chain and superoxide and reactive quinones generation, promoting oxidative stress in human glioblastoma GL-15 cells. In this work, we used the cytotoxicity of catechol to GL-15 cells as an experimental model to demonstrate the neuroprotective effects of coumarins obtained from Zantoxylum tingoassuiba. Approximately 1.2 liters of the water used in the hydro distillation, after cooled, was extracted, for 3 times, with 250 mL of the chloroform and to proceed with ethyl acetate. The solvents were evaporate under reduce pressure. This material, after chromatography on column, afforded the furanocumarins xathotoxin and isopipinelin. Catechol and coumarins were dissolved in HCl (0.01 M) and DMSO (1 %), respectively. Cells were cultured and treated for 48 hours with increasing concentrations of each substance in order to determine the concentration that kills 50 % of cell (IC₅₀). Cell viability was measured using MTT method. Data were fitted to nonlinear regression plots to determine the IC₅₀ for catechol and coumarins (Fig.1 a-b), that was 912 µM and 73 µg/mL, respectively. After that, the cells in culture were pretreated with increasing concentrations of coumarins (below toxic concentrations) with 1 mM catechol. The viability was compared with a negative control for cytotoxicity (cells treated with 0.5 % DMSO alone) and a positive control (cells treated with 1 mM catechol and 0.5 % DMSO). Catechol killed 61 ± 3.6 % of cells, differing significantly of the negative control group (p<0.001). A partial but significant protective effect of coumarins was observed when the cells were treated with concentrations in the range of 1 µg/mL - 3 µg/mL (Fig.2).

The development of potential neuroprotective therapies for neurodegenerative diseases can be based on understanding the protective effects of compounds present in natural products. Thus, these data are important to know relevant pharmacological activities of Zantoxylum tingoassuiba.

Refs.
The phospholipases A\(_2\) (PLA\(_2\), E.C.3.1.1.4) are multifunctional enzymes, with specific Ca\(^{2+}\)-dependent catalytic activity on phospholipids, hydrolyzing the 3-\(sn\)-phospholipids 2-acyl ester bond and releasing fatty acids and lysophospholipids. The PLA\(_2\)s are widely distributed in nature and instigate medical-scientific interest due to its involvement in many human inflammatory diseases and in envenomation by bees and snakes. The present work aimed at the partial functional characterization of an acidic phospholipase A\(_2\), Asp49 (BpirPLA\(_2\)), isolated from Bothrops pirajai venom and having its N-terminal sequence determined. Its catalytic activity in the induction of pharmacological effects (hypotensive and antiplatelet action) was evaluated after chemical modification of His48 residue with 4-bromophenacyl bromide (BPB). BpirPLA\(_2\), an acidic phospholipase A\(_2\) previously isolated from Bothrops pirajai snake venom, caused a hypotensive response in the rat and inhibited platelet aggregation \textit{in vitro}. Catalytic, antiplatelet and other activities were abolished by chemical modification with BPB, which is known to covalently bind to His48 of the catalytic site. N-terminal sequence was elucidated (SLWQFGMINYVM-GE5GVLQYLSYGCC GCGLGGQGQPTDADRCCFVHDCC). Although not myotoxic, BpirPLA\(_2\) was catalytically active. These data confirm that myotoxicity does not necessarily correlate with catalytic activity in similar native PLA\(_2\)s and that either of these two activities may exist alone. Besides being a molecular model of relevant catalytic activity, BpirPLA\(_2\) is also promising as a hypotensive agent and inhibitor of platelet aggregation, as these pharmacological effects are potentially interesting for the elaboration of therapeutic agents in clinical-medicine.

References:


Financial support: FAPESP and CNPq.
ANTIBACTERIAL ACTIVITY OF CRUDE HYDROALCOHOLIC EXTRACT AND FRACTIONS FROM *PFAFFIA GLOMERATA* (AMARANTHACEAE) ROOTS AGAINST ORAL PATHOGENS

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The roots of several *Pfaffia* species (Amaranthaceae) are used in the Brazilian folk medicine as antitumoral, tonic, aphrodisiac, and for the treatment of diabetes and rheumatism¹. Because of their popular use and form, the roots of *Pfaffia* species are popularly known as “Brazilian ginseng”, similar to the Asian ginseng (*Panax sp*, Araliaceae)¹. According to literature data, the hydroalcoholic extract of *Pfaffia glomerata* roots displays anti-inflammatory¹ and antileishmanial² activities. The aim of this work was to evaluate the *in vitro* antibacterial activity of the crude hydroalcoholic extract of *Pfaffia glomerata* roots (RhE) and its organic fractions against several oral pathogens. Air-dried and powdered roots were exhaustively extracted with EtOH: H₂O (95:5 v/v) at room temperature. The filtered extract was concentrated under vacuum to afford the RhE, which was dissolved in MeOH: H₂O (7:3 v/v) solution, followed by partition with hexane, DCM, EtOAc, and n-BuOH in sequence. The results showed that the crude extract (RhE) displayed antibacterial activity mainly against *Streptococcus mitis* (ATCC 49456) (MIC: 60 µg mL⁻¹), *Streptococcus sanguinis* (ATCC 10556) (MIC: 70 µg mL⁻¹), and *Streptococcus salivarius* (ATCC 25975) (MIC: 90 µg mL⁻¹). The hexane (RH) and ethyl acetate (RE) fractions were active mainly against *Streptococcus sobrinus* (ATCC 33478), showing MIC values of 60 µg mL⁻¹ and 170 µg mL⁻¹, respectively, while the dichloromethane fraction (RD) was moderately active against *Enterococcus faecalis* (ATCC 4082) (MIC: 350 µg mL⁻¹). The best antibacterial results were obtained to the aqueous fraction (RA), which was active mainly against *Streptococcus sobrinus* (ATCC 33478) (MIC: < 50 µg mL⁻¹), *Streptococcus mitis* (ATCC 49456) (MIC: < 50 µg mL⁻¹), and *Streptococcus mutans* (ATCC 25175) (MIC: < 50 µg mL⁻¹), while the butanol fraction (RB) was inactive against most of the tested pathogens. This study encourages further investigations of the chemical constituents which are responsible for the antibacterial activity against oral pathogens of the hydroalcoholic extract of *Pfaffia glomerata* roots and its active fractions.

Refs:
With the increase in productivity in the last 40 years old, the agriculture became dependent of chemical, insecticides, fungicides, herbicides and fertilizers. Since the men recognize the toxicity of some synthetic herbicides, the use of vegetal extracts and compounds of vegetal origin increases and is studied. These studies are related to allelopathy, which is all beneficial or harmful interferences among plants, including microorganisms, provoked by the release of chemical compounds elaborated by them, through alive or dead tissues. Among these substances there are the chemical composites present in the plants, resulting from the primary and secondary metabolisms. In the first group, there are the indispensable substances to the plants, formed through the photosynthesis, which is divided in: carbohydrates, proteins, lipids and nucleic acid. In the second group, there are the substances formed in the sequence, from the metabolism energy of the primary compounds that have the mainly function of vegetable protection. Nowadays, 10,000 secondary products are known, and are divided into groups according to the structural class, in: phenolic acid, flavonoids, terpenoids, alkaloids and so on. The allelopathy is very common in vegetables, and the most known ones are: Allium cepa (garlic), Coffea arabica (coffee), Glycine max (soybean), Helianthus annuus (sunflower), Nicotina tabacum (tobacco), Zea mays (corn) and others. Due to this fact, in the past years, the University of Ribeirão Preto has been interested in evaluating the allelopathic potential of the purified fractions and isolated substances from vegetal species, described as allelopathic, looking for results to produce a biotechnology product. The aim of this work is evaluating the allelopathic potential of Gomphrena globosa, Tabernaemontana catharinensis and Tithonia diversifolia, using Bidens pilosa and Brachiaria brizantha as weed plant. For the germination tests of the weed plants, acrylic boxes (7.5 x 7.5 x 7.0 cm) were used, and phenolic triturated foam was the substrate, with doses of 0%, 1 %, 2 % and 4 % of vegetal extracts being tested in each box separately. The experiments were kept to 23 ºC, 80 % of relative humidity and photoperiod of 13 h/day. The datas of germination percentage, germination velocity, dry weight and the survival of them were the collected in inspections on the 7th, 14th and 21st days, after the sowing. The results showed that the three plants used as allelopathic in the study presented inhibition activity and delay in the development of the weeds, in special the Tithonia diversifolia against Brachiaria brizantha and the Gomphrena globosa against Bidens pilosa.


Support: UNAERP / CAPES
As part of our on-going research on plants from Brazilian plant species, we select six species of *Senna/Cassia* sp., which have been reported to accumulate bioactive piperidine alkaloids, in different plant organs.\(^1\)\(^-\)\(^3\) Our preliminary studies showed high concentration of the piperidine alkaloids in flowers of *S. spectabilis*, and this evidence may be used for further study on the ecological role of piperidine alkaloids for the taxon Fabaceae, since this class of secondary metabolite is rare in plants.\(^4\) Aiming to select the species that accumulate these metabolites, the extracts obtained from the flowers of *S. multijuga*, *S. macranthera*, *S. velutina*, *S. spectabilis*, *C. fistula* and *C. leptophylla* were evaluated by tandem mass spectrometry, using direct injection in order to have a metabolomic profile of all species analyzed. Additionally, a bioautographic enzyme assay on TLC plates was carried out for evaluation of these extracts as potential acetylcholinesterase (AChE) inhibitors.\(^5\) Among the evaluated species only *S. spectabilis* and *S. multijuga* showed inhibition of AChE in the bioautography screening assay, and the metabolites responsible for the activity was identified as piperidine and pyridine alkaloids respectively, by tandem mass spectrometry (Figure 1). The tandem mass methodology allied with biological screening was fast and efficient on the dereplication of the known bioactive alkaloids as well as for map the metabolomic profile from the six species evaluated.

**Figure 1.** (a) piperidine alkaloid identified in *S. spectabilis*, and (b) pyridine identified in *S. multijuga*.

Refs.

ANTIOXIDANT CAPACITY OF SOME MORACEAE LATTICE SAMPLES

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Reactive oxygen species, whether produced endogenously or derived from external sources, can result in severe damage to DNA, protein, and lipids. The importance of oxidative damage to the pathogenesis of many diseases as well as to degenerative processes of aging has becoming increasingly apparent over the past few years (1). Many natural products had been tested as antioxidants in order to confirm their preventive action. In this study, the antioxidant capacity of Ficus cremersii C.C.Berg and Perebea mollis (Planch. & Endl.) Hubbers ssp. mollis lattices (Moraceae) were evaluated for the first time.

Lattice samples (± 500 mL) collected in Ducke Reservation (AM, Brazil) had been mixed into ethanol 98% (2). After filtration and complete ethanol elimination, filtrated fractions were lyophilized. DPPH assay and Folin-Ciocalteu (FC) assay were performed as described in literature (3, 4). EC50, time needed to reach EC50 (TEC50) and antiradical efficiency (AE) were determined and FC results were expressed as mg gallic acid/g sample. In cell culture, human fibroblasts (10⁴ cell/mL, 100 µL/well) were seeding in a 96-well plate 24h before treatment with successively sample dilutions (100 µL/ well, 0.25 to 250 µg/mL), in triplicate. Twenty-four hours later, medium was removed and oxidative stress was induced by H2O2 (0.5 mM in DMEM/FBS 5%, 100 µL/ well) during 90 min. Cells were allowed to recover in fresh medium for 48h after stress before MTT assay. Trolox was used as antioxidant control.

In DPPH assay, trolox (EC50 = 2.7 ± 0.2 µg/mL, AE = 3.7 ± 0.1) were more active than F. cremersii filtrated extract (51.3 ± 1.4 µg/mL, 0.195 ± 0.005) and P. mollis filtrated extract (93.5 ± 4.8 µg/mL, 0.107 ± 0.005). In the order hand, the Folin-Ciocalteu experiment presented P. mollis filtrated extract (620.3 ± 13.0 mg gallic acid/g sample) more active than Trolox (116.6 ± 36.4 mg gallic acid/g sample) and F. cremersii filtrated extract (73.4 ± 7.1 mg gallic acid/g sample). In the oxidative stress model using human fibroblasts, at 250 µg/mL, trolox reduced in 51.2 ± 4.2% and P. mollis filtrated extract in 34.5 ± 2.7 % the oxidative damage while F. cremersii filtrated extract showed none protective action. In conclusion, FC assay results were better correlated with those obtained in cell culture. This evaluation demonstrated that P. mollis filtrated extract is more promissory as antioxidant than F. cremersii filtrated extract.

Refs.
Screening of sesamine analogues as inhibitors of human and T. cruzi glycer aldehyde-3-phosphate dehydrogenase enzymes

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The development of screening methods to identify new biologically active compounds in complex mixtures is a challenging task. One promising approach is the use of immobilized enzymes onto solid support creating immobilized enzyme reactors (IMERs), which are a helpful tool to select specific inhibitors bye the use of rapid on-line screening of natural and combinatorial library, and also for the direct determination of drug-receptors binding interactions. The enzyme glyceraldehyde-3-phosphate dehydrogenase plays an important role in the cycle life of the T. cruzi. Structural differences between the human and T. cruzi GAPDH might offer the possibility of developing a drug to treat Chagas’s disease by the selection a specific inhibitor of T. cruzi GAPDH.¹² The human GAPDH displays a number of diverse activities unrelated to its glycolytic function, including its role in membrane fusion, nuclear RNA export, DNA replication and DNA repair. Moreover, it is reported that GAPDH might be involved in apoptosis, age-related neurodegenerative disease, prostate cancer and viral pathogenesis.³

This work reports the use of IMERs based on the human and T. cruzi GAPDH enzymes for the screening of GAPDH inhibitors for theses two enzymes. Thus, the inhibitory activities of a collection with twelve compounds of sesamine analogues were analyzed with the free human GAPDH enzyme in solution and in the IMER format. The same collection was screened using the IMER of T. cruzi GAPDH enzyme. The assays with both IMERs identified a reversible active compound selective to the human enzyme. The assay with free human GAPDH indicated that the activity of the selective compound is time dependent.

Fig 1. Sesamine structure.

Refs.
Plants of the family Solanaceae are known to produce a series of C28-steroidal lactones, structurally based on the ergostane skeleton, designated as withasteroids. Among this class of compounds, the withaphysalins, isolated from *Acnistus arborescens*, showed an antiproliferative activity against various tumor cell lines\(^1\). As part of the study on the mechanisms involved in the cytotoxic effects of withaphysalins F and O, the cell viability (membrane integrity), cell cycle distribution, DNA fragmentation and the mitochondria transmembrane potential of HL-60 cells (human leukemia) treated with these compounds, were analysed using flow citometry. HL-60 cells (0.3 x 10\(^6\) cells/mL) were treated with withaphysalins O and F at concentrations of 1, 2.5 and 5\(\mu\)g/mL. The vehicle (DMSO) and doxorubicin (0.3\(\mu\)g/mL) were used as negative and positive controls, respectively. After 24 hours of incubation, aliquots of 50 \(\mu\)L were added to 100 \(\mu\)L of propidium iodide (50\(\mu\)g/mL) to analyze the membrane integrity, and added to 100 \(\mu\)L of propidium iodide containing citrate (0.1%) and Triton X-100 (0.1%) to analyze the cell cycle distribution and DNA fragmentation. To analyze the mitochondria transmembrane potential, cells were incubated with rhodamine 123 (1\(\mu\)g/mL). In all experiments, cell fluorescence (5000 events) was determined by flow citometry. Withaphysalins O and F, only at the highest concentration tested (5\(\mu\)g/mL), reduced the number of viable cells to 60 and 40 \% respectively, when the negative control showed 96\% of viable cells. In the cell cycle analysis, both withaphysalins led to a cell cycle arrest at G2/M, at the concentration of 2.5\(\mu\)g/mL. Cells treated with withaphysalin F also showed a significant increase in DNA fragmentation (4.8, 11.9 and 13.2\% for 1, 2.5 and 5\(\mu\)g/mL respectively) comparing to the negative control (2.1\%). Withaphysalin O caused 10.2 and 13.4\% of DNA fragmentation at concentrations of 2.5 and 5\(\mu\)g/mL respectively. Results of the mitochondria transmembrane potential shows that depolarization varies in accordance to the concentration tested (1, 2.5 and 5\(\mu\)g/mL) with 4.7, 17.5 and 9.1\% for withaphysalin O and 7.6, 16.6 and 5.6\% for withaphysalin F. Withaphysalins O and F, caused a cell cycle arrest at G2/M, leading cells to apoptosis.

Key words: withaphysalin, flow citometry, HL-60, *Acnistus arborescens*

Refs.
STACHYTARPHETA CAYENNENSIS (RICH.) VAHL. -DERIVED GLYCOSYLATED PHENYLPROPANOIDS WITH SYNERGISTIC ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

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S. cayennensis (Rich.) Vahl, Verbenaceae, is commonly known in Brasil as “Gervão- roxo”, “Gerbão”, “Verbena”, etc. The roots and leaves of this plant are used in Brazilian folk medicine as diuretics, analgesics and to treat sore skin, wounds, bronchial problems and rheumatism.¹ In this work the crude ethanolic extract and the partitioned fractions obtained from hexane, dichloromethane, ethyl acetate (as well as the isolated substances of this fraction, glycosylated phenylpropanoids: verbascoside, isoverbascoside, martinoside) and butanol from roots of S. cayennensis were assayed for their potential antioxidant activity against DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free-radical and several Gram-positive and Gram-negative bacteria by using the agar diffusion method.²,³ The greatest antioxidant activity was exhibited by the ethyl acetate fraction (IC₅₀ = 18,0 µg/ml). Antibacterial activity was demonstrated against Gram-positive bacteria, especially Streptococcus pyogenes, in the EtOAc fraction. The estimation of minimal inhibitory concentrations (MIC) from this fraction was MIC = 0,080 mg/ml. The compounds isolated from the EtOAc fraction exhibited very strong antioxidant properties and synergistic antibacterial activity against S. pyogenes.⁴,⁵,⁶

Refs.

5. Tomassini,I.C et al., Fitoterapia, 64, 3 (1993)
STUDY OF ANTIFUNGAL ACTION IN VITRO OF METHANOLIC EXTRACT OF
Pothomorphe umbellata TO STRAINS OF Trichophyton rubrum

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Pothomorphe umbellata, species of the flora Brazilian, belonging the family Piperaceae, is a shrub erect, perennial, very ramified, with articulate stems, with 1.0 to 2.5 m of height. Known popularly as caapeba, pariparoba, malvaísco, capeva, caapeba-verdadeira, it can be found from the South of the Brazil until Amazonas, mainly in the states of São Paulo, Minas Gerais, Espírito Santo and Bahia. It is used in the popular medicine as anesthetic, diuretic, anti-spasmodic agent, against gastrointestinal illnesses and asthma, for inflammatory disorders and as antimalaric. The root decoction is indicated for diseases of the liver and vesicle. It presents action anti-PAF and antioxidant in vitro. Of their leaves they were isolated antioxidant substances, as the 4-nerolidylcatechol, steroids as sitosterol, besides essential oil, mucilage, phenolic substances and pigments. Trichophyton rubrum is a dermatophyte fungi, filamentous, well adapted the human being that causes infections in the skin, hair and nail. In last two decades the incidence of infections caused by dermatophytes and other fungi has been increasing considerably, besides observation of the inefficiency of the drugs to these agents, needing, for this reason, the search for new drugs. The objective of this work was to evaluate the antifungal activity of the methanolic extract of P. umbellata to the strains of T. rubrum: H6, clinical isolated from patient with dermatophytosis and ∆TruMDR2 obtained from the H6 strain, after disruption of TruMDR2 gene, involved in the multiple resistance to drugs. The tests were made according to adaptation of the norm NCCLS M38-P, using plates of microtitration of 96 wells for the determination of the minimum inhibitory concentration (MIC) for visual reading after incubation of 7 days to 28°C. A sample of each well was transferred for Petri plates containing Sabouraud agar for determination of the minimum fungicidal concentration (MFC). The tests for the extract methanolic presented action antifungal in vitro, obtaining values of CIM of 625 µg/mL and CFM of 1250 µg/mL for the strain H6 while for strain ∆TruMDR2 the values of CIM and CFM were of 1250 µg/mL. This difference can be due to the fact of the strain ∆TruMDR2 to express a gene of multiple resistance that was disrupted. It was demonstrated antifungal action of the extract that can be due to several substances contained in this. Our preliminary results suggest that the antifungal activity should be more explored to characterize fractions or compounds that possess this property.

Support: UNAERP
DNA DAMAGE INDUCED BY WITHAPHYSALINS O, M AND N ON MYELOID LEUKEMIA CELLS AND PERIPHERAL BLOOD MONONUCLEAR CELLS

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Withaphysalins O, M and N are C28 - steroidal lactones isolated from \textit{Acnistus arborescens}. These three compounds possess a selective cytotoxicity against leukemia cell lines when compared to peripheral blood mononuclear cells (PBMC)\textsuperscript{1}. The aim of this study was to evaluate the genotoxic (DNA damage) effect of withaphysalins O, M and N on human leukemia cell line (HL-60) and PBMC using the alkaline comet assay\textsuperscript{2}. PBMC were obtained from the peripheral blood of healthy volunteers after centrifugation on a Ficoll gradient. PBMC and HL-60 cells were treated for 24 hours with 0.45, 0.9 and 1.8\textmu g/mL, 4.85, 9.7 and 19.4\textmu g/mL, 2.3, 4.6 and 9.2\textmu g/mL (values corresponding to 1/2IC\textsubscript{50}, IC\textsubscript{50} and 2IC\textsubscript{50} in HL-60 cell line), respectively to withaphysalins O, M and N. The vehicle (DMSO) and doxorubicin (0.3 \textmu g/mL) were used as negative and positive controls, respectively. After treatment, slides were stained with ethidium bromide and cells were scored visually into five classes, according to tail size (from undamaged-0, to maximaly damaged-4), and a value (Damage Index, DI) was assigned to each sample according to its class. Relatively to the negative control, the tested concentrations induced DNA damage in a dose-dependent manner, increasing DNA damage indexes and frequencies. Comparatively, HL-60 demonstrated to be more sensitive to DNA damage than PBMC, as withaphysalins O and N were more genotoxic against HL-60 in all three concentrations tested. For HL-60, 0.9\textmu g/mL of withaphysalin O was able to present a DNA damage index (DI) of 135.00 \pm 6.08, while N only achieved approximately the same DI (134.66 \pm 12.42) with a 10-fold increase in concentration (9.2\textmu g/mL). For all treatments in HL-60 and PBMC, O, M and N caused less DNA damage than the positive control doxorubicin (0.3\textmu g/mL), except M at 19.4\textmu g/mL in HL-60, whose DI was 255.00 \pm 14.73 (DI\textsubscript{positive control}: 205.66 \pm 14.97). In conclusion, withaphysalins O, M and N display a genotoxic activity in HL-60 cell line and PBMC.

Key words: withaphysalin, comet assay, HL-60, \textit{Acnistus arborescens}

Refs.
MIMOSINE ASSOCIATED WITH CYCLOPHOSPHAMIDE IS EFFECTIVENESS TO REDUCE ERLICH TUMOR GROWTH

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Introduction: Mimosine (MI) is an amino acid derived from plants from Leguminosae family, as that from generous Mimosa and Leucaena. These sorts of plants cause natural intoxication on livestock, mainly on dry season. One of the mechanisms of toxicity of this compound is by interfering on protein and DNA synthesis. Recently, it has been demonstrated that MI can inhibit the cell cycle, specifically on G1 fase, consequently it appears to interrupt the renovation of cells from esophagus and intestine. The MI also causes the occurrence of alopecia in intoxicated animals. In addition, it has been reported that MI inhibited pancreatic tumor development in vivo, and in vitro studies showed that this amino acid can repressed uterine cancer growth. However to the best of our knowledge, no studies are found in the literature showing the effects of this amino acid on growth of the Ehrlich tumor cell. Aim: To evaluate the effects of MI on growth of ascitic Ehrlich tumor cells, as well as the effect of the association of this amino acid with a classic chemotherapeutic drug, the cyclophosphamide (CY).

Methodology: The dose of MI employed in the present study was 30.0mg/kg and 12.5mg/kg of CY. Sixty Swiss mice were at random divided into 6 groups (n=10) that received, during 20 days, the follow treatments by ip injection: PBS (control group – Co), Ehrlich cells (E), Ehrlich cells plus MI (EM), Ehrlich cells plus CY (EC), Ehrlich cells plus MI and CY (EMC) and finally, MI alone (M). In the last day of treatment all animals were killed to perform the ascitic cells collection. The aspect of the ascitic fluid was evaluated and volume was measured. Total tumor cell numbers, as well as, dead and live Ehrlich tumor cells were counted. It was employed the Tukey statistical test to analyze significant differences among groups. Results: In relation to body weight it was interestingly to verify that MI when administered alone did not promote toxicity on mice. The statistical analyses employed here revealed that animals from EMC group showed a significant reduction on body weight when compared to those mice from E and EC groups. Corroborate with this data, the results obtained from ascitic volume measurement in which it was noticed that mice from EMC group showed a diminished ascitic volume when compared to E group; however, EC group showed the same results when compared to animal that received only the tumor. When total cell number was evaluated EMC group revealed a reduction on cell number when compared to all groups that received the tumor and the different treatments. Discussion: CY is one of the most important alkylating drugs employed to treat cancer which reacts to DNA interrupting, in this manner, the mitosis process. Many studies reported that MI toxic mechanism of action could be related to its iron chelator property. It is well known that iron deficiency alter folate metabolism in mammals and can interfere on tumor cell growth. The results here obtained clearly shown that the association of CY and MI is effectiveness to avoid ascitic tumor development. It could be hypothesize that MI shows a synergetic effect when administered with CY, probably due to its antimitetabolic property. More studies have been conducting in our lab to better evaluate the chemothertapeutic activity of MI. Conclusion: MI can promote synergetic effect on cancer therapy when administered in association with alkylating drugs.
BIOLÓGICAL ACTIVITY OF THE ROOTS FROM TEPHROSIA TOXICARIA PERS.

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Species of the genus Tephrosia are known as a source of rotenoids and flavonoids. Previous reports described the antibacterial, hepatoprotective and potential cancer chemopreventive effects.1,2 In this study, bioassays of toxicity against Artemia salina (Leach)3, antifungal4 and antioxidant activities5,6 of the ethyl acetate extract (hexanic - TTAH and methanolic - TTAM fraction) of the roots of Tephrosia toxicana were examined. The bioassays were performed as described elsewhere.3-6 For DPPH• assay, 100mM acetate buffer pH 5.5, ethanol, 250µM DPPH• and TTAH and TTAM in different concentrations were added, and then the absorbance was measured after 10min at 517nm. For lipid peroxidation assay, reaction mixture (65 mM KCl and 10 mM Tris-HCl, pH 7.4), TTAH and TTAM in different concentrations, mitochondria (1mg of protein), 50µM (NH4)2Fe(SO4)2 and 2mM sodium citrate were added, and kept for 30 min at 37 °C. MDA formation was estimated using the thiobarbituric acid method and the absorbance was measured at 535 nm. The LC50 value obtained through mortality of the A. salina was 124 µg/mL for the TTAH and total mortality was verified in the 5 mg/mL for the TTAM. The antifungal activity was examined by TLC bioautography using Cladosporium herbarum. Four antifungal spots were observed at RF 0.33, 0.4, 0.46 and 0.73 for the TTAM and RF 0.56, 0.86, 0.9 for the TTAH. Standard isolated C-prenylflavonoids such as 7-methylglabranine and tephrowsatin B are being tested to verify which flavonoid is responsible for the antifungal activity. The TTAH and TTAM presented concentration-dependent effect in scavenging DPPH• radical and lipid peroxidation. The IC50 value for hydrogen-donating ability of TTAH and TTAM were 1.43 and 0.29mg/mL, respectively. The maximum percentage of inhibition of malondialdehyde (97%) was obtained using 160µg/mL of TTAH. The results showed that the extracts of the T. toxicaria presented great pharmacological potential.

IN VITRO ANTIPROLIFERATIVE ACTIVITY OF Arctium lappa L. ROOT EXTRACTS

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Arctium lappa L. (Asteraceae) also known as “burdock” is a traditional Chinese medicine (1). The root has long been cultivated as a popular vegetable for dietary use and folk medicine (2, 3). Several studies have reported that the root possesses various pharmaceutical activities including antibacterial, desmutagenic, antioxidant, anti-inflammatory activity, and hepatoprotective efficacy (3). Ming et al reported anti-cancer activity in prostate cancer cell lines for this plant species (1). Recently, researches on natural products have increased and resulted in the discovery of more efficient drugs for cancer treatment. The aim of this study was to evaluate the in vitro antiproliferative activity of eight different extracts from A. lappa. The roots were extracted sequentially with dichloromethane, ethanol and water in soxhlet apparatus and at room temperature with agitation, also extracting sequently with dichloromethane, ethanol and water. The direct extraction with 70 % hydro alcoholic solution under reflux and aqueous crude extract by soxhlet apparatus were also prepared. The extracts were concentrated under vacuum and were further freeze-dried to yield the crude extracts. The in vitro antiproliferative assay was performed using nine human cancer cell lines: leukemia (K-562), prostate (PC0-3), kidney (786-0), ovary (OVCAR-03), melanoma (UACC-62), colon (HT-29), lung (NCI-460), breast (MCF-7) and multi-drug resistant ovarian cells (NCI-ADR) which response was determined by the Sulforhodamine B assay (SRB). Cells were treated with at least four different concentrations levels ranging from 0.25 - 250 µm/ml to determine growth inhibition and cytotoxic properties. The results showed that burdock root extracts did not have antiproliferative activity. According to Zheng (4), phytochemicals with antioxidant capacities, such as Arctium lappa L., are associated with lower incidence and lower mortality rates of cancer in humans, although they are not cytotoxic to cancer cell lines.

Refs.
2. S. Lin et al., Journal of Biomedical Science, 9, 401 (2002)
IN VIVO ANTICANCER POTENTIAL OF A CUCURBITANE TRITERPENE FROM *Cayponia racemosa* (CUCURBITACEAE)

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**Introduction:** Historically, natural products have served as the most significant source of new leads for pharmaceutical development and plants have been the most important source of human medicines. *Cayaponia racemosa* is a plant commonly found in northeastern Brazil known as “guardião”. It had been shown that some cucurbitacins from its fruits have cytotoxic activity against tumor cell lines. Deacetylpicracin, cucurbitacin P and 2,3,16,20(\(R\)),25-pentahydroxy-22-oxocurbita-5-en presented IC\(_{50}\) values ranging from 0.64 to 4.97µg/mL against leukemia, colon, breast, and melanoma cell lines (1). Nevertheless, those compounds have never been investigated concerning their *in vivo* effects. Thus, in the present study we evaluated the *in vivo* antitumor effects of the cucurbitacin 2,3,16,20(\(R\)),25-pentahydroxy-22-oxocurbita-5-en obtained from *C. racemosa*. **Experimental:** Anticancer effects of the isolated cucurbitacin were evaluated in Sarcoma 180 tumor. On day 0, mice received injections s.c. with 2 x 10\(^6\) cells/animal in mice left hind limbs. Animals were treated with the cucurbitacin (25 mg/kg) i.p. for 8 days. Negative control group was treated with the vehicle (4% DMSO) used for diluting the tested compound. The 5-fluorouracil (25 mg/kg/day) was used as positive control. On day 9, the mice were sacrificed and tumors and organs (liver, spleen and kidneys) were excised, weighed and submitted to histopathology analysis in order to assess toxicity. **Results and Discussion:** The *in vivo* effects of cucurbitacin (25 mg/Kg) were examined in the Sarcoma 180 tumor model. 8-day treatment led to 47.7% tumor suppression by day 9. Histopathological analysis showed no hepatotoxicity. Kidneys were only weakly affected by the cucurbitacin treatment. On the other hand, spleen weights increased after treatment (\(\bar{x}\) 0.8350g) compared to control group (\(\bar{x}\) 0.6580g) (p<0.01). In conclusion, the cucurbitacin 2,3,16,20(\(R\)),25-pentahydroxy-22-oxocurbita-5-en from the fruits of *C. racemosa* showed a moderate *in vivo* anticancer effect in Sarcoma 180 model. The enlargement of the spleen of treated animals could suggest an immunomodulatory property. **Financial Support:** CNPq, CAPES, BNB, FINEP, InCB

Refs.
SEARCH FOR INHIBITORS OF CATHEPSINS FROM BRAZILIAN PLANTS

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Key words: natural products; Zanthoxylum; cathepsins K, V, L and S.

Introduction: Mammalian lysosomal cysteine proteases, the cathepsins, are generally regarded as enzymes that randomly degrade proteins in lysosomes. However, this concept has been reviewed, and it was demonstrated that some of the human cathepsins are also involved in selective and controlled processes and have specific functions associated with their restricted tissue localization.¹

Human cysteine proteases are implicated in the pathogenesis of several diseases including rheumatoid arthritis, osteoporosis, tumor metastasis, periodontal disease, asthma and autoimmune diseases.² Therefore, potent and selective inhibitors of the cysteine proteases are expected to be good candidates for therapeutic agents.³

We have been interested in finding natural products as inhibitors of cathepsins K, V, L and S. A small library of natural products isolated from Brazilian plants and species of genus Zanthoxylum were tested as inhibitors using a high-throughput fluorogenic substrate assay.

Experimental Procedure: Assays of cathepsins (K, V, L and S) were determined spectrofluorometrically using the fluorogenic substrate Z-FR-MCA. The compounds and extracts used for HTS were diluted in DMSO. The enzymes were preactivated for 5 min in a buffer 100 mM sodium acetate pH 5.5, 5 mM EDTA, 2.5 mM DTE. Before the addition of substrate, all compounds were preincubated 5 min with the active enzyme, directly in the black microplate (96 well). After that, the substrate Z-FR-MCA (in DMSO) was added and the hydrolysis was followed continuously by increase of fluorescence at λ_em 460 nm and λ_ex 355 nm (emission and excitation wavelengths for MCA). The positive control was E-64, an irreversible inhibitor for cysteine peptidase. Enzyme inhibition data were calculated using the program SigmaPlot 9.0.

Conclusions: Through inhibition assays of cathepsins we found that some compounds can potently inhibit cathepsin V and L. We could also observe different selectivities for the inhibition of cathepsins L. From all tested compounds, terpenes, cumarins, alkaloids and flavonoids are the more promising ones. The extracts of Zanthoxylum species showed to be active against different cathepsins.

Reference:
IN VITRO ANTIBACTERIAL ACTIVITY OF *Senecio crassiflorus* (Poir.) DC. VAR. *crassiflorus*

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BACKGROUND: Several *Senecio* species (Asteraceae) have been used in folk medicine for the treatment of infections\(^1\). *Senecio crassiflorus* (Poir.) DC. var. *crassiflorus*, commonly known as “margarida-das-dunas” among others, is native from the south coast of Brazil\(^2\) and is known for containing pyrrolizidine alkaloids\(^3\) and sesquiterpenes.\(^4,5\)

OBJECTIVE: Evaluation of the antibacterial activity of the CH\(_2\)Cl\(_2\) extracts of the fresh leaves and stems of *Senecio crassiflorus* var. *crassiflorus*.

METODOLOGY: The plant was collected in Capão da Canoa, RS, Brazil, in April 2006 and identified by Prof. Dr. Nelson Matzembacher (UFRGS). Voucher specimen nº SMDB 10132 is preserved in the Herbarium of the Department of Biology, UFSM. Leaves (50g) and stems (12g) of *S. crassiflorus* var. *crassiflorus* were extracted by maceration with CH\(_2\)Cl\(_2\). The extracts were concentrated in vacuum at 25°C. The evaluation of the antimicrobial activity of the extracts was accomplished through the broth microdilution method based on M7-A6/CLSI/NCCLS\(^6\) document for anaerobic bacteria. The tested concentrations varied between 0.016 and 8.2 mg/mL, and the strains assayed were *Staphylococcus aureus* ATCC 25922, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 14579, and the clinical isolates *B. cereus* and *S. aureus* β-hemolytic.

CONCLUSION: Both extracts were tested for bactericidal effect. The CH\(_2\)Cl\(_2\) extract of the stems showed good inhibitory activity against *Staphylococcus aureus* ATCC 25923 (MIC of 0.064 mg/mL and MBC of 0.512 mg/mL) and *B. cereus* ATCC 14579 (MIC and MBC of 0.128 mg/mL). The leaves extract exhibited a weaker activity than the stems extract and showed activity against *S. aureus* ATCC 25923 (MIC of 0.512 mg/mL and MBC of 2.050 mg/mL) and *B. cereus* ATCC 14579 (MIC of 2.050 mg/mL and MBC greater than 4.100 mg/mL). Only the stems extract showed activity against Gram-negative strains.

KEY-WORDS: *Senecio crassiflorus* var. *crassiflorus*; antibacterial activity; CH\(_2\)Cl\(_2\) extract.

Refs.
There is a continuing need for new antibiotics, mainly due to the increasing number of resistant microbes. Microbial natural products have been the most prolific source of antibiotics suggesting that efforts to discover new natural lead compounds are likely to be fruitful. We have focused our bioprospecting program based on three main approaches, in order to increase the chances of success in the discovery of new antibiotics: i) selecting endophytic microorganisms to be studied, because they are untapped sources of biodiversity and live in a complex web of interactions with the host plant and other microbes; ii) using a recently new in vivo developed assay with the nematode Caenorhabditis elegans infected with Enterococcus faecalis, in order to get in vivo active compounds and to find new mechanisms of action, and iii) co-culturing endophytic fungi, since the competition for nutrients might induce the production of bioactive compounds. The endophytes were isolated from the Brazilian Asteraceae host plants, and cultured in liquid (Czapek, malt extract) and solid (rice) media. The in vivo antibiotic activity is measured by the rescue of worms from the E. faecalis infection. A total of 114 endophytic extracts have been screened in the C. elegans assay resulting in five active extracts (4.4%), an excellent hit rate compared to the previously screened ChemBridge Library of compounds (0.3%) and NCI collection of plant and marine extracts (0.8%). The best results were found for Guignardia mangiferae (VA-15, malt extract), Alternaria sp. (VR-2, Czapek and rice cultures), Diaporthe phaseolorum (VR-4, rice culture), and Glomerella sp (VA-28, rice culture), which were able to rescue the worms (50%) from the E. faecalis infection at different concentrations up to 100 µg/mL in 168 hours. Mixed cultures have been carried out on malt extract liquid medium with VR-2 and VR-8, VR-2 and VR-10, VR-8 and VR-10, and the three fungi were also cultured alone as an experiment control. All the obtained extracts were in vitro screened against Candida albicans using the serial dilution method in 96-well plates. Most active extracts were obtained from single cultures of Alternaria sp. (VR-2, MIC 100 µg/mL) and Chaetomium globosum (VR-10, MIC 10 µg/mL). We have not observed better antifungal activity for mixed cultures. HPLC analyses of the extracts showed that when mixed together, the endophytic fungi completely change their metabolite production. Our results have shown endophytes from Asteraceae as good sources of antibiotics, and chemical investigation of the active extracts is in progress in our laboratory.

Refs.
* Both authors contributed equally.
MECHANISM OF ACTION OF SELECTED NATURAL PRODUCTS ON SPINACH CHLOROPLASTS PHOTOSYNTHESIS

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Inhibitors may block photosynthesis at different sites along the electron transport chain [1]. Electron acceptors may divert electron flow at the level of different redox enzymes. In isolated chloroplasts, studied commercial herbicides inhibit the reducing side of photosystem II (PSII) or accept electrons at the reducing side of photosystem I (PSI). Continuing with our studies [2-4] of natural products as inhibitors of photosynthesis on spinach chloroplasts, we found that ocotilone (1), anacardic acid (2) and 1,3,5-trihydroxy-2,8-di-(3-methyl-butenyl-2)-10-methyl-9-acridone (3) have different mechanisms of action on photosynthesis process.

The mechanisms of action have been characterized through several reactions: Measurement of ATP Synthesis (Hill’s reaction), Measurement of Non-cyclic Electron Transport Rate, Uncoupled photosystem II (PSII) and photosystem I (PSI) electron flow determination (including partial reactions) and studies of chlorophyll a [3-4]. The table 1 shows typical results from experiments measuring the effects of purified compounds 1 to 3 on the rates of ATP synthesis by freshly lysed spinach chloroplasts with MV as electron acceptor. The I₅₀ values for (1), (2) and (3) were 16.0, 38.0 and 24.0 µM, respectively.

The compounds studied in this work behaved as a Hill reaction inhibitors. Compounds (1) and (3) interact at PSII of spinach chloroplasts because act as inhibitors of donator side of PSII. Compound (2) interacts at PSI and PSII of spinach chloroplasts. Oxygen evolution measurement indicates that the site of interaction of compound (2) is at level of QA to QB. Chl a fluorescence measurements confirmed that electron transport is blocked at the QB site.

Table 1. Effect of 1 to 3 on the ATP synthesis, PSII (H₂O to DCPIP) and PSI (TMQH₂ to MV).

<table>
<thead>
<tr>
<th>Compound</th>
<th>ATP (µM)</th>
<th>PSII (µM)</th>
<th>PSI (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>16.0</td>
<td>93.0</td>
<td>N.E.</td>
</tr>
<tr>
<td>(2)</td>
<td>38.0</td>
<td>26.0</td>
<td>81.0</td>
</tr>
<tr>
<td>(3)</td>
<td>24.0</td>
<td>23.0</td>
<td>N.E.</td>
</tr>
</tbody>
</table>

Acknowledgment. The authors gratefully acknowledge financial support from grants DGAPA-UNAM, IN 205806; CNPQ; FAPESP and the author T.A.M.V. thanks to CAPES for the scholarship support.

MUTAGENIC ACTIVITY FROM *Indigofera suffruticosa* MILLER (FABACEAE) AND CYTOTOXIC ACTIVITY FROM INDIGO-GLU

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*Indigofera suffruticosa* (Fabaceae, IS), known in Brazil as anil and jiquelite, is used for the treatment of some diseases, and also as antimicrobial, purgative, sedative and antiulcer. The ethnobotanic information, integrated with the pharmacologic and chemistry studies, is the starting point of our research concerning the discovered of phytoterapeutics from the Brazilian flora. To be used as a phytomedicines, the evaluation of its adverse effects is essential. Therefore, we evaluated the mutagenic activity of the MeOH extract from IS as well as a flavonoid and alkaloid fraction obtained from this extract. The MeOH extract have 3 new substances: quercetin 3-O-[4‴‴-O-α-L-rhamnopyranosyl-β-D-xylpyranosyl(1→2)β-D-galactopyranoside], indigo 3-O-β-glucopyranoside and 6‴-metoxi-2,5,6-trihydroxindirubin, and quercetin 3-O-[β-D-apiofuranosyl(1→6)β-D-glucopyranoside], quercetin 3-O-[α-L-rhamnopyranosyl(1→6)β-D-glucopyranoside], quercetin 3-O-[β-D-glucopyranosyl(1→2)β-D-galactopyranoside], quercetin 7-O-β-D-glucopyranoside, indican, indigo, indirubin, allantoin, gallic acid, pinitol and sitosteryl 3-O-β-D-glucopyranoside.

Assays were performed by the Ames test, using *Salmonella typhimurium*, TA100, TA98, TA102 and TA97a strains, with and without S9 mix. The cytotoxic assays with indigo, alkaloid isolated from IS, were performed by colorimetric sulforodamina-B method, using human mammary tumor cells (MCF7 line), in specific experimental conditions. Only the MeOH extract exhibit mutagenic activity in the TA98 (-S9) strain. Flavonoid fraction presented signs of mutagenic activity for the strain TA98 (-S9) strain. Flavonoid fraction presented signs of mutagenic activity only for the highest dose used (1.5 µg/plate), with MI 1.7 and p≤0.05. The cytotoxic assays showed interesting indigo-glu activity, with reduction of 30-40% of cell viability and the IC50% 84, 4µg/mL. These results are important for a better understanding of the biologic assays of phytomedicines extracted from the Brazilian biodiversity and take information about these new substance, the alkaloid indigo-glu.

Nota: Biota-Fapesp, CNPq.
Some reports correlate the pharmacologic properties of several medicinal plants with their C-glycosyl flavonoids profile. The aim of this study was to compare the C-glycosyl flavonoids chromatographic profiles by TLC and HPLC from three plants that had been largely used in popular medicine in Brazil: Passiflora edulis form flavicarpa Degener, Cecropia cattarinensis Cuatrecasas e Wilbrandea ebracteata Cogn. P. edulis, known as “maracujá” is used in the folk medicine mainly as sedative; C. cattarinensis, traditional named as “embaúba”, is used for the asthma treatment, cough and as anti-inflammatory while W. ebracteata, know as “taiuiá” is traditionally used as laxative, skin affections and also as anti-inflammatory. Herein, the n-BuOH fraction from the crude aqueous extract from the P. edulis and C. cattarinensis leaves and W. ebracteata roots were compared by TLC, using silica F254 as adsorvent and ethyl acetate:formic acid:water (80:10:10,v/v/v) as mobile phase. The HPLC analysis were performed with a C18 column, a gradient of acetonitrile:acetic acid 1% as the mobile phase (1.0 mL/min) and UV detector (330 nm). The flavonoids orientin, isoorientin, vitexin, isovitexin, vicenin-2, spinosin and swertisin were used as reference substances. The chromatographic analysis by TLC showed that n-BuOH fractions from P. edulis, C. cattarinensis and W. ebracteata are rich in C-glycosyl flavonoids, according to the intensity of the fluorescence observed after revelation with NP Reagent. Additionally, the comparison with the C-glycosides standards by TLC and HPLC suggest the presence of orientin, isoorientin, vitexin, isovitexin and vicenin-2 in all fractions with exception of C. cattarinensis, where vitexin is absence. Furthermore, spinosin and swertisin were verified only in n-BuOH fraction from W. ebracteata.
INSECTICIDAL ACTIVITY OF *Trichilia* sp. (MELIACEAE) EXTRACTS AGAINST *Atta sexdens rubropilosa*

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The family Meliaceae includes many plants that are sources of valuable timber and many of them have wide ranging of uses in ethnomedicine. Numerous secondary metabolites that exhibit significant insecticidal activity were already found in *Trichilia* genus⁵. The leaf-cutting ants of the genera *Atta* and *Acromyrmex* use mostly fresh plant fragments to raise their symbiotic fungi² and are the cause of considerable economic damage, due to defoliation that they cause³. Control of this pest is still problematic, presenting only temporary effects and is sometimes, harmful to the environment, to man and other animals. Consequently, an extensive search for alternate methods to control these insects has been made in an attempt of substitute traditional agrochemicals with high specificity and, therefore, causing less damage to the environment. In this context, this work evaluated the toxic effect of dichloromethane and methanol extracts of leaves, stem, bark, branches and thin branches of *Trichilia* sp against leaf-cutting ants *Atta sexdens rubropilosa* as source for less toxic compounds that could be used as a soft control method. The extracts were incorporated in artificial diet for the ingestion toxicity test for the ants. For each treatment were used 50 ants distributed in 10 Petri plates, lined with filter paper. The Petri plates were maintained in B.O.D, at 24 ºC and R. U.H. above 70 %. The ants mortality were accomplished daily, during 25 days and the obtained data of the bioassay were analyzed through the comparison of the survival curves by the test "log rank" (Prism 3.0). The dichloromethane and methanol extracts of bark, branches, thin branches and methanol extracts leaves and stem of *Trichilia* sp showed high activity against leaf-cutting ants *Atta sexdens rubropilosa*, while leaves and stem dichloromethane extracts showed lower rates of activities. The dichloromethane extract of stem was fractionated with the objective of testing the pure compounds and led to isolation of steroids, triterpene, acid cinamic derived and coumarin characteristics of this genus. These compounds were identified by comparison of their NMR, MS, UV and IR data.


FAPESP, CNPq, CAPES
The term “endophyte” is an all-encompassing topographical term which includes all organisms that, during a variable period of their life, symptomlessly colonise the living internal tissues of their hosts. The colonization and propagation of endophytes may in some ways offer significant benefits to their host plants by producing a plethora of substances that provide protection and survival value to the plants. These facts indicate that endophytes play an important role in ecological community.

This work aimed the chemical study and mainly the evaluation of the biological potential of the compounds isolated from the endophytic fungus AM-04, associated with the plant species of Cerrado *A. macrophylla*.

The fungus was cultivated using two different culture media: PDB (potato dextrose broth, 5.0L) and maize (810.0 g). The crude AcOEt extract obtained in maize (10.0 g) was fractionated by CC using silica gel C18 and eluted with a H2O-ACN gradient (90:10-100% ACN) affording 10 fractions (A-J). The fraction B (0.024g) afforded the compound 1 and the fraction D (0.033g) afforded the compound 2. The crude AcOEt extract (0.501 mg) obtained in PDB was fractionated by CC using silica gel C18 and eluted with a H2O-ACN gradient (90:10-100% ACN) affording 5 fractions (A-E). The fraction B (0.040g) was further purified using reversed-phase prep. HPLC [λ = 254 nm, 12.0 ml/min, H2O-ACN (90:10, 40 min)] and supplied the substance 4 (12.4 mg, Rt = 3 min). The fraction C (0.020g) presented crystalline aspect and after recrystallization with MeOH led to the isolation 3 (0.021g).

The compounds 1-4 were evaluate for antifungal activity against the phytopathogenic fungi *Cladosporium cladosporioides* and *C. sphaerospermum* as well as for anticholinesterase inhibition using Ellman’s reagent.

The chemical study of AM-04 allowed the isolation of the known compounds 2,4,6-trihydroxytoluene (1), orcinol (2), cyclo(L)-Pro-(L)-Val (3) and uracil (4). Their structures were stablished by spectrometric methods (1H, 13C, 1D and 2D NMR and MS-ESI) and comparison with the literature. The compounds 1 - 4 presented moderate activity at 100 µg against the assayed fungi and weak acetylcholinesterase activity at 60µg. To our knowledge this is the first report of the antifungal and anticholinesterase activity for 1 - 4.

The results evidence the endophytic fungi as an important source of bioactive compounds and suggest that they exert a role of defense of the plant species, against possible phytopathogens.

**Refs.**


Financial support FAPESP and CNPq
ANTIFUNGAL ACTIVITY OF THREE EUGENIA SPECIES

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In humans, fungal infections range from superficial to deeply invasive or disseminated, and have increased dramatically in recent years. Therefore, a search for new antifungal drugs is extremely necessary¹. *Eugenia brasiliensis* Lam., *Eugenia beaurepaireana* (Kiaerskou) Legrand and *Eugenia umbelliflora* Berg are trees that grows in the coastal Brazilian forests and is commonly know as “grumixama”, “ingabai” and “baguacu”, respectively. Previous studies reported antifungal activity of species of *Eugenia* genus.² The aim of this study was to test the antifungal activity of these species. The aerial parts of plants (leaves and stems) were collected in Santo Amaro da Imperatriz and Florianópolis (SC), in August 2004 and identified by Prof. Dr. Daniel Falkenberg (Botany Department, UFSC). The air-dried plant material was powdered and extracted separately by maceration with ethanol to yield the crude ethanol extract. The crude ethanol extract was re-suspended in aqueous ethanol solution (70 %), and partitioned with solvents of increasing polarity, to give hexane, dichloromethane, ethyl acetate, butanol and aqueous fractions. Extracts and fractions were submitted of antifungal assays against *Candida albicans* (ATCC 18804), *C. glabrata* (ATCC 2001), *C. krusei* (ATCC 200298), *C. parapsilosis* (ATCC 22019), *C. tropicalis* (ATCC 22019), *Cryptococcus neoformans* (ATCC 32608). The antifungal activity was determined by the microbroth dilution assay following the guidelines of NCCLS for yeasts M-27A³ in 96-well microplates. MICs values were determined in RPMI 1640 (Sigma) buffered to a pH 7.0 with MOPS (Sigma). Extracts solutions in DMSO (100 µL) were tested in several concentrations, from 1000 to 7.8 µg.mL⁻¹. Incuba and extracts were incubated at 35 °C for 48 h for *Candida* and for 72 h for *C. neoformans*. Tests were performed in triplicate. The same tests were performed simultaneously for growth control (RPMI + yeast) and sterility control (RPMI + extract). Amphotericin B (Sigma) was used as reference compound with concentrations ranging from 25 to 0.03 µg.mL⁻¹. The MIC was calculated as the highest dilution showing complete inhibition of tested strain. Extracts with MIC values below 1000 µg.mL⁻¹, were considered active⁴, and below 100 µg/ml as very potent⁵. Of 35 samples tested, 27 showed some activity against strains tested. The most potent sample was crude extract of stems of *E. brasiliensis*, that presented MIC value of 7.8 µg.mL⁻¹. The results of this screening investigation confirm the great antifungal potential of *Eugenia* species. The phytochemical characterization of the extracts and the identification of responsible bioactive compounds are necessary.

⁵Rios, J.L.; Recio, M.C. *Journal of Ethnopharmacology* 100, 80 (2005).
ANTIBACTERIAL ACTIVITY OF SUBSTITUTED METHYLENE DIOXI LIGNANS OBTAINED FROM TOTAL SYNTHESIS

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Lignans, one class of the oldest known natural products, have attracted much interest over the years on account of their broad range of biological activities. This growing interest in lignans is due to their application in cancer chemotherapy and their several pharmacological activities.¹ The aim of this work was to evaluate the structure-antibacterial activity relationship of the lignans against oral pathogens. Starting from the Stobbe condensation reaction between piperonal and methylssuccinate, 7-hidroxiinoquin (1) was obtained, being used as the start material for the synthesis of other ten lignans 2-11. All synthesized compounds were evaluated (MIC: 50 a 400 µg/mL) against the oral pathogens causers of the caries. The compound 10 displayed the best activity against Lactobacillus casei (MIC: 80 µg/mL), while compound 9 showed activity against Streptococcus sanguinis (MIC: 100 µg/mL). Since the structural difference between compound 9 and 10 is the presence of the hydroxyl group at C-8 in the compound 10, this polar group may be related to the higher antibacterial activity of the compound 10 against L. casei. The antibacterial results showed that compounds 4-8 were inactive in the band of tested concentration, against all evaluated microorganisms. The compounds 2 and 3 were active against most of the evaluated microorganisms, with exception to the activity of compound 2 against Enterococcus faecalis (MIC> 400 µg/mL). The compound 11 was active against S. sobrinus (MIC 400 µg/mL), while the racemic compound 1 (1a+1b) was active against S. mutans (MIC 300 µg/mL). It is important to point out that the cis isomer 6b showed better antibacterial activity against S. mutans (MIC: 190 µg/mL), and S. mitis (MIC 300 µg/mL). Analyzing the chemical structures of all evaluated compounds and their antibacterial activity, it was possible to suggest that introduction of oxygen at C-7 and C-8, as well as any other structural modifications how ciclization may affect the antibacterial activity of 7-hidroxiinoquinin (1).

Refs.
TRYPANOCIDAL ACTIVITY OF COUMARINS AND STYRYL-2-PYRONES FROM POLYGALA SABULOSA A W. BENNETT (POLYGALACEAE)

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Bioactivity of fractions and compounds obtained from Polygala sabulosa against Trypanosoma cruzi epimastigote, blood trypomastigote and amastigote forms were evaluated in vitro. Dichloromethane and ethyl acetate fractions showed a strong trypanocidal activity on epimastigotes (IC₅₀ ≤ 10.4 µg mL⁻¹). Chromatographic analysis by TLC of these fractions confirmed the presence of previously described compounds (dihydrostyryl-2-pyrones, styryl-2-pyrones and 7-prenyloxy-6-methoxycoumarin)(1, 2). The dichloromethane fraction was fractioned by silica gel column chromatography to afford the compound α-spinasterol and the ethyl acetate fraction obtained apigenin, quercetin and a quercetin-3-O-glucoside, being the first description for the Polygala genus. 4-methoxy-6-(11,12-methylenedioxy-14-methoxydihydrostyryl)-2-pyrene, 4-methoxy-6-(11,12-dimethoxystyryl)-2-pyrene, 7-prenyloxy-6-methoxycoumarin and quercetin-3-O-glucoside showed a weak activity against blood trypomastigotes (IC₅₀ ≤ 1008.6 µg mL⁻¹). The coumarin was the compound most active against both epimastigote and trypomastigote forms, IC₅₀ 10.5 and 88.2 µg mL⁻¹, respectively. The hemolytic activity and cell toxicity of each active compound was also assessed. Furthermore, 4-methoxy-6-(11,12-methylenedioxy-14-methoxystyryl)-2-pyrene and 7-prenyloxy-6-methoxycoumarin reduced 4 times the T. cruzi infection rate for Vero cells at 100 and 50 µg mL⁻¹, respectively. These results show for the first time active compounds against T. cruzi in P. sabulosa.

Refs.
MEASUREMENTS OF THE INHIBITORY EFFECT OF FRACTIONS FROM *Terminalia catappa* LEAVES IN THE ACTIVITY OF FISH ACETYLCHOLINESTERASE

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Several plants present inhibitory effect in acetyl cholinesterase (AChE). Aiming to diminish the cholinergic deficit found in Alzheimer disease, it has been increasing the number of research on the development of new anticholinergic drugs from plant extracts. Plants of the genus *Terminalia* (Combretaceae) are known as a rich source of secondary metabolites, such as pentacyclic triterpenes and their glycoside derivatives, flavonoids, tannins and other aromatic compounds, some of them with antibacterial, antifungal, anticancer and hepatoprotective activities. The Malaysian tree *Terminalia catappa* L. (Combretaceae), known popular ‘chapéu de sol’ in Brazil, have been used on folk medicine for dermatitis and for antipyretic and homeostatic purposes. Recently, the fallen leaves of this plant have been used for preventing hepatoma and treating hepatitis in India, the Philippines and some other countries. Previous studies showed that the water extract of the leaves exert antioxidant, hepatoprotective and anti-inflammatory activities and could prevent carcinogenesis. Crude extract from *T. catappa*, collected in Maceio-Brazil, was prepared using leaves and then it was extracted with ethanol and concentrated to dryness under reduced pressure. This material was partitioned with n-hexane (C₆H₁₄), chloroform (CHCl₃), ethyl acetate (AcOEt), n-butanol (BuOH) and water (H₂O). It was measured the inhibitory effect of CHCl₃, AcOEt, BuOH and H₂O fractions on activity of acetylcholinesterase-AChE from fish *Magil cephalus* brain. Stock solutions of these fractions were prepared in water containing 5% DMSO to give a final concentration of 3 mg/mL. Acetylcholinesterase activity was measured in spectrophotometrically at λ= 412 nm. Concentrations of 5.10⁻³ to 4.10⁻¹ mg/mL of the CHCl₃ fraction were used for the determination of IC₅₀. Although all the fractions tested presented inhibitory effect on the enzyme activity, they shown different degree of inhibition that was evaluated by the percentage of the drop on the AChE activity value, CHCl₃ (57,32%), AcOEt (21,99%), BuOH (15,24%) e H₂O (19,41%). In order to identify the isolated compound(s) responsible for anticholinergic activity in the CHCl₃ fraction further analysis have been made.

Keywords: *Terminalia catappa*; Combretaceae, Acetylcholinesterase inhibitors

Acknowledgments: FAPEAL and CNPq-PIBIC.

Refs.
Sugar esters (biosurfactants) are amphiphilic compounds naturally synthesized by microorganisms and enzymes which can reduce surface and interfacial tensions. They are important biotechnology products for industrial applications due to their specific modes of action, low toxicity, relative ease of preparation and applicability. They can be used in the agrochemicals, foods and beverages, cosmetics and pharmaceuticals industries. For agricultural applications, biosurfactants, in many instances enhance the effects of the microbial biocontrol agent. They have mechanisms action include facilitation of penetration or infection by the control agent, or its products or coformulated components into the cells/tissues of the target organism. Surfactants may also have a direct antagonistic effect on organisms. For example, soil organisms may have the potential to degrade an added chemical control agent, such as an insecticide, and surfactants may be exploited to inhibit insecticide degraders. Surfactants can employ several mechanisms in rumen biology, when used as growth enhancers. The insecticidal activity of many biological systems appears to be enhanced by use of surfactants. For example, sugar esters are believed to be responsible for observed entrapment or repulsion of insect predators on wild potato species. Therefore, new researches have been showed that the esters of sugar are new class of “natural” insecticides that should be developed, particularly, for the control of the insect which has developed resistance to commercial insecticides. In this work, we describe the synthesis of long-chain sugar ester derivated of the castor oil/D-glucose and the its investigation for insecticidal application in fruit fly, due to caused serious damages for the exporting of native fruits of the Brazilian northeast. The castor oil was hydrolyzed and purified for obtaining of the ricinoleic acid. The D-glucose was dissolved in dimethylformamide (DMF), on following was added ricinoleic acid. The reaction was initialized by addition of 40 mg.mL⁻¹ of the catalyst (alkaline proteinase from *Bacillus subtilis*), and the reaction was accomplished in an incubator under constant agitation and temperature in the range at 30-60 °C for 3-7 days, which were terminated with the removal of the catalyst and evaporating the DMF until to obtain a final mixture constituted of two layers. The first layer was constituted of the unreacted ricinoleic acid and the second layer with D-Glucose and glucose ester that was soluble in acetone for its purification.

FTIR, ¹H and ¹³C NMR measurements were determined with objective to evaluate the chemistry changes possible occurred and its structural characterization. Insecticidal test of the product was realizated thought the bioassays accomplished with *Ceratitis Capitata* adult (fruit fly). They were trapped with assay tube, refrigerated for 3 minutes at 0°C, sprayed with various concentrations (0.1-10%) of the sugar esters and after 2 hours the mortality percent was determined. Thus, FTIR measurements were used to characterization of the formation of products through absorption in 1730 cm⁻¹ related carbonyl group typically of ester. This product was tested on bioassay with fruit fly, showing toxicological efficiency with 1% of concentration and obtaining high mortality rate (80%) using 10% sugar esters solution.

The authors acknowledged the Biovet JSC (Bulgaria) and Novozyme for gift of enzymes and ANP/PRH-30 for financial support.

Refs.
POTENTIAL ANTIMALARIAL AND ANTIOXIDANT ACTIVITIES OF GALLIC ACID AND ITS SEMI-SYNTHETIC ESTERS

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For more than a decade a bioassay-guided approach is used in our laboratory to search for bioactive agents of plant origin. Besides the isolation of various active novel compounds, this methodology has also led to the discovery of new interesting properties of known plant constituents. Gallic acid, a widespread natural compound, was found to possess several pharmacological activities, such as scavenging activity towards DPPH, anticarcinogenic, antimutagenic, anti-inflammatory, and recently antimalarial activity\(^1-3\). In this study, gallic acid was used as model compound for antimalarial analogues design, and a series of eight compounds (1-9) have been prepared and evaluated for their ability to interact with heme (FP) using a spectrophotometric assay. Compounds 1 and 9 induced spectral changes in the UV spectrum of FP showing an absorbance decrease at \(\lambda\) 405 nm, which evidenced their ability to complex with FP. Compounds 1 and 9 exhibited activities by 25% and 50% of the standard chloroquine. Additionally, these compounds were screened for antioxidant activity, using DPPH and ABTS spectrophotometric tests, evidencing high scavenging activity of compounds 1-8 relative to the antioxidant standard rutin. In contrast, compound 9 displayed weak scavenging activity, due to the lack of free hydroxy groups. The results obtained for both antimalarial and antioxidant activities are of interest, since Plasmodium-infected erythrocytes are under constant oxidative stress induced by exogenous and endogenous reactive oxidant species (RONs, byproducts of hemoglobin digestion, etc). This leads to various free-radical-induced noxious effects to the erythrocyte and suggests that compounds exhibiting both potential antiplasmodial and antioxidant activities might be interesting as leads in the search for new antimalarial drugs\(^4,5\).

1. L.C. Woodsom et al., Molecular Pharmacology, 24, 471 (1983)
Eclipta alba, popularly known as “erva de botão”, is rich in flavonoids, with antiophydic, antitumoural and hepatotoxic actions. The aim of the work was to analyse and prove the antioxidant activity from Eclipta alba. Antioxidant is a group of heterogenic compounds formed by vitamins, minerals, natural pigments and other vegetal compounds and even enzymes, that avoid the harmful effect of free radicals. It is believed that the antioxidant compounds help in the prevention of chronic diseases such as cancer, heart diseases, cerebral spills and others. Phytochemical studies show it is found in Eclipta alba, mainly flavonoids and terpenes. The ethanolic extract (56.2g) was dissolved in methanol:water (85:15) and submitted to successive partition with hexane and ethyl acetate, resulting in three fractions: FH (21.3g), FAc (3.0g) and Faq (30.4g). These fractions were submitted to assays at concentrations 10, 2.5, 1.0, 0.5, 0.1, 0.05, and 0.01mg/ml, in MeOH. The antioxidant activity was evaluated using the colorimetric method with DPPH (free radicals). The test evaluates the antioxidant potential of the substance in the reduction of DPPH and consequent down of absorbance of this compound at 517 nm, analyzed by spectrophotometer, and it was used as control a rutin solution (1.0mg/ml). The colorimetric assay with DPPH gives a percentual variation of absorbance of the DPPH solution to each tested sample. The results were compared among themselves and with the reference control through the shown statistics parameters. The fractions showed antioxidant activity when compared with the rutin that showed 93% of activity. The acetate fraction (2.5mg) showed 87% and the aqueous (2.5mg), 75% of activity. The acetate fraction (1mg and 0.1 mg) showed 81% and 34% respectively, while the aqueous fraction, in the same concentrations, showed 54% and 7%. Since the fractions are not purified yet, we cannot state which substances are antioxidant.

Refs.
1. A. Çakir et al., Turkish Journal of Chemistry, 30, 483 (2006)
In this work we evaluated the bioactivity of the leaves ethanolic extract of *Pterogyne nitens* in order to discovering potentially antimalarial lead compounds. The crude extract and fractions, obtained by liquid-liquid partition with hexane, EtOAc, MeOH/water, and *n*-BuOH, have been assayed in their capacity of inhibit *in vitro* β-hematin formation. The crude extract and the EtOAc fraction showed the highest activity in the hematin polymerization assay in the range of 59.6 ± 6, and 92.4 ± 1 % at 2.5 mg/mL, respectively. This assay may provide information about compounds that are able to interfere in heme (FP) detoxification pathways, which is crucial to the malaria parasite, and may be considered as potential targets for new antimalarial drug discovery. The *Plasmodium* infects erythrocytes, ingesting and degrading hemoglobin, a process which liberates an enormous quantity of toxic heme. A heme polymerization reaction serves to detoxify this molecule by connecting heme monomers together to form the insoluble hemozoin or malaria pigment, which was found to be identical to β-hematin, a synthetic polymer of heme linked by the ferric iron and the porphyrin carboxylate side chain. Continuous detoxification of heme is necessary for uninterrupted growth and proliferation of the parasite, since heme causes oxidative damage to cell membranes and other biomolecules. The phytochemical study of the EtOAc fraction afforded compounds 1–11, which have been evaluated for interaction with heme by spectrophotometry in a microplate reader using 6 µM heme bovine suspended in 40 % DMSO, and 0.02 M HEPES pH 7.5. Compounds 1, 3, 6, 7, and 9 induced spectral changes in the UV spectrum of FP showing absorbance decrease at λ 405 nm, which evidenced their ability to complex with FP. Guanidine alkaloids 1 and 3 activities showed moderate interaction with FP, by 50% of the antimalarial standard compound chloroquine. On the other hand, flavonoids 6, 7, and 9 showed a very small influence in the FP absorbance, by ca. 20% of chloroquine evidencing the need of further specific studies such the ones using malaria parasite cell culture with the isolated alkaloids and flavonoids from *P. nitens*.

CORDIAQUINONE J, A QUINONE FROM *Cordia leucocephala*, INDUCES INTERRUPTION OF THE CELL CYCLE PROGRESSION TRIGGERING APOPTOSIS IN HL-60 CELLS

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*CORDIAQUINONE J,* Moric, popularly known as “Maria-preta”, is a native plant from caatinga used in folk medicine to treat several diseases. The plant is used in infuse or decoct against rheumatism, indigestion, and general tonic [1]. The aim of this study was to investigate the cytotoxic effects of the cordiaquinone J, a cordiaquinone from *C. leucocephala*. Cordiaquinone J was isolated from the extract obtained from the roots of *C. leucocephala* and identified based \textsuperscript{13}C and \textsuperscript{1}H on NMR spectra. The cytotoxicity of cordiaquinone J was tested against HL-60 (leukemia), MDA-MB-435 (melanoma), SF-295 (brain), and HCT-8 (colon) human cancer cell lines by MTT assay. The inhibition of proliferation was also determined by trypan blue dye exclusion test and DNA synthesis, based on the reduction of BrdU incorporation, using HL-60 as model. To further investigate the mechanisms involved in the cytotoxic activity, the effect of cordiaquinone J on membrane integrity, cell cycle distribution, and DNA fragmentation were evaluated by flow cytometry. The differential morphology with hematoxylin-eosin and acridine orange/ethidium bromide (AO/EB) staining of treated cells were also analysed. Cordiaquinone J displayed potent cytotoxicity against all cancer cell lines tested, showing IC\textsubscript{50} values in the range of 0.89 to 2.13 \( \mu \)g/mL in HL-60 and SF-295, respectively. At all concentrations tested (0.25, 0.5, and 1.0 \( \mu \)g/mL), cordiaquinone J reduced cell viability by the trypan blue exclusion test, however it only caused significant increase in the number of non-viable cells in higher concentration. Cordiaquinone J inhibited by 55.7 \%, 66.0 \%, and 87.1 \% the DNA synthesis at the concentrations of 0.25, 0.5, and 1.0 \( \mu \)g/mL, respectively. At concentrations of 0.25 \( \mu \)g/mL, the cells of the G\textsubscript{0}/G\textsubscript{1} and S phase remained constant, however, there were fewer cells in the G\textsubscript{2}/M phase (12.06 \% against 17.96 \% of the control). At concentrations of 1.0 \( \mu \)g/mL, all phases were decreased. Additionally, cordiaquinone J caused significant DNA fragmentation at all concentrations tested. Cordiaquinone J induced disruption of membrane integrity at higher concentration. In morphological analysis of treated cells, an increasing number of apoptotic cells were observed. Our findings suggest that cordiaquinone J reduce tumor cell proliferation, triggering apoptosis in HL-60 cells. Supported by: CNPq, CAPES, BNB, FUNCAP, FINEP, Claude Bernard Institute.

Key words: Cordiaquinone J, *Cordia leucocephala*, Apoptosis, HL-60.

Refs.
Cryptocarya moschata is an arboreal species of some 25-30m height that is widely spread in the Atlantic forests of Brazil, source of interesting polyketides know as styrylpyrones. This communication reports the evaluation of anti-fungal and acetylcholinesterase inhibitor activities of four compounds: methyl ester of the acid 3,5-dihydroxy-7-phenyl-hept-2-enoic (1), goniothalamin (2), 4′-hydroxy- 6′-phenyl-1′,5′-hexadienyl-5,6-dihydro-2-pirone (3) and asymmetric photodimer of component 6-[(E)-styryl]-piran-2-one (4). These compounds were isolated from dichloromethanic extract of leaves, sequentially by liquid-liquid extraction, low pressure column chromatography (Silica gel) and finally preparative reverse phase (C18) HPLC. The results for anti-fungal assays showed that the compound (2) was more potent against Candida albicans, Candida krusei, Candida parapsilosis and specially against Cryptococcus neoformans with MIC of 15.6 µg/mL. Compounds (3) and (4) showed activity against Cryptococcus neoformans, with MIC of 62.5 µg/mL. For phytopathogen fungal assay, the compound (2) was more potent against Cladosporium cladosporioides and Cladosporium sphaerospermum with MIC of 1.0 µg. Compound (1) showed activity against Cladosporium cladosporioides and Cladosporium sphaerospermum with MIC of 25 and 100 µg, respectively. Compound (3) showed activity against Cladosporium cladosporioides and Cladosporium sphaerospermum with MIC of 5 and 25 µg, respectively. Compound (4) showed activity against Cladosporium cladosporioides with MIC of 100 µg and had no activity against Cladosporium sphaerospermum. For acetylcholinesterase inhibitor assay, the compounds (2), (3) and (4) showed inhibitor activity, being the compound (2) the most active of them. In conclusion, goniothalamin (2) was the most active of these four styrylpyrone related compounds, showing expressive anti-fungal and acetylcholinesterase inhibitor activities.

Ref.
The species *Lotus corniculatus* belongs to Fabaceae family and is popularly known as “cornichão”, from the Mediterranean and European origins. The species of this genus is a forage legume and was introduced in Brazil as food supplement for lambs, ewes and cows due to the presence of condensed tannins. The aim of this study was to determine the antimicrobial activity of this species. The vegetable material was collected at Lages – SC at the Center of Research of EPAGRI, in November 2006. The hydroalcoholic crude extract was prepared by maceration of the whole plant for fifteen days using 1 Kg of the dried plant with ethanol 96%. The crude extract was partitioned into hexane and ethyl acetate soluble fractions. The ethyl acetate fraction was chromatographed in a column of silica gel and eluted with a mixture of hexane:ethyl acetate in order of increased polarity. Sixteen fractions were obtained from this procedure, and the fourteenth fraction was subjected to a flash chromatograph yielding a diglycoside flavonoid identified as kaempferitrin. The compound was identified through the analysis of spectroscopy data NMR \(^{1}H\), NMR \(^{13}C\) and experiments in 2D (COSY, HMQC e HMBC) and was compared with a standard that was previously isolated. The crude extract, fractions and isolated compound were submitted for antimicrobial activity against the strains *Escherichia coli* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923). Broth microdilution method was used to determine the MIC (Minimum Concentration Inhibitory). The value of MIC for the kaempferitrin was compared with other two glycosidic flavonoids that contain the same aglycone. Gentamicin was used as an inhibition control. The plates were then aerobically incubated at 35°C. After incubation for 18–24 hrs, bacterial growth was evaluated by the addition of a solution of methanol (5 mg/mL) and 2,3,5 triphenyltetrazolium chloride (TTC; Vetec) which was used to detect bacterial growth by a color change to red. The best results obtained was that for the flavonoids against the strain *Pseudomonas aeruginosa* exhibiting the MIC 0.35mg/mL for kaempferol monoglycoside; 0.17 mg/mL for the kaempferitrin and 0.16 mg/mL for kaempferol triglycoside.

1. R. Bras. Zootec., v.33, n.6, p.1654-1661, 2004
Oxidative profile of piplartine through synthetic metalloporphyrins


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Piplartine is an alkaloid/amide isolated from roots and stems of Piper species. It has been reported to display significant cytotoxic activity on tumor cell lines, as well as antifungal properties and anti-platelet aggregation activity. It also possesses in vitro antimitotic activity. There are still few studies regarding the metabolism of natural products to prove their efficacy, security and toxicity, and this could difficult their use in therapeutics. An alternative approach for these studies is the use of synthetic metalloporphyrins to mimic the in vivo reactions of the metabolism by the enzimes of cytochrome P450 (CYP 450). The aim of the present study was evaluate the oxidative profile of piplartine through different synthetic metalloporphyrins (MeP) in homogeneous media. The oxidation of piplartine was carried out in organic solutions, using iodosylbenzene as oxygen donor. The catalysts used were FeTPPCl, MnTPPCl, FeTFPPCl and MnTFPPCl and the solvent for the reaction medium was dicloroethane (2 mL). Reaction mixtures contained 6.0 mM piplartine, 0.3 mM metalloporphyrin and 9.0 mM PhIO. The reaction media were analyzed by gas chromatography with mass spectrometry detector in a DB-5MS column and helium was the carrier gas. The GC-MS spectra from the reaction mixtures, in comparison with the spectra of the standart, showed that all metalloporphyrins studied lead to oxidized products, recognized through theirs fragmentation patterns. The oxidation of piplartine, with m/z 317, occurs preferentially in the lactame ring, and results on the formation of the peak with m/z 333, which corresponds to the hydroxylation of piplartine. It was also observed the presence of the peak with m/z 303, which corresponds to an oxidation resulting in a ketone in the lactame portion of the molecule, followed by a ring contraction and loss of carbonile. The comparison among the metalloporphyrins used in this study showed that the best system to obtain the oxidized products of piplartine was FeTFPPCl. These results can contribute for the study of alternative ways to obtain derivatives of natural products by mimicking the reactions of CYP450, in sufficient amounts for testing their efficacy, security and toxicity.

Refs.
1 D.P. BEZERRA et al., Toxicology in Vitro, 21, (2007).

Financial support: CAPES, CNPq, FAPESP
Supervisor: Norberto Peporine Lopes
SCREENING ORGANIC HYDROPEROXIDE RESISTENCE INHIBITORS FROM Xylella fastidiosa IN THE SPECIES Lippia salviaefolia (VERBENACEAE)

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Xylella fastidiosa is a fastidious, xylem-limited bacterium that causes a range of economically important plant diseases. Citrus variegated chlorosis (CVC), which was first recorded in Brazil in 1987, affects all commercial sweet orange varieties, leading to great losses to this country, which is the biggest exporter of citrus juice. Affected fruits are small, hardened and of no commercial value. CVC control is at present limited to removing infected shoots by pruning, the application of insecticides and the use of healthy plants for new orchards. In addition to CVC, other strains of X. fastidiosa cause a range of economically important plant diseases, affecting coffee crops and other important agriculture crops to many countries¹. One of the defense mechanisms of plants against microorganism infections is the induction of an oxidative burst, which enhances the production of Reactive Oxygen Species (ROS) on the cell. To oppose the oxidative species produced in the oxidative burst and other oxidative mechanisms of the host, antioxidant systems were developed by the pathogens. Recently, a new gene related to the antioxidant defense of bacteria was isolated. The deletion of this gene enhances the cells sensitivity to organic peroxides, but not to H₂O₂ or super oxides generators². So, this gene was called organic hydroperoxide resistance (OHR). The present study thus investigates potential Xylella fastidiosa OHR inhibitors in Lippia salviaefolia, which is native in Cerrado of São Paulo State. The activity of OHR in test samples was measured by “Ferrous Xylenol Orange” method³, which is based in the oxidation of Fe²⁺ to Fe³⁺ by peroxides in acid media. In the presence of xylenol-orange reagent (a Fe³⁺ chelating agent), the formation of Fe³⁺/xylenol orange complex occurs and it can be spectrophotometrically measured at 560nm. After tannin removal, the Ethanol Extract of Lippia salviaefolia Leaves (EELLs) and its fractions, obtained from several separation procedures (Liquid-Liquid extraction - LLE, Vacuum Liquid Chromatography-VLC, and Preparative Thin Layer Chromatography-PTLC) were submitted to OHR preliminary inhibition test. The ethyl acetate fraction, obtained by LLE, was the most active, surpassing the activity observed by the crude extract. Subfractions PF2+PF3 and F5, obtained by VLC, also showed Ohr inhibitory activity and were purified by PTLC, leading to isolation of flavonoids 1 and 2, respectively. Compound 1, identified as 5,4′-dihydroxy-7-methoxyflavanone, showed strong activity whereas compound 2, identified as 7,4′-dihydroxy-5-methoxyflavanone, showed weaker Ohr inhibitory activity than the starting material. These results suggest the presence of other bioactive compounds in the tested fractions, which are now under further chemical/biological investigation.

The authors would like to thank the FAPESP, CNPq and CAPES agencies for scholarships and financial support.

Refs

BPS-98
Coumarins are an important category of secondary plant exhibiting various biological activities such as anticancer, inhibition of platelet aggregation, antiviral, antibacterial and so on (1). The antimicrobial activity of a series of twenty 4-R$_1$-coumarins was evaluated against different species of bacteria. Coumarins were obtained by Pechmann reaction, where different substituted phenols were condensed with $\beta$-keto esters in the presence of acid catalytic generating coumarins in good yield with substituents in the phenolic nucleus and/or in the heterocyclic ring (2). The antimicrobial activities of the compounds were determined using the broth dilution technique measuring their MICs against gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *S. aureus* methicillin-resistant- MRSA (clinically isolated) and gram-negative bacteria: *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) (3). Gentamicin was used as an antibiotics standard in order to control the sensitivity of the test organisms. Through the antimicrobial screening, twenty tested compounds showed a broad diversity regarding growth inhibitory activity. Eleven compounds [2,6,8,9,10,12,13,15,16,17,19] among the tested ones appeared to be significantly active against all tested bacteria (MIC values 0.084–1.35 mg/mL). Eight others showed moderate activity (1.36–2.75 mg/mL) while one coumarin [1] did not show any antibacterial activity. The coumarin 6 was the most active compound against *S. aureus* and MRSA with MIC 0.16 and 0.082 mg/mL, respectively. The results of the assays suggest that the activity of the tested coumarins can contribute to the search for new antimicrobial agents, mainly against *S. aureus*, including MRSA, that shows resistance to many known antibiotics.

Refer:
SEARCH FOR ANTIOXIDANT COMPOUNDS IN THE SPECIES *Lippia salviaefolia* (VERBENACEAE)

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The *Lippia* genus comprises ca. 200 species and is the second largest in the family Verbenaceae. It is distributed in South and Central Americas and in tropical Africa (about 70-75% of the known species are widespread in Brazil)\(^1,\)\(^5\). It is employed in folk medicine worldwide, mainly in the treatment of gastrointestinal and respiratory dysfunctions and hypertension, having just two toxic species registered (*L. rehmani* H.H.W. Pearson e *L. javanica* N.L. Burm.)\(^2,4\). Few pharmacological studies have been reported for *Lippia* species, mainly focused in antimicrobial, repellent and larvicidal effect of their essential oils, which are also the focus of chemical studies. The *oxidative stress* has a central role in the development of degenerative processes related to ageing and several diseases\(^3\). So, the search for natural antioxidant compounds can be a strategy for the discovery of new drug leads. The present study thus investigates antioxidant compounds in *Lippia salviaefolia*, which is native in Cerrado of São Paulo State. Samples obtained from each fractionation step were submitted to antioxidant tests towards β-carotene (Thin Layer Chromatography on Silica gel; eluted with EtOAc-MeOH-H\(_2\)O 100:13,5:10) and DPPH free radical (spectrophotometric assay), for selection of the most active subfractions and further investigation. The Ethanol Extract of Leaves (EELLs) was dissolved in MeOH-H\(_2\)O 8:2 and extracted with hexane, ethyl acetate, n-butanol and water. The ethyl acetate fraction, the most active of the series, was fractionated by Vacuum Liquid Chromatography (VLC) (EF: C-18; MF: H\(_2\)O:MeOH 1:0; 4:1; 3:2; 1:1; 2:3; 1:4; 0:1), giving 6 fractions. Among them, “SF2+SF3” was the most active and was submitted to Size-Exclusion Chromatography (SEC) over Sephadex LH-20, eluted with MeOH. Fractions “PF2+PF3” and F5 were fractioned by Preparative Thin Layer Chromatography (PTLC). Nine out of fourteen SEC fractions showed stronger activities than fraction “SF2+SF3”, especially S17, which scavenged 50% of DPPH free radical at 3,33 µg/mL and 90% at 10 µg/mL, against 20,4 e 59% exhibited by “SF2+SF3”. Flavonoids 5,4'-dihydroxy-7-methoxyflavanone and 7,4'-dihydroxy-5-methoxyflavanone were isolated by PTLC from fractions “PF2+PF3” and F5, respectively. These results indicated that chromatographic procedures have afforded semi-purified fractions with higher radical scavenging activities than the crude extract. Additionally, it has been evidenced that the antioxidant activities toward β-carotene and DPPH free radical were related to the most polar compounds in the EELLs and in its semi purified fractions.

The authors would like to thank the FAPESP, CNPq and CAPES agencies.

Refs
IN VITRO INHIBITION OF PLASMODIUM FALCIPARUM BY NEOSERGEOLIDE AND 12-ACETYL NEOSERGEOLIDE

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Plants of the family Simaroubaceae are used in traditional medicine for the treatment of malaria, cancer, dysentery, and other diseases¹. Quassinoids are degraded triterpenes which are found exclusively in Simaroubaceous plants and have proven antimalarial, cytotoxic and antitumor properties, among others². In the present study, a quassinoid, neosergeolide (1) (1.14 g), was isolated from dried, ground roots and stems (6.5 kg) of the Amazonian species Picrolemma sprucei³,⁴ (local name: caferana). Isolation involved maceration in hexanes (to remove apolar compounds), then continuous H₂O extraction, followed by continuous CHCl₃ extraction of the H₂O extract, and finally fractional precipitation of 1 from the CHCl₃ extract. 2 was prepared (in 85 % yield) from 1 using Ac₂O/pyridine. In vitro inhibition of multidrug-resistant Plasmodium falciparum K1 strain was evaluated as described previously by our group⁵. Quassinoid 1 exhibited significantly high inhibition of human malaria parasite (IC₅₀ = 2.0 nM) and was more active than chloroquine and quinine⁵. Semi-synthetic derivative 2 presented less significant inhibition of parasite growth (IC₅₀ = 50 µM). The in vitro sensitivity of the P. falciparum strain to the compounds tested was similar and reproducible in assays in duplicate on separate occasions. Thus, in vitro antimalarial activity of 1⁵ and 2 is demonstrated for the first time. These compounds are potential candidates for pre-clinical tests as novel lead structures for new antimalarial prototypes.

\[
\begin{align*}
(1) \quad R &= H \\
(2) \quad R &= \text{COCH}_3
\end{align*}
\]

Refs.
ANTIFUNGAL ACTIVITY OF *Serjania erecta* Radlk

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*Serjania erecta* Radlk, commonly known as “retrato-de-teiú” and “cinco-folhas”, is a shrub plant of up to 2m height. Endemic in the *sensu stricto* cerrado, it presents hoop-bent down caule, composed leaves with oval wide foliole, apical inflorescence with white flowers, cordado and rounded fruit. *Serjania erecta* Radlk is popularly used as menstrual regulator, cicatrizant, antiseptic, in the treatment of ulcers, and in the combat of hipertension, presenting saponin as the main active principle. Ahead of its pharmacology activities exists interest in verifying its action in fungal infections of which the treatment its restricts to few therapeutically options and presents some collateral effect to the man, beyond inefficacy against of course resistant fungal. The objective of this study was to evaluate the antifungal activity of the aqueous extract of the high parts and respective fractions get from the column chromatograph of the study species against *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258) and *Cryptococcus neoformans* (ATCC90012). The analysis of the antifungal activity was determined by the minimal inhibitory concentration (MIC) through the microdilution of compounds in accordance with the CLSI (M27-A2) guidelines and the determination of the minimal fungicidal concentration (MFC) was performed in the sequence, after 24 hours. Assays using Fluconazole were made simultaneously for methodology control purposes. The results showed that the extract and the a fraction had presented activity against the tested yeasts with MIC and MFC values of 62.5 µg/mL for all four strains of yeasts, probably due to the concentration of flavonoids proven by TLC. Due to proven antifungal activity in the study, the chemical characterization of the probable components with the action must be explored to allow their use in formulations with therapeutically purposes.

Support: UNAERP
EVALUATION OF THE ANTIOXIDANT ACTIVITY OF *SERJANIA ERECTA* RADLK (SAPINDACEAE) OBTAINED OF TWO DIFFERENT HABITATS.

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The *Serjania erecta* Radlk (Sapindaceae) plant, popularly known as “Cipó cinco folhas” and “retrato de teiú”, is used in the popular medicine in the region of cerrado-Brazil, for diverse indications among them anti-inflammatory, antiulcerogenic and against infectious diseases. This plant is endemic to the cerrado-region which has a focus of deforestation. This therefore called our attention to this medicinal plant and caused us to grow it in another habitat, the region of Campo Mourão-PR. The anti-inflammatory activity of this medicinal plant can be explained, among other reasons, for its antioxidant activity. This action is normally associated with the presence of phenol and flavonoids. This work evaluates the antioxidant activity of extracts, the resin and different fractions gotten from the liquid-liquid partition (with solvent of increasing polarity) of aerial parts of the *S. erecta*; those collected from the cerrado (CE) and those cultured in Paraná (PR). They were evaluated through the DPPH free radical scavenging activity, determination of reducing power, as well as the total phenolic and flavonoid content. The total phenolic content was estimated colorimetrically using Folin-Ciocalteu reagent. The standard curve was drawn using 1.6-50 μg/mL of pyrocatechol. Flavonoid contents were determined by colorimetric assay using AlCl₃. Quercetin was used to calculate the standard curve (1.6-25 μg/mL). The dried materials (PR) and (CE) were reduced to medium powder and extracted with 70% ethanol at room temperature for 7 days and re-extracted twice at the same conditions. The extract was evaporated under reduced pressure in a rotary evaporator and dehydrated afterwards in a vacuum desiccator, over silica gel, to remove the remaining moisture from the concentrated extract. The crude extract was dissolved in an ethanol/water mixture (2:8) and filtered to separate the resin fraction which was then subjected to sequential extraction with hexane, dichloromethane, ethyl acetate, and butanol. Each fraction thus obtained, including the final aqueous fraction was evaporated to dryness, resulting the following fractions: hexane (HX), dichloromethane(DIC), ethyl acetate (EtOAc), butanolic (BU) and aqueous. The *S. erecta* of the CE, had the highest content of phenolic compounds where we can salient the EtOAc and BU fractions with 16,80mg and 11,16mg of pyrocatechol for 100mg of extracts respectively. For the test for flavonoids, the fraction with the highest content was the EtOAc with 8,9mg of quercetin for 100mg of extract. It was this fraction that presented good antioxidant activity in the tests of reducing power and DPPH. Moreover, the CE extracts were found to possess the strongest antioxidant activities. In this work, we can verify significant alterations in the amount of total Phenols and flavonoids between some fractions of the sample from the CE and that of Paraná. In general, a correlation between higher amounts of antioxidants and larger amount of total phenolics and flavonoids were found. This indicates that ambient factors can interfere with the phenolic and flavonoid metabolite synthesis, as well as their antioxidant activity. Taking these into consideration, it suggests that, these extracts should be further investigated in order to determine the possible alterations in the quality of its secondary metabolites.
The present work is a part of our project on naturally occurring species in Brazilian stretch of the Upper Paraná River. The general purpose is the investigation of the biological and chemical potentiality of plants present in this area, contributing for the preservation of its biodiversity. A floristic study on the diversity of the Paraná River floodplain, area of Porto Rico (PR, Brazil), showed a high floristic heterogeneity characterized by forest formations [1]. In the survey of vascular plants, 117 families and 652 species were registered [1]. In this work, we report the results of the chemical investigation and of the radical-scavenging activity evaluation of *Urvillea ulmacea* Kunth (Sapindaceae), a plant present in the area of our interest. The genera *Urvillea* is a group of perennial climbers with 13 species distributed in tropical and subtropical areas of the American continent [2]. Phytochemical studies reported on this genus are restricted to the fatty acids and cyanolipids composition analysis for *U. uniloba* [3].

For isolation of the chemical constituents, the concentrated methanolic extract from aerial parts of *Urvillea ulmacea* was dissolved in MeOH: H₂O 1:1 and successively extracted with *n*-hexano and EtOAc. The EtOAc fraction was subjected to repeated columns chromatography on Sephadex LH-20. The resulting sub-fractions were purified by silica gel CC or Sephadex LH-20 filtration to afford the N-methyl-5-hydroxy-pipelic acid (1), epicatechin (2), procyanidin A2 (3) and a new natural compound identified as 3-carbomethoxy-3,4-diydro-1-naphtol (4). The structures were elucidated by NMR techniques and comparison with literature data [4, 5]. The radical-scavenging activity was determined by using the DPPH assay. Our results showed a potent free radical-scavenging activity for methanolic extract, ethyl acetate fraction and procyanidin A2 (3), with IC₅₀ values of 26.7, 16.3 and 11.5, respectively.

Ref.
EFFECT OF SOME COUMARINS, FLAVONOIDS, LIMONOIDS AND ALKALOIDS ON THE GROWTH OF XYLELLA FASTIDIOSA.

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*Xylella fastidiosa* is an important fastidious plant pathogen. This Gram-negative bacterium is transmitted by xylem-feeding leafhoppers (Homoptera, Cicadellidae), and colonizes the xylem of plants causing diseases on several economic important crops such as citrus variegated chlorosis (CVC) in various citrus species, Pierce’s disease (PD) of grapevine and coffee leaf scorch (CLS). Rangpur lime (*Citrus limonia*) is the key rootstock for citriculture in Brazil and it may be grown in orchards in the presence of high disease and insect pressure and they do not show foliar symptoms of the disease. Our earlier phytochemical studies on *Citrus sinenesis*, grafted on Rangpur lime, showed the presence of xanthyletin, a linear pyranocoumarin, in great amount stimulating us to test some roots coumarins, flavonoids, limonoids, alkaloids, and synthetic alkaloid derivatives on growth of *X. fastidiosa*. The results could clarify the basis of resistance to *X. fastidiosa*. The 9a5c strain were obtained from petioles and stems of CVC-affected sweet orange (*Citrus sinensis*) (6 months) maintained in a protected green-house. The 9a5c strain was PCR positive for specific *X. fastidiosa* primers. The best results on exponential phase are summarized below:

If it is assumed that the MIC required for continued investigation is ≤ 1500 µg/mL, the MICs of some compounds are meaningful. Thus, clearly these compounds need further investigation as potential bactericidal agents.
Screening for achetilcolinesterase inhibitors and antioxidant activity of microorganisms extracts from rhizosphera’ corn

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The usefulness of acetylcholinesterase (AChE) inhibitors as insecticides and as a treatment for symptoms of the early stages of Alzheimer’s disease has stimulated much research into finding natural products with this activity in recent years.¹ The enzyme acetylcholinesterase (AChE) plays a pivotal role in the nervous system and rapidly hydrolyzes the active neurotransmitter acetylcholine (Ach) into the inactive compounds choline and acetic acid. Amongst others, low levels of acetylcholine in the synaptic cleft are associated with Alzheimer’s disease. Achetilcolinesterase inhibitors are currently the only approved therapy for the treatment of Alzheimer’s disease, only a limited number of drugs are commercially available. Acetylcholinesterase (AChE) from electric eel, in a free or immobilized state, can be used for the detection of insecticides. This system is convenient because of the selectivity and specificity of the inhibition of AChE by organophosphorus and carbamate insecticides.²

The fact of free radicals are implicated in a number of pathological processes including aging, inflammation, reoxygenation of ischemic tissues, atherosclerosis, and cancer have been stimulated the great interest for antioxidant compounds. Thus, natural antioxidant components, can stabilize free radicals, which are highly reactive and potentially harmful.³

The aim of this work is the screening of microbial extracts, which are an interesting source of biological compounds as selective Ache inhibitors, antioxidants, and investigate their activity as insecticides.¹,⁴ Based on this, 250 extracts were tested using a TLC assays by achetilcolinesterase enzyme, DPPH and β-carotene. In this work we related only the screening of fifty four extracts of microorganisms from rhizosphera’ corn, which thirty showed Ache inhibition, ten showed antioxidant activity and one of them showed activity for both assays. All active extracts were characterized by chromatography and mass spectrometric techniques. However, only two extracts were selected to isolation and identification of compounds responsible to the biological activity. Subsequently, the microorganisms of each active extracts will be identified.

References
IN VITRO DETERMINATION OF THE ANTIBACTERIAL ACTIVITY OF NATURALS PRODUCTS AGAINST ORAL PATHOGEN

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Natural products present a vast variety of molecules with great diversity in its structures. The interest in investigating efficient molecules with herbicidal, insecticidal, fungicidal lesser toxicity includes the potential biological as bactericidal and/or pharmacolgy. In this context, the aim of the present work was to evaluate the therapeutical properties of the secundary metabolites, ricinin, guianin and lasiodiplodin against the oral bacteria being aimed at the biological active compounds search that can be used in odontology. The antibacterial activity was carried through by the microdilution broth method, for the determination of Minimum Inibitory Concentration (MIC). Type standard selected had been Streptococcus sanguinis (ATCC 10556), S. sobrinus (ATCC 33478), S. salivarius (ATCC 25975), S. mutans (ATCC 25175), S. mitis (ATCC 49456), Enterococcus faecalis (ATCC 4082) and Lactobacillus casei (ATCC 11578). The best results had been against the S. sobrinus, L.casei, S. mitis, S. sanguinis and S. mutans for compounds guianin and lasiodiplodin (MIC=0,3mg/mL). Natural products with properties antibacterial in the care of the oral health constitute an important new compounds source.
ANTILEISHMANIAL CONSTITUENTS FROM THE LEAVES OF ARISTOLOCHIA CYMBIFERA

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Medicinal plants have been the basis for the treatment of various diseases in traditional methods. In Brazil, populations in rural areas rely on traditional medicines for the treatment of many infectious diseases. Some species includes prescriptions for therapeutic purposes, like healing wounds, inflammation due to microbial or parasitic infections, skin lesions, ulcers and others¹. Leishmaniasis is a protozoan tropical disease which ranges from a single cutaneous ulceration to a progressive and fatal disease². Besides of the limited and toxic chemotherapeutic arsenal, composed mainly by pentavalent antimonials, amphotericin B and pentamidine, the relapses due to resistant parasites, demonstrate the urgent need for new drug candidates and natural products represent a rich source of new chemical entities for the development of drugs for neglected diseases. Aristolochia cymbifera, a member of the Aristolochiaceae family, has been used as abortifacients, emmenagogeous and against snake bit poisoning³ and was object of this work. We have analyzed crude extract and fractions from the leaves of Aristolochia cymbifera against the most dramatic and fatal disease form of Leishmaniasis, the visceral form (VL). Methanolic extract from the leaves killed 100% of Leishmania (L.) chagasi promastigotes, the etiologic agent of Visceral Leishmaniasis in Brazil, at the highest concentration of 500 µg/mL. In as much as extract from the leaves showed significant antileishmanial activity with an EC₅₀ of 89.17 µg/mL, determined by MTT assay, this was submitted to bioguided fractionation. Activity was concentrated in fractions 7 to 12 and after fractionation was possible to isolate three furofuran lignans from the fractions 7 and 8 that killed about 50 to 100% of L. chagasi promastigotes, determined by light microscopy. These substances were previously isolated from A. Cymbifera, but no antileishmanial activity was described⁴. New fractionation can lead to others active compounds that could be a useful tool for the development of new therapeutic agents for VL.

Refs.
LARVICIDAL ACTIVITY OF *Piper tuberculatum* Jacq. (Piperaceae)

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Malaria is a tropical infectious disease affecting around 400 million people with high level of morbidity, estimated in 1 million deaths annually¹. Piperaceae species are well-known for their insecticidal activity. In a previous study, we have identified the efficacy of *P. tuberculatum* extracts against *Aedes aegypti* L. larvae². With the purpose to evaluate the larvicidal activity against *Anopheles nuneztovari* Gabaldón (Diptera: Culicidae), one of the vectors of malaria in the brazilian Amazon; different methanolic extracts from different parts of the plant (leaf, stem and fruit), from fruits at different growth phases, as well different extracts of mature fruits (hexanic, chloroformic, methanolic and aqueous) were prepared and tested against third instar larvae of *A. nuneztovari*. From this initial screening, we have identified that the apolar extracts from mature fruits have much more significant activity. The crude chloroform extract has shown 50% mortality at 8.0 µg/mL. A biomonitored isolation has been carried out by using different chromatographic steps, leading to the isolation of two amides identified on the basis of their NMR spectra, as being pellitorine (1) and piplartine (2). However, pellitorine has shown moderated activity and piplartine none, even though the previous fraction that led to the isolation of pellitorine had 93.0 % efficacy at 5.0 µg/mL; indicating a possible synergism. This fraction has been purified again with RP-HPLC in isocratic mode with MeOH/H₂O (65:35) collecting the pellitorine (average retention time of 21 min) in one fraction and all of remaining components in a second fraction. After concentrated, both fractions were tested, isolated and combined and it could be observed a synergistic effect between due to pellitorine (figure 1).

![Figure 1. Dose-response of different components of *P. tuberculatum*](image)

Figure 1. Dose-response of different components of *P. tuberculatum*

Refs.
ANTIOXIDANT AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY FROM STEMS OF ABUTA GRANDIFOLIA

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Abuta grandifolia (Mart.) Sandw is a Menispermaceae species widely spread in the Amazon, popularly known as “abuta-branca”. It is used in the traditional medicine as analgesic, for the treatment of ulcer, rheumatism and malaria1. This work describes the antioxidant activity and in vitro acetylcholinesterase (AchE) inhibitory activity of extracts, fractions and an isolated alkaloid, palmatine1, from stems of A. grandifolia. The plant material was collected 23 km from Manaus (AM), in the Reserve Adolpho Ducke and a voucher is deposited at INPA herbarium. Stems dried powdered were extracted by Soxhlet in methanol for 6 h (3 fold) and the extracts were partitioned with hexanes, chloroform and ethyl acetate and concentrated under vacuum. The antioxidant activity of the extracts and fractions were evaluated by two methods (radical scavenger capacity with DPPH2 and ferric reduction antioxidant power3). The total phenolic content was also determined by the Folin-Ciocalteu method4. The AchE inhibitory activity was determined spectrophotometrically at 412 nm using Ellman’s method5. The antioxidant activity of methanolic extract was determined as CS50 = 53.0 µg/mL (CS50 = 6.2 µg/mL of quercetin, used as positive control). The antioxidant activity was concentrated in the acetate and hidroalcoholic fractions. The inhibitory AchE activity of methanolic extract, hidroalcoholic and acetate fractions from stems were determined as IC50 = 29.5, 31.6 and 23.1 µg/mL, respectively. The alkaloid palmatine have shown good inhibitory effect 2.0 µg/mL (4.3 µM) near to the values described by Mukherjee et al (2007)6. The results indicate that the extracts and fractions of A. grandifolia have potential anti-inflammatory effect. Further investigations are being undertaken to confirm the anti-inflammatory activity in vivo.

NOMe
OMe
MeO
MeO
palmatin iodide

Eclipta alba (L) Hassk (sin. Eclipta prostrata) is an herbaceous species of annual cycle, member of the Asteraceae family, commonly known as “erva-botão”. This native species to Brazil and other tropical and subtropical regions presents white flowers, constituting a source of several combinations as flavonoids, phytosteroids and cumestanes. As application of the secondary metabolites, this plant has in evidence anti-inflammatory and anti-hepatotoxic activities, activity in the hepatic infections, arthritis rheumatoid, acting also as antidote against serpent poisons. Beyond its antimicrobial activity, an interest in verifying its antifungal action also exists. The synthetic drugs present some side effects to humans, inefficacy against naturally resistant fungus and development of resistance due to selection. Fungal infections caused by Candida still remains as an emergent problem, especially among immunodeficient patients. The treatment is restricted to few therapeutic options, and so it becomes necessary to search for new active molecules of natural and human-safe sources. The objective of this study was to evaluate the antifungal activity of the ethanolic extract and respective fractions of Eclipta alba against Candida albicans (ATCC 90028), Candida parapsilosis (ATCC 22019), Candida krusei (ATCC 6258) and Cryptococcus neoformans (ATCC 90012). The analysis of the antifungal activity was determined by the minimal inhibitory concentration (MIC) of the extract and fractions through the microdilution on microplates in accordance with the CLSI (M27-A2) guidelines and the determination of the minimal fungicidal concentration (MFC) was performed in the sequence, after 24 hours. Fluconazole was used as positive control. The results showed that the ethanolic extract presented activity against the tested microorganisms, and the ethyl acetate fraction showed better results with MIC and MFC values of 125 µg/mL and 62.5 µg/mL, respectively, for all four strains of yeasts. Due to its pharmacology properties, the chemical characterization of the major active components must be carried out to allow the use of this plant species in formulations with antifungal properties.

Support: UNAERP
ANTIFUNGAL ACTIVITY OF *Tabernaemontana catharinensis*

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*Tabernaemontana catharinensis* belongs to *Apocynaceae* family and is originary from Americas (LEEUVENBERG, 1994). It is known for the accumulation of alkaloids indolic responsible for many pharmacological activities, such as: anti-leishmanicidal, antitumoral, hipoglicemic, analgesic, cardiotonic, antimicrobial (VAN BEEK & VAN GESSEL, 1988). Ahead of its pharmacological activities the interest in verifying its antifungal action in the infections caused by yeasts, in which the treatment is restricted to few therapeutical options and presents some collateral effects to humans, beyond inefficacy against naturally resistant fungus. The objective of this study was to evaluate the antifungal activity of the aqueous extract and respective fractions of *Tabernaemontana catharinensis* against *Candida albicans* (ATCC 90028), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258) and *Cryptococcus neoformans* (ATCC 90012). The analysis of the antifungal activity was determined by the minimal inhibitory concentration (MIC) through the microdilution of the compounds in accordance with the CLSI (M27-A2) guidelines and the determination of the minimal fungicidal concentration (MFC) was performed in the sequence, after 24 hours. Assays using Fluconazole were made simultaneously for methodology control purposes. The results showed that the extract presented activity against *Cryptococcus neoformans* with MIC and MFC values of 125µg/ml and *Candida krusei* with MIC and MFC values of 250µg/ml. This activity was probably due to active substances present in the fractions, respectively, as founded by CCDC. Due to its diverse properties, the chemical characterization of the probable components must be explored to allow the use in formulations with therapeutical purposes.

Support: UNAERP
Casearia sylvestris is a shrub species, member of Flacourtiaceae family, commonly known as “guacatonga” and “chá-de-bugre”, among other popular denominations. It is a South America native species and presents diterpenes, flavonoids, flavones, essential oils, saponins, tannins and anthocyanosides as secondary metabolites. Casearia sylvestris is used as febrifuge, depurative, anti-diarrhea and cardiotonics, and also acts like diuretic, analgesic, aphrodisiac, anesthetic, anti-hemorrhagic, anti-ophidian, anti-septic, blood depurative, circulation stimulant, hemostatic, antiviral, cicatrizant, bactericidal and antifungal. These two last activities are basic in this study, therefore the existing synthetic antifungal drugs in the market present some collateral effects to the man and inefficacy. The objective of this work was to evaluate the antifungal activity of the ethanolic extract and respective fractions of Casearia sylvestris against Candida albicans (ATCC 90028), Candida parapsilosis (ATCC 22019), Candida krusei (ATCC 6258) and Cryptococcus neoformans (ATCC 90012). The analysis of the antifungal activity was determined by the minimal inhibitory concentration (MIC) through the microdilution in the microplates in accordance with the international guidelines (CLSI) and the determination of the minimal fungicidal concentration (MFC) was performed in the sequence, after 24 hours. Assays using Fluconazole were made simultaneously for methodology control purposes. The results showed that the extract and a fraction had presented activity against the tested yeasts with MIC and MFC values of 125 µg/mL for all four species, probably due to the concentration of flavonoids proven by CCDC. Due to its diverse properties, the chemical characterization of the probable components must be explored to allow the use in formulations with therapeutical purposes.

Support: UNAERP
ANTIBACTERIAL ACTIVITY OF VOLATILE CONSTITUENTS FROM AERIAL PARTS OF POLYGALA SABULOSA A.W BENNETT

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Phytochemical and biological studies of species of the genus Polygala revealed the presence of a broad spectrum of secondary metabolites with promising biological activity. The presence of a bisanthraquinone in P. sabulosa, a prenyloxycomarin, as well as the styril- and dihydrostyril-2-pyrone, a new class of compounds for the genus Polygala, were recently described (1,2). In a previous work, we also described the composition of essential oil from aerial parts of this species where the major compounds were methyl salicylate (31.5%) and an unknown compound (47.0%) (3). In this work we reported the antibacterial activity and a proposal for the structure of this unknown compound (UC). The volatile oil was obtained by hydrodistillation (0.3 mL/100 g) for two hours in a modified Clevenger-type apparatus and analyzed by GC/MS. The antibacterial activity was determined using the dilution technique MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) against the bacteria Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853) and Escherichia coli (ATCC 25922) (4). The oil had a strong inhibition of the growth of bacteria S. aureus (MIC = 0.15 mL.mL-1) and P. aeruginosa (MIC = 0.15 mL.mL-1) and a moderated activity against E. coli (MIC = 0.63 mL.mL-1). These results can be related with a presence in high concentration of the methyl salicylate and of the UC. This fact led us to elucidate the molecular structure of this compound (UC). In the process of the styril- and dihydrostyril-2-pyrone synthesis, compounds with an important anxyolitic effect reported (5), we found that the fragmentation profile on mass spectrometry of 4-methoxy-6-methyl-2-pyrone was similar to the fragmentation of UC [(m/z) = 196 (M+, 25%), 140 (23%), 125 (100%), 112 (23%), 98 (27%), 69 (53%)] present in the essential oil. Analyzing the fragments we observed that the UC had a basic skeleton of the 4-methoxy-2-pyrone, plus a side chain, with five carbons, in the C6. So, we proposed that the structure of UC is 6-(2-methylbutyl)-4-methoxy-2-pyrone. This compound is still in the process of synthesis for the confirmation of its structure and for the determination of its antibacterial activity.

Most of the snakebite incidents in the Amazon region involve the Bothrops atrox, whose venom presents the most potent edematogenic and necrotic activities in the genus.¹ This work describes the studies of isolation of the chemical constituents and antiedematogenic activity of the species Peltodon radicans (Lamiaceae),² which is used in the treatment of snakebites and scorpion stings in the region.³ The extracts presented aliphatic hydrocarbons, 3β-OH,β-amirin, 3β-OH,α-amirin, β-sitosterol, stigmasterol, ursolic acid, 2α,3β,19α-trihydroxy-urs-12-en-28-oic acid (tormentic acid), methyl 3β-hydroxy,28-methyl-ursolate, sitosterol-3-O-β-D-glucopyranoside, and stigmasterol-3-O-β-D-glucopyranoside. The flower extracts presented the largest antiedematogenic activity. This is the first report on the study of the flowers, stem, and roots of this plant.

Refs.

ACKNOWLEDGMENTS
Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), and Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM).
EXTRACTS OBTAINED FROM THE ROOTS OF *Stachytarpheta cayennensis* (Rich.) Vahl PROTECTS AGAINST OXIDATIVE STRESS

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Oxidative stress, caused by an accumulation of reactive oxygen species (ROS), is frequently associated with aging and numerous diseases (cancer, cardiovascular and chronic diseases). Among ROS, generated as normal by-products of aerobic metabolism, are superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and the hydroxyl radical (OH$^*$. Superoxide dismutase, catalase and peroxidases provide a defense against the potential cytotoxicity of ROS. The purpose of this work was to evaluate the antioxidant capacity of the crude ethanolic extract obtained from roots of *S. cayennensis* through *in vitro* and *in vivo* methods: the capacity to inhibit the reduction of the free radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and the ability to protect *Saccharomyces cerevisiae*, an eukaryotic cell model, against a lethal oxidative stress. Topical application of the macerated leaves and roots of this Brazilian plant of the Verbenaceae family is recommended in ethnomedical usages to treat sore skin wounds and other diseases. We also analyzed the antioxidant activity of two fractions obtained from the ethanolic extract (ethyl acetate and chloroformic extracts) and of a substance verbascoside isolated from the ethyl acetate fraction. All extracts and the isolated substance showed a DPPH activity similar or higher than that obtained from *Ginkgo biloba*, a reference plant with well documented antioxidant activity. For the *in vivo* assays, first exponential cells were submitted to 0.1 mg/ml of extract for 1 hour at 28°C. In the case of the isolated substance, we treated the cells with 0, 01 mg/ml. Following, cells were exposed to lethal concentrations of H$_2$O$_2$ or menadione (a generator of superoxide radical), for more 1 hour and then plated. All extracts were able to increase cell tolerance against both oxidants. With exception of the treatment with chloroformic extract during the menadione stress, the increase in tolerance caused by the extracts seems to be correlated to a protective effect against lipid peroxidation. The verbascoside was able to protect yeast cells against menadione. The next step of ours research will analyze the verbascoside's antioxidant activity in H$_2$O$_2$ presence. Based on these results, it is possible to consider the roots of *S. cayennensis* as a potential source of antioxidant agents.
COMPOSITION, TRYPANOCIDAL AND ANTILEISHMANIAL ACTIVITIES OF APOLAR EXTRACTS FROM *BOSTRYCHIA RADICANS* (RHODOMELACEAE)

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Marine environment has been investigated with the purpose of discovering new chemical structures and bioactive compounds. In this context, secondary metabolites isolated from marine red algae (Rhodophyta) has been associated to a variety of biological and pharmaceutical activities, including antitumoral, antibacterial and antifungal activities¹. Red algae from *Bostrychia radicans* species were collected in Praia Dura, Ubatuba – São Paulo State (Brazil). The dried material was milled with liquid nitrogen and submitted to extraction by maceration with hexane, dichloromethane, and methanol. Considering the biological potential of red algae, the extracts were evaluated *in vitro* against *Trypanosoma cruzi* and *Leishmania amazonensis* (Table 1). The volatile fraction of dichloromethanic and hexanic extracts were analyzed by HS-SPME-GC/MS (DB-5 column). The samples (200 mg) were put into headspace vial (4 mL) coupled to the PDMS fiber and were heated to 60 °C, with extraction time of 30 minutes. The identification of the constituents was based on comparison of the retention indices (RI) with literature date and library mass spectra. The major constituents of dichloromethanic extract were *n*-hexadecane (1.10%), methyl hexadecanoate (1.85%), 1-heptadecene (2.10%), 6,10,14-trimethyl-2-pentadecanone (2.76%), isopropyl myristate (3.40%) and heptadecane (55.59%); while the major constituent of hexanic extract was heptadecane (67.92%). The fatty acids and steroids composition of these extracts was determined by GC/MS (DB-Wax and DB-17 columns, respectively). Fatty acids, derivatized with sodium methoxide, were identified by co-injection with authentic standards and comparison with library mass spectra. The major compounds found in the dichloromethanic extract were methyl octadecanoate (7.42%), methyl hexadecanoate (8.21%), methyl oleate (10.30%), 1-eicosanol (15.57%), cholesterol (18.12%) and α-cholestanl (19.01%); while in the hexanic extract, it was observed mainly methyl hexadecanoate (3.43%) and heptadecane (4.35%). It must be mention that there are few works about chemical composition from genus *Bostrychia*, which justify this chemical evaluation.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Trypanocidal IC₅₀(µg/mL)</th>
<th>Antileishmanial IC₅₀(µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dichloromethanic extract</td>
<td>8.7</td>
<td>0.6</td>
</tr>
<tr>
<td>Hexanic extract</td>
<td>60.7</td>
<td>7.9</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>12.0</td>
<td>568.3</td>
</tr>
</tbody>
</table>

Data are presented as IC₅₀ values and 95% confidence interval obtained by nonlinear regression;

*²Positive Control: gentian violet (IC₅₀: 31µg/mL); ¹Positive Control: Anfotericine B (ED: 304µg/mL)

Acknowledgements: CNPq, CAPES and FAPESP for financial support.
MYELOPEROXIDASE INHIBITORY FLAVONOIDS FROM FRUITS AND LEAVES OF *PTEROGYNE NITENS* (FABACEAE)

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*Pterogyne nitens* (Fabaceae) is widely distributed in Cerrado and Atlantic Forest in Brazil, and other subtropical regions of South American countries, and is the sole species of *Pterogyne* genus¹. Although no reports on ethno uses of this species have been found, our preliminary studies on the EtOH extract of its leaves and fruits, and their respective EtOAc and *n*-BuOH partition phases, indicated potent cytotoxic and antioxidant activities. Therefore, *P. nitens* was chosen for detailed chemical investigations with the main goal of discover the potential active compounds. The EtOAc and *n*-BuOH phases were further purified using Sephadex LH-20 gel and silica gel RP-18 column, and led to isolation of seven flavonols (1–7) and five flavones (8–12) including the new 3”-O-4’’-methylgalloyl-afzelin (*Pterogynoside-A, 1*) and 6”-O-rhamnosyl-3’-deoxypedaliin (*Pterogynoside-B, 8*), which had their structures elucidated by extensive use of 1D and 2D NMR techniques and HRESIMS. All isolates were tested for their scavenging activities towards DPPH [2,2´-diphenyl-1-picrylhydrazyl], and ABTS [2,2´-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] free radicals, in those assays, the activities were determined by the decrease in the absorbance at λ 515 and 734 nm, respectively, and for their myeloperoxidase (MPO) inhibitory potential through a guaiacol oxidation/hydrogen peroxide method. Vitamin E, Trolox, and quercetin were used as positive controls. Highest MPO inhibitory activities were obtained for quercetin (5), isoquercetrin (6) and rutin (7) (IC₅₀ 1.22, 3.75 and 3.60 nM), whereas flavones (8–12) and kaempferol-derived flavonols (1–4) exhibited 4 to 20 fold weaker MPO inhibition when compared with the compounds 5. The radical scavenging effect measured in the DPPH and ABTS assays displayed by these compounds showed correlation with the MPO activity. Additionally, those results may be associated with the well established antioxidant potential of flavonoids, due to their hydrogen-donating ability, thus increasing the number of hydroxyls or catechol groups results in a more efficient effect in the assays.


The family Rutaceae includes many plants that are sources of valuable medicines and uses in ethnomedicine. Numerous secondary metabolites that exhibit significant insecticidal activity were already found in Rutaceae genera. The fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), is a major pest of many crops in the Americas. In Brazil, it is one of the most important pests of maize plantations, with production losses reaching 34%. Control of this pest is still problematic and is sometimes, harmful to the environment, to man and other animals. Consequently, an extensive search for alternate methods to control these insects has been made in an attempt of substitute traditional agrochemicals with high specificity and, therefore, causing less damage to the environment.

In this context, this work evaluated the toxic effect of leaves and stem hexane, dichloromethane and methanol extracts of *Rauia* sp against *S. frugiperda*. Larvae of *S. frugiperda* (J. E. Smith) were obtained from the Insect Bioassay Laboratory of Universidade Federal de São Carlos, Brazil, and reared on artificial diets. They were maintained in an incubation chamber with a photo phase of 12:12h L:D, 70 (±5) % relative humidity and 25 (±1) °C. For each treatment and control, 30 neonate larvae of *S. frugiperda* were used. A solution of extracts were added to ascorbic acid (1.56 g; an ingredient of the diet), after evaporation, the mixture was incorporated to the artificial diet in which bean and wheat germ are the basic ingredients at final concentrations of 1.0, 10.0, and 50.0 mg kg⁻¹. Diet for the control was prepared similarly but without the extracts. The bioassays and statistic treatment were done as described by Kasten et al.². For evaluation of the mortality of the larval and pupal phases, the experimental unit was constituted by the mean of five tubes with one larva each, with six replications by treatment. The leaves extracts of *Rauia* sp showed higher activity against *S. frugiperda* than stem extracts. Stem dichloromethane and methanol extracts showed lowest activity. The stem dichloromethane extract was fractionated with the objective of testing the pure compounds and to contribute to chemosystematics of Rutace and led to isolation of the coumarin rauianin and a mixture of glycosylflavonoids having rhamnosyl as one of the glycosyl unit. These classes of compounds are characteristics of this genus. Rauianin was identified by comparison of their NMR, MS, UV and IR data with literature and the flavonoids are in process of complete identification of the second sugar unit and their positions in the flavanone skeleton.


FAPESP, CNPq, CAPES
EFFECT OF THE ETANOLIC EXTRACT OF *Endopleura uxi* HUBER (HUMIRIACEAE) IN THE PRODUCTION OF HYDROGEN PEROXIDE AND NITRIC OXIDE IN MURINE MACROPHAGES

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Introduction: From a historical perspective, medicines production and the pharmacological treatment of diseases started with the use of medicinal plants. In order to adapt to environmental insults, plants produce many natural products that have antimicrobial and immunomodulating potential. *Endopleura uxi* is a common tree in the Amazon region pertaining the family Humiriaceae. The tea of its rinds is used in the popular medicine as a powerful natural composition in the combat of the renal infection, uterine inflammation, prevention of the cancer amongst others (1).

Objective: The purpose of this work was to study the effects of the etanolic extract of *Endopleura uxi* in peritoneal macrophage cells from Swiss mice by determination of production the NO and H\textsubscript{2}O\textsubscript{2}.

Material and Methods: The etanolic extract was obtain by maceration during 7 days in ethanol 70% (w/w). The exudate peritoneal macrophages (PEC) were harvested from Swiss mice (6-8 weeks old, 18-25 g). For the determination of the cell viability, PEC (5x10\textsuperscript{6} cells/mL) were re-suspended in medium RPMI-1640 C. The suspension (100 µL) and the samples in different concentrations (100 µL) were added to each well of a 96-well tissue culture plate and incubated for 24 h. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was performed and absorbance measured at 540 nm with a 620 nm reference filter. The nitric oxide production was determined by measuring nitrite. 50 µL of cell supernatant was removed from each well and incubated with an equal volume of Griess reagent at room temperature for 10 min; the absorbance was measured at 540 nm. For H\textsubscript{2}O\textsubscript{2} measurement, PECs (2x10\textsuperscript{6} cells/ml) were suspended in a solution of phenol red complete buffer; 100 µL of this suspension was added to each wells of a tissue culture plate and added 50 µL of aqueous extract in the concentration to viability of 50%. After incubation for 1 h, the reaction was stopped with 50 µL of NaOH 5M and absorbance measured at 620 nm. Results: The cytotoxicity of the etanolic extract in vitro on peritoneal macrophages, it was IC\textsubscript{50} = 15,20 mg/mL and IC\textsubscript{25} = 4,00 mg/mL. In concentrations of 4,00 mg/mL the NO production was low (31,51 ± 1,32 µmlos/5.10\textsuperscript{6} células, however in concentration of 15,20 mg/mL the production was greater that LPS (positive control). The H\textsubscript{2}O\textsubscript{2} production was 483,17 ± 18,92 nmoles/5.10\textsuperscript{6} (15,20 mg/mL) and 138,48 ± 3,84 nmoles/5.10\textsuperscript{6} (4,00 mg/mL). These values suggest a imunoestimulate potential of this species so used by the population. Future studies will be necessary to evidence the effect of this extract in the production of other involved mediators in the immune reply.


FINANCIAL SUPPORT: CNPq
EVALUATION OF TOPICAL ANTIINFLAMMATORY AND WOUND HEALING ACTIVITIES OF *Arrabidaea chica* EXTRACTS

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*Arrabidaea chica* Verlot, (Bignoniaceae) is known by the common names carajiru, crajiru, pariri and chica-red. It is found in Central and South America, mainly in Amazon region. The leaves are used as astringents, feminine genital disinfection, in the treatment of inflammation, skin disease and wounds, intestinal cramps, bloody dysentery, leucorrhoea, anemia and leukemia in traditional medicine¹. The red pigmentation of *A. chica* leaves is due to the presence of 3-deoxyanthocyanidins. These rare substances differ from the known anthocyanins by the lack of a hydroxyl group and for its higher stability. The objective of this work was to evaluate topical antiinflammatory and wound healing activities of *A. chica* extracts and isolated compound using croton oil-induced mouse ear edema and mouse healing excision models, respectively.

Two extracts were prepared from dried, crushed *A chica* (type I) leaves. Maceration in ethanol-water solution, followed by filtration and evaporation yielded crude extract EtOH/AC. For the second extract, sequential maceration in water (19ºC), hexane and finally methanol/acid solution was performed. The latter fraction (MeOH/H+/AC) was concentrated deoxyanthocyanins. A characteristic red-colored deoxyanthocyanidin, carajurin², was obtained using the following procedure: sequential maceration in water, hexane and dichloromethane, followed by filtration of the dichloromethane fraction on C18 cartridges to yield concentrated substance. Two extracts, EtOH/AC, MeOH/H+/AC, and isolated carajurin were tested.

Two models were used as means to evaluate the extracts: the ear oedema and the healing incision model. The ear oedema model was induced by croton oil through topical application (0.4mg/ear) followed by the extracts application (0.01-1mg/ear). Ear thickness was measured and the oedema was expressed after inflammation induction. In the wound healing excision model, animals were submitted to dorsal incision and treated with the extracts (0.2mg/wound, twice a day) during 14 days. The healing was assessed by the rate of wound contraction. MeOH/H+/AC extract caused a dose-related inhibition of oedema (DI₅₀=0,45 mg/ear; Imáx=60±8%) while EtOH/AC extract showed no effect. Carajurin demonstrated a slight antiedematogenic effect (Imáx=30±5%). Extracts did not alter the rate of wound contraction when compared with control group. These results suggested that the concentrated deoxyanthocyanins extract has an interesting topical antiinflammatory effect, being more effective than carajurin alone. Further studies are required to investigate other deoxyanthocyanins other than carajurin as well as the mechanism of action.

Refs.
¹Mors, W. B. et al. *Medicinal plants of Brazil* (2000)
ANTIMICROBIAL ACTIVITY OF \textit{Arrabidaea chica} EXTRACTS

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\textit{Arrabidaea chica} Verlot, (Bignoniaceae) is known by the common names carajiru, crajiru, pariri and chica-red. It is found in Central and South America, mainly in Amazon region. The leaves are used as astringents, feminine genital disinfection, in the treatment of inflammation, skin disease and wounds, intestinal cramps, bloody dysentery, leukorrhoea, anemia and leukemia in traditional medicine\textsuperscript{1}. The red pigmentation of \textit{A. chica} leaves is due to the presence of 3-deoxyanthocyanidins. These rare substances differ from the known anthocyanins by the lack of a hydroxyl group and for its higher stability\textsuperscript{2}. The objective of this work was to evaluate the antimicrobial activity of extracts of \textit{A. chica} leaves.

\textit{A. chica} leaves were extracted with ethanol solution at room temperature for one hour, after filtration the solution was partitioned with four solvents of increasing polarity, hexane, methylene chloride, ethyl acetate and butanol. The ethanol extract and resulting fractions were subjected to antimicrobial susceptibility tests. The most active fraction was separated on PTLC and the resulting fractions were again submitted to antimicrobial tests. The antimicrobial tests were conducted using filamentous fungi (dermatophytes), yeasts and bacteria. The broth microdilution technique was used for the tests involving yeasts and dermatophytes. Tests were conducted using NCCLS reference method for broth dilution for yeasts and filamentous fungi. Strains of yeasts evaluated were \textit{Candida albicans} ATCC 90028 and ATCC 76615, \textit{Candida krusei} ATCC 6258 and \textit{Candida parapsilosis} ATCC 22019. The most active fractions presented MIC (Minimum Inhibitory Concentrations) ≤ 100 µg/mL and MFC (Minimum Fungicidal Concentrations) ≤ 1500 µg/mL. Dermatophytes used were \textit{Trichophyton rubrum} (LIF/FCM 142), \textit{T. mentagrophytes} (LIF/FCM 10) among 20 others from Unicamp’s fungi hospital library. The results for dermatophytes showed MIC and MFC ≤ 100 µg/mL for the most active fractions. To evaluate the activity against bacteria, the technique of disk diffusion was used, according to NCCLS (antimicrobial disk susceptibility tests), using strains of \textit{Staphylococcus aureus} ATCC 25923 and \textit{S. hominis} HC-12432-0 (both present in skin infections), the best results were obtained presented inhibition zones varying from 12 to 18 mm. These results indicate that \textit{A. chica} extracts have potential as antimicrobial agents\textsuperscript{3}. The observed inhibition of \textit{C. krusei} is important given its resistance to fluconazole.

Refs.
\textsuperscript{1}Mors, W.B. et al. \textit{Medicinal plants of Brazil} (2000)
\textsuperscript{3}Barata, L.E.S. et al., Patente de Invenção PI0600943-3. Depósito: 23/02/2006, INPI, Brasil
Species of the genus *Eugenia* have been demonstrating scientific interest. We have recently shown that the water extract of *E. punicifolia* influences the cholinergic neurotransmission in rat diaphragma. In the present work we aimed to verify the antioxidant activity of the dichloromethane extract (DCME) of *E. punicifolia*, since oxidative stress is associated to several neurodegenerative diseases. Two experimental methodology were applied, namely thin layer chromatography associated to revelation with the 2, 2-diphenil-1-pycril-hydrazil (DPPH) radical, and also a test with DPPH and the extract in methanolic solution. The colorless rate of the radical in solution (decay of $A_{517\text{nm}}$), was evaluated. This parameter indicates the scavenger capacity from the free radical for the compounds in the extract.

**Results:** Through thin layer chromatography (hexane: ethyl acetate 9:1) of DCME from *E. punicifolia* and the revelation with vaniline:sulfuric acid, it was possible to observe 10 distinct stains, and among them 3 demonstrated a fast reaction for the revelation with DPPH, thus indicating the presence of active antioxidants substances within. In order to verify the antioxidant activity of the extract in solution, previous dissolution in methanol: dichloromethane (1:1) was required, for the low solubility in methanol, followed by mixing with methanolic solution of DPPH radical and careful observation of $A_{517\text{nm}}$ decay for 30 minutes. The DPPH percentage that remained in solution was calculated based on the $A_{517\text{nm}}$ decay. In order to perform the test the following concentrations of the extract were employed: 200, 400, 600, 800, 1,000, 1,200, 1,400 and 1,600 ppm. In order to compare the results, it was verified the antioxidant activity of terc-butyl-hydroquinone (TBHQ), as standard solution, in the concentrations of 50 and 100 ppm. The results showed that DCME of *E. punicifolia* displayed antioxidant activity in DPPH radical in all concentrations employed, since a reduction of the concentration of the radical after incubation with the extract was obtained. Results were proportional to tested concentration. We observed that 800 ppm extract displayed equivalent activity to 50 ppm TBHQ; meanwhile, 1,600 ppm extract showed activity close to 100 ppm of synthetic antioxidant utilized as standard. Antioxidant activity demonstrated for DCME of *E. punicifolia* indicates that this fraction of the plant may be of interest in the search for new candidate molecules for possible employment in diseases that share oxidative stress as basic phenomenon.

**Keywords:** *Eugenia punicifolia*, oxidative stress, DPPH, TBHQ.

**Grants:** FAPERJ, CNPq
Marine organisms are an important source of bioactive secondary metabolites, presenting a high incidence of cytotoxic and antimicrobial compounds. Despite the recency of this field of study, the oceans have already yielded interesting natural products with confirmed or highly promising pharmacological applications and have yet awakened the industrial interest. The aim of this work was to perform a bioguided fractionation for determination of cytotoxic activity of a hydromethanolic extract (PV-HM) obtained from the zoanthid Protapalythoa variabilis collected at Paracuru and Taíba beaches (Ceará State, Brazil). The cytotoxic activity of both PV-HM and derived fractions was evaluated by the MTT assay against 4 human tumor cell lines: HL-60 (leukemia), MDA MB-435 (breast cancer), SF-295 (CNS glioblastoma) and HCT-8 (colon cancer). The PV-HM of specimens collected at Taíba beach did not show activity, however the PV-HM of Paracuru specimens was very toxic to all cell lines tested (IC\textsubscript{50} values ranging from 0.36 to 4.24 µg/mL). The hemolytic potential was evaluated on mouse erythrocytes after 1, 2 and 4 h incubation. The PV-HM from Paracuru beach induced hemolysis only after 4 hours incubation (EC\textsubscript{50} = 68.83 µg/mL). The bioactive PV-HM was diluted in methanol and partitioned with n-hexane, dichloromethane and ethyl acetate, yielding 4 fractions: PV-Hex, -DCM, EtOAc and -MeOH. All fractions were cytotoxic, being the PV-Hex and -MeOH the strongest (IC\textsubscript{50} values ranging from 0.2 to 0.4 µg/mL for PV-MeOH, from 0.09 to 0.7 µg/mL for PV-Hex, from 1.4 to 2.9 µg/mL for PV-EtOAc). Only PV-MeOH induced hemolysis on mouse erythrocytes and, like PV-HM, after 4 h incubation. To further investigate the mechanisms involved in the cytotoxic activity, the effect of fractions on membrane integrity, cell cycle distribution and DNA fragmentation was evaluated on HL-60 cells using flow cytometry. Morphological alterations of treated cells were analyzed with hematoxylin-eosin staining. The results suggest that PV-Hex, -DCM and -MeOH induced apoptosis, while PV-EtOAc seemed to induce necrosis. Purification of the fraction PV-EtOAc was efficiently performed using high performance liquid chromatography, and activity of the resulting fractions was slightly improved (IC\textsubscript{50} values ranging from 0.34 to 2.73 µg/mL). It is worthwhile to mention that several other chromatography methods used for separation (Silica gel 60 column, Sephadex-LH 20 column, TLC for PV-Hex, -MeOH and -DCM respectively) resulted in inactive fractions. Purification of others fractions are already in progress.

Supported by: CNPq, CAPES, BNB, FUNCAP, FINEP, Claude Bernard Institute.

Keyworks: Protapalythoa variabilis, cytotoxicity, bioguided fractionation.
Antifungal and acetylcholinesterase inhibitors compounds of *Harpagopytum procumbens* (Devil’s Claw).

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*Harpagopytum procumbens* (Burch) DC. Ex Meisn. (Pedaliaceae) is a medicinal plant used by indigenous to the northwestern parts of Southern Africa. This plant is used by population to treat fevers, blood diseases, arthritis and rheumatism. In this study the dried and crude hydroethanolic extract of secondary roots, contained 1.6 to 2.0% of harpagoside was provided by Pro-formula, São Paulo, Brazil. The extract was fractionated through the normal phase silica bed using a short vacuum chromatographic method¹. A stepwise gradient of increasing polarity of mobile phase (200mL) consisting of a mixture of CHCl₃: CH₃OH ratio 100:0, 99:01, 98:2, 96:4, 94:6, 92:8, 90:10, 88:12, 85:15 and 80:20) was employed to obtained the iridoids presents in this extracts. Iridous detection was performed with sulfuric acid/vanillin reagent by TLC and HPLC (Varian Pro Star) using reverse phase C18 (150mmx 4.6mm), isocratic system mobile phase pump A. H₂O, pump B. CH₃OH (1:1), flow rate 1mL/min and detection was carried out at 278nm, during 30min. The degree of purity (%) was evaluated by HPLC and characterization by hydrogen nuclear magnetic resonance (¹HNMR). The extracts, fractions and pure compounds have been evaluated towards phytopathogen fungi *Cladosporium cladosporioides* and *C. sphaerospermum* using bioautography and acetylcholinesterase inhibitors were performed on TLC plates¹. The cinnamic acid show an antifungic effect in this bioassay and harpagoside showed a strong inhibitory activity for the acetylcholinesterase. Financial support. FAPESP.

SEARCH FOR INHIBITORS OF XILELLAIN - AN Xylella fastidiosa PROTEASE

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Key words: Xylella fastidiosa; xylellain; natural products; inhibitors.

Introduction: Xylella fastidiosa is a xylem-limited, Gram-negative bacterium responsible for a large number of economically important plant diseases, such as Pierce’s disease in grapevines, citrus variegated chlorosis (CVC) in sweet oranges and leaf scorch diseases in other plants, including almond, plum, oleander, mulberry and coffee. In all cases, X. fastidiosa infects the plant xylem and impairs fruit production. CVC is considered to be potentially the most devastating citrus disease. Proteins such as proteases, cellulosases and lipases may be involved in the infection process by disrupting plant tissue and allowing the spread of bacteria throughout the vascular system. The interest in cysteine proteases as chemotherapeutic targets stems from the recognition that they are critical to the life cycle or pathogenicity of a large number of parasites. Cysteine proteases from parasites play key roles in immunoevasion, enzyme activation, virulence, tissue and cellular invasion, as well as excystment, hatching and molting. Recent studies described an X. fastidiosa cysteine protease (dubbed Xylellain). This protein may be involved in X. fastidiosa pathogenicity and therefore constitutes a probable target for combating CVC and other diseases. We have been interested in finding natural products as inhibitors of xylellain¹. A small library of natural products isolated from Brazilian plants was tested for their inhibitory activity using a high-throughput fluorogenic substrate assay. Some of the compounds were shown to be potent inhibitors of xylellain.

Experimental Procedure: The compounds used for HTS screening were diluted in DMSO and tested in concentration of 25 µM/well. Assays were performed in 50 mM sodium acetate buffer, pH 5.0. The xylellain was preactivated in the presence of 2.5 mM DTE for 5 min at 25 °C. Before the addition of substrate, all compounds were preincubated 5 min with the active enzyme, directly in the plate (96 well; 200 µL/well). After that, substrate Z-FR-MCA (in DMSO) was added (10 µM/well) and the hydrolysis was followed continuously by increase of fluorescence at λem 460 nm and λex 355 nm (emission and excitation wavelengths for MCA). The positive control was E-64, an irreversible inhibitor for cysteine peptidase. Enzyme inhibition data were calculated using the program SigmaPlot 9.0.

Conclusions: From all tested compounds it was possible to conclude that the flavonoids, cinnamic acid derivatives and alkaloids are the more promising ones followed by the coumarins. These compounds are very widely spread in many plants. Based on these observations we have already tested a library of synthetic compounds and have also found good inhibitory activity. This is the first time that natural products from plants have been tested on xylellain inhibitory activities.

References:
Species of the Piperaceae family are well known for their insecticidal properties\(^1,2\). Steam distillation of dry *Piper aduncum* leaves yields a volatile oil from which dillapiol (1) can be isolated by fractional vacuum distillation in 72% yield (v/v). Ethers 2-6 were prepared by stirring 1 with mercury(II) acetate under nitrogen using, respectively, methanol, ethanol, propanol, butanol and octanol as solvents (0 °C to r.t., over 2-168 h, reaction time increased with increase in alcohol chain length). After this initial reaction, the organo-mercurate intermediates were reduced by addition of alkaline sodium borohydride solution. Mercury metal was removed by filtration and chloroform was used for extraction. The chloroform phase was separated, washed with water, then saturated sodium chloride solution and was finally dried over magnesium sulfate. After removal of solvents, crude ethers were obtained. Purification was performed using flash chromatography providing 2 (41.8 mg, 78%), 3 (37.4 mg, 62%), 4 (149.7 mg, 77%), 5 (166 mg, 42%) e 6 (78.5 mg, 17%) whose identities were confirmed by \(^1\)H and \(^{13}\)C-NMR. Mosquito larvae (*Aedes aegypti*) were bred in the laboratory under controlled temperature (27 °C) and humidity (60%) conditions. Tests were performed according to the WHO protocol. For each treatment and control, 10 third instar larvae were transferred to 10 mL plastic cups, having a final volume of 5 mL water. The evaluation of mortality rate was performed 24 h after the beginning of the experiment, verifying the number of dead larvae. Data were evaluated through probit analysis to determine the LC\(_{50}\) representing the concentrations in µg/ml that caused 50% mortality. Compounds 1, 2, 3, 4 and 5 exhibited CL\(_{50}\) = 36.2, 100.6, 50.3, 28.0, 19.7 µg/mL. Compound 6 exhibited no larvicidal activity.

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**Refs.**

The genus *Erythrina* is known in Brazilian folk medicine for the treatment of central nervous system (CNS) illnesses. Its stem barks are recommended for nervous system excitation, sleepiness, convulsions and nervous coughs[1]. Most of the scientific work on *Erythrina velutina* describes important pharmacological effects for crude extracts of the plant as the anti-nociceptive activity of the aqueous extract of the leaves [2] and the antibacterial activity of bark ethanolic extract [3]. *E. velutina* extracts have also CNS depressant activity showed through the potentiation of pentobarbital-induced sleeping time [1;4] and through the anticonvulsant action in strychinine-induced seizure model [4]. In the literature the erythrinian alkaloids were reported as responsible for the pharmacological properties on CNS. In a recent work, the phytochemical study of *E. mulungu* flowers afforded three erythrinian alkaloids, which were found to have anxiolitic effect in an elevated T-Maze test [5]. In continuing our work for the search for bioactive compounds, the phytochemical study led to the isolation and characterization of two known erythrinian alkaloids from stem bark of *Erythrina velutina*. The isolation steps were carried out on HPLC system using water/methanol as mobile phase and the identification of the structures isolates were based on NMR and ESIMS data. Preliminary evaluation of the anxiolitic activity was performed on the Ethanolic extract and alkaloidic fraction using elevated T-Maze assay. Additionally, were evaluate acute toxicity.

Refs.
The genus *Kielmeyera* belongs to the family Clusiaceae (Guttiferae), subfamily Kielmeyeroideae, and is endemic to South America, with the vast majority of the species occurring exclusively in Brazil. Here, nearly 50 species are found, chiefly in the ‘restinga’ (sand dunes), ‘campos rupestres’ (rocky savannas) and ‘cerrado’ (savanna) vegetation south of the Amazon. Some species are commonly known in Brazil as ‘pau-santo’ and are traditionally used in Brazilian folk medicine to treat several tropical diseases, including schistosomiasis, leishmaniasis and malaria as well as fungal and bacterial infections. Phytochemical studies with Brazilian *Kielmeyera* species showed mainly 4-alkyl and 4-phenyl coumarins and xanthones. We have previously reported the isolation and structure identification of 4-phenyl and 4-n-propyl coumarins from *K. rugosa* fruits and leaves collected in a ‘restinga’ of Sergipe State, Brazil. In a continuation of our study on this plant, we now report herein the isolation and structure elucidation of another 4-n-propyl coumarin (1), 4-phenyl coumarin (2) (Figure 1) from the CHCl₃ extract of fruits and stem bark, respectively, along with a mixture of the two well-known triterpenes lupeol and α-amyrin isolated from the hexane extracts of leaves. For structure elucidation of these natural compounds one- and two-dimensional NMR techniques (¹H, ¹³C, DEPT, HMBC, HSQC, and COSY) were used besides IR spectroscopy. In addition, the extracts and some fractions from the stem bark, fruits, and latex of *K. rugosa* were tested for their enzymatic inhibitory activities against glicososomal GAPDH from *Trypanosoma cruzi* and APRT from *Leishmania donovani*. Some Extracts and fractions displayed relevant inhibitory activity (% inhibitory activity > 80%), suggesting that the plant extracts and fractions may have tripanocidal and leishmanicidal agents.

**Figure 1:** 4-n-propyl and 4-phenyl coumarins isolated from *K. rugosa*

Refs.
ACTIVITY OF EXTRACTS FROM *Melia azedarach* AGAINST *Nezara viridula* (HEMIPTERA: PENTATOMIDAE) - PEST OF FORAGE LEGUMES AND SOYBEAN. DESIGN AND STANDARIZATION OF BIOASSAYS.

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Soybean –and other legumes- production has grown during the last years in Uruguay, leading to different sanitary problems, among which the damage caused by pentatomids is an important one. Not only these pests have been difficult to control by conventional pesticides, but also they have developed resistance against conventional pesticides. Furthermore, the trend worldwide is to decrease the use of traditional pesticides, since its overuse has caused not only the development of resistance, but also the appearance of adverse effects on the environment and on non-targeted organisms. Within this framework we started a program aiming to find alternatives for controlling pentatomids of high economic incidence in Uruguay. The present work reports on the standardization of bioassays to screen for toxic activity against nymphs and adults of pentatomids, using *Nezara viridula* as a model. The standardization was performed exposing the insects by contact and by ingestion to a commercially available insecticide used to control them (Engeo®). Reproducible results were found only in the contact bioassay (Table 1). Using this design, extracts from different organs of *Melia azedarach* (Meliaceae) were tested against *N. viridulans*. After 24 hours of exposure, results showed good activity of extracts from fruits and twigs against nymphs, but not of the extract from leaves (Table 1). Although LD₅₀ for fruits’ and twigs’ extracts are greater than the positive control (Engeo®); in the case of LD₉₉, figures are comparable. Interestingly, the same extracts did not seem to affect adults when exposed for 24 hours- at least up to 1500 µg/cm² (Table 1). In the case of adults, the lethal effect is reached only after 72 hours of exposure, although with greater doses (LD₉₉ = 850 ± 280 µg/cm²; p < 0,001), indicating a greater susceptibility of immature stages than of adults.

Table 1: Lethal doses [50% and 99%, mean ± standard error (µg/cm²)] after 24 hours of exposure of Engeo® and extracts from *M. azedarach* under the conditions of the standardized bioassay (activity by contact).

<table>
<thead>
<tr>
<th>Products</th>
<th>LD₅₀</th>
<th>LD₉₉</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nymphs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engeo®</td>
<td>6,7E-03 ± 6,1E-04 (p&lt; 0,001)</td>
<td>1,6E-02 ± 1,3E-03 (p&lt; 0,001)</td>
</tr>
<tr>
<td><em>M. azedarach</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits</td>
<td>2,1E-02 ± 8,2E-03 (p &lt; 0,05)</td>
<td>5,0E-02 ± 2,2E-02 (p &lt; 0,05)</td>
</tr>
<tr>
<td>Twigs</td>
<td>1,9E-02± 8,2E-03 (p &lt; 0,001)</td>
<td>4,3E-02 ± 3,5E-03 (p &lt; 0,001)</td>
</tr>
<tr>
<td>Leaves &gt; 700</td>
<td>&gt; 700</td>
<td></td>
</tr>
<tr>
<td><strong>Adults</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Engeo®</td>
<td>3,7E-03 ± 1,2E-03 (p&lt; 0,005)</td>
<td>1,1E-02 ± 3,5E-03 (p&lt; 0,005)</td>
</tr>
<tr>
<td><em>M. azedarach</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits &gt; 1500</td>
<td>&gt; 1500</td>
<td></td>
</tr>
<tr>
<td>Twigs &gt; 1500</td>
<td>&gt; 1500</td>
<td></td>
</tr>
<tr>
<td>Leaves &gt; 1500</td>
<td>&gt; 1500</td>
<td></td>
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</tbody>
</table>

BPS-130
The vasorelaxant effects induced by the leaves methanolic extract (ExMeOH01, 78.50 g, 15.7%) of *Hyptis fruticosa*, ethyl acetate (ExAcOEt1, 29.50 g, 37.6%), dichloromethane (ExDCM1, 5.40g, 6.9%) and petroleum ether (ExEP1, 8.43g, 10.7%) fractions of this extract, and 16-ent-kauranol 1 [Rf=0.64 , Hex:AcOEt 6:4/anisaldehyde, mp 210-211°C, $^1$H NMR (200 MHz, CDCl$_3$) 0.78(3H,s,Me); 0.82 (3H,s,Me); 1.00 (3H,s,CMe) e 1.3 (3H,s,Me) e 1.5 (2H, m), $^{13}$C NMR 33.24 (C, C-4), 45.37 (C,C-8),39.35 (C, C-10) , 79.31 (C, C-16), 56.23 (CH, C-5), 56.90 (CH, C-9), 49.06 (CH, C-13), 40.36 (CH$_2$, C-1),18.61 (CH$_2$, C-2),42.08 (CH$_2$, C-3), 20.41 (CH$_2$,C-6), 42.07 (CH$_3$, C-7, 17.97 (CH$_2$, C-11), 26.91 (CH$_2$, C-12), 37.70 (CH$_2$, C-14), 58.10 (CH$_2$, C-15), 24.44 (CH$_3$, C-17), 33.57 (CH$_3$, C-18), 21.54 (CH$_3$, C-19), 17.75 (CH$_3$, C-20)] were studied in isolated rings of rat superior mesenteric artery. In intact rings (control), the extract, fractions (1-1000 µg/mL, n=6, cumulatively) and 16-ent-kauranol (0.01 – 10 µg/mL) induced concentration-dependent relaxations of tonus induced by phe 10 µM ($E_{max}$ = 83±5.4%; 26±4.3%; 71±4.6%; 99±0.4% and 58±5.7%; respectively). In endothelium-denuded rings, the concentration-response curves to methanolic extract, dichloromethane, petroleum ether fractions and 16-ent-kauranol were not changed ($E_{max}$ = 90±4.3%; 88±13.3%; 99±0.4%; 43±3.2% respectively). The curve to ethyl acetate fraction was increased in the final concentration ($E_{max}$ = 52±6.3%). The petroleum ether fraction was more efficacious than others ones and the 16-ent-kauranol was the most potent. These results show that all products from *H. fruticosa* tested in this work were able of relaxation in mesenteric artery. The citotoxicity in vitro of extracts and fractions were also tested to determine the toxic concentrations for mammal cells using BALB/c mice splenocytes cultured in 96-well plates as normal cells. This method is efective for avaluation of specific T lymphocyte cytotoxicity. All samples are considered as non-cytotoxic (Table 1).

### Table 1- Results of citotoxicity

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ExMeOH01</td>
<td>25</td>
<td>18.53</td>
</tr>
<tr>
<td>ExEP01</td>
<td>10</td>
<td>12.14</td>
</tr>
<tr>
<td>ExDCM01</td>
<td>25</td>
<td>27.16</td>
</tr>
<tr>
<td>ExAcOEt01</td>
<td>5</td>
<td>11.74</td>
</tr>
<tr>
<td>16-ent-kauranol</td>
<td>50</td>
<td>22.85</td>
</tr>
</tbody>
</table>

(a) = the highest non-toxic concentration on spleen cell of BALB/c mice; (b) percentage of growth inhibition was determined comparing the percentage of the percentage of [H]- thymidine incorporation of drug-treated wells in relation to untreated wells.

Refs.
PROLIFERATIVE EFFECT OF AQUEOUS EXTRACT OF HYPTIS FRUCTICOSA ON LIVER REGENERATION AFTER PARTIAL HEPATECTOMY IN RATS

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The *Hyptis fructicosa* is a plant from the Lamiaceae family and is popularly known in Brazil as “Alecrim do Campo” or “Alecrim de Vaqueiro”. Although there are some studies about other species of the Hyptis gender, few publications about this species are found in literature. The Liver has the property of regeneration under influence of different stimuli due to hepatocyte proliferation. Some studies show that this proliferation process may be affected by different factors such as drugs, growth factors or phitotherapics. This present study aims to evaluate the effect of aqueous extract of *Hyptis fructicosa* on hepatic regeneration after partial hepatectomy in rats. In order to do this, 16 rats were divided into two groups: C (Control Group, whose rats received water daily for 4 days) and HF (Whose rats received aqueous extract of *Hyptis fructicosa* during 4 days using the dose of 100 mg/kg/day). On the consecutive day of this treatment, the animals of both groups underwent hepatectomy of about 67% of liver. Twenty four hours later, they were sacrificed, and the remaining mass of liver was removed and prepared to be studied through the PCNA immunohistochemical technique, using monoclonal antibody PC-10. The glass slides were analysed through optical microscopy at high power (400 X). Nuclear labeling index for PCNA (positive nuclei/total number of counted nuclei) were determined by evaluation of at least 1,000 hepatocyte nuclei. The liver regeneration index of C group was 53.56 ± 18.91 %, while index of HF group was 21.12 ± 8.29 %. These results show that the administration of aqueous extract of *Hyptis fructicosa* using the dose of 100mg/kg/day increased the hepatocyte proliferation in the group HF (p=0.0027) (Figure 1).

![Figure 1 – Hepatic Regeneration](image)

Lignans have been used as lead compounds for the development of new drugs. *Podophyllum hexandrum* has an economic importance as a source of the aryltetralin lignan podophyllotoxin, which is used as a precursor for the semi-synthesis of the anticancer drugs etoposide, teniposide and etopophos (1). The successful introduction of these drugs and the development of new derivatives have created a demand for podophyllotoxin. To date, it is commercially obtained from the rhizomes and roots of wild populations of *P. hexandrum*, and thus the availability of this natural product is limited (2). The search for alternative sources of podophyllotoxin and related compounds remains a key area of extensive research. The establishment of cell and organ cultures from lignan-accumulating plants opens up new approaches for the production of lignans (3). This work reports on the isolation and identification of podophyllotoxin and 4'-demethylpodophyllotoxin from the rhizomes and roots of *P. hexandrum*. Dried rhizomes and roots were powdered and extracted by stirring with hot ethanol. The filtered extracts were combined, evaporated to dryness and redissolved in acetone. This extract containing the lignan aglycones was separated by preparative TLC on silica gel using CHCl₃/MeOH (25:1). Lignans were isolated, purified and crystallized from EtOH, according to a published method (4). The identities of the isolated compounds were confirmed by spectroscopic methods (¹H NMR and MS) and supported by comparison with the literature data (4). The lignans podophyllotoxin and 4'-demethylpodophyllotoxin obtained were further used as a marker compounds in the phytochemical analysis of whole plants and tissue culture materials of species within the Berberidaceae.

References
Lupane triterpenoids are pentacyclic compounds with 30 carbon atoms, and a vast class of natural products whose structural diversity includes a wide array of functional groups\(^1\). Compounds of this class are reported to be bioactive with cytotoxic\(^2\), antitumor-promoting\(^3\), antiviral\(^4\), and anti-inflammatory\(^5\) activities. The biotransformation of lupeol was investigated by using submerged shaken liquid cultures of *Glomerella cingulata* and *Aspergillus ochraceus*. The fungi were cultivated in a pre-fermentative medium\(^6\) at 30°C and 120 rpm for 24 and 48 hours, respectively. The resulting mycelia were harvested and transferred to different fermentative media: Czapek and Koch’s K1\(^7\). Lupeol was added as solution in dimethylsulfoxide and the cultures were incubated at 30°C and 120 rpm for 10 days. Samples of each culture were taken in each 24 hours period, extracted with ethyl acetate, and analyzed by GC/MS. Experiments were also run with control flasks, which contained sterile culture broths that were not inoculated and to which the substrate was added. The control flasks were incubated under the same conditions. GC/MS analyzes revealed two different peaks (Retention times of 46.583 and 48.817 minutes) in the chromatograms of the extracts from *Aspergillus ochraceus* cultures developed in the Koch’s K1 medium for more than 7 days. The mass spectra of these peaks displayed a series of ions similar to those observed for lupeol that indicated that the main portion of the molecule was not modified. However, both the mass spectra showed molecular ions at \(m/z\) 384, suggesting that the difference between the compounds should be small. Considering the difference between the molecular ion of lupeol at \(m/z\) 426 and the ones of other compounds at \(m/z\) 384, we suggest that successive oxidations in the isopropenyl side chain of lupeol, followed by decarboxylations, resulted at least in one of these compounds. Further investigations will be carried out in order to identify the products of biotransformation of lupeol by *Aspergillus ochraceus*.

Refs.
5. T. Geetha *et al*., *J. Ethnopharmacol.*, **76**, 77 (2001)
GLYCOSYLATED PHENYLPROPANOID FROM STACHYTARPHETA CAYENNENSIS IMPAIRS HYDROGEN PEROXIDE FORMATION FROM MICE BRAIN MITOCHONDRIA.

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S. cayennensis, Verbenaceae, is commonly known in Brazil as “Gervão-roxo”. The roots and leaves of this plant are used in Brazilian folk medicine for rheumatism, back pain and as diuretics, analgesics and tranquilizers. Plants of this family Verbenaceae are known to be rich in both phenylpropanoids and iridoids (1). This study describe the antioxidant activity of one glycosylated phenylpropanoid, called verbascoside, against endogenous reactive oxygen species (ROS) produced in mice brain mitochondria during respiration. The tests were performed using brain homogenates as mitochondria source, and demonstrate that the antioxidant activity of the verbascoside display a dose-dependent increase, without altering mitochondrial bioenergetics parameters like oxygen consumption and mitochondrial membrane potential (ΔΨₘ). The mitochondrial electron transport chain is widely viewed as the main site of ROS generation in the cell. The partial reduction of molecular oxygen, during oxidative phosphorylation, leads to a constant flux of superoxide and hydrogen peroxide radicals production in cells mitochondria, which may exceed cellular antioxidant defenses (2). Increasing evidence in the literature indicates that the imbalance between ROS production and detoxification is associated with several pathological conditions, such as diabetes, sepsis and neurodegenerative disorders (3-6). Thus, under a pro-oxidant mitochondrial respiration, it is expected that the oxidative damage and cell death in nervous system could be prevented by antioxidants.

Refs.
SYNTHESIS AND SAR STUDIES OF ANTIFUNGAL ANALOOGUES OF GALEGINE

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Galega officinalis (popularly named Goat’s Rue, French Lilac, Italian Fitch or Professor-weed) has been known since the Middle Ages for relieving the symptoms of diabetes mellitus.¹ Upon analysis, it turned out to contain galegine (1), a alkaloid that display several pharmacological activities such as hypoglycemic, antimicrobial, weight reduction, and inhibition of platelet aggregation.²⁻⁵ Galegine, a guanidine compound, provide the template for the synthesis of metformin and opened up interest in the synthesis of other biguanide-type antidiabetic drugs.⁶ Using galegine as lead compound for antifungal analogue design, a series of 40 compounds (prenylguanidines, prenylthioureas, prenylureas and prenylpseudothioureas) have been synthesized and evaluated for in vitro antifungal activity against a panel of the fungi: Candida albicans, C. tropicalis, Saccharomyces cerevisiae, Cryptococcus neoformans, Aspergillus fumigatus, A. niger, A. flavus, Microsporum gypseum and Tricophyton rubrum. In conducting the structure-activity relationship (SAR), we chose to concentrate on two region of the molecule: the guanidine core and the prenyl group. In general, geranylguanidines showed more effective than isoprenyl and farnesylguanidines. The replacement bioisosteric of the guanidine core by thiourea, pseudo thiourea or urea nucleus decreased fungitoxic activity. Furthermore, the addition of the second prenyl unit on the guanidine displayed an enhancement significant of the activity. The analogs are shown to be potent inhibitors of the in vitro growth of the fungi (MIC below micromolar level) and promising as hits for further optimization.

\[
\begin{align*}
\text{Addition of the second prenyl unit (R}_1^1\text{)} & \quad \text{Replacement bioisosteric (X= O, S and SMe)} \\
\text{Elongation of prenyl unit (R}_2^2\text{= geranyl and farnesyl)} 
\end{align*}
\]

2. A.T. Atasanov et al., Folia Medica (Plovdiv), 41, 46 (1999)
4. G. Reuter, Archiv der Pharmazie, 8, 516 (1963)
In several cases plants secondary metabolites act as a barrier against herbivores due to their toxicity. On the other hand, several detoxifying mechanisms have been used by insects in order to overcome toxic compounds and to use them as part of their own defense against predators. The biotransformation to more polar or easily extractable compound has been suggested to be one important mechanism developed by insect to cope with the toxicity. Recent studies have demonstrated the interaction between phytophagous insect and Piper species (Dyer 2004; Ramos, 2006). The sequestration of prenylated benzoic acids from \textit{P. gaudichaudianum} by \textit{Naupactus bipes} was shown to occur at early stage of development of larvae which is maintained until adult phase. Additionally, the demethylation of tetrahydrofuran lignans and oxidative cleavage of dihydrobenzofuran neolignans such as conocarpan from \textit{P. solmsianum} and \textit{P. regnelli} by caterpillars of \textit{Heraclydes brasiensis} and \textit{H. hectorides} have previously been described (Ramos, 2006).

Herein we describe the interaction between caterpillars of the Lepidopterae \textit{Quadrus u-lucida} feeding on the \textit{Piper solmsianum} cultivated in our garden. This species contain the neolignans eupomatenoid-5 and 6 (1a and 1b), as described previously (Freixa et al., 2001).

The chromatographic analysis by HPLC of the extracts from feces of \textit{Quadrus} caterpillar indicated a complete depletion of the neolignans during the digestion. The NMR data of feces extract displayed singlets at $\delta$ 8.30 and 8.05, assigned to aldehyde hydrogens which were associated to the deshielded hydrogens at $\delta$ 7.68 and 7.5 of the conjugated H-6’ indicated the presence of aldehydes which are compatible with the structures 2a and 2b. The role of such biotransformation reaction during the digestive process of \textit{Q. u-lucida} requires further investigations.

STRUCTURE AND SYNTHESIS OF CYCLIC PEPTIDES ISOLATED FROM JATROPHA GOSSYPIFOLIA L. AND J. CURCAS L.


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Latices of Jatropha species (Euphorbiaceae) have been shown to be a rich source of bioactive cyclic peptides, and the novel jatrophidin I (1), the known cyclogossine A (2) and cyclogossine B (3), isolated from J. curcas and J. gossypifolia respectively, were synthesized using Fmoc strategy, aiming to confirm the natural peptide structure, and to have more amount of these peptides for further pharmacological evaluation. The linear precursors of peptides 1-3 were prepared using glycine in C-terminal position to prevent racemization during the cyclization process. Each precursor was synthesized using solid-phase technique based on the 9-fluorenylmethoxycarbonyl (Fmoc) amino protection employing chlorotriyl resin (cleavable with dilute TFA). After complete chain assembly, the linear peptide was cleaved from the solid support followed by precipitation of the crude material in diethyl ether. The cyclization was accomplished in DMF under high dilution conditions (1mM) with 1.5 equivalents of hexafluorophosphate (HBTU) and 10 equivalents of DIEA. The final cyclic peptide was extracted with AcOEt, purified in HPLC and analyzed by mass spectrometry and NMR experiments, comparing spectral data with the natural peptides. Additionally, computational MD calculations and analysis were performed using the GROMACS programs package version 3.3.1 and the OPLS/AA force field to establish the lowest energy conformations of the new peptide 1 (Fig. 1). (FAPESP, CNPq, CAPES)

Figure 1: Superimposition of the 10 conformations of cyclic peptide 1, in two different orientations with fewest violations in H—H restraints as obtained from SA/MD.
PYRROLIZIDINE ALKALOID PROFILES IN CROTALARIA SPECIES FROM BRAZIL: CHEMOTAXONOMIC SIGNIFICANCE

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The genus \textit{Crotalaria} L. (Leguminosae, Papilionoideae, Crotalarieae) includes about 600 species distributed throughout the tropics and subtropics. The centres of diversity are eastern and southern tropical Africa and India, with two additional centres found in Mexico and Brazil. The genus was subdivided into eight sections, based on floral morphology of African species. This study reports the occurrence of pyrrolizidine alkaloids (PAs) in the genus \textit{Crotalaria} occurring in Brazil. Thirty-eight PAs were detected via GC-MS in seeds of 27 species and one subspecies of \textit{Crotalaria} from Brazil, belonging to the sections \textit{Calycinae}, \textit{Crotalaria}, \textit{Chrysocalycinae} and \textit{Hedriocarpae}. PAs were reported for the first time in twelve native Brazilian species: \textit{C. flavicoma}, \textit{C. grandiflora}, \textit{C. hilariana}, \textit{C. martiana} subsp. \textit{mohlenbrockii}, \textit{C. martiana} subsp. \textit{martiana}, \textit{C. otoptera}, \textit{C. tweediana}, \textit{C. velutina}, \textit{C. vespertilio} (sect. \textit{Calycinae}), \textit{C. harleyi}, \textit{C. holosericea}, \textit{C. miottoae}, \textit{C. rupipila}, \textit{C. vitellina} (sect. \textit{Chrysocalycinae}). The sections \textit{Calycinae} and \textit{Crotalaria} were characterized by eleven-membered macrocyclic monocrotaline-type PAs (1). In the section \textit{Chrysocalycinae}, the single representative of subsection \textit{Incanae}, \textit{C. incana}, showed as main PA, integerrimine, a twelve-membered macrocyclic senecionine-type (2). The group close to subsection \textit{Stipulosae}, represented by \textit{C. micans} and \textit{C. maypurensis}, showed a distinctive PA pattern: \textit{C. micans} presented integerrimine, and \textit{C. maypurensis} had the unusual 7-hydroxy-1-methylene-8-pyrrolizidine as main PA (3). Into the species group close to subsection \textit{Glaucae}, the PAs with otonecine as necine base (secopyrrolizidine-like, 4) were the main PAs, except in \textit{C. rupipila} which showed an assamicadine-like PA, monocrotaline-type. Into the section \textit{Hedriocarpae} the twelve-membered macrocyclic senecionine-type were the main PAs, as well as in part of \textit{Chrysocalycinae} species. The section \textit{Grandiflorae}, not represented in the Neotropics, showed as characteristic alkaloids the madurensine-like compounds (5). As well as in the genus \textit{Senecio} (Compositae)\textsuperscript{1}, PAs provide for \textit{Crotalaria}, a good maker in the infrageneric level

![Reaction images](image1.png)

Ref.s.
The genus Uncaria (Rubiaceae) is represented in Central and South America for two species: U. tomentosa and U. guianensis, known as cat’s claw. They have been used medicinally by indigenous peoples for at least two thousand years for several diseases. The species U. guianensis showed experimentally anti-inflammatory and anti-oxidant activities and U. tomentosa is noted for its immunostimulant activity, as well as cytotoxic, anti-inflammatory and antioxidant action. These bioactivities have made the species valuable plant materials and led to them being commercialized in natura or as phytopharmaceutical derivatives. Both species contain, in different proportions oxindole and indole alkaloids, triterpenoid glycosides, sterols and flavonoids that isolated or synergistically contribute for their therapeutic properties. They have some morphological differences and U. tomentosa can be further identified for their oxindole alkaloid profile and content. However, U. guianensis has a very low concentration of those alkaloids and does not have so far an efficient chemical marker. Until now only the polyphenolic compounds found in aqueous extracts of U. tomentosa have been reported. In a previous work we described the isolation for the first time in those species, from the bioactive EtOH extract of the leaves of U. guianensis, of the also bioactive kaempferol-3,7-O-(α)-dirhaminoside (kaempferitrin). Now we described the screening for kaempferitrin in the EtOH extracts from leaves and stems of U. tomentosa (Acre) and U. guianensis (Amazonas and Mato Grosso) through TLC and HPLC-DAD-MS by comparison to kaempferitrin previously isolated. The leaves and stems of both species were dried, milled and exhaustedly and sonically extracted with EtOH. The dried extracts were partitioned with hexane and MeOH/H2O 9:1 and the fractions were submitted to silica gel TLC with mobile phase systems for polyphenolic compounds and UV and NP/PEG reagent to visualize the spots. The MeOH/H2O fractions that contained polyphenolic compounds were then submitted to filtration on Sephadex LH-20 CC and the MeOH sub-fractions were analyzed by HPLC-DAD-MS in C18 reverse phase system and gradient mode using MeCN/H2O/HCOOH, pH=3. The results showed the presence of kaempferitrin only in U. guianensis with concentrations in the leaves almost five times greater than those observed in the stems (m/z 287 area). Besides, U. tomentosa had a polyphenolic profile with an overall content around four times lower than U. guianensis (TIC area). The results show the selectivity of U. guianensis to produce this bioactive flavonoid glycoside and suggest this compound as a potential chemical marker for the species.

3M.V. Carvalho, C. Penido, A.C. Siani et al., Inflammopharmacology 14, 48 (2006).
4M.B.S. Almeida, L.M.M. Valente, P.J.C. Benevides et al., X Encontro Regional de Química da Sociedade Brasileira de Química, 2005.
EFFECT OF ESSENTIAL OIL OF *Melampodium divaricatum* (Rich. In Pers.) IN THE PRODUCTION OF HYDROGEN PEROXIDE AND NITRIC OXIDE IN MURINE MACROPHAGES

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Introduction: *Melampodium divaricatum* is a member of the family Asteraceae known as false-calendula in Brazil where is used in a folk medicine, as anti-inflammatory, diuretic and against leucorrhea \(^{(1)}\). In order to adapt to environmental insults, plants produce many natural products that have antimicrobial and immunomodulating potential \(^{(2)}\). Macrophages release more than one hundred compounds into the extracellular environment. Among these, there are intermediate oxygen compounds, such as NO and H\(_2\)O\(_2\). Objective: The purpose of this work was to study the effects of the essential oil of *M. divaricatum* in peritoneal macrophage cells from Swiss mice by determination of production the NO and H\(_2\)O\(_2\). Material and Methods: The oil was obtained from the powdered leaves from *Melampodium divaricatum*, using Clevenger-type and the exudate peritoneal macrophages (PEC) were harvested from Swiss mice (6-8 weeks old, 18-25 g). For the determination of the cell viability, PEC (5x10\(^6\) cells/mL) was re-suspended in medium RPMI-1640 C. The suspension (100 µL) and the samples in different concentrations (100 µL) were added to each well of a 96-well tissue culture plate and incubated for 24 h. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was performed and absorbance measured at 540 nm with a 620 nm reference filter. The nitric oxide production was determined by measuring nitrite. 50 µL of cell supernatant was removed from each well and incubated with an equal volume of Griess reagent at room temperature for 10 min; the absorbance was measured at 540 nm. For H\(_2\)O\(_2\) measurement, PECs (2x10\(^6\) cells/ml) were re-suspended in a solution of phenol red complete buffer; 100 µL of this suspension was added to each wells of a tissue culture plate and added 50 µL of aqueous extract in the concentration to viability of 50%. After incubation for 1 h, the reaction was stopped with 50 µL of NaOH 5M and absorbance measured at 620 nm. Results: The data indicated that essential oil of *M. divaricatum* has a high cytotoxicity in vitro on peritoneal macrophages (IC\(_{50}\) = 0,075 µL/mL e IC\(_{25}\) = 0,040 µL/mL). In this study, the essential oil could strongly inhibit NO production and H\(_2\)O\(_2\) production in macrophages stimulated by LPS and PMA respectively. The inhibition percentage ranged from 80,33 ± 4,76% (NO) and 76,22 ± 8,87% (H\(_2\)O\(_2\)) in the concentration 0,075 µL/mL and 40,58 ± 6,16% (NO) and 70,59 ± 1,19% (H\(_2\)O\(_2\)) in the concentration 0,040 µL/mL. According to this work, it is possible to suggest that essential oil obtained from *M. divaricatum* presents anti-inflammatory activity.


FINANCIAL SUPPORT: CNPq
Burseraceae family has a great importance in Brazilian folk medicine. Its oleoresin, popularly known as “Breu”, has several ethnopharmacological indications (1). Species from this family are usually hard to identify (2) and only few botanical specialists are able to identify them, but only through observation of its flowers, resulting in several taxonomic doubts. The genus Protium is the most common specie in Brazil, with chemistry and pharmacology recently reviewed (3). The present study proposes that an extensive chromatographic profile of oleoresin extracts from this family could be used to correlate chemical and taxonomic data, in a chemosystematic research. About 37 oleoresins, from Protieae (Crepidospermum, Protium and Tetragastris genus) and Canarieae Tribes (Dacryodes and Trattinnickia genus) were collected at South, Southeast, Northeast and North regions of Brazil. The majority of the oleoresins were obtained in the Amazon Region, where endemism and biodiversity of this family are the greatest in the New World. Thin layer chromatography (TLC), gas chromatography (GC-FID) and gas chromatography coupled with mass spectrometry (GC-MS) were applied to the apolar (hexane) and medium polarity (ethyl acetate) extracts. The yield from each oleoresin from the several extracts shows a clear division between some genus, and inside Protium genus. The comparative TLC showed several profiles that fit on the Burseraceae species phylogenetic distribution (4,5). Crepidospermum genus showed the greatest variation, with unusual low concentrations of the very common triterpenes α- and β-amirin, easily obtained from the Burseraceae breu. Using GC analyses we observed that the triterpene retention time region could be a fingerprint region, together to the monoterpene/sesquiterpene/triterpene yield relationship. Again, Crepidospermum showed a quite different profile, with some diterpenes that are not common in Neotropic Burseraceae species.

Refs.
INFLUENCE OF THE CHIRALITY OF (R)-(-)- AND (S)-(+) -CARVONE IN THE CENTRAL NERVOUS SYSTEM: A COMPARATIVE STUDY

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Many terpenes are used therapeutically, and as flavor and fragrance materials. (R)-(-)-Carvone, the main constituent of spearmint oil, and (S)-(+) -carvone, found as major component of caraway and dill seed oils, have several applications and are used in cosmetic, food, and pharmaceutical preparations. In this study, the effect of enantiomers of carvone on the central nervous system (CNS) was evaluated in mice. The LD50 value was 484.2 mg/kg (358.9–653.2) for (S)-(+) -carvone, and 426.6 (389.0–478.6) mg/kg for (R)-(-)-carvone. Both enantiomers caused depressant effects, such as decrease in the response to the touch and ambulation, increase in sedation, palpebral ptosis, and antinociceptive effects. (S)-(+) - and (R)-(-)-carvone caused a significant decrease in ambulation. (R)-(-)-Carvone appeared to be more effective than its corresponding enantiomer at 0.5 and 2.0 h after administration. However, (S)-(+) -carvone was slightly more potent at 1 h. In potentiating pentobarbital sleeping time, (R)-(-)-carvone was more effective than (S)-(+) -carvone at 100 mg/kg, but was less potent at 200mg/kg compared to the (+)-enantiomer, indicating a sedative action. (S)-(+) -Carvone at the dose of 200 mg/kg increased significantly the latency of convulsions induced by PTZ and PIC, but (R)-(-)-carvone was not effective against these convulsions. These results suggest that (S)-(+) -carvone and (R)-(-)-carvone have depressant effect in the CNS. (S)-(+) -Carvone appears to have anticonvulsant-like activity.

Refs.
1. D.P. De Sousa et al., Chirality, 19, 264 (2007)
OIL PROPOLIS EXTRACT FOR PHARMACEUTICAL PURPOSES: AN ALTERNATIVE TO ETHANOLIC EXTRACT

Lilian Buriol¹, Daiane Finger¹, Eduardo Morgado Schmidt¹, Julio M. T. dos Santos¹, Marcos Roberto da Rosa¹, Sueli Péricio Quináia¹, Yohandra Reyes Torres¹, Alexandra Christine H. F. Sawaya², Marcos Nogueira Eberlin².

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Propolis is a natural resinous product used as hydroalcoholic extract in pharmaceutical, cosmetic and hygienic products. Propolis pharmacological properties such as antibiotic, antifungal, cytotoxic, antioxidant and anti-inflammatory have been related to the phenolic composition in flavonoids and phenolic acids¹. The amount of these compounds is indicated as a parameter for quality control of extracts and crude propolis². The presence of ethanol in the formulation provokes rejection by some consumers. That’s why there has been a growing number of patents dealing with new solvents for preparing propolis extracts³. In this work we aimed to prepare edible oil propolis extracts (OEP) and compare their composition with ethanolic propolis extracts (EEP) using Electrospray Ionization Mass Spectrometry (ESI-MS) and the spectrophotometric method for qualitative and quantitative analyses, respectively. By visual inspection of the ESI-MS spectra we have concluded that both extracts have similar chemical composition. Twelve compounds have been identified by comparison of their ESI-MS spectra with those of standards: p-coumaric acid, caffeic acid, 2,2-dimethyl-2H-1-benzopyran-6-propenoic acid, 3-prenyl-4-hydroxycinnamic acid, 3,4-dihydroxy-5-prenylcinnamic acid, kaempferide, 3,5-diprenyl-4-hydroxycinnamic acid, dihydrokaempferid, 4-hydroxy-3(E)-(4-hydroxy-3-methyl-2-butenyl)-5-prenyl cinnamic acid, betuletol, 3-prenyl-4-dihydrocinnamoyloxy-cinnamic acid and dicaffeoylquinic acid. Total phenolic substances and flavonoids were measured by spectrophotometric method. Compared with EEP, the OEP had lower yield but higher phenolic and almost similar flavonoids content, although it was necessary a longer extraction period. These results have encouraged us to create a new methodology of preparing oil propolis extracts for pharmaceutical purposes which is currently being patented.

Keywords: ethanolic and oil propolis extracts, phenolic acids, flavonoids, Electrospray Ionization Mass Spectrometry, spectrophotometric method.

Refs.
The genus *Piper* belongs to the Piperaceae and has over 1,000 species of herbs, shrubs, small trees and hanging vines distributed in both hemispheres. The *Piper* species have high commercial, economical and medicinal importance, widely distributed in the tropical and subtropical regions of the world where are used in folk medicine as, for example, stomachic and carminative in digestion, diuretic and toothache. Chemical studies have shown that the genus *Piper* has many components including unsaturated amides, flavonoids, lignans, terpenes, steroids, phenols and alkaloids. This work presents the chemical composition from essentials oil of two *Piper* species, *P. demeraranum* (Miq.) C. Dc. and *P. duckei* C. Dc. that were collected in the Reserve Ducke, Manaus (Amazonas state) and the voucher specimens were deposited at herbarium of INPA (AM). The essential oils from leaves of these species were analyzed by a combination of GC/FID and GC/Mass Spectrometry using a capillary GC column HP-5 (30 cm). The essential oils were obtained from dry leaves (40g) of both plants using a Clevenger type apparatus for 3h. The oils were separated from water, dried with Na$_2$SO$_4$ and stored in sealed vials at low temperature before analysis. The yields of the oils were 1.8% (*P. demeraranum*) and 1.2% (*P. duckei*). The main constituents found in the oil of *P. demeraranum* were β-elemene (34.50 %), limonene (20.56 %), germacrene B (9.11 %), β-pinene (7.07 %). In the oil of *P. duckei* the main compounds found were trans-caryophyllene (29.62 %), 1H-cycloprop[e]azulene (19.20 %), germacrene-D (15.92 %). Although being the first study about the essential oils of these *Piper* species, the most frequently identified compounds were non-oxygenated monoterpenes and sesquiterpenes, which are similar to other essentials oils of this analyzed genus. Financial support: Fapeam; CNPq/PADCT
ELECTROPHYSIOLOGICAL RESPONSES OF **THYRINTEINA ARNOBIA** MALE AND FEMALE MOTHS TO **PSIDIUM GUAJAVA** ESSENTIAL OIL

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The Brazilian eucalyptus brown looper, *Thyrinteina arnobia* (Lepidoptera: Geometridae), is the most harmful of the *Eucalyptus* pests in Brazil.\(^1\) It also feeds on native plants such as *Villaresia congonha*, *Ilex paraguaiensis*, *Campomanesia* spp., *Eugenia* spp. and *Psidium guajava*.\(^2\) In this way, an efficient methodology for this insect control in different cultures is necessary and becomes essential to understand the interactions mediated by semiochemicals.

In this work were evaluated the electroantennographic responses (EAG) of male and female moths of *T. arnobia* to essential oil of white guava plants (*P. guajava* L.). The experiments were carried out using young (sprouts) and old leaves, collected from two different plants, which were submitted to steam distillation, using a Clevenger apparatus. For EAG, antennae from virgin male and female created in artificial diet and later transferred to feeding with plant leaves had been used. Three different oil concentrations were evaluated. It was observed, for both male and female, a maximum antenna depolarization at concentration of 10 mg/mL of either young or old leave essential oils, with no significant difference. EAG responses in both sexes were significantly higher than that for the control (hexane). These results indicate that *T. arnobia* adults recognize some volatile compound in the *P. guajava* essential oils; that is, in the antennae of both sexes there are neuron receptors (chemoreceptors), present in sensillas, which detected compounds in the essential oil. In order to confirm these results further studies will be necessary, followed by semiochemicals identification by using GC-EAD and GC-MS techniques.


Acknowledgements: FAPESP, CNPq, CAPES and IFS.
LIPOSOMAL DELIVERY SYSTEM FOR ENCAPSULATION OF Zanthoxylum tingoassuiba ESSENTIAL OIL: PREPARATION, CHARACTERIZATION AND IN VITRO ANTIMICROBIAL ACTIVITY

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Essential oils are made up of many different volatile compounds. In general, essential oils have a high content of terpenes easily prone to oxidation and resinification. It seems that the antimicrobial effects are the result of many compounds acting synergistically. In addition, oils have low solubility in aqueous media, limiting their pharmaceutical applications. In order to circumvent those disadvantages, microencapsulation has been proposed as an alternative to improve stability, solubility and biological activity of essential oils (1). In recent years, liposomes have been extensively studied as a delivery system which can improve the activity and safety of many molecules. Further, liposomes are regarded as suitable carriers because they can serve as a depot system for the sustained release of an associated compound and enhance the molecules targeting to cells (2). Pursuing our interest in medicinal products obtained from Rutaceae plants, in this work we have studied the preparation and characterization of multilamellar liposomes entrapping essential oil (EO) from Zanthoxylum tingoassuiba. Essential oil from Z. tingoassuiba loaded into multilamellar liposomes was successfully produced through thin film hydration method with mean diameter of 9.4 µm. The liposome-incorporated EO showed good sphericity and narrower size distribution than empty liposomes. Results of GC-MS and UV-VIS spectrophotometer revealed that EO incorporation efficiency in liposomes was approximately 50%. A qualitative analysis by Thin Layer Chromatography revealed that essential oil was successful incorporated into liposomes with no exclusion of the essential oil components. Differential scanning calorimetry (DSC) was applied to further investigate interactions of essential oil with a liposome. Changes in the liposome DSC endotherms occurring in the presence of essential oil suggest that it is situated in lipid bilayer of liposomes. The antimicrobial activity of incorporated and free EO against some ATCC bacteria, mushroom and multiresistant clinical isolates of S. aureus was evaluated. Free EO inhibited the growth of all microorganisms tested. Results obtained clearly indicate that essential oil from Z.tingoassuiba has been successful incorporated into multilamellar liposomes. Liposomes can be useful in enhancing the solubility and antimicrobial activity.

Refs.
2. C. Sinico et al., European J. of Pharmaceutics and Biopharmaceutics, 59, 161 (2005)
Abstract

Hyptidendron canum is a specie belonging to the savannah biome of the Lamiaceae family, which in turn has great economic standing for being the source of aromatic essential, volatile oils and ornamental plants. The aim of this study is to analyse the chemical constituents of the essential oil from leaves and inflorescences of H. canum from population samples representative of four towns in the state of Goiás - Brazil, and compare the chemical variability among them. For this purpose the essential oils from the material collected were obtained through hydrodistillation in a Clevenger apparatus and analysed by GC/Ms. Sesquiterpene hydrocarbons constituted the main group of compounds in all the populations. Nonetheless a few differences were noted in the quantities of the main constituents, namely of (E)-cariophyllene and amorpha-4,7(11)-diene, with the greatest contents in Bela Vista, and of bicyclogermacrene with high percentages in the Silvânia population, sabinene and β-copaen-4-α-ol with a large amount in the Silvânia population, as well as δ-3-carene and carotol with greater contents in the Hidrolândia population, suggestive of chemical polymorphism. Results obtained from Principal Component Analysis (PCA) and cluster analysis indicate great variability in the chemical composition of essential oils in the populations.
Maytenus obtusifolia Mart. is a small tree found in the South America and Asia. In Brazil, where it is commonly known as “bom-nome” and “carne-de-anta”, its leaves are largely used in the folk medicine as anti-ulcer. Previous studies reported the presence of pentacyclic triterpenes from the roots extracts and flavonoids in the leaves extracts of Maytenus species. This work describes the antimicrobial activity of the essential oil of Maytenus obtusifolia. The plant was collected in May 2002, near the city of Santa Rita, State of Paraíba, Brazil, a coastal area around the Atlantic Forest. The voucher samples (Agra et Góis 3230) were deposited in the Herbarium Prof. Lauro Pires Xavier (JPB) and in the reference collection of the Laboratório de Tecnologia Farmacêutica from Universidade Federal da Paraíba, Brazil. Fresh leaves of Maytenus obtusifolia (1000 g) were cut into small pieces, and subjected to steam distillation in a Clevenger-type apparatus. For the bioassays 5 bacteria and 8 fungi were used. The essential oil was tested “in natura” (100%) and in dilutions from 32 until 2%, according to Allegrini et al. (1973). The present work was conducted in order to evaluate the antimicrobial activity using the agar well diffusion assay. The biological activity of the oil was considered positive when the media of the inhibition zone were equal or superior to 10 mm in diameter. The initial evaluation (in natura) showed inhibition growth against: S. epidermidis (ATCC 12228), S. aureus (ATCC 6538), E. coli (ATCC 18739), P. aeruginosa (ATCC 27853), L. monocytogenes (ATCC 9610), C. albicans (ATCC 90028), C. albicans (ATCC 13803), C. tropicalis (LM 37), A. flavus (LM 247) and C. guilliermondii (LM V70) affording inhibitions zones between 10-45 mm diameters. In the dilution of 32% presented inhibition growth against: S. aureus (ATCC 6538), E. coli (ATCC 18739), L. monocytogenes (ATCC 9610), C. albicans (ATCC 13803), C. tropicalis (LM 37), C. guilliermondii (LM V70), and A. flavus (LM 247). In the dilution of 16% against: S. aureus (ATCC 6538), E. coli (ATCC 18739), L. monocytogenes (ATCC 9610), C. albicans (ATCC 13803) and C. tropicalis (LM 37). The dilution of 8% presented activity against Staphylococcus and Candida. In summary, the study of volatile constituents showed that the oil inhibited the growth of ten of the microorganisms, when tested with the oil at the concentration of 100%. These results led to the conclusion that the essential oil tested has a weak activity against the tested microorganisms.

Refs.
2. J. Allegrini et al., Société de Pharmacie de Montpellier, 33, 73-86 (1973)
THE SMELL OF Melipona quadrifasciata INFLATED VIRGIN QUEENS
(HYMENOPTERA: APIDAE, MELIPONINI)

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Melipona quadrifasciata is a native social stingless bee belonging to the tribe Meliponini, which occurs in tropical regions.\(^1, 2\) Mating in Meliponini generally occurs during the flight, and can be observed under laboratorial controlled conditions.\(^3\) Nevertheless, several questions concerning to virgin queens’ attractiveness to males remain to be answered. Attractive virgin queens inflate their abdomens in the colonies calling a court of workers, that allow them to survive for a while in their nests, testing their attractiveness to supersede the physogastric queen or to depart with a swarm.\(^3\) Inside their nests, they do not attract males, what occurs during their nuptial flights or in experimental arenas, with solitary gynes.\(^3\) Melipona quadrifasciata virgin queens liberate a characteristic smell after inflating their abdomens, and this smell is perceived by humans. Therefore, the aim of this work was to characterize the compounds responsible for this smell. Inflated virgin queens (2 to 3 days old) and normal virgin queens (1 day old) were submitted to dynamic headspace using porapack Q resin to trap the volatiles. The substances were eluted from the polymer with a hexane/ethyl acetate mixture and analyzed by gas chromatography-mass spectrometry. Inflated virgin queens produced a mixture of nerol and geraniol (2:3), while these substances were not observed in normal virgin queens. Biological assays aiming to discover whether these substances play any role on male attraction are currently being carried out. It was not found in literature any other report concerning the characteristic smell of inflated virgin queens.

\[\text{Geraniol} \quad \text{Nerol} \]

Refs.
CASTE SPECIFIC ALKALOIDS OF *Solenopsis saevissima* (HYMENOPTERA: FORMICIDAE)

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The ants belonging to the genus *Solenopsis* are referred to as *fire ants* because of the potency of their venoms, which exhibit pronounced necrotic, hemolytic, antibiotic, and toxic properties.\(^1\) The venoms of *Solenopsis* species are characterized by a predominance of a complex mixture of 2-methyl-6-alkylpiperidines with a low concentration of proteinaceous constituents.\(^2\)\(^,\)\(^3\) These piperidine alkaloids have been assigned the trivial name solenopsins by MacConnell et al. 1971 and differ from each other by the relative configuration of their substituents, and the length and unsaturation position of the alkyl chain.\(^3\)\(^,\)\(^4\)\(^,\)\(^5\) The relative proportions of these in the venom may differ between castes within a species and individuals of a particular caste.\(^3\) *Solenopsis invicta* is the most studied species, however, it was originally described as *Solenopsis saevissima*, however nowadays *Solenopsis saevissima* is the name of a new particular species. To date, in the literature there is no comparison between the alkaloids produced by the different castes of *Solenopsis saevissima*, therefore this work aims at analyzing the alkaloids some workers and queens of a colony of *Solenopsis saevissima* collected in the state of Rio de Janeiro, Brazil. Gas chromatography-mass spectrometry analyses showed that the queens of *Solenopsis saevissima* mainly produced cis-2-methyl-6-undecylpiperidine, while the workers mainly produced trans-2-methyl-6-undecylpiperidine. It clearly indicates that alkaloid production in this species is caste-specific, as was also observed in *Solenopsis maboya* and *Solenopsis torresi*,\(^6\) confirming that the relative configuration of these alkaloids might play an important role in caste discrimination in this genus. The absolute configuration of these alkaloids will be determined shortly.

\[ \text{2-methyl-6-undecylpiperidine} \]

Refs.
Activity of the essential oil from *Coriandrum sativum* L. against different oral *Candida* spp

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Different *Candida* spp. are commonly found in the oral microbiota and can act as opportunist producing superficial clinical manifestations, since candidoses until serious and invasive system infections. *Coriandrum sativum* L. (Umbelliferae/Apiceae) known as “coentro” is a popular condiment in the Brazilian cooking and also is used as digestive, carminative, diuretic and tonic. The objective of this work was to evaluate the activity of the essential oil of *C. sativum* L. against different oral *Candida* spp: *Candida albicans* CBS 562, *C. krusei* CBS 573, *C. parapsilosis* CBS 604, *C. dubliniensis* CBS 7987 and *C. tropicalis* CBS 94. The major oil constituents were also identified. The essential oil was obtained from leaves by hydrodistillation in Clevenger system. Subsequently, the oil was separated from the water phase by liquid/liquid extraction with dichloromethane and diethyl ether, and fractionated in dry column to supply six fractions for different polarities. The oil and fractions composition were determined by GC and GC–MS analyses. The minimal inhibitory concentration (MIC) from oil and fractions was determined by microdilution method (NCCLS, 2002), until concentration of 1.0 mg/mL. MIC results of the crude oil and most active fractions are shown in Table 1. The results shown that fractions 4 and 6 presented the best activities against *C. albicans* CBS 562, *C. dubliniensis* CBS 7987, and *C. parapsilosis* CBS 604, with MIC values between 0.007 and 0.031 mg/mL. These values are similar or lower from that commonly observed for conventional antibiotics.

**Table 1:** Anti-*Candida* activity of the essential oil and fractions from *Coriandrum sativum*.

<table>
<thead>
<tr>
<th><em>Candida</em> spp.</th>
<th>Crude essential oil</th>
<th>Fraction 4</th>
<th>Fraction 6</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em> CBS 562</td>
<td>0.500</td>
<td>0.031</td>
<td>0.031</td>
</tr>
<tr>
<td><em>C. krusei</em> CBS 573</td>
<td>0.250</td>
<td>0.063</td>
<td>0.063</td>
</tr>
<tr>
<td><em>C. parapsilosis</em> CBS 604</td>
<td>0.125</td>
<td>0.007</td>
<td>0.007</td>
</tr>
<tr>
<td><em>C. dubliniensis</em> CBS 7987</td>
<td>0.250</td>
<td>0.007</td>
<td>0.015</td>
</tr>
<tr>
<td><em>C. tropicalis</em> CBS 94</td>
<td>*</td>
<td>0.063</td>
<td>0.063</td>
</tr>
</tbody>
</table>

* MIC > 1.0 mg/mL;

The major compounds identified in the crude essential oil were alcohols and aldehydes (80.76%): n-decanal (24.17%), 2-decenol (18.05%), 2-dodecenal (17.55%), 3-hexenol (Z) (10.34%), decanal (4.76%), dodecanal (3.02%) and 2-dodecenal (2.88%). The fraction 4 presented dodecanal (39.88%), 2-decenol (30.12%), n-decanol (11.41%), and 2-tetradecenol (E) (8.38%), while the constituents of the fraction 6 were: n-decanol (54.78%), 3-hexenol (Z) (16.29%) and 2-hexenol (E) (6.22%) and 2-undecenol (Z) (6.15%). The analysis revealed that fractions 4 and 6 are more enriched in alcohols than in aldehydes. The present study revealed a strong activity from *C. sativum* essential oil and fractions against oral *Candida* spp., and its potential use in the treatment of the oral candidoses.
Analysis of volatile components of aerial parts from *Mikania pilosa* Baker (Asteraceae) by HS-SPME and GC-MS.

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The genus *Mikania* (family Asteraceae, tribe Eupatorie, subtribe Mikaniinae) contains more than 450 species that grow in tropical regions of Africa, Asia and America, with 150 species occurring in Brazil [1]. Several *Mikania* species, particularly those known under the common name “guaco”, are used in Central and South American folk medicine as antiinflammatory agents, to treat respiratory tract diseases, rheumatism, influenza and against snake bite [2].

*Mikania pilosa* Baker is a Brazilian species, which is found mainly in the states of MT, MG, SP, RJ, SC and PR [3]. However, still there is no previous report of the chemical composition of *Mikania pilosa* Baker. The aim of this study was to evaluate the volatile components of the aerial parts from *Mikania pilosa* Baker by the technologies HS-SPME and GC-MS. The plant *Mikania pilosa* Baker was collected in Costa Rica, MS, Brazil, in October 2004 and were identified by Dr. Roberto Lourenço Esteves (Departamento de Biologia Animal e Vegetal, Universidade Estadual do Rio de Janeiro, Brazil). The sample (400 mg) was put into headspace vial (4 mL) coupled to the PDMS fiber and was heated to 60 ºC, with extraction time of 30 min. GC-MS analysis were performed with a Shimadzu GC-2010 Chromatograph equipped with a DB-5 column (30 m x 0.25 mm, coating thickness 0.25 µm) and a GCMS-QP2010 quadrupole mass detector. Analytical conditions: injector 250 ºC; oven temperature programmed from 60 ºC to 240 ºC at 3 ºC/min; carrier gas helium at 1.33 mL/min; injection splitless mode; fiber desorption time 5 minutes. The identification of the constituents was based on comparison of the retention indices (RI) with literature data [4] and library mass spectra. Forty-eight components were identified of which spathulenol (31.4 %), δ-cadinene (11.2 %), β-selinene (6.0 %) and ledol (4.2 %) were major.

Acknowledgements: CNPq, FAPESP and CAPES for financial support, Prof. Dr. Roberto Lourenço Esteves for plant identification.

spathulenol  δ-cadinene  β-selinene  ledol

Refs.
ASSESSMENT OF ESSENTIAL OIL YIELDS FROM Cymbopogon citratus (DC) STAPF, AND ANTIMICROBIAL ACTIVITY RELATED TO SEASON AND CULTIVATION WITH Achillea millefolium

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The essential oil of the plant Cymbopogon citratus (DC) Stapf cultivated in the Medicinal Garden of Paranaense University in the city of Umuarama, Paraná, was obtained by steam distillation. Yield and seasonal variation were determined, and correlated with the ambient temperature and precipitation. Plants harvested in the morning of days with high temperatures and rainfall, gave better yields from plants cultivated alone and also together with Achillea millefolium L. Microbiological analysis of the essential oil of C. citratus was carried out by the broth microdilution method against the yeasts Candida albicans and C. tropicalis, and the bacteria Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The essential oil from plants cultivated alone and together with A. millefolium showed moderate activity against all yeasts tested (0.63 - 1.25 mg/mL⁻¹). The essential oil from plants cultivated alone showed high activity against Staphylococcus aureus (0.08 mg/mL⁻¹), but no activity against the other bacteria.
CHARACTERIZATION AND ADULTERATION OF ROSEWOOD OIL BY SYNTHETIC LINALOOL USING ELECTROSPRAY IONIZATION MASS SPECTROMETRY

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Rosewood oil (obtained from *Aniba rosaeodora*) is one of the most important commercial essential oils produced in the Amazon region. A common practice is to adulterate fine rosewood oil (US$80/Kg) with synthetic linalool (US$8/Kg). Earlier studies have applied alternative techniques to characterize essential oils\(^1\). In this work, a systematic analysis with different blends of rosewood and synthetic linalool was used to establish a simple and fast methodology to trace contaminants, based on direct infusion of the samples in the electrospray ionization source of the mass spectrometer (Electrospray Ionization Mass Spectrometry ESI-MS). Five individual samples of each essential oil (wood and leaf) and synthetic linalool were analyzed. The polar compounds were extracted from each sample with methanol/water 1:1 (0.1% strong acid) solution and analyzed in positive ion mode. For the adulteration detection, leaf and wood oils were mixed with synthetic linalool. The three pure samples presented similar profiles in positive ion mode, \(m/z\) 137, 274 and 292, although in distinct proportions. The three samples could be differentiated from one another, by the presence, absence or different proportions of marker ions. For instance, the intensity ratio of \(m/z\) 137 and 153 ions in crude wood oil was 1.2:1.0, in crude leaf oil it was 3.1:1.0 and for synthetic linalool the \(m/z\) 153 ion was not detected. The intensity ratio of \(m/z\) 203 and 221 ions for crude wood oil was 1.0:2.0, for crude leaf oil it was 2.3:1.0 and in the analysis of synthetic linalool these ions were not detected. Furthermore, there were some characteristic ions found in each of the three samples. In addition to this, when synthetic linalool was mixed with crude wood oil, a change in ion intensity ratio was observed.

This analytical technique proved to be very versatile and fast, and can often be applied with simple sample preparation to immediately establish chemical characterization of polar ionizable compounds.

Refs.

CARACTERIZATION OF FATTY ACIDS OF BRASILIAN OIL PALM (BUTIA CAPITATA, BUTIA YATAY AND SYAGRUS ROMANZOFFIANA) FRUITS

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Brazil, a tropical country, possesses an enormous diversity of palm fruit, most of which are excellent sources of oil. However, the potential of the Butia capitata (Mart.) (Becc.), Butia yatay (Mart.) (Becc.) and Syagrus romanzoffiana (Cham.) Glassman (Arecaceae) fruits and kernels are still little exploited. These species are native in Brazil, Uruguay and Argentina and their fruits are popularly used in beverages or as food. In this way, the proposal of this work was to analyze the fatty acids composition in kernel of the fruits in the species described; to check possible alterations in the composition of the oil from B. capitata in three different year collections (2005, 2006, 2007) and to compare three different derivatization techniques. Transesterification using three different catalysis was employed to obtainment of fatty acid methyl ester (FAME). The fruits were collected from different cities from Rio Grande do Sul (Brazil): B. yatay in São Francisco de Assis; S. romanzoffiana in Porto Alegre and B. capitata in cultures of Embrapa in Pelotas. The kernel oil from palm fruits was extracted with n-hexane and suffered transesterification with: NaOH/CH₃OH (1), HCl/CH₃OH (2) and BF₃/CH₃OH (3). Thereafter, the fatty acid compositions were determined qualitatively and quantitatively by GC-MS. Statistical analysis of the results was done using ANOVA and Tukey, with multiple range significance test (p < 0.05). The oil content ranged between 200-280 g/kg of kernel. In all the samples the levels of medium-chain saturated fatty acids (71.84- 91.01%) were higher than the levels unsaturated FAME. The qualitative composition for the FAME was quite similar for all samples analyzed; however, quantitative differences were observed among the techniques employed. Thirteen compounds were identified and the predominant fatty acids found were dodecanoic acid, octadecanoic acid, decanoic acid and 9-octadecenoic acid, ranging 25.27-48.29%; 9.21-19.55%; 8.47-20.55% and 7.4-26.6%, respectively, of the content oil, taking in account all the catalysis agents. Transesterification with BF₃/CH₃OH and NaOH/CH₃OH provided a higher FAME unsaturated content while HCl/CH₃OH transesterification provided a higher medium-chain saturated FAME content. For all the samples the dodecanoic acid was the main compound in the three different derivatization methods. Except for 2005 collection of B. capitata, no statistical difference in the predominant compounds was observed between 2006 and 2007 samples; this result can be related to the age of the samples. Therefore, considering the total content oil and the characteristics of FAME composition oil, it is possible to suggest that the oils of Butia species and Syagrus romanzoffiana can be an alternative source for industrial exploitation as well as for medical usages and specific consumption.

1. ANVISA. Métodos físico-químicos para a análise de alimentos 4 ed (2005).
THE CHEMICAL ASSESSMENT OF *Genipa americana* L. (Rubiaceae) FRUITS ESSENTIAL OIL

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*Genipa americana* L. (Rubiaceae) is a widely distributed species in Brazil, from Amazônia to São Paulo and popularly known as “jenipapo”, “janipaba”, “jenipapeiro”, “jenipapinho” and “jenipá”. Its edible fruit is used in the folk medicine as antiasthmatic, aphrodisiac, antianemic, tonic, diuretic and useful in the spleen and liver affections and jaundice. When still unripped it supplies a bluish colored dye used by the food and cosmetic industry. Chemically, “jenipapo” is characterized by the presence of iridoids (like genipin, geniposidic acid and genamesides A-D). Some of them display anti-tumor, anti-angiogenic, anti-inflammatory and antioxidant activities. Previous works state the high amounts of carboxylic acids (butyric, 2-methylbutyric and hexanoic) found in the volatile fraction of “jenipapo” as the main responsible constituent for the characteristic fruit odor\(^{(1,2,3)}\). Although its relevant properties, research at Chemical Abstracts don’t provide much work on “jenipapo”. The postgraduate dissertation project, in which this work is inserted, is to investigate the chemical constituents and pharmacological activities of *G. americana*, aiming to the improvement of the family chemical systematic understanding and the investigation of its medicinal properties. The present work deals with the analysis of the volatile fraction of the collected fruits at different times. The fresh, riped and sliced fruits of *G. americana*, collected in 2006 and 2007 at Nova Iguaçu (Rio de Janeiro) were submitted, separately, to steam distillation using modified Clevenger apparatus. The yellow oil was analyzed by gas chromatography-mass spectrometry for comparison of possible chemical changes during storage of fruit under refrigeration. The components’ separation was performed in a capillary column (ZB-5ms, from 60 to 240°C with increment of 3°C/min). Hydrocarbons standards of C8-C26 series were injected. The substances’ identification was done on basis of their mass spectra fragmentation patterns, with the aid of the Kováts’ indices and by comparison to the literature data. Esters and alcohols were identified as main components, as well as octanoic acid as the major compound in the oil.

Refs.
ACARICIDE ACTIVITY OF LEAF ESSENTIAL OIL FROM *Schinus terebinthifolius* Raddi ON THE TWO-SPOTTED SPIDER MITE (*Tetranychus urticae*).

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*Schinus terebinthifolius*, commonly known as the Brazilian pepper tree, pertains to the family Anacardiaceae. It is a South American tree, native to Brazil, Paraguay and Argentina. In Brazil, it occurs naturally from the state of Ceará to Rio Grande do Sul. In folk medicine, the stem bark is used as a stimulant and astringent. A lotion made from the leaves and fruit is used for cleaning wounds and sores. Previous investigation of fruit essential oil collected in Rio Grande do Sul State, Brazil, showed antimicrobial activity and α-3-carene as a main component. One of the current alternatives in the control of arthropods is the search for insecticide properties in medicinal plants. This type of investigation is promising with regard to the discovery of new sources of natural insecticides. As part of a systematic study on the assessment of the acaricide potential of flora in the state of Pernambuco, the aim of the present study was investigate the chemical composition of the leaf essential oil in *S. terebinthifolius* and its fumigating action on the two-spotted spider mite (*Tetranychus urticae*), an important pest in tomato and bean cultivation that causes considerable harm to small agriculturalists in Pernambuco. The essential oil (EO) was obtained through hydrodistillation and submitted to GC/MS analysis. Identification of the components was performed through a comparison with a computerized databank as well as a comparison of the retention indices to those described in the literature. Retention indices were calculated by the co-injection of a homologous series of n-alkanes and the use of the Van Den Dool equation. The fumigating action was performed in glass receptacles (2.5 L) used as fumigation chambers (FC). Three disks (2.5cm) from jack bean leaves (*Canavalia ensiformis*) were placed on paper filter disks saturated with water in Petri dishes. Ten adult female mites were placed on each leaf disk. Each Petri dish, containing 30 mites, was then placed inside the FC. The EO was applied to strips of filter paper (5x2cm) attached to the lower surface of the FC lid. EO exposure was 24 hours. CL50 was calculated using the MicroProbit program. Three repetitions were performed. Thirty-three components were identified, representing 95.5% of the EO. The main components were p-cymen-7-ol (22.5%); 9-epi-(E)-cariophyllene (10.1%), carvone (7.5%) and verbenone (7.4%). The EO tested on the two-spotted spider mite proved toxic and the CL50 value was 6.84 µL/L of air. This result suggests that the essential oil of *S. terebinthifolius* has a fumigating potential. Further studies are needed before this essential oil can be used in the management of this pest. This was the first record of acaricide activity of the essential oil from the Brazilian pepper tree.

Refs.
EVALUATION OF VOLATILE COMPONENTS IN VEGETABLES OF THE BRASSICACEAE FAMILY

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A great number of research papers concerning the antioxidant and anticarcinogenic properties of species of the Brassicaceae family are related to the presence of glucosinolates. The vegetal tissue damaged by chew, cut or processing releases mirosinase, an enzyme found in specialized cells that hydrolize these compounds in a variety of products including isothiocyanates and indols, that can be identified in the volatile fraction of parts of the vegetable. In order to provide adequate information to the consumer to the benefits of functional foods, it is necessary to know the variation on the content of bioactive components. Moreover, some works have pointed out to the variability of glucosinolates due to post-harvest manipulation, processing, storage or preparation of species of Brassicaceae. The present work is inserted in the project that aims to evaluate the variability of volatile glucosinolates and its antioxidant properties in three species of Brassicaceae: Eruca sativa L. (“salad rocket”), Brassica rapa L. (“turnip”) and Raphanus sativus L. (“radish”), purchased directly from Rio de Janeiro’s food stores via gas chromatographic /mass spectrometry profiles (GC/MS). B. rapa, with and without peel, and E. sativa, fresh and cooled by 5 days, had been submitted, separately, to the hydrodistillation in modified Clevenger apparatus. The colourless volatile fractions were analysed by GC/MS in the following conditions: ZB-5ms column; temperature ranging from 60 to 240°C, with increment of 3°C/min; injector temperature: 260°C. Standards of hydrocarbons of the series from n-8 to n-26 were injected. The components of the volatile fraction were identified after interpretation of their mass spectra and comparison with literature data and Kovats´ index. Results pointed to the presence of nitriles and isothiocianates (2-methyl-5-hexenonitrile, phenyl-(ethyl-2-isothiocyanate), butyl-2-isothiocyanate and 4-(methylthio)-butanonitrile, among others) as major compounds in the four analysed fractions. The chromatographic pattern of samples (“turnip” with or without peel and fresh and cool “salad rocket”) displays a similar qualitative composition but also shows quantitative variations among the identified substances.

Refs.
STINGLESS BEES’ VOLATILE COMPOUNDS

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The social insects use different mechanisms of communication to organize the tasks and the internal and external functioning of the colonies. Bees, wasps, ants and termites have well organized and impressive colonies which fascinate chemists and biologists. This lifestyle depends on an efficient communication system to exchange information among the colony individuals. This social language can occur through visual, acoustic and chemical signals ¹. The insects, in general, are rather sensitive to floral fragrances. The males Euglossini and Meliponini bees, for example, are attracted to the orchid floral fragrances that resemble the odour emitted by the queen bees of the pollinators species, and actually collect the floral volatiles ², ³.

In this work the volatiles from cultivated stingless bees’ colonies (Meliponinae) trapped by dynamic headspace using solid phase extraction were analyzed. The analyzed Meliponinae colonies are cultivated in the Entomology Department of the College of Philosophy, Sciences and Letters of Ribeirão Preto - USP. The solvent extracts of the captured volatiles were analyzed by GC-MS. The constituents were identified by comparison between the calculated Retention Index, obtained through Van den Dool & Kratz equation, and the values from literature and their mass spectra.

Major constituents of the colony of Geotrigona sp. Moure volatile were the monoterpenes α-pinene (62%) and β-pinene (33%), which are responsible for its citric aroma. The colony of Frieseomelitta varia Lepeletier has a sweet aroma and its major constituents were the sesquiterpene germacrene D. (24%) and the monoterpene α-pinene (58%). These distinct volatile constituents for species living in the same habitat may reveal that bees from different species have different visiting and foraging preferences, leading to relatively specialized plant-pollinator interactions.

The constituents identified in the analyzed bees’ colonies are often found in essential oils, and have already been identified as kairomones (molecules exhaled by one species that attract or repel other species). These compounds may even have the function of protection, since it is known that some monoterpenes act, for example, as ant repellent.

Refs.
CHARACTERIZATION OF THE ESSENTIAL OIL AND BIOCIDAL ACTIVITY OF EXTRACTS FROM FIFTEEN MESOAMERICAN SPECIES OF PIPER

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In Guatemala it has been described about 88 species of the genus Piper, but the native ones (about 30) have never been studied chemically or pharmacologically. Fifteen species were collected from three regions; from Suchitepéquez 4 species (Piper jacquemontianum, P. oradendrum, P. patulum and P. umbellatum); from Alta Verapaz 7 species (P. geniculatum, P. hispidum, P. obliquom, P. phytolaccifolium, P. sempervirens, P. shippianum and P. variabile) and from Izabal, 4 species (P. diandrum, P. donnell smithii, P. fallens and P. peltatum).

Essential oils were obtained by hydrodistillation (yields 0.12-1.32%) and dichloromethane (yields 1.67-9.32%) and methanol (yields 2.75-14.05%) extracts by percolation followed by concentration in rotary evaporator. Activity to bacteria, yeast, fungi, protozoa and cancer cell lines were determined by micrometric methods. Five species showed some antibacterial activity at 0.5 mg/mL; four species showed antiprotozoal activity with GI50 <10 µg/mL or against cancer cell lines. Most interesting results were shown by P. jacquemontianum and P. variabile with activity against bacteria, Leishmania promastigotes, Trypanosoma epimastigotes, Plasmodium falciparum and cancer cell lines at <10 µg/mL.

Constituents from the essential oil were identified by GC-MS showing that P. phytolaccifolium had the larger number of constituents (42), mainly germacrane D (18%); P. umbellatum showed 39 constituents, mainly E-nerolidol (23.4%); P. variabile showed 36 constituents, mainly camphor (28.4%); P. oradendron showed 35 constituents, mainly β-pinene (30.3%); and, P. jacquemontianum showed 34 constituents mainly linalool (69.4%).

Piper species showed important biocidal activities and are quite diverse in the chemical composition of its essential oils. Due to its abundance in tropical and subtropical environment, and relative easy cultivation, they could be important sources of new materials for the chemical, aromatic and pharmaceutical industry.

Acknowledgement – The authors wish to thank the financial support from Dirección General de Investigación (DIGI) and Consejo Nacional de Ciencia y Tecnología (CONCYT), Guatemala, and, RIBIOFAR Network from Ibero-American Program of Science and Technology for Development (CYTED), Brasil-Spain
SECONDARY METABOLITES FROM *Humicola grisea* var. *thermoidea*

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The antibiotics have been useful in our battles against infectious bacteria and fungi for over 50 years. However, many antibiotics are used commercially, or are potentially useful, in medicine for activities other than their antibiotic action. They are used as antitumor agents, immunosuppressive agents, hypcholesterolemic agents, enzyme inhibitors, antimigraine agents, and antiparasitic agents. A number of these products were first discovered as antibiotics which failed in their development as such, or as mycotoxins. In addition to the above alternative applications, new powerful antibiotics have been discovered and commercialized in recent years and others are in clinical testing at the moment. A few successful secondary metabolites appear to have no antibiotic activity (DEMAIN, 2003). The aim of this work was to evaluate the antimicrobial activity and applications of secondary metabolites produced by *Humicola grisea* var *thermoidea*. The production of secondary metabolites was carried out by cultivating \(10^6\) spores/g in solid medium (rice) at 40ºC for 60 days. The culture was filtered and submitted to the process of liquid-liquid partition with organic solvents. Then, the dichlorometanic extract was concentrated under vacuum, and submitted to vacuum liquid chromatography technique, followed by preparative thin layer chromatography for the isolation of dimethyl tereftalate (DMT), which was elucidated by spectroscopy means (NMR). This compound was submitted to antibacterial assay to determine the minimum inhibitory concentration (MIC) against *Escherichia coli*, *Kocuria rhizophila*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The interval of concentrations evaluated was from 50 \(\mu\)g/mL to 400 \(\mu\)g/mL. The obtained MIC values against *K. rhizophila* and *E. coli* were 350 \(\mu\)g/mL. The dimethyl tereftalate is used for production by biopolymer polytrimethyltereftalate (PTT), when polymerized with 1,3-propanediol. Nowadays DMT is obtained from the petroleum. Therefore, the production of this compound from fermentative means, using microorganisms would be a promising strategy.

\[
\begin{align*}
\text{CH}_2\text{OH} & + \\
\text{CH}_2\text{OH} & \rightarrow \\
\text{PDO} & \rightarrow \\
\text{MeOH} & \\
\text{HO}-\text{O}-\text{O} & \text{HO} \rightarrow \\
\text{HO} & \text{HO} \\
\text{CH}_3 & \text{CH}_3 \\
\text{HO} & \text{HO} \\
\text{O} & \text{O} \\
\text{O} & \text{O} \\
\text{O} & \text{O} \\
\text{HO} & \text{HO} \\
\text{CH}_3 & \text{CH}_3
\end{align*}
\]

1,3-propanodiol (PDO) Dimethyl tereftalate (DMT) Polytrimethyltereftalate (PTT)

Refs.

Financial Support: FAPESP (Grant 01/07935-6) and FCFRP/USP.
A NEW SAPONIN FROM *ALIBERTIA EDULIS* (RUBIACEAE)

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*Alibertia edulis* (Rich.) A. Rich. ex DC, commonly known as “marmelada-bola”, is widely spread in the Brazilian “Cerrado”. *Alibertia* genus is known for the occurrence of iridoids, triterpenoids and phenolic derivatives [1]. We report the isolation and structure elucidation of a new saponin 3\(\beta\)-O-[\(\alpha\)-L-rhamnopyranosyl-(1→2)-O-\(\beta\)-D-glucopyranosyl-(1→2)-O-\(\beta\)-D-glucopyranoside 28-O-\(\beta\)-D-glucopyranoside pomolate (5). The ethanolic extract prepared from dried and powdered stems of *A. edulis* was submitted to partition in hexane, ethyl acetate and *n*-butanol. EtOAc extract was subjected to open-column chromatography over silica RP-18 using water with a gradient of methanol. Fraction 3 (84.4 mg) was submitted to a preparative RP-18 HPLC (MeOH-H\(_2\)O 18:82, v/v) to afford compounds 1 (5.7 mg), 2 (8.4 mg), 3 (5.3 mg) and 4 (8.7 mg). Fraction 6 yielded compound 5 (31.1 mg) with no purification step. Compound 5 was obtained as a brown amorphous powder. The HR-ESI-MS showed a peak at \(m/z\) 1127.5641 [M + Na]\(^+\) corresponding to the molecular formula C\(_{54}\)H\(_{88}\)O\(_{23}\). The \(^1\)H and \(^13\)C NMR spectra and the long-range correlations observed in the gHSQC spectrum suggesting that the aglycone was consistent with pomolic acid [2]. The presence of four sugar moieties was established from the observations of four anomic hydrogens. The carbon signals observed to C-3 and C-28 are consistent with a bidesmoside saponin moiety. In order to confirm our assumption regarding the glycosidic composition of this compound, an ESI-IT-MS\(^a\) experiment was performed. Fragmentation led to the loss of one deoxysaccharide and three hexose moieties, which structures were deduced using 1D-TOCSY and 2D NMR experiments: a \(\alpha\)-L-rhamnopyranosyl and three \(\beta\)-D-glucopyranosyl units. The positions of the sugar moieties were unambiguously defined by the gHMBC experiment and this completes the identification of compound 5. This is the first report of this compound in literature and it is the first report of the isolation of a saponin from the *Alibertia* genus in Rubiaceae.

Ref.
Chemical studies carried on Piperaceae species have revealed the occurrence of pyrones, lignoids, chromenes, terpenes, prenylated benzoic acids and amides several of which showing biological potential\(^1\). In the course of our studies on *Piper* species, the crude extract from leaves of *P. laevicarpu* was submitted to phytochemical investigation. The dried leaves (210 g) were extracted with CH\(_2\)Cl\(_2\) to afford 1.2 g of crude extract. Part of this extract (800 mg) was subjected to chromatographic separation on silica gel using gradient of CH\(_2\)Cl\(_2\) in MeOH to afford seven groups (I – VII). Group IV (587 mg), after purification on Sephadex LH-20 (MeOH), gave eight groups (IV-1 – IV-8). After spectrometric analysis (\(^1\)H NMR and LREIMS), the flavonoids naringenin (31 mg) and sakuranetin (27 mg) were detected in the groups IV-4 (31 mg) and IV-5 (27 mg), respectively. Group IV-3 (30 mg) was composed by an amorphous powder, whose \(^1\)H NMR spectrum showed the presence of two aromatic rings due to the AA'BB' system at \(\delta\) 6.95 (d, \(J = 8.5\) Hz) and 7.20 (d, \(J = 8.5\) Hz) and the ABX system at \(\delta\) 6.69 (d, \(J = 8.2\) Hz), 6.53 (dd, \(J = 8.2\) and 2.1) and 4.58 (d, \(J = 2.1\) Hz). These signals, associated to two coupled triplets of methylene hydrogens at \(\delta\) 2.09 (\(J = 5.2\) Hz, 2H), 2.72 (\(J = 5.2\) Hz, 2H), 2.83 (\(J = 6.2\) Hz, 2H) and 3.51 (\(J = 6.2\) Hz, 2H) were indicative of a dihydrocoumaroyltamamine derivative\(^2\). The \(^{13}\)C NMR spectra (BBD and DEPT 135°) showed 16 resonance lines consisting of one methoxyl, four methylenes, five methines (two equivalents), and six quaternary carbons (including a carbonyl group at \(\delta\) 172.0). The molecular formula was established as C\(_{18}\)H\(_{19}\)NO\(_3\) by HRESIMS based on the molecular ion peak at \(m/z\) 320.1258 [M + Na]\(^+\). Finally, analysis of HMQC, HMBC and NOESY spectra indicated an ether linkage between the two aromatic rings, a rare feature among secondary compounds isolated from Piperaceae species. The structure of this amide, named as laevicarpin, is derived from dihydrocoumaroyl and tyramine and has previously been described as synthetic product\(^3\) but not as natural product.

ISOLATION AND CHEMICAL CHARACTERIZATION OF A PHENOLIC MARKER IN ACQUEOUS EXTRACT OF *PHYLLANTHUS* SPECIES

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The species belonging to the genus *Phyllanthus* (Euphorbiaceae) are widely distributed throughout most tropical and subtropical countries. In Brazil, these species are popularly known as “quebra-pebra”, and used for the treatment of many diseases, such as: hepatitis, kidney stones, intestinal and urinary infections. It is reported that a great variety of secondary metabolites were isolated and identified, including flavonoids, tannins and lignans\(^1\). The characterization of the chemical profile by High Performance Liquid Chromatography (HPLC) for different extracts of *Phyllanthus* species can be used for differentiate them and applied in quality control/authentication of vegetable raw materials for pharmaceutical purposes\(^2\).

This work reports the isolation by semi preparative HPLC from the aqueous extract of *Phyllanthus niruri*, a hydrolysable tannin. The structure of this substance was identified by Nuclear Magnetic Resonance experiments (\(^1\)H, \(^{13}\)C, DEPT 135, HSQC and HMBC) and it was characterized as corilagin\(^3\) (Fig 1). This compound was analyzed by Mass Spectrometry eletronspray negative mode of ionization in ion trap analyzer. The ionization condition for the isolated substance was used as reference for the characterization of lyophilized aqueous extract samples using HPLC reversed mode of six *Phyllanthus* species: *P. niruri*, *P. tenellus*, *P. amarus*, *P. caroliniensis*, *P. stipulatus* and *P. urinaria*.

![Chemical structure of corilagin](image)

Fig 1. Chemical structure of corilagin.

Refs.


Acknowledgement
To FAPESP and CNPq for the grants and financial support.
NEW PENTACYCLIC TRITERPENES FROM COMBRETUM LAXUM

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Amongst the 18 genera comprising the Combretaceae, the genus Combretum is the largest with 250 species, which are mostly native to tropical and subtropical regions. Plants of this genus are known as a rich source of triterpenes and their glycosides, flavonoids, phenanthrenes, bibenzyls, stilbens, in addition to alkaloids and other aromatic compounds, some of which with anticaner, antimicrobial and hepatoprotective activities, among others. Combretum laxum Jacq., popularly known as “pombeiro branco”, is a woody shrub, which grows in the “Pantanál” of Mato Grosso do Sul, Brazil. In a previous phytochemical study on the stems of this species we obtained arjunolic (1) and asiatic (2) acids and their glucosides arjunglucoside II (3) and quadranoside IV (4), respectively.¹ Further work has now led to the isolation of one new pentacyclic triterpene, 2α,3β,24-trihydroxyolean-12-ene-28-O-β-D-glucopyranoside (5) and four known ones, 2α,3β,6β-trihydroxyolean-12-ene-28-O-β-D-glucopyranoside (6), betulinic acid (7), quadranoside I (8) and bellericoside (9), of which 9 is being described for the first time in the genus Combretum. Air-dried and powdered stems of C. laxum (4375 g) were extracted at room temperature with EtOH. After concentration in vacuo, the residue obtained from the EtOH extract was subsequently partitioned between MeOH/H₂O 9:1 and hexane; MeOH/H₂O 1:1 and CH₂Cl₂; and MeOH/H₂O 1:1 and n-BuOH. After a combination of column and flash chromatography on silica gel, gel filtration on Sephadex LH-20 and reversed phase CC and HPLC separations of the EtOAc (7.7 g) and n-BuOH (6.75 g) phases, triterpenes 5, 6 and 8 were isolated from the former while 3, 4 and 9 were obtained from the latter. Betulinic acid (7) was isolated from the CH₂Cl₂ phase. The antifungal activities of compounds 5, 6, 8, 9 and a mixture of 1 and 2 against Candida albicans, C. krusei, and Cryptococcus neoformans were determined using the broth microdilution method (Table 1).² Although showing weak or no activities against the strains tested, it is noteworthy the specificity of the mixture of 1 and 2 against Candida krusei and also of 5, 6 and 8 against C. albicans and Cryptococcus neoformans.

Table 1-MIC values (µg/mL) of compounds isolated from C. laxum against Candida and Cryptococcus strains

<table>
<thead>
<tr>
<th>Strains</th>
<th>Compounds 1</th>
<th>5</th>
<th>6</th>
<th>8</th>
<th>9</th>
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I: inactive
Reference compound: amphotericin B (MIC = 0.125 µg/mL)

References
3. F. R. Garcez et al., 29th Annual Meeting of the Brazilian Chemical Society, Águas de Lindóia, SP, Brazil, 2006 (PN-052).
NEW LABDANES FROM COPAIBA OLEORESIN

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The copaiba oleoresin is an exudates obtained from the trunk of many species of the Copaifera genus (Caesalpinioideae, Leguminosae) and is popularly used as cicatrizing, anti-inflammatory, in the treatment of bronchitis and for skin diseases, also in the cosmetic industry as fixative of the perfumes and as solvent for inks and varnishes. Although there is an extensive and recent study on the chemical composition of the oleoresin in the literature, the absolute configuration of many of them still remains unknown. In order to characterize and to determine the absolute configurations of some components, the commercial copaiba oleoresin was reinvestigated. The oleoresin was submitted to an acid-base extraction supplying two fractions: neutral fraction (81.7%) and acidic fraction (18.3%). The purification of neutral fraction by column chromatography allowed the isolation of eleven sesquiterpenes {α-trans-bergamotene, γ-murolene, β-trans-caryophyllene, β-bisabolene, caryophyllene oxide, humulene oxide, 7(11)-selenin-4-ol, torreyol, α-cadinol and two epimeric mixture of humulene dioxides} [1], three dinorlabdanes 1-3 and ent-clerodane 4 [2, 3]. The absolute configuration of the dinorlabdanes 1-3 was established through the synthesis starting from known ent-3-hydroxy-copalic acid [2, 3]. The acidic fraction furnished known ent-labdane {copalic acid, 3-acetoxy-copalic acid, 3-hydroxy-copalic acid and ent-agathic acid}, ent-clerodane diterpenes {hardwickiic acid, 7-acetoxy-hardwickiic acid} [1] and two new ent-labdanes as methyl ester after treatment with CH₂N₂ {methyl 4-hydroperoxy-18-norcopalate (5) and methyl 3,19-dihydroxy-copalate (6)}. The structures of these terpenes were established on the basis of one- and two-dimensional NMR experiments and absolute configuration was established by biogenesis correlation and by comparison of the optical rotation reported in literature [4].

References
NEW ALKALOID OF Psychotria stachyoides Benth.

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Psychotria (Rubiaceae) is one of the largest genera of flowering plants whose specimens are used in the traditional medicine against dizziness, hallucination, dementia and rubella\(^1\). A number of studies have cited Psychotria as a promising source of alkaloids with cytotoxic\(^2\), inhibitor of the human platelets\(^3\), antiprotozoal\(^4\) and analgesic\(^5\) properties. Species of this genus are widespread in Brazil, and the phytochemical works of several Southern species have revealed the presence of indole monoterpane alkaloid glucosides. As part of our investigative studies of alkaloids from Psychotria species from the northeastern Brazil flora, we have investigated Psychotria stachyoides, a native species from Guaramiranga Mountain, a Atlantic Forest spot at Ceará State. Acid-base extraction of the crude ethanol extract from the stems of P. stachyoides yielded an alkaloid fraction with a major compound. The alkaloidic fraction was submitted to preparative chromatography using the mixture of CH\(_2\)Cl\(_2\)/MeOH/NH\(_4\)OH (90:10:0.1)as eluente to afford the pure compound 1, in addition to the coumarin scopoletin (2). Spectroscopic analysis using 1D and 2D NMR experiments including COSY, HMQC, HMBC and NOESY sequences for compound 1, revealed structural elements of a new unusual terpenoid alkaloid glucoside containing a secologanin and a tetrahydro-β-carboline moiety.

\[\text{(1)}\]

\[\text{(2)}\]


CNPq/CAPES/FUNCAP/PRONEX
Species of Rubiaceae have widespread occurrence in Brazil and are well known by their economic and therapeutic importance, but the phytochemical analysis of plants belonging to the genus *Alibertia* have been limited to a few species. Previous reports reveals *Alibertia* species as a rich sources of fungitoxic iridoids, flavonoids, caffeic acid and its esters, and pentacyclic triterpenes\(^1,2,3\). In our work with *A. myrciifolia*, we have reported the isolation of cytotoxic flavonoids and iridoids, besides oleanane and hopane triterpenes\(^4,5\). As part of the investigative efforts to study *Alibertia* species from the northeastern Brazil flora, it is presented the chemical study of *A. rigida*. The crude ethanol extract from the leaves was partitioned with hexane, CHCl\(_3\), AcOEt and n-BuOH. The CHCl\(_3\) fraction was submitted to Si gel chromatography to afford the cafeic acid (1). Successive exclusion chromatography Sephadex LH-20 of the AcOEt fraction yielded the 1,2,4-trihydroxi-benzene (2), the flavonoids kaempferol (3) and quercetin (4), in addition to the proanthocyanidin A-6 (5). The flavonoid rutine (6) was obtained from the n-BuOH fraction after recrystallization in MeOH. The complete proton and carbon assignments of the isolated compounds were accomplished by the use of 1D and 2D NMR experiments including COSY, HMQC, HSQC, HMBC and NOESY pulse sequences.
FLAVONOIDS AND SESQUITERPENOIDS FROM *Acritopappus pintoi* (Asteraceae)


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The Chapada Diamantina presents one of the richest ecosystems in the state of Bahia, Brazil, with many endemic and new species of plants. *Acritopappus pintoi* Bautista & Hind, from Asteraceae family, was collected in September 2004, at Capada Diamantina, in the municipal district of Piatã, Bahia. The importance attributed to this study came up due to few studies on this species is reported. Thus, from the chemical and biological perspective *Acritopappus* genera has been evaluated. The dichloromethane fraction of *A. pintoi* leaves ethanolic extract was active against gram-negative, gram-positive bacteria and fungi *Micrococcus luteus* and *Streptococcus mutans*.

The dichloromethane phase of *A. pintoi* leaves ethanolic extract was submitted to a silica gel chromatography separation. The first fractions eluted with CHCl$_3$–MeOH (1%) gave after recrystallization in hexane and ethanol two sesquiterpenes, 1 and 2. The further fractions gave, after a Sephadex LH-20 column chromatography five flavonoids (3-7). The isolated compounds will be submitted to bioassays.

Compound 1 was obtained as colorless crystals, PF 224-225°C in ethanol. The $^{13}$C NMR spectrum showed twenty signals revealing an aliphatic structure. The $^1$H NMR confirmed the supposition and suggested the structure of a terpene compound. The number of methyl groups was incompatible with a diterpene structure, and an isoprenyl sesquiterpene was proposed. The presence of two trisubstituted double bonds, one carbonyl, two carboxyl groups, three oxygenated carbons and two hydroxyl groups was inferred by the NMR spectra. The mass spectrum showed a signal at $m/z$ 362, which is in agreement with the loss of a H$_2$O molecule of C$_{20}$H$_{28}$O$_7$. HSQC, HMBC, COSY and NOESY spectra were used to propose the structure of 1, a new rearranged sesquiterpene. The structure of 2 (PF 198-199°C in ethanol) was proposed comparing its spectral data with those of 1.

\[
\begin{align*}
1 & \quad R \cdot \text{CO-CH(CH}_3\text{)}_2 \cdot \text{CH}_2\cdot \text{CH}_3 \\
2 & \quad R \cdot \text{CO-CH}_2\cdot \text{CH(CH}_3\text{)}_2 \\
3 & \quad R=\text{Me}; R_1=\text{H}; R_2=\text{OMe} \\
4 & \quad R=\text{Me}; R_1=\text{H}; R_2=\text{H} \\
5 & \quad R=\text{Me}; R_1=\text{H}; R_2=\text{OH} \\
6 & \quad R=\text{H}; R_1=\text{OMe}; R_2=\text{H} \\
7 & \quad R=\text{H}; R_1=\text{H}; R_2=\text{O-beta-D-} (6''-E-p-cumaroyl glucopiranosyl)
\end{align*}
\]

BIFLAVONOIDS AND TERPENOIDS ISOLATED FROM THE LEAVES OF OURATEA MICRODONTA Engl. (OCHNACEAE)

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In the course of our phytochemical and pharmacological investigation of the genus Ouratea (Ochnaceae) we reported the presence of terpenoids, isoflavonoids, flavonoid glycosides and biflavones,¹ ² besides their DNA topoisomerase inhibition, citotoxic and antitumoral activities.³ ⁴ In this study we have also reported some reviews and spectral investigation of biflavonoids and their new derivatives⁵ ⁶. Continuing the phytochemical study of Ouratea species we are describing the first phytochemical study of Ouratea microdonta Engl. (Ochnaceae). The leaves and branch of this plant were collected in the Ilha de Marajó, municipality of Soure, Pará state, Brazil. Voucher specimen (Nº IAN-21438) is deposited at the Herbarium Embrapa Amazônia Oriental, Pará, Brazil. The dried and powdered leaves (1.0 Kg) were extracted with methanol at room temperature. The solvent was removed under vacuum furnishing a residue OMFM (250.0 g). The crude methanol residue (200.0 g) was filtered on silica gel with C₆H₁₂, CHCl₃, AcOEt and MeOH yielding the fractions OMFH (3.0 g), OMFC (20.6 g), OMFAc (6.5 g) and OMFM (60.0 g). These fractions were analyzed by TLC plate and fractionated on silica gel and/or sephadex LH-20 column eluting by the each adequate eluent. The subfractions that were analyzed until this time let us to identify steroids, triterpenes, a benzoic acid and three biflavonoids. From the fraction OMFH were isolated a mixture of lupeol, α- and β-amyrin (30.5 mg) and β-amyrin (50.6 mg), from OMFC were isolated a mixture of sitosterol and stigmasterol (20.0 mg), lupeol (10.0 mg) and 4-methoxy,2,5-dihydroxy-benzoic acid; from the fraction OMFAc were isolated three biflavonoids, agathisflavone (15.0 mg), 7"-methylagathisflavone (20.0 mg) and amentoflavone (12.0 mg); the agathisflavone was also isolated from the fraction OMFM. The structures of these compounds were deduced by the ¹H and ¹³C-NMR spectra data analysis and comparison with literature.¹ ²

References
CNPq, CAPES, FAPERJ.
Over the past decade drug discovery from natural products, especially medicinal plants, has continued effectively to provide new drugs and drug leads against various pharmacological targets such as tumors, viruses, bacteria and fungi. Plants of the Amaryllidaceae family are amongst the top 20 in the most widely applied medicinal plant families. As primary constituents, more than 400 structurally diverse alkaloids have been isolated and most of them have shown significant biological activities, particularly antiviral, antitumoral and psychopharmacological. *Hippeastrum morelianum* (Lem.) is found throughout Brazil and our interest is to investigate biologically active alkaloids synthesized by members of the *Hippeastrum* genus. Therefore, an ethanolic extract from fresh bulbs of *H. morelianum* which was pooled, dried and the residue was partitioned in light petroleum and HCl (10%). The HCl phase was washed with CH₂Cl₂ and the acid phase thus obtained basified with NH₄OH (pH 9) and extracted first with CH₂Cl₂ and subsequently with n-butanol. The CH₂Cl₂ fraction was re-suspended and chromatographed on silica-gel using vacuum liquid chromatography (VLC) technique using an increasing polarity of solvents (200ml) pentane, ethyl ether, acetone, dichloromethane, n-butanol, ethyl acetate and, finely, methanol. Methanol fraction was chromatographed on silica-gel reverse-phase using the medium pressure liquid chromatography (MPLC) technique using an increasing gradient of methanol-water (volume rate from 0-100 to 100-0, in 8h). The fractions were purified with column chromatography as well as preparative chromatography. The potential antioxidant of the isolated alkaloids and extracts were investigated for DPPH method. Twelve alkaloids have been isolated but only four were identified using spectroscopic methods until the moment. From VLC technique dichloromethane fraction resulted in 6 mg of pretazettine and n-butanol fraction, after purification, resulted in 110 mg of candimine. The methanolic fraction, after purification techniques, generated 40 mg of tazettine and 7 mg of haemanthamine or crinamine. The potential antioxidant was observed, at 10 mg/ml, for all fractions of acid-basic extraction while none isolated alkaloid demonstrated positive result up to 3 mg/ml. The genus *Hippeastrum* is one important source of Amaryllidaceae alkaloids and the extracts presented important antioxidant potential. Moreover, the occurrence of candimine alkaloid in high quantity is detached a time that none pharmacological investigation was related at the moment opening perspectives for future works.

Refs.
New Steroidal Constituents of *Ganoderma lucidum* from Ceará-Brazil.

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*Ganoderma lucidum* (Ganodermataceae) is a type of fungi popularly known in the northeast of Brazil as “orelha-de-pau”, name originated for the similarity to an ear linked on trees in decomposition. From it hexane extract of *G. lucidum* after chromatographic procedure in silica gel and sephadex LH-20 was possible to characterize three steroids compounds with ergostane skeleton (G-1, G-2, G-3). Structures elucidation were done using spectroscopy techniques including 1 and 2D NMR. The compounds were also evaluated for their scavenging activity, using DPPH assay and showed high inhibition percentage determined as 73.5 %, 65.7% and 63.2%, respectively at 1 µg/µL, with IC$_{50}$ 0.021 µg/µL, 0.056 µg/µL, and 0.052 µg/µL.

Ref.

CHEMICAL CONSTITUENTS FROM *ANNONA AMAZONICA* (ANNONACEAE)

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*Annona* (Annonaceae) comprises about 120 species reported in South and Central America, Africa, Asia and Australia. Previous chemical and pharmacological investigations on species of this genus have indicated the presence of important bioactive compounds, exhibiting various pharmacological activities, including antiparasitic; in particular, against *Leishmania* sp., *Plasmodium falciparum* and *Trypanosoma cruzi*.¹ Therefore, in continuation of our studies of Amazon medicinal plants and search for novel antiparasitic natural products, we have undertaken the chemical investigation of *Annona amazonica* R.E. Fries. This annonaceous plant is a tropical tree distributed from Panamá to South America. In Brazil this species is commonly found in the Brazilian Amazon, particularly in the states of Amazonas and Pará.² The powdered air-dried bark of *A. amazonica* (1.1 Kg) was extracted successively with hexane (15.0g), CH₂Cl₂ (6.2g) and MeOH (40.0 g), at room temperature. The CH₂Cl₂ extract (6.0g) was redissolved in CH₂Cl₂ and submitted to acid-base extraction to yield the neutral (5.0g) and alkaloidal fractions (1.0g). The neutral fraction (4.8g) was initially subjected to silica gel column chromatography eluted with increasing concentrations of CH₂Cl₂ in hexane, EtOAc in CH₂Cl₂ and MeOH in EtOAc. The eluted fractions were evaluated and pooled by TLC analysis. Additional chromatographic separation of the fractions by preparative TLC resulted in the isolation of one sesquiterpene (caryophyllene oxide, 1); two steroids (β-sitosterol and stigmasterol, ² and ³); one monounsaturated fatty acid (oleic acid, ⁴) and tree methyl esters of fatty acids (oleic, linoleic, and linolenic acids, methyl esters, ⁵, ⁶ and ⁷). The compounds were identified by spectroscopic methods and by comparison with literature data. This is the first report of isolation these substances in this species. The identification by GC/MS of others sesquiterpenes and methyl esters has been described in our previous work.³ The antileishmanial activity and cytotoxicity against human tumor cells will be investigated for purified compounds.

Refs.
ALKALOIDS FROM LEAVES OF *ANONOA SERICEA* (ANNONACEAE)

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*Annona* (Annonaceae) comprises about 120 species reported in South and Central America, Africa, Asia and Australia. Previous chemical and pharmacological investigations on species of this genus have indicated the presence of important bioactive compounds, exhibiting various pharmacological activities, including antiparasitic, in particular against *Leishmania* sp., *Plasmodium falciparum* and *Trypanosoma cruzi*.¹ Therefore, in a search for novel antiparasitic natural products we have studied the leaves of *Annona sericea* Dunal. This annonaceous plant is a tropical tree distributed in South America rain forest² and known as “aráticum do Pará”. In Brazil this species is commonly found in the Brazilian Amazon, particularly in the states of Amazonas and Pará.² The powdered air-dried leaves of *A. sericea* (500g) was extracted successively with hexane (24.0g) and MeOH (35.0 g), at room temperature. The MeOH extract (33.0g) was redissolved in CH₂Cl₂ and subjected to acid-base extraction to yield the neutral (8.0g) and alkaloidal fractions (0.55g). The alkaloidal fraction (0.5g) was initially subjected to silica gel column chromatography, having been previously treated with a 10% NaHCO₃ solution and eluted with increasing concentrations of CH₂Cl₂ in hexane followed by EtOAc in CH₂Cl₂ and MeOH in EtOAc. The eluted fractions were evaluated and pooled by TLC analysis. Additional chromatographic separation of the fractions by preparative TLC, resulted on the isolation of two oxoaporphine alkaloids, oxonucoferine (1) and oxonantenine (2), and two aporphine alkaloids, nornuciferine (3) and nornantenine (4). The compounds isolated were identified by spectroscopic methods and by comparison with literature data. The antileishmanial activity will be investigated for purified compounds. This is the first report of these alkaloids in this species. The results obtained in this study confirm that plants of this genus have strong ability to produce oxoaporphine and aporphine alkaloids, a class of compounds typically found in plants of Annonaceae.

![Chemical Structures](image1.png)

Ref.
Cropocarya mandioccana is an arboreal species of some 25-30m height that is widely spread in the Atlantic forests of Brazil. It is recognized as an important food source for primates such as Brachyteles arachnoids E. Geoffroy, 1806 (mono-carvoeiro or muriqui). Cropocarya plants have components like alkaloids, flavonoids and styrylpyrones. Phytochemical studies of the bark of C. mandioccana have shown that styrylpyrones are the typical secondary metabolites present in this species\(^1\). Flavonoids glycosides, as well as styrylpyrones, have been detected in leaves and the qualitative and quantitative intra-specific variability of these secondary metabolites has also been determined\(^2\). However, a phytochemical study of other Cropocarya genus species suggested the presence of alkaloids\(^3\). This present paper deals with the isolation and structural determination of quaternary aporphine alkaloids from the bark of this plant. The powdered dry bark of C. mandioccana was submitted to liquid-liquid extraction by sonication with hexane and methanol:acetic acid 10% (1:1 v/v). The crude extract was partitioned with chloroform to remove the lipophilic compounds. The hydroalcoholic extract was divided into two parts by solubility in chloroform:methanol (70:30). Each part was submitted to normal phase chromatography (25.0 x 1.2 cm i.d; 40-63µm) eluted using a step gradient from chloroform:methanol (70:30) with 10% of ammonium hydroxide to chloroform:methanol (63:37) with 10% of ammonium hydroxide to yield two quaternary aporphine alkaloids, the (+) menisperine and the (+) xanthoplanine. The structural determination these components were assured by UV, \(^1\)H and \(^13\)C NMR uni and bidimensional. The stereochemistry was elucidated by the optical rotation. The data were compared with those describe in the literature\(^3,4\). In addition, the quaternary aporphine alkaloids, (+) menisperine and (+) xanthoplanine, were isolated from the C. mandioccanna for the first time.

Refs.
NEW ACETOGENINS FROM ANNONA CORNIFOLIA A. ST. -HIL.

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Annonaceous acetogenins are a group of secondary metabolites, until now isolated only from fourteen out of the 130 genera of Annonaceae family, that possess important biological activities as cytotoxic, antitumoral, antimicrobial, insecticide and immunosuppressive1. *Annona cornifolia* is a tropical shrub, typical of Minas Gerais “cerrado” whose fruit is popularly known as “araticum das caatingas” and “araticum mirim”. In our recent studies of the seeds of *Annona cornifolia* we have described four bis-tetrahydrofuran γ-lactones belong to this very interesting natural products: cornifolin2, annofolin3, desacetyl uvaricin and squamocin M4. We describe here the isolation and structural elucidation of a new acetogenin, folanin B (1) along with a mixture of two known compounds, glaucanisin (2) and parviflorin (3) from the ethanolic extract of *A. cornifolia* seeds. The phytochemical study of the ethanolic extract from *A. cornifolia* seeds was guided fractionation by brine shrimp test (BST). The cleaned seeds were dried at 40 °C, powdered (150.0 g) and extracted with ethanol in Soxhlet, yielding the ethanolic extract (F01, 10.0 g). This extract was successively extracted with hexane and ethylacetate. Solvent removal under reduced pressure provided hexanic (F02, 6.3 g) and ethylacetate (F03, 3.5 g) fractions. F02 was fractionated by silica gel column and reverse phase HPLC, resulting in the isolation of folanin B (9.5 mg, 1), a mixture of glaucanisin and parviflorin (34.6 mg, 2 + 3). The structure and relative or absolute stereochemistry of the acetogenins were elucidated based on 1D and 2D NMR spectroscopy techniques, including 1H NMR, 13C NMR, COSY and HMQC, and ESIMS of the parent compound. These results suggest the great potential of this plant in yielding cytotoxic, antitumoral and insecticide leader-compounds.

Refs.
As part of our ongoing studies on medicinal plants, we investigated the constituents of *Ptychopetalum olacoides* Bentham (Olacaceae), popularly known as “Marapuama and Muirapuama”, collected in the Brazilian Amazon. Voucher specimen is preserved at the Emílio Goeldi Museum (Pará state), collection number MG 170.021. The ethanolic extract of the root bark was suspended in H$_2$O and then partitioned with Et$_2$O, EtOAc and BuOH. The fractions were performed on precoated plates Si gel 60 F$_{254}$ and visualized by spraying reagents for alkaloids (IP), flavonoids (NP-PEG), essential oil (VS) and phenolic compound (FBS-KOH)$_1$. The residue of the EtOAc fraction, after evaporation to dryness was purified by Flash Chromatography on Si C-18 with H$_2$O-MeOH solvents system (9:1 to 100% MeOH). The 80% aqueous MeOH fraction provided a plumeria iridoid skeleton$^2$. The Plumieride structure (I) was elucidated by spectroscopic methods, mainly 1D and 2D NMR$^{3,4}$. This iridoid has been reported to have antidermatophytic$^5$, antifertility$^6$ and anticancerous activities$^7$. Despite phytochemical investigation has been carried out, this is the first isolation of iridoid from *P. olacoides*.
PEPTIDES DERIVATIVES FROM TWO SPECIES OF LEGUMINOSAE

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Cenostigma gardnerianum Tul. and Cratylia mollis Benth. are two Leguminosae species that occur in the brazilian semi-arid region. They are popularly known as “canela-de-velho” and “camaratu”, respectively. Species of Cenostigma are employed by the local population as herbal medicine and the leaves of C. mollis is employed for cattle feeding during the drought periods in Brazilian northeastern. Previous studies with the wood and leaves of these plants indicated the presence of flavonoids and terpenoids1,2.

This work describes the chemical study of the CHCl₃ fractions which were obtained from partition between CHCl₃/MeOH:H₂O (9:1) of the MeOH extract of the leaves of C. gardnerianum and C. mollis. These fractions were submitted to CC over Si gel and eluted with mixtures of hexane and ethyl acetate of different polarities. The fractions obtained were further purified employing Sephadex LH-20 permeation and Si gel PTLC. These procedures permitted to obtain compound 1 (aurentiamide acetate) from C. mollis and 1 and 2 from C. gardnerianum. The structures of the peptides were elucidated by ¹H and ¹³C NMR (uni and bidimensional), IR and MS.

The presence of the peptide derivative aurentiamide acetate (1) in the two Leguminosae species is not a surprise because it was already found in different natural sources. It was firstly isolated in Aspergillus spp. but, it was also detected in algae and different plant species of the families Piperaceae, Euphorbiaceae and Malvaceae3. In Leguminosae family, aurentiamide acetate was only obtained from Medicago polymorpha. However, besides compound 2 was a known compound it can be considered an unusual plant peptide which was previously obtained only from Croton hieronymi3.

(CNPq, FAPESB, CAPES, PRONEX).

Refs.
2. C. Q. Alves, Flavonóides antioxidantes e derivados de ácido gálico isolados de Cenostigma gardnerianum Tul. Dissertação de Mestrado, Instituto de Química, UFBA (2007)
APORPHINE TYPE ALKALOIDS FROM BARK OF GUATTERIOPSIS FRIESIANA (ANNONACEAE)

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Plants of the family Annonaceae are known to elaborate a variety of alkaloids, some of which are reported to have interesting pharmacological properties.1 Despite the importance of the members of this family in folk medicine, the number of species that have been chemically investigated is still small.1 Guatteriopsis is a small genus, comprising about five species of small trees whose majority is distributed in South America.2 It has been reported only one phytochemical study of essential oils from leaves of G. blepharophylla.3 Continuing our work in a search for novel antimicrobial natural products we have studied the bark of Guatteriopsis friesiana. This plant, commonly known as “envireira”, is a tropical native small tree found in Brazil and Colombia Amazon rainforest.2 The powdered air-dried bark of G. friesiana (1.9 Kg) was successively extracted, at room temperature, with hexane and MeOH to afford 27.11g and 260.62 g, respectively. Antimicrobial activity was found for MeOH extract against Staphylococcus aureus (500 µg/mL). TLC investigations revealed the presence of alkaloids in the MeOH extract. The MeOH extract of bark (250 g) was redissolved in CHCl3 and subjected to extraction with 3% aqueous HCl. This aqueous solution had the pH adjusted to 12 with NH4OH, followed by extraction with CHCl3 to yield an alkaloid fraction (6.20 g). This fraction (6.0 g) was subjected to several chromatography columns and preparative TLC yielding four known aporphine type alkaloids: liriodenine 1 (60.0 mg), lycaspartine 2 (13.6 mg), guadiscine 3 (8.8 mg) and guadiscidine 4 (7.0 mg). The isolated compounds were identified by spectroscopic methods (UV, IR, EIMS, 1H, 13C 2D NMR experiments) and comparison with literature data.4

![Structures of alkaloids](image)

(1) R1-R2 = OCH2O  
(2) R1-R2 = OCH3  
(3) R1 = OCH3  
(4) R1 = OH

Refs.
Galianthe Griseb. (Rubiaceae, tribe Spermacoceae) is a genus of about 50 species endemic to South America\(^1\), with the main center of diversity in Central and southern Brazil. The only chemical study in the genus is about *Galianthe brasiliensis* (Cham. & Schltdl.) E.L. Cabral, where some iridoids\(^2,3\) were reported. In order to expand the understanding of the diversity and possible systematic significance of the secondary compounds of the genus, the present study focuses on *Galianthe ramosa* E.L. Cabral, a species endemic of Central Brazil.

**Experimental:** Plant material was collected and identified by Delprete. The aerial parts of the plant were air-dried and extracted (by maceration) with methanol at room temperature. The extract was concentrated in vacuum, and the residue was fractionated by chromatography column over silica gel, eluted with \(n\)-hexane, \(n\)-hexane:CHCl\(_3\) (1:1), CHCl\(_3\), CHCl\(_3\):ethanol (1:1) and ethanol. The CHCl\(_3\):ethanol (0.9 g) fraction was subjected to CC over silica gel and yielded the alkaloid \(1\) (5.6 mg). Identification and structural elucidation of this alkaloid were performed with \(^1\)H NMR and \(^{13}\)C NMR 1D and 2D (HMQC, HMBC) spectroscopy. Additionally, HMBC data shows \(\delta_H\) of methyl at 1.74 ppm (\(\delta_C\) 21.3) is correlated with quaternary C\(sp^2\) at \(\delta_C\) 147.6 and methylene C\(sp^2\) at \(\delta_C\) 109.8 ppm. This data suggests that alkaloid \(1\) could be an analog of chrysotricine \(2\), an alkaloid previously isolated from *Hedyotis chrysotricha*\(^4\), by elimination of H\(2\)O.

Further HRMS data will be sought in order to confirm the structure of alkaloid \(1\).

This finding indicates that alkaloid \(1\) might be useful chemotaxonomic marker supporting the systematic position of these two genera. In fact, *Hedyotis* is positioned within tribe Hedyotideae, sister tribe of the Spermacoceae, and *Galianthe* in tribe Spermacoceae, both belonging to the subfamily Rubioideae.

**Refs.**
2. V.M. Moura et al., *Quimica Nova*, 29, 452 (2006);
IDENTIFICATION OF THE C-GLYCOSYL FLAVONOIDS IN THE BUTANOLIC FRACTION FROM THE ROOTS FROM Wilbrandia ebracteata COGN.

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Introduction: Wilbrandia ebracteata is a Brazilian plant from the Cucurbitaceae family popularly named “Taiuiá”. The Taiuiá roots are used in the folk medicine for the treatment of gastric ulcer, acute and chronic rheumatism, among other diseases. Studies with the Wilbrandia ebracteata roots showed the presence of cucurbitacins in the dichloromethane fraction and C-glycosyl flavonoids in the butanolic fraction. Objective: Identify the C-glycosyl flavonoids in the butanolic fraction from the roots of Wilbrandia ebracteata. Methods: The butanolic fraction was analysed by Thin Layer Chromatography (TLC) (silica gel plates; ethyl acetate: formic acid: water – 8:1:1 and ethyl acetate: methanol: acetic acid – 11:4:0.3; natural products reagent/UV 366 nm) and High Performance Liquid Chromatography (HPLC) (phenyl column; acetonitrile: acetic acid 1% - gradient; UV-detection (330 nm). As reference compounds were used the C-glycosyl flavonoids orientin, vitexin, isovitexin, isoorientin, vitexin-2’-O-rhamnoside, spinosin, 6,8-di-C-glicosil-crisin and vicenin-2. Results: The TLC and HPLC analysis indicated the presence of vitexin, isovitexin, spinosin and vicenin-2 in the butanolic fraction of the roots. The HPLC analysis demonstrated that the major compounds of the butanolic fraction are spinosin, isovitexin and swertisin. Conclusions: The presence of vitexin, isovitexin, spinosin, vicenin-2 and swertisin was characterized in the butanolic fraction from Wilbrandia ebracteata roots by TLC and HPLC analysis. Isovitexin, spinosin and swertisin are the major compounds from this fraction.
The chemical communication mechanism or quorum-sensing is now a widely recognized phenomenon in Gram-negative bacteria, in which the acyl-homoserine lactones (acyl-HSL) play a key role. It was demonstrated in many species that important phenotypes are under the quorum sensing control, as expression of exoenzymes, secondary metabolites and biofilm formation. The aim of this work is to characterize the acyl-HSL produced by the Gram-negative bacterium *Methylobacterium mesophilicum*, which occurs endophytically in citrus plants and seems to be associated to citrus variegated chlorosis disease. One of the most interesting characteristic of this microorganism is the use of methanol as the sole carbon source. Therefore, the production of acyl-homoserine lactones by this microorganism under methanotrophic conditions was investigated. Initial β-galactosidase bioassays with reporter *Agrobacterium tumefaciens* NTL4(pZLR4) indicated the possible presence of acyl-HSL in *M. mesophilicum* extracts, stimulating more accurate chemical studies. GC-MS and 1H NMR analyses of fractionated extracts allowed the identification of six acyl-HSLs in a complex fraction (1.1 mg) obtained from 8 L of fermentation media. Such small amount prompted us to employ chemical derivatization techniques as dimethyl-disulfide reaction, catalytic hydrogenation and synthetic methods for chemical identification. Moreover, the catalytic hydrogenation procedure allowed the absolute configuration determination of five substances in just one experiment employing gas chromatography-flame ionization detection technique with chiral column. In summa, we report herein the detection and first synthetic studies for new (1) and unusual (2). Studies concerning to characterization of compound (3) and the biological activities of these metabolites are being carried out currently. Financial support FAPESP 05/02934-4.

*Absolute configuration not determined - trace amounts*

Refs.
The genus *Salacia* (Celastraceae) has many species distributed in Brazil. Extracts from *Salacia* species has been used during thousand years in Ayurvedic medicine for oral diabetes treatment. The inhibition of intestinal α-glucosidase activity has been demonstrated using roots extracts of *S. oblonga*. Therefore, it holds potential as a natural method to mitigate the blood glucose response of people with diabetes. This work shows the study of *Salacia elliptica*, known as “Bacuri”, “Saputá” and others folk names. The plant was collected in “Mata Samuel de Paula”, in Nova Lima region, Minas Gerais state. Dried and powdered leaves (394 g) were submitted to exhaustive extraction with hexane (H), ethyl acetate (EA) and methanol (M), at room temperature. After solvent withdrawn under reduced pressure, the H (7.97 g), EA (8.80 g) and M (36.55 g) extracts were obtained. Each extract was submitted to silica gel column chromatography using appropriate eluent polarity. From H extract were isolated a mixture of long chain saturated hydrocarbons, with C_{29}H_{60} (1) as a principal constituent, a mixture of pentacyclic triterpenes constituted by 3β-stearyloxy-ursan-12-ene (2) and 3β-stearyloxy-olean-12-ene (3), 1,4-trans-polisoprene (4) (gutta-percha), friedelin (5), β-friedelinol (6) and β-sitosterol (7). From EA extract (6), canofilol (8) and β-sitosterol-3-O-β-D-glucopyranoside (9) were isolated. Dulcitol (10) was isolated from M extract. The structure of these constituents was confirmed by HRGC (comparison with standard) and spectrometric methods (IR, $^1$H and $^{13}$C NMR with 2D experiments).

1. B.W. Wolf et al., *Food and Chemical Toxicology*, 41, 867 (2003)
2. A.M. Flammang et al., *Food and Chemical Toxicology*, 44, 1868 (2006)

Thanks FAPEMIG for financial support.
Compounds from bark of Maytenus salicifolia Reiss. (Celastraceae)

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Maytenus salicifolia is encountered in forests of Minas Gerais state, Brazil. It is popularly known as “Cafezinho” and is used in medicine folk for stomach ulcer treatments (1). The decoct is topically employed in the form of bath to alleviate itches and allergic symptoms. The pharmacological activities attributed to different Celastraceae species (2) and the popular use of M. salicifolia induced its phytochemical study. A sample (1.25 g) of dried and powdered bark wood was submitted to exhaustive hot extraction with hexane, ethyl acetate and methanol. After filtration, the solvent was removed under reduced pressure and the resulted extracts were submitted to fractionation through silica gel G-60 (70-230 Mesh) column chromatography (CC), with graduating polarity of hexane, chloroform, ethyl acetate and methanol, as eluents. Sephadex LH-20 CC eluted with methanol also was used to isolate and/or purify compounds. Lupeol (I), 4’-O-methylepigalocathechin (II), 2-ethyl-1-(4-diethyl)-octyl phthalate (III) and a mixture of proanthocianidins (IV) (Fig. 1) were isolated from ethyl acetate extract. These compounds were identified by CCD comparing with authentic samples and through spectrometry [IR, ¹H and ¹³C NMR including 2D experiments]. Until this moment, a triterpene I, the flavonoid II, the asymmetric phthalate III and a mixture of proanthocianidins IV were isolated from bark. The continuity of studies certainly will become possible the isolation of new compounds, that permits the establishment of the biosynthetic routes of different M. salicifolia constituents.

References
PHYTOCHEMICAL STUDY OF Bowdichia virgilioides Kunt. (FABACEAE)

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Bowdichia virgilioides Kunt. (Fabaceae) is a medium size tree found in the tropical forests of South America. In Northeastern Brazil it is popularly known as “sucupira”, and its bark is used for healing of wounds, as anti-ulcer and anti-diabetic while the seeds are used in the treatment of rheumatism, arthritis, and skin diseases. The importance of this plant promoted its inclusion in the first Brazilian Pharmacopoeia. Various bioactivities, including antimalarial, hypoglycemic and inhibitor of the enzyme acetylcholinesterase, of crude extracts from this plant were reported. Previous chemical investigation resulted in the isolation of flavonoids, benzo furanoids, essential oil, triterpenoids and alkaloids. This study reports the structural identification of flavan-3-ol from the acetic phase. The plant was collected in December 2005, near the city of Santa Rita, State of Paraíba, Brazil, a coastal area around the Atlantic Forest and identified by Maria de Fátima Agra from Laboratório de Ciências Farmacêuticas in the Universidade Federal da Paraíba. This material was submitted to dry heat around 45°C and powdered until 3 kg and exhaustively extracted with ethanol (95%), obtaining the ethanolic extract (EE). The EE was partitioned in hexane, chloroform and ethyl acetate. The acetic phase was analyzed with Sephadex LH-20, in mixtures of chloroform and methanol 1:1, obtaining 42 fractions organized and visualized with ultraviolet light. The sample 18-19 was also submitted to successive purifications resulting in a red solid compound. The identification was based on experiments using 1D and 2D NMR (200MHz) in methanol-d4 and comparison with literature data to identify Bv-1 as 3,3’,4’,5,7-pentahidroxiflavan known as epicatechin. Through the phytochemical study of Bowdichia virgilioides Kunt, flavonoid epicatechin, already known in the family, yet isolated for the first time in genus Bowdichia.

Keywords: Fabaceae, Bowdichia virgilioides, Epicatechin.

Refs.
TWO NEW FLAVONOLS FROM THE LEAVES OF *ARRABIDAEA BRACHYPODA* (BIGNONIACEAE)

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The Bignoniaceae family is constituted by 120 genera and 800 species distributed throughout tropical regions of South America and Africa⁴, and species from the genus *Arrabidaea* have been used in traditional medicine for wound asepsis and treating intestinal disorders⁵. Our previous work on *Arrabidaea samydoides* led to the isolation of six xanthones, which showed moderate free radical scavenging activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH)³. As part of our continuing interest in exploring the chemistry of Brazilian medicinal plants species, an ethanolic extract of the dried leaves of *A. brachypoda* was partitioned with solvents with different polarities and the EtOAc fraction was analyzed by HPLC-UV. Further, the EtOAc fraction was purified by preparative HPLC to give two new flavonols arrabidoside A (1) and arrabidoside B (2) along of the known, isoquercetrin (3) and rutin (4). The analysis of the antioxidant activity was performed with DPPH and the determination is done through the percentage of free radical scavenging. Their structures were determined on the basis of spectroscopic analysis, and all the isolates exhibited antioxidant activity in the DPPH assay, using rutin as positive control.

![Chemical structures](image)

(1) R = feruloyl  
(2) R = sinapicoyl  
(4) R = H

References.
Flavonoids from *Plinia cauliflora* leaves

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*Plinia cauliflora* is a plant belonging to Myrtaceae family that occurs in Brazil, Argentine and Paraguay and has comestible fruits already studied. However, their leaves are still without chemical and biological evaluation. The aim of this work was to evaluate *Plinia cauliflora* leaves composition. The leaves extract was obtained with 70% ethanol by percolation and dried under reduced pressure. Liquid–liquid partition of the extract (15.0 g) was done with 500 mL of water and 500 mL of ethylacetate. After dried, ethylacetate fraction was dissolved in methanol and chromatographed on Sephadex LH 20 column (72.0 x 2.5 cm) using methanol as mobile phase. The fractions obtained were analyzed by thin layer chromatography and similar samples were grouped according their Rf values. Purified flavonoid fractions were dissolved in deuterated DMSO and analyzed by NMR spectrometer operating at 500 MHz. Using the comparison of their 1D and 2D NMR spectroscopic and physical data with those from previous reports in the literature, the isolated compounds were assigned to be the flavonoids quercetin-3-O-β-glucopyranoside, myricetin-3-O-β-glucopyranoside, myricetin-3-O-β-galactopyranoside and myricetin-3-O-β-allopyranoside. Flavonoids could be responsible for many biological activities, as anti-inflammatory, antimicrobial and antioxidant. The knowledge of *P. cauliflora* leaves composition together with biological activity studies could result in efficient therapeutic uses for people of this brazilian widely known vegetable species.

Support: PADC-UNESP.

Refs.
PHYTOCHEMICAL STUDY OF PIPER MALACOPHYLLUM (PIPERACEAE)

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The genus *Piper* is a source of several classes of bioactive secondary metabolites including alkaloids, amides, flavonoids, benzoic acid derivatives, terpenes, and cyclopentanediones.1 Recently, two butenolides (1 and 2) were isolated from leaves of *Piper malacophyllum* in a bioactivity-guided phytochemical investigation due to the strong activity observed against *Cladosporium cladosporioides* and *C. sphaerospermum*.2 However, an HPLC analysis of the crude extract showed that there are other derivatives, which were not detected in the activity-guided fractionation. Thus, in the present work we report the complete phytochemical analysis from the leaves of *P. malacophyllum*. Additionally, the composition of fruits, stem and roots extracts have been described by use of HPLC and HRGC/MS experiments.

The dried and powdered leaves of *P. malacophyllum* were extracted with MeOH and the crude extract was concentrated and partitioned between MeOH/H2O and CH2Cl2 to yield the CH2Cl2 residue. An aliquot of this residue was subjected to successive chromatographic procedures to afford six compounds, which were identified by spectral analysis: 4,6-dimethoxy-5-\(E\)-phenylbutenolide (1), 4,6-dimethoxy-5-\(Z\)-phenylbutenolide (2), 1,2-methylene dioxy-4-(oct-3-\(E\)-enyl)benzene (3), shizuka-acoradienol acetate (4), shizuka-acoradienol (5) and 4-(dec-3-\(E\)-enyl)phenol (6).

The dried and powdered fruits, stem and roots were individually extracted with MeOH and the crude extracts were treated in C18 sep-pak (MeOH) and analyzed by HPLC and HRGC/MS. In all extracts was verified the predominance of 6, a different profile observed to the leaves extracts, in which the butenolides 1 and 2 were the major compounds. Indeed, in the fruits and stem extracts was also detected the new compound 3. Butenolides and alkylbenzene derivatives are rarely found in the *Piper* genus while sesquiterpenes have been detected in several species1. However, this is the first occurrence of acorane sesquiterpenes in *Piper* genus being compound 4 a new natural product.

A NEW TETRAHYDROFURAN LIGNAN AND OTHER METABOLITES FROM *Peperomia blanda*

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*Piper* and *Peperomia* represent the most important genera of the Piperaceae family with 2000 and 1700 species, respectively. Species of *Peperomia* are well known as ornamental plants and have found application in folk medicine for the treatment of inflammation, asthma and gastric ulcers, and as analgesic and antibacterial agents. *Peperomia blanda* is a perennial herb that typically grows in wet rock crevices. To date, the only chemical informations available are on the composition of essential oil and on the structures of two chromenes isolated from apolar fraction of its aerial parts. In the present work, we report on the isolation and structural elucidation of one tetrahydrofuran lignan (1), two secolignans (2-3), two flavones (4-5) and one polyketide (6). The EtOAc extract derived from aerial parts of *P. blanda* was submitted to partitions and chromatographic column to yield six compounds. The structures of the isolates were elucidated by interpretation of their spectral data, including gHMQC and gHMBC, while the relative and absolute configurations were determined from NOESY data and optical properties, respectively. The secolignans, flavones and polyketide have been isolated from other *Peperomia* species but the tetrahydrofuran lignan is a new natural product apparently resulting from a mixed coupling between sinapyl alcohol and propenylphenol unities.

![Chemical structures of compounds](image)

Refs.
EVALUATION OF PHENOLIC COMPOSITION FROM SOUTH BRAZILIAN CECROPIA SPECIES

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Department of Pharmaceutical Science, Universidade Federal of Santa Catarina – SC/BR

Introduction: Cecropia catherinensis Cuatrec. (sin.: C. pachystachya Mart.) and Cecropia glaziovii Sneth. are abundant in South of Brazil, and its infusion are used in the folk medicine as cardiotonic, diuretic and for the treatment of inflammation and cases of asthma. Although they have morphological differences in their aerial parts, there are few phytochemical analysis with C. catherinensis and, as far as we are aware, there are no report comparing the chemical composition of these species. Considering that these species are used as medicinal plants, often without correct identification, the development of thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) methodology may contribute significantly to the differentiation between these plants and to their control quality as raw materials.

Objective: The aim of this study was to compare the phenolic composition from different extracts of C. catherinensis and C. glaziovii. Methodology: Aerial parts from both species were crushed and extracted, by aqueous infusion and aqueous decoction. The extracts obtained, after evaporated to dryness under reduced pressure, were analyzed by TLC, HPLC and UV-spectrometry (total phenolic content). In order to evaluate the flavonoid fingerprint, the aqueous infusions were successively partitioned with AcOEt and n-BuOH, and these last one were analyzed by TLC and HPLC. Results: The extracts of plants were prepared in order to obtain polar compounds, considering their popular uses. The TLC and RP-HPLC analysis for crude extracts did not shown qualitative differences in the phenolic composition between extraction methods, but allowed to difference between species. The total phenolic content higher values were verified for aqueous decoction from C. glaziovii, e for aqueous infusion from C. catherinensis. The analysis of butanolic fractions from both species aqueous infusion extracts by TLC, using AcOEt:MeOH:H2O (10:1,5:1 v/v/v) as mobile phase and RP-HPLC, with a linear gradient of ACN:H2O, suggest that majors flavonoids presents in C. glaziovii were isovitexin and isoorientin, while in C. catherinensis were predominant orientin and isoorientin. Additionally, rutin and chlorogenic acid were verified in both species. Conclusion: It is possible to differentiate C. glaziovii and C. catherinensis species by their total phenolic content and by their C-glycosil flavonoid fingerprint.

Refs.
Great variety of secondary metabolites, such as alkaloids, flavonoids, coumarins, limonoids, etc, is Rutaceae characteristic. These classes of metabolites present biological activities of pharmacological and agronomical importance, that make relevant to study plants of this family [1].

The Helietta genus (Rutaceae) is distributed in neotropical region and plants of this genus can be obtained in México, USA, Cuba, Venezuela, Colombia, Peru, Paraguay, north of Argentine and Brazil. This genus is few studied chemically. For example, the specie H. apiculata presents activity in the central nervous system and this fact justify studying other plants of this genus. Nothing is known about other species [2].

The aim of this communication is to present further compounds obtained from the hydroalcoholic partition of branch ethanol extract of Helieta puberula (Rutaceae). This extract was fractioned by column chromatography with silica gel yielding coumarins: 3'- (1',1'-dimethylallyl)-isoescopoletin, graveliferone methyl ether and alkaloids: N-methyl-4-methoxy-2-quinolone, dictamin, maculin and, in mixture, rutaecarpin and 7,8-dehydrorutaecarpin, previous isolated from H. puberula by Simote [3] and the furthers alkaloids: flindersin, N-methylflindersin, evolitrin, maculosidin, robustin and edulitin; coumarin: bergapten and Limonoid: limonin. The identification of these compounds was done by analysis of their 1D and 2D NMR (1H and 13C NMR, 1H-1H COSY45, HSQC and HMBC), MS, IR and UV data.


FAPESP, CNPq, CAPES
**COMPLETE $^1$H AND $^{13}$C NMR STRUCTURAL ASSIGNMENT FOR TWO NEW SESQUITERPENE LACTONES FROM *Tithonia diversifolia.*

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*Tithonia diversifolia* is a Mexican shrub from Asteraceae family naturalized around the tropics. Its crude extracts and secondary metabolites present several biological activities. Sesquiterpene lactones (STL) were detected in glandular trichomes of the leaves and inflorescences of a Brazilian population. The dichloromethane rinse extracts of the leaves and inflorescences were investigated. In this work, we describe the total elucidation and NMR assignment of two novel STL isolated from *T. diversifolia*: a furanheliangolide derivative (1) and another with an unusual seco-guaianolide skeleton (2). As part of our work on the NMR complete assignment of STL, both structures were clarified using $^1$H-NMR, $^{13}$C{$^1$H}-NMR, DEPT135, gCOSY, gHMQC, gHMBC and NOEdiff techniques. All NMR experiments were performed on a Bruker Avance DRX500 spectrometer. Most chemical shifts and scalar coupling constants were measured and assigned after careful analysis of the $^1$H-NMR spectra. 2D NMR information also contributed to the total $^1$H assignments.

### Table 1: $^1$H and $^{13}$C NMR data and structures of 1 and 2. (CDCl$_3$ – 500 MHz)

<table>
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<th>Position</th>
<th>$^1$C</th>
<th>$^1$H (J in Hz)</th>
<th>$^1$C</th>
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<td>123.3</td>
<td>6.38 dd (3.4)</td>
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<tr>
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<td>dd (2.5)</td>
<td>56.1</td>
<td>dd (3.0)</td>
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<td>23.6</td>
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<td>175.6</td>
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<td>34.1</td>
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<tr>
<td>3</td>
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<td>1.05 d (7.0)*</td>
<td>18.9</td>
<td>1.10 d (7.0)*</td>
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<tr>
<td>4</td>
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<td>1.04 d (7.0)*</td>
<td>19.0</td>
<td>1.13 d (7.0)*</td>
</tr>
<tr>
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<td>59.1</td>
<td>3.38 s</td>
<td>59.1</td>
<td>3.38 s</td>
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</table>

*integrated to $^3$H

The gHSQC experiments were enough to assign most $^{13}$C signals, but for quaternary carbons, an extra help was provided by gHMBC. The novelty, attributed to structure 2 made the elucidation of this compound severally harder than the other, which has an known kind of structure. Only with NOE experiments data and detailed mixing of NMR information, the structures of 1 could be confirmed and of 2 could be finally assigned.

**Refs.**


BPS-193
**IRIDOID AND SECOIRIDOIDS FROM GUETTARDA Pohliana**

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*Guettarda pohliana* Müll Arg. (Rubiaceae) is popularly know as “angélica-domato”. In a previous work we have reported the isolation of 3-**O-βD-quinovopyranosyl-28-O-β-glycopyranosyl-quinovic acid and its aglycone from the roots of specie [1]. Further investigation of the AcOEt root extract of *G. pohliana* led to the isolation of iridoid glycosides loganic acid (1), sweroside (2), the seco-iridoid secoxyloganin (3), 4,5-**O-dicaffeoylquinic acid (4) and 28-**O-β-glycopyranosyl-quinovic acid (5). These compounds were isolated by successive silica gel column chromatography and Sephadex LH 20 filtration, and their structures were characterized by one and bi-dimensional $^1$H NMR and $^{13}$C NMR spectroscopy and comparison with literature data [2, 3, 4,5]. The compounds loganic acid, sweroside and secoxyloganin were previously reported in *G. platypoda* [6] and their occurrence in *G. pohliana* may be of chemotaxonomic significance, since iridoids have been used as chemotaxonomic markers in the Rubiaceae family plants [7].

![Chemical structures](image)

**Refs.**
The family Rubiaceae, one of the largest among the Angiospermae comprises around 637 genera and approximately 10,700 species, mostly spread in tropical regions of Brazil. Its species are very used as eatable plants, as ornaments purposes and in the pharmaceutical industry. *Richardia brasiliensis* Gomes is commonly known as “poaia branca” which is native from south region of Brazil and also utilized as anti-emetic and to treat diabetes. Phytochemical analysis demonstrated the presence of alkaloids, terpenoids, steroids and phenolic compounds, including flavonoids. This class of compounds is known for their properties as anti-oxidants and anti-ulcer. This study reports the structural identification of glycoside flavonoids from the ethyl acetate phase. The botanic material was collected in Santa Rita- PB and identified by Maria de Fátima Agra from Laboratório de Tecnologia Farmacêutica of the Universidade Federal da Paraíba. This material was dryed and powdered to obtain 2.3 kg and exhaustively extracted with ethanol (95%), obtaining the ethanol extract (EE). The EE was partitioned in hexane, chloroform and ethyl acetate. Chromatography methods (ethyl acetate phase) were carried out to obtain 14 fractions organized by their Rf’s using TLC. The samples 12 and 13 were joined and chromatographed over Sephadex LH-20, eluted with chloroform and methanol 1:1, obtaining 9 fractions. The sample 12-13.2 was also submitted to successive purifications resulting in a yellow solid compound. The identification was based on experiments using 1D and 2D NMR (500MHz) in methanol-d4 and comparison with literature data what led to identify Rb-1 as Isorhamnetin-3-O-Rutinoside, already known in the family, but isolated for the first time in its genus *Richardia*.

**Keywords:** Rubiaceae, Richardia brasiliensis, Isorhamnetin-3-O-Rutinoside

**Figure 1. Isorhamnetin-3-O-Rutinoside**

Refs.

A New Bidesmosidic Oleanane Saponin from *Chiococca alba*

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*Chiococca alba* is a tropical and sub-tropical shrub spread all over the American continent. Commonly known as “cainca”, the infusion of its roots is used in traditional medicine for the treatment of several illnesses¹. Previous works on *C. alba* reported the isolation of lignans, coumarins, ketoalcohols², triterpenes³, quinoline alkaloids⁴, a bioactive ent-kaurane and a new nor-seco-pimarane⁵, three new iridoids and a new seco-iridoid⁶, flavonoids⁷ and saponins⁸. In this report we present the isolation and structural elucidation of a new saponin from *C. alba*.

The ethanol extract of the roots of *C. alba* was evaporated and dissolved in water. After several partitions with dichloromethane and butanol, the butanol fraction was evaporated and the residue resuspended in methanol. This solution was subjected to controlled precipitation with ethyl ether. The precipitate was fractionated by mpc yielding several saponin rich fractions. Further purification was made by preparative reversed phase hplc.

The high resolution electrospray mass spectrum of the saponin displayed quasi-molecular peaks at m/z 1039.5122 ([M-H]⁻; negative mode) and at m/z 1063.5084 ([M+Na]⁺; positive mode) consistent with a molecular formula C₅₂H₈₀O₂₁. ¹H NMR (methanol-d₅, 400MHz): 0.63; 0.85; 0.88; 0.90; 0.93; 1.06; 1.16; 1.27; 2.93; 3.17; 4.38; 5.06; 5.33; 5.46; 5.44; 5.55; 5.66; ¹³C NMR (methanol-d₅, 100MHz): 16.0 (CH₃); 16.9 (CH₃); 18.3 (CH₃); 18.5 (CH₃); 19.4 (CH₂); 23.5 (CH₃); 24.3 (CH₂); 25.0 (CH₃); 27.1 (CH₂); 28.4 (CH₃); 31.3 (C); 33.6 (CH₃); 33.6 (CH₃); 33.6 (C); 33.8 (CH₂); 33.6 (CH₃); 38.2 (C); 39.7 (CH₂); 40.2 (C); 41.2 (C); 43.4 (CH); 44.6 (CH₂); 45.5 (C); 48.7 (CH); 57.0 (CH); 64.0 (CH₂); 65.5 (CH₂); 67.4 (CH); 68.9 (CH); 71.7 (CH); 72.5 (CH); 73.2 (CH); 74.4 (CH); 75.1 (CH₂); 75.3 (CH₂); 77.7 (CH); 78.4 (CH); 79.9 (CH); 80.6 (C); 91.1 (CH(OH)); 94.1 (CH); 101.5 (CH); 107.0 (CH); 111.6 (CH); 124.6 (CH); 128.7 (CH); 137.5 (CH); 141.6 (C); 176.1 (C=O); Acid hydrolysis of the saponin afforded 3β-hydroxiolean-12,15-dien-28-oic acid and the monosaccharides rhamnose, arabinose and apiose (1:1:1) based on GLC analysis. The structure of the new saponin (28-O-α-D-apiofuranosyl (1→3)-α-L-rhamnopyranosyl(1→2)-α-L-arabinopyranosyl 3-O-β-D-glucopyranuronosyl-3β-hydroxyolean-12,15-dien-28-ate) is proposed based on chemical and spectral evidences (COSY, HSQC, TOCSY, TROESY and HMBC)².

Piperaceae comprise approximately 3000 species distributed among four genera (Jaramillo et al., 2004). One of the largest genera of this basal angiosperm family, *Peperomia*, includes both terrestrial and epiphytic species. In Brazil, 154 herbaceous species have been described, and several of them are widely cultivated as ornamental plants. A number of traditional medicine uses for *Peperomia* species has been reported in South America and China. The phytochemical investigations carried out on species of *Peperomia* have revealed unusual secolignans such as peperomins. The biosynthetic origin of peperomins is unknown and their occurrence in the plant kingdom is quite restricted. The peperomins A - F were previously isolated from *Peperomia japonica* (Chen et al., 1989), *P. dindigulensis* (Govindachari et al., 1998), *P. glabella* (Monache and Compagnone, 1996) and *P. pellucida* (Xu et al., 2006). The biological activities described to these compounds include anticancer and anti-HIV (Zhang et al., 2007).

In this study we isolated and characterized three new peperomins, denominated G, H and I, besides peperomins A, D, and E from *P. glabella* (Sw.) A. Dietrich and *P. glabella* var. *nervulosa* (C.DC.) Yunck, cultivated in facilities at IQ-USP. The methanol and ethyl acetate extracts of the fresh whole herbs were chromatographed on a silica gel column, and the peperomins had their structures elucidated by analysis of spectrometric data.

GLYCOSIDIC IRIDOIDS FROM *MOLOPANTHERA PANICULATA TURCZ* (RUBIACEAE, POSOQUERIEAE).

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Rubiaceae is a large family of about 650 genera and more than 13,000 species, many of them being important medicinal plants¹. In Brazil, the Rubiaceae are represented about 110 genera and 1700 species². The tribe Posoquerieae was recently described by Delprete³, based on general morphology, palynology, floral morphology and phylogenetic evidence, to include *Posoqueria* and *Molopanthera*. The two genera have been traditionally positioned in the two distantly related tribes: *Posoqueria* in the Gardenieae, and *Molopanthera* in the Rondeletieae². In order to examine the chemical relationships between the two genera, *Molopanthera* is examined, and preliminary results are here presented. *Molopanthera* is a rare, monotypic genus of trees endemic to the Atlantic forests of Brazil, mainly in the states of Bahia, Espírito Santo and Minas Gerais.

Experimental: Plant material of *Molopanthera paniculata* Turcz. was collected and identified by Delprete. Voucher specimens are deposited at UFG Herbarium. Leaves (40 g) were powdered, air-dried and submitted to extraction with ethanol by cold percolation and the crude extract was extracted with hexane, ethyl ether, AcOEt, propanone and MeOH. The different fractions were concentrated to dryness under reduced pressure. The ethyl ether fraction (0.9 g) was subjected to silica gel CC eluted with CH₂Cl₂ and MeOH, resulting in 43 fractions. Pooled fraction (116-128, 269 mg) was purified by preparative TLC yielding pure iridoids 1 (66 mg) and 2 (6.5 mg). Identification and structural elucidation of isolated compounds were performed using spectroscopic analysis of 

$$\text{O}$$

$$\text{COOCH₃}$$

$$\text{OH}$$

$$\text{H}$$

$$\text{H}$$

$$\text{1=R=H; 2=R=COCH₃}$$

$$\text{4}$$

$$\text{5}$$

$$\text{8}$$

$$\text{9}$$

$$\text{10}$$

$$\text{O}$$

$$\text{Gly}$$

$$\text{RO}$$

$$\text{1=R=H; 2=R=COCH₃}$$

$$\text{RO}$$

$$\text{1=R=H; 2=R=COCH₃}$$

Glycosidic iridoids in Rubiaceae are rather common. However, in *Posoqueria latifolia* were only isolated two non-glycosidic iridoids⁴, and this report presents the isolation of glycosidic iridoids 1 and 2 in *Molopanthera*.

Refs.

The genus *Myrcia* is one of the largest American genera of the Myrtaceae and comprises more than 300 species spread from Mexico to southern region of Brazil. Some species of this genus have presented microbicide, antidiabetic and antitumor activities. The chemical evaluation of *Myrcia guianensis*, harvested in the sand dunes of Salvador, Bahia state, northeastern region of Brazil, has presented relevant results which contribute to the chemical understanding of this family. Until now were isolated two new compounds: a C-methylflavone, 5,4′-dihydroxy-7,3′-dimethoxy-6,8-dimethylflavone and a chlorinated sesquiterpene, along with the known compounds 5,4′-dihydroxy-7-methoxy-6,8-dimethylflavone, 3,4′,5′,7-pentahydroxyflavone, ursolic acid, bourbonenone and 4-hydroxy-4,7-dimethyl-1-tetralone. The sesquiterpenes 1 and 2 were isolated from the dichloromethane phase of the ethanolic-extracted protocol after separation by repetitive silica-gel column chromatography. Their structures were characterized by 1H NMR, 13C NMR, HMBC, HMQC, MS and by comparison with published data. The 1H NMR spectrum of compound 1 revealed the presence of a prenyl group (δ 0.74, d, 3H and δ 1.01, d, 3H) and signals for two methyl groups (δ 2.05, 3H and δ 2.25, 3H). Besides these signals, it presented two doublets at δ 6.68 (1H) and δ 6.84 (1H) and one doublet of doublets at δ 6.88 (1H) indicating one aromatic ring trisubstituted. The 13C NMR spectrum presented 15 signals, being four of them methyls carbons (δ 20.7, δ 20.8, δ 21.2 and δ 30.0), two methylenes (δ 26.6 and δ 41.8), five methines (δ 32.9, δ 44.2, δ 115.6, δ 127.2 and δ 128.5), three quaternary carbons (δ 129.7, δ 129.8 and δ 152.0) and a carbonyl carbon at δ 210.6. The comparison to literature indicated it as the sesquiterpene sesquichamaenol. The compound 2 exhibited similar 1H and 13C NMR spectrum to that of 1. The differences in the 1H NMR spectrum of compound 2 were the presence of two singlets at δ 6.8 and δ 7.0 (such, 1H) indicating the presence of an aromatic ring tetrasubstituted. The 13C NMR spectrum confirmed the presence of an aromatic ring tetrasubstituted by the two signals of methine carbons at δ 126.6 and δ 127.8. The mass spectrum analysis of compound 2 presented, besides the molecular ion peak at m/z 268, a peak M + 2 presenting the third part of the intensity of the molecular ion, indicating a chloro atom presence. The occurrence of halogenated compounds is unusual in higher plants and there is no report of their presence in the Myrtaceae family. The chlorinated sesquiterpene isolated in this study was described for the first time.

CHEMICAL STUDY OF THE ENDOPHYTIC FUNGI *Xylaria* sp. AND *Colletotrichum crassipes*, ASSOCIATED TO *Casearia sylvestris*.

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Endophytic fungi live in the internal tissues of plants for at least part of their life cycle; they can also inhabit the plant for a lifetime, being transmitted, in some cases, to future generations through the seed of the host. The endophytic fungi *Xylaria* sp. and *Colletotrichum crassipes* were isolated from healthy leaves of the plant *Casearia sylvestris*, popularly known as "chá de bugre" and "guaçatonga". These fungi were cultivated separately in plates containing PDA (potato dextrose agar) medium for one week. Following, they were inoculated in flasks containing PDB (potato dextrose broth) liquid medium, where they were maintained under agitation (in shaker) during 28 days, at 25°C. Elapsed this time, filtration was accomplished to separate the mycelium from the fermented broths. The filtered broths were submitted to liquid-liquid partition with ethyl acetate. Evaporation of the organic phase resulted in the crude extracts. The crude *Xylaria* sp. extract was submitted to column chromatography using normal phase silica and eluted with hexane, ethyl acetate and methanol, in increasing order of polarity. The collected 54 fractions, after analyzed by TLC were joined in 10 sub-fractions. From the sub-fractions E (10.0 mg) and I (3.0 mg) were isolated the compounds 1 (3.0 mg) and 2 (3.0 mg), respectively. The crude *Colletotrichum crassipes* extract was submitted to column chromatography in reverse phase C18 silica and resulted 14 fractions, which were analyzed by HPLC-DAD. The fraction 7 (12.7 mg) was submitted to preparative HPLC, resulting in the isolation of the compound 3 (2.0 mg). A fraction of the crude extract was also submitted the column chromatography using normal phase silica and resulted in the isolation of the compound 4 (2.5 mg) and 5 (3.0 mg). Analysis of the spectrometric data of the compounds 1, 2, 3, 4 and 5 allowed to identify them as Cytochalasin C, carboxylic 6-hydroxy-3-methyl-3,4-dihydroisocoumarin-5-acid, (6-methyl-3-(phenylethoxy)-1,4-dioxan-2-yl)-methanol, tyrosol and 1-hydroxy-1-phenylethyl-tyrosol, respectively. Searching the available databases resulted in no report about the compounds 3 and 5, evidencing that they are new. Concerning the compound 2, this is the first description of it as natural product. The results evidence how promising the endophytic fungi are in producing new metabolites with diverse and complex chemical skeletons. This fact justifies the urge to study chemically this niche of microorganisms, once it constitutes a source in potential for new compounds and is still little explored.

Ref.

Financial support FAPESP and CNPq
TRITERPENES AND STEROID ISOLATED FROM LEAVES OF _EUGENIA BEAUREPAIREANA_ KIAERSK.

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The _Eugenia_ is one of the largest genera of the Myrtaceae, and comprises around 350 native species¹, and some are used in folk medicine for their anti-diarrheic (_E. uniflora_) and antidiabetic (_E. jambolana_) properties². _Eugenia beaurepaireana_ (Kiaerskou) Legrand is a tree that grows in the Brazilian coastal forest and is commonly known as ingabaú. The popular use of this specie is recommended for the treatment of inflammatory and ulcerative diseases and used as astringent.

The aerial parts of _E. beaurepaireana_ were collected in Santo Amaro da Imperatriz (SC), Brazil, in August 2004 and identified by Prof. Dr. Daniel de Barcellos Falkenberg (Botany Department, UFSC) - SC, Brazil. The air-dried plant material (leaves) was powdered and extracted by maceration with ethanol to yield the crude ethanol extract. The crude ethanol extract was re-suspended in aqueous ethanol solution (70 %), and filtered to give a fat fraction, called resin (15.4 g). The resin was chromatographed on silica gel column, with eluents of increasing gradient of polarity (hexane/ethyl acetate/ethanol), to afford two white amorphous compounds, 1 (1.2 g) and 2 (17.7 mg).

Compounds 1 and 2 were analyzed by spectroscopic methods IV (KBr), NMR ¹H (400 MHz, CDCl₃), ¹³C (100 MHZ, CDCl₃) and capillary gas chromatography (GC-FID, GC Shimadzu 14 B; N₂, flow rate 1.0 mL min⁻¹; column DB-1, 30 m × 0.25 mm, I.D. × 0.25 µm film thickness; T °C column: 00 °C, increasing rate of 10 °C min⁻¹ to 300 °C; T °C injector: 300 °C, T °C detector: 290 °C; injection volume: 1.0 µL). Individual components were identified by co-injection and comparison with authentic standards and comparison of NMR literature data³. The quantitative data were obtained by electronic integration of the GC-FID peak areas.

Compound 1 showed melting point of 184.9 – 187.4 °C. The analysis of the gas chromatogram showed to be about the mixture of two substances, and its structure was confirmed as mixture of α and β-amirin (in 7,1:2,9 proportion). Compound 2 showed melting point 142.5 – 144.1 °C, characteristic of steroids, and identified as β-sitosterol. The identification of these compounds were given through the spectroscopic data of IV and NMR, in comparison to the data literature.

References
Bignoniaceae comprises 113 genera and 800 species. Species of this taxon are found throughout the tropical regions of the world and frequently occur in the Americas. The most representative members of this family are Tabebuia alba (“ipê amarelo”), T. avallanedae (“ipê roxo”) and Jacaranda brasiliiana (“jacarandá”). The genus Tabebuia has been the subject of a number of investigations and is well known by the occurrence of naphthoquinones, furanonaphthoquinones and iridoids, among other classes of secondary metabolites. We have recently reported the isolation and structure elucidation of 2-(4-hydroxyphenyl)-ethyl-1-O-β-D-apiofuranosyl-(1→6)-β-glucopyranoside, 2-(4-hydroxyphenyl)-ethanol, 6-O-E-p-coumaroyl-catalpol, coumaric acid and sitosterol from the trunk bark and of 6-O-E-cafeiroyl-catalpol, kaempferol, kaempferol-3-O-β-D-glucopyranoside, ursolic and betulinic acids and sitosterol from the leaves of Tabebuia insignis.

In continuing our investigations on the chemical constituents of the trunk bark of this species, we herein report the isolation of betulinic acid (1) and catalpol (2). Air-dried and powdered trunk bark of T. insignis (1200 g) was extracted at room temperature with ethanol. After concentration in vacuo, the ethanol extract (154.9 g) was successively subjected to column chromatographic separations on silica gel, Sephadex LH-20 and RP-18 silica gel to afford compounds 1 and 2. The structure elucidation of 1 and 2 was accomplished mainly on the basis of the interpretation of NMR spectroscopic data. The antibacterial activities of 1 and 2, as well as of the substances previously isolated from the trunk bark and leaves against Staphylococcus aureus were also evaluated in the present work, but nevertheless, all of them were devoid of any activity in this assay.
Introduction: *Luffa operculata* (L.) Cogn (Cucurbitaceae) is popularly known as buchinhapaulista, buchinha-do-norte and cabacinha. It occurs mainly in the North, Northeast and Southeast of Brazil. Their dry fruits are commonly used in popular medicine as nasal decongestant, sinusitis treatment. This species also is used improperly as abortifacient. The main compounds identified in *Luffa* genus are cucurbitacins, saponins and proteins. A qualitative assay (CCD with blood agar) showed a hemolytic activity for cucurbitacin B.

Objectives: The aim of this study was isolate and identify the cucurbitacins from *Luffa operculata* fruits. Material and methods: The mesocarp from *Luffa operculata* fruits were macerated with ethanol 96% during seven days. The dry extract was resuspended in water and successively extracted with petroleum ether and dichloromethane. The dichloromethane fraction (DF) were chromatographed on successive silica gel column with petroleum ether:ethyl acetate:isopropanol (20:5:0.5, v/v/v) as mobile phase. From DF were isolated three compounds named LO1-LO3. The compounds LO4 and LO5 where also isolated by silica gel column with methylethylketone:dichloromethane:isopropanol:water (10:2:2:5, v/v/v/v, organic phase) and dichloromethane:methanol (80:10, v/v) as mobile phase. For detection were used phosphoric vanilin, tetrazo1 blue and ferric chloride. LO6-LO9 were isolated through HPLC, using column reverse silica C18 (28cm X 2,5cm), mobile phase: acetonitrila:water (20:30, v/v), detector UV (230nm) and flow: 4mL/min. The compounds were identified on the basis of spectral data (NMR, UV and IR), comparison with authentical sample and, if necessary the compounds were submitted to acid hydrolyse with HCl 10% to identified the sugar. Results: LO1, LO2 and LO3 were identified as cucurbitacin B, isocucurbitacin B and cucurbitacin E, respectively. Through of CCD, IR and acid hydrolyse was observed that LO4 and LO5 are glycosides. Their structures are being elucidated by NMR spectroscopy. The compounds LO6 and LO7 were identified as cucurbitacin I and cucurbitacina D, through of comparison with authentic sample. Finally, LO8 was identified as isocucurbitacin D using $^1$HNMR, $^{13}$CNMR, HMBC, COSY, ROESY and HSQC.
Chemical constituents from *Palicourea rigida* (Rubiaceae)

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*Palicourea* Aubl. (Rubiaceae), besides the closely related genus *Psychotria*, are known as a prolific source of micromolecules such as indoles alkaloids\(^1\), pyrrolidinoindoline alkaloids\(^2\), terpenoids, benzoic acids and coumarins\(^3\) which possess a biological and chemistry diversity. Recently, the genera *Palicourea* and *Psychotria* were selected by a US NCI anticancer screening as a “hot” genera within Rubiaceae family\(^4\). In our ongoing search for new biologically active secondary metabolites from Brazilian Cerrado species, the *Palicourea rigida* was studied because the crude ethanolic extract showed significant cytotoxicity to human melanoma cancer cells line (SK MELL 37).

The aerial parts of *P. rigida* were collected in Goiânia/GO, Brazil, air-dried, ground and extract with ethanol three times at room temperature. After removal the solvent in vacuo the residue was sequentially partitioned with hexane, chloroform, ethyl acetate and water. The ethyl acetate fraction was chromatographed over silica gel 60 using hexane, ethyl acetate and methanol mixtures in increasing polarity. The ethyl acetate-methanol 10\% fraction was submitted to purification by Sephadex filtration with methanol and the resulting was the flavonoid (1) and the iridoid (2). Besides, the chloroform fraction was chromatographed over silica gel 60 using hexane/ethyl acetate gradient system to furnish a coumarin (3). The identification of the known compounds 1-3 involved the analysis of 1D and 2D NMR (\(^13\)C NMR, \(^1\)H NMR, DEPT, \(^1\)H-\(^1\)H COSY, HMQC, HMBC) spectral data and comparison with literature values\(^5,6,7\).

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Species belonging to the Aristolochiaceae family have been used in Brazilian traditional medicine as stomachic, anti-inflammatory, antiasthmatic, and abortifacient. Flavonols, dihydroflavonols and isoflavonols have been isolated from these species. However, the occurrence of bi- and tetraflavonoids is restricted to Aristolochia ridicula Brown. This work deals with the isolation and structural elucidation of three new flavonoids: a biflavone (1), a chalcone-flavone dimer (2), and a tetraflavonoid (3) from the leaves of A. ridicula. The natural occurrence of tetraflavonoids is rare and limited to A. ridicula, Lophila alata, Lophila lanceolata (Ochnaceae), and Cephalotaxus wilsoniana (Cephalotaxaceae).

Compounds 1-3 were isolated by partition and chromatographic procedures from the acetone extract, and were analyzed by HPLC-UV-MS and spectrometric methods (IR, UV, NMR, and MS). Compounds 1 and 2 were determined as biflavonoids based on the HRMS and NMR analyses. The HRMS spectra displayed quasi-molecular ions [M−H]− at m/z 567.0913 (C31H21O11) for 1 and at m/z 595.1228 (C33H24O11) for 2, and the IR spectra showed a characteristic absorption band for aromatic ketones at 1626 ± 2 cm−1. The 1H and 13C NMR spectra of both compounds suggested the presence of 1,4-disubstituted, 1,3,4-trisubstituted, tetrasubstituted, and pentasubstituted aromatic rings in the structures. Moreover, these spectra showed a signal for a methoxyl group and two signals for similar carbonyl groups for 1, whereas, three signals for methoxyl groups and two signals different for carbonyl groups were observed for 2. Furthermore, the correlations observed by gHMBC and gNOESY experiments allowed us to establish a biflavone structure for 1, which the monomer units should be linked through positions C-3 and C-6", and a chalcone-flavone dimer for 2 with furanic aromatic ring linked through position C-6 of the flavone. The structure of 3 was also determined based on the HRMS and NMR experiments as a tetraflavonoid constituted of two flavones and two chalcones unities. The HRMS spectra of 3 displayed a quasi-molecular ion [M-H]− at m/z 1192.2462 (C66H48O22), and the 1H and 13C NMR spectra suggested the presence of pairs of 1,4-disubstituted, 1,3,4-trisubstituted, tetrasubstituted, and pentasubstituted aromatic rings in the structure of 3. In addition, these spectra showed six signals for methoxyl groups and four signals for carbonyl groups. Based mainly on MS, gHMBC, and gNOESY experiments it was possible to establish a new carbon skeleton for 3.

Refs.
Acosmium dasycarpum is a plant characteristic from the Brazilian savannah. It is found almost exclusively in the Central and Northeastern states, especially in Bahia, Minas Gerais, São Paulo, Mato Grosso and Goiás. This plant is popularly known as “perobinha do campo, chapada, pau-paratudo, unha d’anta and genciana”. It has been used as tranquilizer, hipotensive, antineoplastic, antisiphylis, antirheumatic and in the treatment of cutaneous problems. Based on the information above we have performed a phytochemical study of the roots of A. dasycarpum. So far, we have isolated and characterized from the methylene chloride extracts the following compounds: lupeol (1) and lupenone (2), (E)-6-(4-hydroxystyryl)-4-methoxy-2H-pyran-2-one (3), bowdenol (4) and alkaloid bowdichine (5), a diaza-adamantane skeleton containing an N-acetyl enamine moiety. All these compounds have previously been isolated from other plants belonging to Acosmium and Bowdichia genera.

Refs.
PHYTOCHEMISTRY OF ROOT AND LEAF EXTRACTS OF Coccoloba mollis

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Coccoloba mollis [Polygonaceae] is a medicinal herb widely used in Londrina as memory modulator and energetic. No studies have been found on bibliographical research about this species. Extractives of roots and leaves were subjected to bioassay as for their cytotoxicity [MTT] and genotoxicidad [Comet assay]. The results obtained from the roots extracts in preliminary investigation were more cytotoxic than the ones obtained from the leaves extracts. This work presents chemical evaluation of the ethanolic extracts of C. mollis. Root and leaf extracts were fractioned by column chromatography using solvents of increasing polarity. These fractions where submitted to other purification techniques such as preparative thin-layer chromatography, crystallization, centrifugation and filtration. These procedures led to the isolation of compounds. Through spectroscopy methods (NMR, \textsuperscript{1}H/\textsuperscript{13}C, CG-MS, IV) the compounds identified in the extracts of the roots were: antraquinones [fission, emodin], hydrocarbons and esters of long chains. In the leaves, hydrocarbons [C-16 to C-31] except C-17 were identified. The isolation of antraquinones in this plant is very important in therapeutic and medicinal chemistry. These compounds present several actions: anti-mutation antibacterial antifungal, active against Schistosoma mansoni, anti-inflammatory, laxative and anticancer. In spite of the benefits, the indiscriminated use of this plant as phytoterapic can be harmful to the health if the results of the preliminary tests of cytotoxicity and genotoxicidad are taken into consideration.
A NEW BIPHENYL FROM Clusia burle-marxii (Clusiaceae).

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The Family Clusiaceae comprise more than 1200 species distributed in 49 genera. The species of Clusia was known as source of benzophenones, xanthones, biphenyls, steroids, terpenes, flavonoids, and tocotrienols [2]. The species Clusia burle-marxii was collected in the neighbourhood of Cachoeira do Fraga in Rio de Contas, Chapada Diamantina, BA.

Phytochemical investigation of the leaves and trunk of Clusia burle-marxii (Clusiaceae) led to isolation of nine compounds. From ethyl acetate partition of methanolic extract of trunk were isolated the compounds 3-β-hydroxylup-20(29)-en-28-oic acid, 3-oxo-fridelane, 3,4-dihydroxybenzoic acid, lyoniresinol, 2, a 9,9′-dihydroxyariltetralin lignan, reported for the first time in the family [3], and a new biphenyl, 1. From ethyl acetate partition of leaves ethanolic extract were isolated the compounds 3-α-L-rhamnopyranosylquercetin [1,4], 3-α-L-rhamnopyranosylkaempferol [1,4], 3. The structural elucidations were based on spectroscopy techniques: GC/MS, ¹H-NMR, ¹³C-NMR, 1D (DEPT 135, NOEDIF), 2D (HMBC and HMQC) and comparison with literature date.

Aspidosperma ramiflorum Muell. Arg. (Apocynaceae) commonly known as “guatambu” is a tree which grows from 12 to 30 m in height and is native to the forests in South-eastern Brazil\(^1\). This study aims to investigate the iridoid content of A. Ramiflorum. Air-dried stems was extracted with methanol at room temperature and after removal of the solvent, the crude extract was submitted to basic-acid extraction. Fractionation of part of the aqueous phase (AP) by preparative thin layer chromatography (PTLC) on silica gel (ethyl acetate / methanol, 40:60) led to the isolation of a mixture of loganic acid (1) and its salt form (2). The salt form of loganic acid was isolated after semi-preparative high performance liquid chromatography of another portion of AP (previously submitted to PTLC on silica gel using methanol in NH\(_4\)OH atmosphere). Another part of the aqueous phase was chromatographed on silica gel column (ethyl acetate / methanol / water / triethylamine, 50:44:5:1) and afforded a new loganic acid derivative (3). The occurrence of iridoids is not uncommon in the Apocynaceae\(^2\), but this is the first time that iridoids are isolated from species of the Aspidosperma genus.

Ref.
1. H. Lorenzi, Árvores Brasileiras, Plantarum, Nova Odessa, São Paulo, Brazil, 21 (1992)
LAGGER AMOUNT OF CABREUVIN FROM *Myroxylon peruiferum*.

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*Myroxylon peruiferum* (L.f.) [syn. *Myroxylon balsamum* (L.) Harms] (FABACEAE) has its dispersion is wide, being spread from the South of Mexico until the north of Argentina. It is a large size tree, with white flowers, winged and aromatic fruits. It is known as cabriuva and cabriuva-vermelha. Its balsam, known as balsam of Peru or Tolu or red oil is rich in vanilla. The oil was used formerly in popular medicine as expectorant for breathing affections and as a sedative in case of cystitis [1].

Previous chemical study of this plant furnished monoterpenoids, sesquiterpenoids, alcohols, phenylpropanoids, isoflavones, pterocarpan, coumestans, flavanone, isoflavanones and arybenzofuran [2,3]. Cabreuvin, 3’,4’,7-trimethoxyisoflavone, isolated from wood present potent anti-*Helicobacter pylori* activity [4].

This communication has as objective to present the isolation of larger amount of cabreuvin from sawdust of *Myroxylon peruiferum*. The sawdust (5 kg) was extracted with dichloromethane yielding (36 g). This extract was fractioned in silica gel chromatography using isocratic mode yielding 1,87 g of pure cabreuvin. Cabreuvin present in its structure groups with potential to produce complex with metals ions (Ca$^{2+}$, Mg$^{2+}$ e Zn$^{2+}$) and polypyridin metals transition complex [Mn(II;III), Co(II;III), Cu(II) and Fe(II;III)] with potential to improve its activity against bacteria. These complexes are in development and they will be tested as insecticidal, fungicidal and antibacterial assays.


FAPESP, CNPq, CAPES
The use of medicinal plants is an ancient practice in human populations. However, there are few scientific studies and the risks of their consumption is no known. The present work aimed chemical evaluation of the ethanol extract of the leaves from the *Serjania sp*. The Sapindaceae are mostly trees and shrubs, and tendril-bearing vines comprising about 140 genera and 1500 species. The *Serjania* genus Will has 200 species and 80 with occurrence in Brazil. Members of the genus *Serjania* have been employed in the folk medicine for the treatment as inflammatory and anti-ulcers. The leaves were collected at Santo Antonio do Leverger/MT State, Brazil. 400.0 g were dried, powdered and percolated at room temperature with ethanol (2 L). The solvent was evaporated under reduced pressure. The ethanol extract (3.0 g) was partitioned between water, ethyl acetate and n-butanol (1:1, v/v 3 times), affording 500 mg of the ethyl acetate, 4.5 g of the n-butanol and 4.7 g of the aqueous phases. An aliquot of the ethyl acetate fraction (400 mg) was fractionated by HSCCC (High Speed Counter-Current Chromatography). The fractions were analyzed by 1D and 2D NMR to afford quercetrin 1, kaempferol-4′-rhamnoside 2 (8 mg) and quercetin 7-methoxy-O-α-L-rhamnopyranoside-(1″→4′′″)-4′′′O-β-D-apiose 3 (10 mg). High-speed countercurrent chromatography (HSCCC) is a leading method for the fast separation of natural products from plants. It was used for the isolation of three flavonoids identified by first time in leaves from *Serjania sp*. The family Sapindaceae is known by the presence of saponins few stories on flavonoids and in the few works they had been told in literature on the chemistry of this genus.

FAPESP/CNPq/CAPES
The Euphorbiaceae is a complex and diverse family with 317 genera and about 8,000 species spread all over the tropics. *Croton* is the second most abundant genus with 1,900 species wildly dispersed at the Antilles, South and North Americas. *Croton* is also a genus very common to the northeastern Brazil flora designated “Caatinga” characterized by plants well adapted to the drastic climatic conditions [just a dry (7-8 months) and a rainy (4-5 months) season occur]. *Croton rhamnifolius*, a shrub, sometimes a small tree, popularly known as “quebra-faca” (Port. lit.: knife breaker) due to the hardness of its wood, grows widely in the semiarid region at the border of Ceará and Pernambuco States. Entire specimens of re-sprouting stalks of *C. rhamnifolius* were collected at Salgueiro County-PE at the beginning of the rainy season in February, 2006. The plant material was divided in leaves, roots, trunk and stems. The leaves yielded an essential oil obtained by hydrodistillation while the other parts, after milling, where extracted with hexane followed by ethanol. TLC and $^1$H NMR analyses of all extracts suggested the root EtOH extract as the candidate to start the research project. Thus, it (60 g) was resuspended in MeOH and liquid-liquid partitioned (by adding water dropwise when necessary to separate the phases) with hexane, CHCl$_3$ and EtOAc. All organics fractions were dried over anhydrous Na$_2$SO$_4$ and rotoevaporated while the residual hydromethanol solution was rotoevaporated and lyophilized. From the hexane fraction (3,18 g), after silica gel column chromatography, lupeol and 12-oxo-stachenone were obtained. Both normal and flash silica gel column chromatography were used to analyse the CHCl$_3$ fraction (32,27 g) affording the triterpene alleuritolic acid, the diterpene stachenone and a novel indol diterpene adduct, designated rhamnifolin (32,1 mg), 1. All structures were elucidated by spectroscopic means, particularly $^1$H and $^{13}$C NMR, applying modern two-dimensional pulse sequences (COSY, HSQC, HMBC, NOESY, etc.). There is no report of indol terpenoids from plants but from endophytic fungi what brings the suspicion that rhamnifolin could indeed be a metabolite from an associated fungus, made by the incorporation of tryptophan to a kaurane type diterpenoid very common in *Croton*. However, rhamnifolin has shown a promising tripanocidal activity at the preliminary tests.

![Chemical Structure](image)

CNPq, CAPES, FUNCAP, FINEP.
The marine ecosystems spread over 70% of the Earth surface and is related to 95% of the biosphere. They have raised the interest of the scientists all over the world as a prolific source of new chemical entities with biological activity. Sponges are marine animals well dispersed through the oceans wild life and have been the subject of several successful research projects on biologically active secondary metabolites of marine origin. The Ceará State, Northeast of Brazil, is located just on the right corner of Brazil near to Africa and thus on a region where the marine currents change their directions. Supposing that this could affect the marine organisms life style, and their metabolism, it was decided to examine the chemical analysis of sponges from that coastal zone.

*Monanchora arbuscula* Duch. & Mich (Crambiedae) is one of the nine sponges species recognized for the genus, characteristic of shallow waters, found in the State Park of “Pedra da Risca do Meio” under ~18 m deepness. After draining the collected material (1.215 kg) was stored in ethanol. Separated from the hydroethanol solution the animal residue was blended with a CH$_2$Cl$_2$/EtOH 1:1 solution and filtered. The organic solution was liquid-liquid partitioned with petrol ether followed by CH$_2$Cl$_2$ and EtOAc. Adsorption chromatography analysis of the petrol ether extractives (4.15 g) revealed the presence of a mixture of steroids not yet identified. From the CH$_2$Cl$_2$ fraction (7.34 g), upon adsorption and gel filtration chromatography analyses, mirabilin B (1; 26.0 mg) and a mixture of the epimers 8$\alpha$ and 8$\beta$ of 1,8a;8b,3a-didehydro-8-hydroxyptilocaulin (2 and 3; 6.0 mg) were isolated. From the EtOAc fraction (2.56 g) after a conjunction of column chromatography over silica gel and HPLC reverse phase ($C_8$) chromatography two other guanidine alkaloids 8$\beta$-hydroxyptilocaulin (4; 34.0 mg) and ptilocaulin (5; 24.5 mg) were obtained. All structures were characterized by spectroscopic analysis, particularly one ($^1$H, $^{13}$C BB and $^{13}$C DEPT) and two dimensional NMR (COSY, HSQC and HMBC), and comparison to the literature data. 1, 2 and 3 have already being reported for *M. unguifera* (Hua et al; 2004), but not for *M arbuscula*. 4 and 5 have been reported previously for *M. arbuscula* (Tavares et al; 1995).

![Chemical structures](image)

Ref:
A NOVEL FLAVANONE FROM POLYGONUM SPECTABILE


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The screening of 10 weed medicinal plant extracts for in vitro antimicrobial activity showed that Polygonum spectabile was active against Staphylococcus aureus and Micrococcus luteus. Bioguided chromatographic fractionation of the ethanol extract from the aerial parts of P. spectabile resulted in an active fraction, that afforded three flavonoids designated PS1, PS2 and PS3. Spectrometric analysis allowed the characterization of PS2 as 7-hydroxy-5-methoxyflavanone (alpinetin) (1) and PS3 as 2',4'-dihydroxy-3',6'-dimethoxychalcone. The latter compound has already been isolated from Polygonum senegalense (2). Characterization of PS1 as 7-hydroxy-5,8-dimethoxyflavanone was accomplished by IR, UV, FAB-HRMS, $^1$H NMR and $^{13}$C NMR, assigned by means 2D NMR experiments (HMQC and ROESY). According to a literature review, the compound PS1 is a novel flavanone.

Brazil has a great diversity on plants that possess non-researched medicinal potential and are promising sources of therapeutic and pharmacological innovations to the most diverse areas of human health\(^1\). The Rubiaceae family is considered the biggest one of the order Gentianales\(^2\) and presents around 637 genera and 10,700 species\(^3\). The species *Richardia grandiflora* (Cham. & Schltdl.) Steud., known popularly as “ervanço”, “poaia” or “ipeca-mirim”, has ethnopharmacological indications to use as decoction against hemorrhoids and as vermifuge\(^4\). Aiming at contributing to the chemotaxonomic study of the family Rubiaceae and considering the absence of data in literature about the chemical constitution of the species *Richardia grandiflora*, the latter was submitted to a phytochemical study to isolate and identify its chemical constituents. The collected plant material (entire plant) was processed yielding the crude ethanol extract which was submitted to low-pressure chromatography giving ten fractions, being three of them submitted to column chromatography using different adsorvents. The resulting fractions were analysed through analytical thin-layer chromatography (TLC) and joined according to their RF’s. Therefore, five constituents were isolated: a mixture of the steroids sitosterol and stigmasterol, orto-benzoic acid, \(m\)-methoxy-p-hydroxy-benzoic acid and phaeophitin a, all of them isolated for the first time from the genus *Richardia* and identified by means of spectroscopic methods such as \(^1\)H and \(^{13}\)C Nuclear Magnetic Resonance, with the add of two-dimensional techniques (COSY, HSQC, HMBC and NOESY), besides comparison with literature data.

Refs.
2 V.P. Coelho et al., Rev Bras Farmacogn, 16 (2), 170-177 (2006).
3 S. Mongrand et al., Phytochemistry, 66, 549-559 (2005)
4 M.F. Agra et al., Rev Bras Farmacogn, 17 (1), 114-140 (2007).
A NEW DAVANONE CHEMOTYPE OF LIPPIA INTEGRIFOLIA

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The genus Lippia (family Verbenaceae) comprises some 200 species growing in the tropical and subtropical regions of South America, Central America and Africa. In the Americas, members of this genus can be found from Mexico to the province of Buenos Aires in Argentina. Because most of the species are aromatic, the studies on the chemistry of this genus are mostly related with the composition of the essential oils, where an outstanding feature of Lippia is the difference observed in the essential oil composition reported for the same species from different geographic origins. However, the mono- and sesquiterpenoids found in the essential oils for all the Lippia species investigated so far are quite common and widespread in the plant kingdom, the exceptions being L. integrifolia (Gris.) Hieron. which produces ketones based on the unique sesquiterpene skeletons named lippifoliane and integrifoliane.

Lippia integrifolia (Griseb.) Hieronymus, commonly known as ‘incayuyo’ or ‘té del inca’, is a woody aromatic shrub native to central and northern Argentina, where infusions of the aerial parts are widely used in traditional medicine (1). L. integrifolia has been shown to be a rich source of uncommon sesquiterpenoids. The unique ketones integrifolian-1,5-dione, lippifoliane-1(6)-en-5-one and closely related derivatives together with sesquiterpenoids based on the rare africanane and asteriscane skeletons, constitute the bulk of its essential oil (2).

In the present study, we report the results of a detailed analysis carried out on the essential oils obtained from two selected populations of L. integrifolia growing wild in northern Argentina: C653 (Santa María Departament, Catamarca Province, voucher specimen LIL 607197) and C660 (Cachi Departament, Salta Provincie, voucher specimen LIL 607276) which allowed the identification of a family of structurally related sesquiterpene compounds, and isomers, belonging to the group of the ketone davanone. These compounds and their distribution in both populations evaluated allowed proposing a new chemotype for L. integrifolia characterized by high contents of davanone and davanol isomers.

Previous reports indicated davanone oil as possessing a promising aromatic profile for perfumery, novel foods and beverages (3). The notes were related to davanona and described as green, pungent, harsh, herbal, intense davana-like, fruity.

The present study focuses on the comparison of sensory odour profile of L. integrifolia essential oil by GC-O, and the odour perception -as an entity- at different retention times, in order to evaluate the aromatic response (aromagram) and the behaviour of davanone and davanol isomers.

Refs.
1. G. Bassols et al., Dominguezia, 13, 7 (1996)
The genus *Caesalpinia* (Caesalpinioideae, Fabaceae), comprised of tropical or subtropical trees or shrubs, contains more than 150 species worldwide. Native Brazilian species such as *Caesalpinia echinata* (pau-brasil) had important economic value in the early colonial period of Brazil. Previous studies of species of this genus report remarkable biological activities for its species such as antimicrobial, antidiabetic (*C. bonducella*), antimalarial (*C. volkensii* and *C. pluviosa*), and antiinflammatory activities (*C. sappan* and *C. ferrea*). *Caesalpinia pyramidalis* Tull is an endemic tree of northeastern region and one of the predominant species in the "caatinga" vegetation. In Bahia State, it is popularly known as "catingueiro" or "pau-de-rato", and its leaves are employed in traditional medicine as diuretic, dyspeptic, digestive, and antipyretic. This work describes the chemical study of the methanol extract wood and leaves of *C. pyramidalis* (Leguminosae). The CHCl$_3$ and EtOAc fractions obtained from the partition of MeOH extract were submitted to different chromatographic procedures over silica gel and Sephadex LH-20. The organic extracts of leaves of *C. pyramidalis* were submitted to different chromatographic procedures which permitted to isolate agathisflavone, podocarpusflavone A, loniflavone (3), glycosil sitosterol besides the mixture of apigenin and kaempferol$^1$ and amentoflavone (1). On the other hand the methanolic extract of the wood of *C. pyramidalis* afforded taxifoline, gallic acid, 5,6,7,8-tetrahydroxy-4'-methoxyflavone, siringaresinol, methyl gallate, pheofitina and 5'-hidroxyamentoflavone$^2$ (2), the unique biflavonoid found in trunk wood of this plant. To date, *C. pyramidalis* is the first species in the genus to present biflavonoids.

All compounds were identified by analysis of IR, UV, MS $^1$H and $^{13}$C NMR data, including bidimensional techniques. The biflavonoid (2) was only obtained from Bartramia ithyphylla (Bartramiaceae) e Rhytidiadelphus squarrosus (Hylocomiaceae).

Ref:
1 M. V. Bahia et al., *Journal of the Brazilian Chemical Society*, 16, 1402 (2005)
2 M. V. Bahia, Tese de Doutorado, Universidade Federal da Bahia, 2007
A NOVEL ESTER FROM HIMALAYAN CEDARWOOD OIL.

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Essential oil extracted from aromatic plants find application in flavour, fragrance industries and in aromatherapy. India with its rich bio and agro diversity is a fertile ground for the production of essential oil and their value added derivatives. However, several natural raw materials consist mainly of hydrocarbons that are not suitable as smelling substance. These hydrocarbons often possess complex carbon skeletons, which are difficult to synthesize, but offer excellent possibilities for functionalization with oxygen containing groups transformations that often lead to interesting new fragrant materials.

In the present work isolation from Himalayan cedarwood oil two sesquiterpenic ketones which were found to be $\alpha$-atlantone and $\gamma$-atlantone. The succesful routes involved the preparation of novel ester molecule of atlantone & study its odour profile. Stability study of these molecules in cosmetic products was carried out.

References:

Piper aduncum L. (Piperaceae) contains a number of chromenes that possess both anti-fungal and anti-tumour activities.1,2 The isoprenoids constitute a large class of natural products comprising more than 29,000 compounds. The prenyltransferases, which are responsible for the key biosynthetic step in the formation of prenylated derivatives, are of evolutionary interest and may be appropriate for further exploitation as a tool for the prenylation of benzoic acid and other aromatic compounds.3 In this work, we tested four protocols for enzymatic extraction of P. aduncum leaves collected in three different sites near Araraquara city. The assay for prenyltransferase activity was based on the conversion of methyl 2,2-dimethyl-2H-1-chromene-6-carboxylate (1) into methyl 2,2-dimethyl-8-(3'-methyl-2'-butenyl)-2H-1-chromene-6-carboxylate (2) using protein extracts and DMAPP as prenyl donor (Figure 1).4 Protein extracts (0.500 mg suspended in 70 µL) were incubated at 37.5 °C with 200 µL of 0.1 M KPi buffer (pH 8.0), 10 mM MgCl₂, 26 µL of 1 (0.13 mM) and 26 µL of DMAPP (0.13 mM). The higher enzymatic activity, with 16.6 % of conversion to 2, was observed with the protein extract from the specimen collected in Basalto Park, using extraction buffer containing 0.5 M KPi buffer (pH 8.0), 10% (w/w) sucrose, 1.5% (w/v) PEG, 20 mM ascorbic acid, 50 mM cysteine and 1 mM EDTA (10 min at 13,000 rpm).

![Figure 1. Conversion of methyl 2,2-dimethyl-2H-1-chromene-6-carboxylate (1) into methyl 2,2-dimethyl-8-(3'-methyl-2'-butenyl)-2H-1-chromene-6-carboxylate (2) by enzymatic extracts from leaves of Piper aduncum using DMAPP](image)

References

FAPESP, CAPES, CNPq
The major constituent of leaves and roots of *Potomorphe umbellata* is the powerful antioxidant, 4-nerolidylcatechol\(^1,2\). Based on such activity, the extract obtained from roots of *P. umbellata* has been recently introduced in the market as active ingredient of an anti-aging lotion\(^3\). The biosynthetic pathway to the formation of 4-nerolidylcatechol in *P. umbellata* involves 3,4-dihydroxybenzoic acid and nerolidol as precursors as demonstrated by incubation of these compounds with cell free extracts prepared from leaves of young plants (Figure 1). Aiming to obtain this compound by green chemistry methodology, the enzyme responsible for 4-nerolidylcatechol formation was purified from leaves of *Potomorphe umbellata*\(^4\). The purification method was optimized as following. The crude extract was obtained from young leaves of *Potomorphe umbellata* using pH 8.0 TRIS-HCl buffer, in a ratio 1:3 of amount of leaves and buffer. The crude extract was submitted to heat denaturation at 70°C for 15 minutes followed by renaturation mixing the solution gently at 4°C. This solution was submitted to centrifugation and the supernatant was submitted to fractionated precipitation with ammonium sulfate. The fraction 20-40% of saturation yielded the enzyme responsible by the formation or 4-nerolidylcatechol with high purity. All processes used during the optimization to purify the enzyme of interest were accompanied by protein determination, enzymatic assay and SDS-PAGE.

![Figure 1: Biosynthetic route to the formation of 4-nerolidylcatechol.](image)

Refs.
PEROXIDASES FROM NEEDLES OF ARAUCARIA ANGUSTIFOLIA
AND THEIR CIRCADIAN RHYTHM

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The Araucaria angustifolia is an endemic coniferae of southern and southeastern Brazil. It composes the “Mata de Araucárias” an important bioma endangered due the extensive loggings. Their leaves contain six major amentoflavone-type biflavonoids, including amentoflavone, ginkgetin and tetra-\(O\)-methylamentoflavone as determined by HPLC-ESI analyses. These compounds are reported to possess a variety of biological activities especially as antioxidants, which, is related to their capacity to suppress singlet oxygen, lipoperoxidation, and DNA oxidation promoted by several oxidant agents. An additional mode of action is related to the ability to quench transition metals involved in free radical formation (Yamaguchi e col. 2007).

The biosynthesis of biflavonoids has been proposed to take place by oxidative coupling of two flavonoids units. The predominance of amentoflavone-type biflavonoid in Araucaria angustifolia indicates the specificity of peroxidase involved in its formation. The peroxidase activity was detected in the enzymatic extract from needles of Araucaria angustifolia. The enzyme convert two molecules of apigenin to amentoflavone and ginkgetin. The products were identified and quantified by its UV-spectrum, LC/mass spectrometry analysis and comparison with reference compound. The circadian rhythm was characterized, as well as, the optimum conditions to enzymatic conversion, such as, pH, temperature and \(H_2O_2\) concentration.

Piperaceae family comprises 4 genera and more than 4000 species and, among them, *Piper* is one of the most abundant genus with approximately 2000 species. *Piper crassinervium* species accumulates antifungal and antioxidant geranylated metabolites derived from benzoic acid and from *p*-hydroquinone. Geranyltransferases are a group of prenyltransferases that catalyze the C-geranylation on several substrates. They have been generally reported as membrane-bound enzymes and have showed strict substrate specificity for geranyldiphosphate (GPP) as the prenyl donor, although accept a variety of substrates as acceptors of GPP moieties. Substrate specificity studies carried out with *P. crassinervium* indicated that 3,4-dihydroxy benzoic acid was selectively geranylated by cell free extracts from its leaves. In this work, we report the substrate specificity studies for geranyltransferase by means of cell free and microsomal fractions from leaves and roots of *P. crassinervium*. Crude protein extracts were submitted to geranyltransferase activity assays with GPP, MnCl₂ or MgCl₂ as cofactors, and the substrates *p*-hydroquinone (HQ), 4-hydroxybenzoic (4-HB) and 3,4-dihydroxybenzoic (3,4-HB) acids. The microsomal fractions were obtained by ultracentrifugation as described. The enzymatic activity was assessed in all fractions by HPLC-UV (table 1). Differences in substrate specificity were observed, suggesting two geranyltransferases in *P. crassinervium*. One in soluble fraction, with affinity to 3,4-HB, and a second in the microsomal fraction with affinity to both HQ and 4-HB. SDS PAGE analysis indicated an electrophoretic band in the microsomal fraction with MW between 45-66 kDa, which is similar to that reported for geranyltransferases.

**Table 1**: Summary of geranyltransferase activity in enzymatic fractions from *P. crassinervium*.

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Roots</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Microsomal</td>
</tr>
<tr>
<td>HQ</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>4-HB</td>
<td>n.d.</td>
<td>+</td>
</tr>
<tr>
<td>3,4-HB</td>
<td>+</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

HQ= *p*-hydroquinone; 4-HB: *p*-hydroxybenzoic acid; 3,4-HB: 3,4-dihydroxybenzoic acid.
n.d.=not detected; + =positive reaction, +/-=weak

**Refs.**
IDENTIFICATION AND CHARACTERIZATION OF AROGENATE DEHYDRATASES IN ARABIDOPSIS: COMPARISON TO A BACTERIAL PREPHENATE DEHYDRATASE

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While both prephenate and arogenate have been reported in plants to undergo decarboxylative dehydration via the action of a dehydratases to afford phenylpyruvate and phenylalanine, respectively, neither enzyme(s) nor encoding gene(s) were isolated and/or identified. In this study, a data mining approach was undertaken to attempt to identify the dehydratase(s) involved in Phe formation. This approach suggested that there were six putative prephenate dehydratase (PDT) homologues in Arabidopsis, based on similarity to bacterial PDTs. However, earlier biochemical analyses of cell extracts detected arogenate dehydratase (ADT) rather than PDT activities. All six putative ADTs/ PDTs were cloned and expressed as Nus-tagged recombinant proteins in E. coli. Three of the resulting recombinant proteins more efficiently utilized arogenate than prephenate, with $k_{cat}/K_m$ values of 1050, 7650 and 1560 M$^{-1}$ s$^{-1}$ for arogenate, vs. 38, 240 and 16 M$^{-1}$ s$^{-1}$ for prephenate, respectively. The remaining three, by contrast, had $k_{cat}/K_m$ values of 1140, 490 and 620 M$^{-1}$ s$^{-1}$ for arogenate, with prephenate not serving as a substrate unless excess recombinant protein (>150 µg/assay) was used. For comparative purposes, a previously characterized PDT from Methanocaldococcus jannaschii was assayed under the same conditions; it was shown to have a very strong substrate preference for prephenate over arogenate. All six Arabidopsis genes, and their corresponding proteins, are thus provisionally classified as arogenate dehydratases and designated ADT1 through ADT6.

MICROENCAPSULATION OF BRAZILIAN CERRADO’S PLANTS

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The extracts used as a microencapsulation purpose have been studied before demonstrated activity against cancer cell lines in vitro. This paper reports the microencapsulation of Brazilian’s plants cerrado, using crude extracts of Aspidosperma tomentosum Mart, Gaylussacia brasiliensis Meiss and Pterodon pubescens Benth. The extracts microencapsulated were evaluated for anticancer activity in UACC62 (melanoma), MCF-7 (breast), NCI 460 (lung, non-small cells), OVCAR03 (ovarian), PC0 3 (prostate), HT-29 (colon), 786-0 (renal) and NCI-ADR (ovarian expressing phenotype multiple drugs resistance) cancer cell lines in vitro. A 48 h SRB cell viability assay was performed to determine growth inhibition and cytotoxic properties of the compounds. Cells were treated with at least four different concentrations levels ranging from 0.25 to 250 µL/mL with determination of total growth inhibition (TGI) parameter. The extracts also were evaluated for minimum inhibitory concentration activity in Staphylococcus epidermides ATCC 12228; Pseudomonas aeruginosa ATCC 13338; Salmonella cholerasuis CCT 4296; Escherichia coli CCT 0547 and Candida albicans CCT 776.

An aqueous suspension containing wall material, crude extract and tween 80 was prepared, homogenized and passed through in a mini spray dryer Büchi B-290 using 175 °C of entrance, 100 °C of exit temperature, 6-7 mL/min of liquid flow rate, 600 L/h of nitrogen pressure and 1.4 mm of nozzle diameter and also using a freezy dryer Virtis 8L.

The microcapsules were obtained from acacia gum, using the proportion wall material/extract (4/1).

The particle size distribution was determined by laser scattering using a Malvern Mastersizer/S MAM-5005.

Microcapsules were characterized by scanning electron microscopy (SEM) in order to verify their structural characteristics. Microcapsules were mounted on a sample holder, sputter-coated with a thin layer of gold and examined with a JEOL JSM-T300 electron microscope.

Both microencapsulated extract by spray dryer and freeze maintained the same anticancer activity of the pure crude extract whereas freeze dried material didn’t produce microcapsules structures.
From raw material to Eucalyptus syrup: quality control of the whole process

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Since ancient times, medicinal plants are used for the treatment of human diseases, and nowadays plants still make an important key for primary health care. In the last years, it has been an increasing interest by the big companies in such products, stimulating the standardization and the development of reliable quality control analytical methodologies\(^{1,2}\). One of the most widely used medicinal plant, *Eucalyptus globulus* (Myrtaceae) has been traditionally used for the treatment of respiratory diseases because of its expectorant, mucolytic and antiseptic effects\(^{4}\). So, this work shows the development of syrup containing *Eucalyptus globulus* extract, its standardization, and the validation of the analytical methodology for its quality control, including the raw material, extracts, essencial oil and the final product. According to ANVISA\(^{3}\), the average daily dosage of 1.8-cineol in formulations containing *E. globulus* is 14.0 to 42.5 mg. So, the syrup was made containing 10% w/w of an eucalyptus fluid extract. The method was developed and validated using a Hewlett-Packard 6890N gas chromatograph (Agilent Technologies) equipped with a split/splitless injector inlet and a flame ionization detector. An HP-5 capillary column was used for the analysis and the non linear increasing temperature gradient was: 75 °C to 200 °C, in 20 min. The identification of the main compounds of *E. globulus* was made using GC-MS. For the qualitative analysis, the fingerprints of 12 *Eucalyptus* species were obtained: *E. botryoides, E. citriodora, E. elba, E. globulus, E. grandis, E. microcorys, E. paniculata, E. resinifera, E. robusta, E. saligna, E. tereticornis and E. urophylla*. The chromatogram of *E. globulus* shows the main peak (1.8-cineol) at 5.23 minutes. The chromatograms of the other species have some significant differences and similarities in the presence and quantity of metabolites, which allows a preliminary identification of each species by this method. Besides, the selectivity and linearity achieved, the recoveries were 99.8% and 100.5% for leaves and syrup, respectively. The repeatability, determined by the relative standard deviation in six replicates at test concentration, were 1.86% and 0.92%, respectively. This result is in agreement with the Brazilian regulatory agency (the maximum value established for ANVISA is 5%). The detection limit was 4.79 µg/mL while the quantification limit was 14.51 µg/mL. Finally, the influence of the main modified parameters for the robustness was determined using the Yuden test. The developed method, with internal standardization, proved to be reliable for the quantification of 1.8-cineol, not only in eucalyptus leaves, extracts and essential oils, but also in syrup formulations containing this plant extract, assuring the rastreability of the entire manufacturing process.

Refs.

Kalanchoe pinnata L. is a perennial plant belonging to the Crassulaceae family. This species is easily cultivated and widely used in folk medicine of tropical regions to treat respiratory infections, bruises, edema of legs and burnings. In Brazil this plant is known as saião, folha da fortuna, coirama branca, among others. According to literature, the main chemical constituents described for this species belong to the class of flavonoids. The objective of this work is to compare K. pinnata cultivation in relation to the edaphic characteristics of a seedbed without soil manipulation with other seedbed treated with a variable amount of calcareous for pH adjusting purposes. Additionally, the chromatographic profiles of these plants extracts were analyzed to compare the flavonoid constitution on carried out experiments. The vegetal species was cultivated in the experimental seedbeds of Farmanguinhos in Jacarepaguá campus (Rio de Janeiro). The aerial parts of plants (leaf, stem and flower) were harvested on flower blooming season. The voucher specimen was deposited at the herbarium of the Botanical Garden of Rio de Janeiro. The extracts of flowers and the mixture of leaves and stems were obtained for maceration process in ethanol 70%. The crude extracts had been compared in regard to flavonoid content using thin layer chromatography (TLC) method. The analyses were carried out on silica gel TLC plates using AcOEt:HCOOH:H₂O (8.8:0.6:0.6) and AcOEt:HOAc:HCOOH:H₂O (10:1.1:1.1:2.7) as solvent systems. The orange spots on TLC were visualized spraying NP/PEG reagent and under UV light (365nm). The plants treated with the calcareous addition (pH=6.8) provided a larger biomass (2.5 kg) when compared to the ones without treatment (pH=5.0) weighting less quantity of biomass (1.4 kg). Nevertheless, the flavonoid composition was similar in both treatments and has not shown qualitative differences in the analyzed extracts. More detailed experiments are necessary to support these results. However, this study under calcareous supply has not shown any relation with flavonoids variation in the K. pinnata aerial parts.
DETERMINATION OF TOTAL FLAVONOID CONTENT FROM LEAVES OF
CULTIVATED Cymbopogon citratus

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Cymbopogon citratus (DC.) Stapf. (Poaceae) is a pleasant-tasting medicinal plant used as tea
(infusion or decoction of its aerial parts) in the folk medicine. This plant is used to treat
digestive disorders, inflammation, nervous disorders and fever. Several literature data have
been supporting its effectiveness in most disorders, which justifies the therapeutic use of this
species. Phenolic compounds are important constituents of leaves and are found in the tea
presentation. Additionally, there are significant data about the antioxidative potential of these
substances obtained by different methods. Considering that this potential is an important
effect, mainly in inflammatory process, this study presents a comparison of the flavonoids
content between the fresh and dried leaves of C. citratus that were collected (4g each) in the
experimental seedbed at Jacarepaguá campus of Fiocruz (Rio de Janeiro state). The total
flavonoid was analyzed according to the Brazilian Pharmacopoea procedure. The content of
flavonoids in the plants leaves, calculated as quercetin, was determined by spectrophotometry
at 426 nm. A Shimadzu UV1601PC spectrophotometer was used for the measurements. The
fresh leaves absorbance was detected at 0.062 nm and for dried leaves at 0.048 nm. These
absorbance values plotted on quercetin calibration curve gave a 1.074 and 0.886 µg/mL of
quercetin content in the fresh and dried leaves. The total concentration of flavonoids in the
fresh and dried plant leaves were calculated at 0.19 and 0.15%, respectively. These
determinations of flavonoid content are in agreement with literature data about antioxidative
potential properties of C. citratus, where these natural compounds are therapeutically related
to this activity. These results can, even partially, attest the popular medicinal use of this plant.
Financial support: CNPq
DETERMINATION OF MINERAL CONSTITUENTS IN PROPOLIS BY FLAME
ATOMIC ABSORPTION SPECTROMETRY

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Propolis, a resin collected by bees from sprouts trees, has viscous consistency, variety of colors ranging from dark brown to red, bitter taste and complex chemical composition. As it is derived from vegetable products, propolis is subjected to environmental contaminations and could accumulate heavy metals in dangerous amounts. Practices such as the paint used in hives and further propolis treatment may influence heavy metals content. The objective of this study was to determine inorganic constituents and the presence of metallic contaminants in samples of crude propolis from Prudentópolis City (States of Paraná-PR). A Varian AA-220 atomic absorption spectrometer (FAAS) equipped with a deuterium-arc lamp background corrector and operating with air-acetylene (Mg, K, Fe, Zn, Cu, Cd, Cr and Pb) and acetylene-oxide nitrous (Ca and Al) flame was used. All chemicals used in the experiments were of analytical-reagent grade. Deionised water (18.2 MΩ cm) obtained from a Human UP 900 System was used throughout the process. All glassware was soaked overnight in 1% v/v nitric acid and rinsed with deionized water before use. Stock solutions of all evaluated species were prepared with concentrations of 1000 mg L⁻¹ (SpecSol, NIST-USA). Figure 1 summarizes the experimental procedure for the determination of metals in propolis. Table 1 shows the average concentration obtained for propolis samples.

Table 1. Metals in propolis (mg/g)

<table>
<thead>
<tr>
<th>Metals</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>2.685±5.10⁻²</td>
</tr>
<tr>
<td>Mg</td>
<td>0.663±4.10⁻⁶</td>
</tr>
<tr>
<td>K</td>
<td>0.138±5.10⁻⁵</td>
</tr>
<tr>
<td>Al</td>
<td>0.114±6.10⁻⁵</td>
</tr>
<tr>
<td>Fe</td>
<td>0.027±5.10⁻⁶</td>
</tr>
<tr>
<td>Zn</td>
<td>0.025±3.10⁻⁴</td>
</tr>
<tr>
<td>Cu</td>
<td>0.005±2.10⁻⁴</td>
</tr>
<tr>
<td>Cd</td>
<td>5.010⁻⁷±0.00</td>
</tr>
<tr>
<td>Cr</td>
<td>&lt;LD⁺⁺⁺</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt;LD⁺⁺⁺</td>
</tr>
</tbody>
</table>

*Detection limit

Figure 1: Procedure for metals determination in propolis (Prudentópolis-PR)

Prudentópolis’s propolis did not show contamination by potentially toxic species and is a good source of Ca, Mg and K. The results obtained for propolis are in agreement with literature. The present study gives information about the mineral content of propolis from Prudentópolis-PR. As heavy metals were below detection limit or in low amounts, this propolis is appropriated for the development of edible products, although other parameters for quality control must be considered as well to abide legislation.

Refs.
ISOLATION OF FLAVANONES FROM Sparattosperma leucanthum BY HIGH SPEED COUNTER-CURRENT CHROMATOGRAPHY

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The family Bignoniaceae is composed of 113 genera and 800 species of trees, shrubs and climbing shrubs. Species of this taxon are located in the tropical regions of the whole world, with frequent occurrence in the American continent¹. In Brazil, plants of this family occur from the Amazon region until Rio Grande do Sul. Sparattosperma leucanthum is popularly known as “Ipê branco”. Naphthoquinones, lignans, triterpenes and flavonoids have been previously isolated from species of Bignoniaceae¹. The only report on phytochemical work on the genus Sparattosperma is that of the isolation of the flavanone pinocembrin-7-O-β-neohesperidoside from fruits of Sparattosperma vernicoseum². The absence of literature data on the chemistry of S. leucanthum allied to the fact that its ethanolic extract possesses high antioxidant activity, motivated the present work. Dried and ground leaves were submitted to extraction with ethanol 96° GL. The crude extract was partitioned between water and hexane, dichloromethane, ethyl acetate and butanol, in this order. All extracts were submitted to antioxidant activity assays with the DPPH radical where the AcOEt extract presented the best results. Preliminary analysis of this extract was made by HPLC (MeOH:H₂O 40:60 → 100:0 in 35 min), showing four major peaks which UV spectra were consistent with flavanone derivatives. The extract was then fractionated by counter-current chromatography (CCC) with the solvent system hexane:AcOEt:MeOH:H₂O 4:10:4:10. About 150mg of the extract were dissolved in both phases of solvent system (5 ml) and injected in a 80ml coil of a PC Inc equipment, organic phase as mobile phase, 2ml/min, 850 rpm. Fractions of 4ml were collected. The rotation was turned off in tube 40 and the stationary phase was, then, fractionated. This procedure resulted in the isolation of the flavanone pinocembrine-7-O-β-(6''-O-acetyl) – neohesperidoside, 1, (F 30-51). HPLC analysis showed that the isolated flavanone has Rf = 16 min. Fractions 52-60 from CCC consisted of a mixture of flavanones with Rf = 6 and 10 min when analyzed by HPLC. These flavanones were separated by preparative HPLC (MeOH:H₂O 40:60 in 75 min). The ¹H-NMR spectrum of the flavanone with Rf = 10 min shows it is a 5,7-diidroxiflavanone diglycoside derivative, probably pinocembrin-7-O-β-neohesperidoside, previously isolated from the fruits of S. vernicosum. Further investigation of the structure of this compound and that of the flavanone with Rf = 6 min are being carried out.

References
COLOR STABILITY OF PIGMENTS OBTAINED FROM THE CYANOBACTERIUM *Nostoc* PCC9205 AS FUNCTION OF STORAGE UNDER DIFFERENT CONDITIONS

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For edible natural pigments, solubility, coloring, stability and safety are important indicative of their applicability. The advantages of producing pigments via microbial fermentation include independence from weather conditions, colors of different shades and the need of small areas for installing bioreactors. Cyanobacteria are photosynthetic microorganisms producers of phycobiliproteins, which are water soluble blue (phycocyanin and allophycocyanin) or red (phycoerythrin) proteins. The aim of the present work was to study the color stability during the storage of an extract of the cyanobacterium *Nostoc* PCC9205 containing phycobiliproteins, which shows a “grape” color.

**Experimental:**

*Nostoc* PCC9205 was grown in BG-11 medium. The water extract from the recovered biomass was used for testing of stability as a function of: pH (4.0, 5.5 e 7.0), temperature (10, 22.5 e 35°C) and storage time (0, 3 and 6 months). The experimental design was multifactorial (full factorial design 2³ with central point). Instrumental color analysis was done with the S & M Suga Color Computer, in the Hunter system. The color indexes measured were: $L^*$ (luminosity), $a^*$ (red index); $b^*$ (blue index) and *haze* (turbidity). Data were analysed using the Statistica 5.0 program.

**Results:**

For the index $L^*$, the most notable effects were caused by the pH and storage time. The highest $L^*$ values are found with pH values of 4 and 7, and the smallest $L^*$ value with pH 5.5, for all storage times. $L^*$ values tend to decrease with the storage time, showing a darkening of the pigments solution. The index $a^*$ is the attribute related with the grade of red, and it is influenced by phycoerythrin. All the factors studied had statistically significant effect on $a^*$. However, the highest effect was due to the pH, and the smallest was due to the temperature. The index $a^*$ tends to decrease with the storage time, demonstrating a tendency of loosing the red color. However, this alteration is more intensive in pH values near 5.5 than 4 or 7. The index $b^*$ is the attribute related with the grade of blue, and it is influenced by phycocyanin and allophycocyanin. The factors pH and time had statistically significant effect on the index $b^*$, but temperature did not have significant effect. In pH values near 4 and 7 the loss of blue color tends to be smaller than that for pH 5.5, showing the highest color stability of the *Nostoc* PCC9205 extract. The index *haze* is related to the turbidity of the pigments solution. The mainly factor with statistically significant effect on this index was storage time. In spite of the little effect of pH on the *haze*, this index tends to be smaller close to the extremes pH values. For all the color parameters considered, the pH values for the higher stability were close to 4 or 7, showing that the *Nostoc* PCC9205 pigments are more interesting for using in acidic foods like beverages, for example, or low acidity foods, like ice cream and others.
COMPARISON OF ANTHOCYANIDINS CONTENT FROM *Arrabidaea chica* VERLOT FROM DIFFERENT BRAZILIAN REGIONS.

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Key word: *Arrabidaea chica*, cultivo, medical plant

*Arrabidaea chica* Verlot (Bignoniaceae) popularly known as Carajuru, Puca Panga, Chica or Pariri is a liana growing in tropical America. Leaves of this plant are used as an anti-inflammatory, astringent agent, as remedy for intestinal colic, sanguine diarrhea, leucorrhoea, anemia, and leukemia in traditional medicine. South American Indians prepare a red pigment from the plants leaves for tattooing.

The use of natural dyes has decreased to a large extent due to the advent of synthetic products. Recently, dyes derived from natural sources have emerged as an important alternative to synthetic ones.

A literature review of *Arrabidaea chica* indicated that this genus is a source of anthocyanins, flavonoids and tannins. The red color has been attributed to carajirun (6,7dihydroxy-5,4’dimethoxy-flavinium) and carajurone.

The scope of this study was to evaluate the red color extract’s chemical composition variation behavior from the plant species obtained throughout Brazil (North, South and West of Brazil) and introduce at CPQBA-experimental field under the same agronomical and climate conditions.

Leaves of *Arrabidaea chica* were obtained from different regions from previously established partnerships and introduced in CPQBA experimental field in January 2005. The leave material was collected, dried and grinned prior to use.

The dry leaves were extracted with methanol / Citric acid 0.3% solution (3X) during periods of 1.5 hours. The solvent was filtered, evaporated under reduced vacuum and freeze dried. The crude extract was cleaned-up on SPE C-18 and further analyzed for carajirun and carajurone by HPLC-DAD (Shimatzu), Phenomenex Gemine C-18 colun (4,6 mm x 250mm i.d. 3 µm), flow rate 1 mL/min, mobile phase Methanol : water phosphoric acid pH 2.00 gradient elution. The samples color values were measured using spectral photometer Color Quest II, Hunter Lab, color CIEL*a* b*, illuminating D 65, angle 10º and calibrates TTRAN (Total Transmittance). Among the three regional samples studied (Manaus-Am; Curitiba-Pr, and Campo Grande-MS) the one from Manaus was the sample that presented the highest carajirun and carajurone ratio, with the most intense red-orange color. The anthocyanin analysis content identified by ESI-MS for the Manaus sample corroborated the above mentioned findings demonstrating higher relative abundance of carajirun (m/z 299) and carajurone (m/z 285) pigments compared to Campo Grande samples, whereas Curitiba sample did not contain those two pigments and presented yellow-green color.

Reference:


Zorn, B. *et al.* Phytochemistry, 2001


Seda Ersus; Unal Yurdagel;. J.of Food Engineering,2006
COUMARIN AND LUPEOL QUANTIFICATION IN GC-MS IN TWO SPECIES OF Mikania spp SUBMITTED TO FOUR LEVELS OF RADIATION INTERFERENCE

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Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs. There are lots of popular homemade medicines that recommend “Guaco” extract (Mikania glomerata and Mikania laevigata) for respiratory and other health problems. Plants of genus Mikania can withstand a tremendous range of light and shade, moisture, and temperature, making it hardy and available to spread to a variety of regions. The present work describes the GC-MS quantitative analyzes of coumarin and lupeol in two Mikania species: M. glomerata Sprengel and M. laevigata Schultz Bip, grown under four levels of radiation: full sun, 25%, 50% and 75% of radiation interference, (I₀, I₂₅, I₅₀ and I₇₅, respectively). It was observed an increase in the concentrations of coumarin and lupeol in both species, as the light intensity decreased. In M. glomerata, the lupeol concentrations were significantly different in the I₇₅ treatment, when compared to the others treatments. In M. laevigata, the concentrations of coumarin were significantly different between the full sun (I₀) and the treatments I₂₅, I₅₀ and I₇₅, but the concentrations of lupeol did not change significantly by effects of any radiation interference treatment. Between the two species, the variations in coumarin concentrations were significant for all treatments. M. laevigata presented 350% more coumarin concentrations than M. glomerata under all levels of radiation. On the other hand, the lupeol concentrations showed an inverse pattern of variation among the species, with M. glomerata presenting concentrations 117% higher than the concentrations found in M. laevigata under all levels of radiation. Physiological parameters as photosynthetic rate, photosynthetic pigments, chlorophyll fluorescence, growth, and dry matter production have been simultaneously studied to determine the possible physiological correlations with these secondary metabolites. (Financial support: FAPESP, CAPES and CNPq)
EXTRACTION OF ACETOGENINS FROM SEEDS OF ANNONA MURICATA L. AND ANNONA SQUAMOSA L. BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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The acetogenins is a class of substances very difficult to separate, despite its large spectra of biological activities. The insectidal properties of this class of compounds which is exclusive of the Annonaceae family has drive our attention to its use against the mosquito *Aedes aegypti*. Structurally the acetogenins are a series of C-35/C-37 natural products derived from C-32/C-34 fatty acids that are combined with a 2-propanol unit. They are usually characterized by a long aliphatic chain bearing a terminal lactone ring, with one, two or three tetrahydrofuran (THF) rings located along the hydrocarbon chain.

The methodology used to extract acetogenins from seeds of *Annona muricata* and *Annona squamosa* crude ethanolic extracts was the preparative High Performance Liquid Chromatography (HPLC) – UV detector. We isolat by HPLC seven acetogenins, two from seeds of *A. muricata* AMS2 (t_R=8,5), AMS3 (t_R=9,5) and five from seeds of *A. squamosa* ASS2A (t_R=4,5), ASS2B (t_R=5,0), ASS2C (t_R=5,5), ASSX (t_R=7,5) and ASS3 (t_R=8,0). The substance AMS2, striking of crude extract from seeds of *A. muricata* was identified as Anonacin. The substance ASS3, striking of crude extract from seeds of *A. squamosa* was identified as Squamocin A.
In our search for bioactive natural products from São Paulo State, Brazil, we have examined the leaves of *Neea* species. These species grow wild in cerrado lands of Brazil, and they are used in folk medicine for the treatment of gastric ulcers and inflammation\(^1\). This work deals with the chemical investigation of the infusion of *Neea theifera*, *N. schwackeana* and *N. pendulina* using HPLC-DAD analyses. The infusions were prepared using a procedure which simulated actual brewing conditions for a cup of tea. The leaves (2 g) were steeped at 80° C for 10 min in 20 mL of water\(^2\). Samples were filtered through a 0.45 µm nylon filter and analyzed directly by HPLC. The analyses showed that *N. theifera* contains mainly flavonoids, whereas *N. schwackeana* contains phenolic acid derivatives and *N. pendulina* showed a poor profile of secondary metabolites, with minor amounts of flavonoids and phenolic acids. The results are important, since they allow the differentiation between these species, mainly *N. schwackeana* and *N. pendulina*, that are hardly differentiated by their morphology and some authors classify these species as synonymy. (BIOTA-FAPESP, CNPq, Capes).

Refs.
DETERMINATION OF RUTIN IN LEAVES AND FRUITS OF FAVA D'ANTA
(Dimorphandra mollis)

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4. Departamento de Química. Laboratório de produtos Naturais. LAPPRONA. Universidade Federal de Ouro Preto. 35400-000.

Dimorphandra mollis Benth. (favela, fava d'anta - Fabaceae), Brazilian species, found in the Cerrado, that constitutes important socio-cultural patrimony as well as economic. Its economic and pharmaceutical importance is in the fruit that has related medicinal use to the presence in pericarp and the pulp of 6 and 30% of rutin (quercetin-3-rutinoside), flavonic glicosidic, that contain, hesperidin and eriodictin, enclosed in the group of the bioflavonoids.

About 50% of the world-wide production of rutin it is proceeding from the fava-d'anta; where approximately 95% of the production destines it the external market, making an annual prescription of 12 million dollar. The objective of this study is to quantify the amount of rutin in leaves and fruits of D. mollis aiming at to the determination and identification of its chemical composition.

This form, leaves (TFO-01 the TFO-12) and fruits (TFR-01 the TFR-12) of D. mollis had been collected with 4 repetitions, in the north of Minas Gerais, may of 2006, after conditioned had been carried, selected, measured, weighed and dry until constant weight. After the drying duly had been homogenized, identified and stored in plastic packing, under the cover of the light and of the heat. The quantification of the flavonoid was made by HPLC having used itself of C18 column and mobile phase methanol/water (pH 3.0) 1:1 (0-10 min) and 7:3 (10-20 min) with flow of 1 mL/min, being monitored the 339 nm. The determination of the concentration of the same one in the analyzed samples was made on the basis of the area of the peaks. The results had been supplied in percentage (m/m) of calculated flavonoids as rutin.

The resulted had been submitted to the analysis variance statistics and the averages to the test of Tukey 5% of probability. The amount of rutin in leaves of D. mollis varies between 10.95 and 3.22 mg/g while that in the fruits it varies between 742.12 and 415.22 mg/g. It can be verified that the rutin in leaves are minors who in the fruits, however, the plant produces great amount of leaves the year entire, while that, the fruits are produced only one time per year. In this way the total amount of rutin in leaves can compared the fruits.

The variations of concentration of the flavonoid in the fruits how much in leaves they can be attributed to the climatic conditions, of the soil, and to the mechanism of protection of the species the inhospitable conditions.

Refs.

Acknowledge
To FAPEMIG and CNPq.
IDENTIFICATION OF SECONDARY METABOLITES OF MICONIA SPECIES (MELASTOMATACEAE) BY ESI/MS/MS ANALYSES

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Miconia is a genus of approximately 1000 species occurring in tropical America and belongs to the Melastomataceae family. Many of these plants are used as medicine by people living in the Cerrado area. There are many works about flavonoids and phenolic compounds isolated from Miconia species. Polyphenols act mainly as antioxidants and radical scavengers, anticarcinogenic and as taxonomic markers in plants. The identification of these compounds by usual methods (e.g. NMR) is difficult, because of their structural complexity. ESI/MS/MS is a sensitive, rapid and convenient technique used to identify plant constituents in complex and mixed plant extracts. The use of this technique affords not only molecular weight information but also provides characteristic structural information that is useful in identifying the compounds. The aim of this study was to identify the main phenolic compounds of M. cabucu and M. rubiginosa by ESI/MS/MS. The methanolic extracts of the leaves were dissolved in methanol (1 mg/mL) and submitted directly to ESI/MS analysis, in negative mode, in a flow rate of 10 µL/min. The capillary voltage was set in -10 V and temperature was 270 ºC. Data were acquired in MS¹ and MS² scanning mode. The study of the fragmentation spectra indicated the presence of quercetin, kaempferol and myricetin derivatives. The nature of interglycosidic linkage can be suggested by the evidence of the [M–132–H]⁻, [M–146–H]⁻ and [M–162–H]⁻ fragments. The nature of the quercetin, kaempferol and myricetin aglycones can be confirmed by MS-MS focused on the m/z 301, 285 and 316 [A–H]⁻. The presence of the diglycoside flavonoids were confirmed by deprotonated ions at m/z (595, 609, 625) [M–H]⁻. The nature of interglycosidic linkage of these compounds can be suggested because the evidence of the loss [M–132–H]⁻ and [M–146–H]⁻ (Rha→Xyll); [M–146–H]⁻ and [M–162–H]⁻ (Gal→Rha); [M–162–H]⁻ and [M–162–H]⁻ (Glu or Gal→Glu or Gal). The CID MS/MS data of these species exhibited diagnostic ions ([M–H]⁻ at m/z 349, 515, 555 and 793) which represents the presence of catechins derivatives (monomer, dimer and trimer) linked with one and two gallic acid unit. The presence of the gallic acid units were confirmed with loss [M–152–H]⁻ fragments. Finally The MS-MS ion negative showed the deprotonated ion [M–H]⁻ at m/z 169 to corresponding gallic acid while a deprotonated ion [M–H]⁻ at m/z 191 represent the quinic acid. In this study it is demonstrated that use of direct injection ESI/MS/MS affords not only molecular weight information about the flavonoids, catechins and others phenolic compounds, but also provides characteristic structural information that is useful in identifying the compounds in Miconia species. (CAPES, FAPESP, CNPq)
ALKALOIDS ISOLATED FROM *Ocotea odorifera* VELL BY COUNTERCURRENT CHROMATOGRAPHY

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The genus *Ocotea* belongs to the family Lauraceae that is widely distributed in the Americas. In Brazil, species of this genus are used in folk medicine as anti-rheumatics, uterine tonics, and antisyphilitics. The chemical profile of *Ocotea* is characterized by the presence of neolignans, pyran-2-one and benzylisoquinoline alkaloids. *Ocotea odorifera* Vell., a tree of 7-8m high, is known as “canela sassafrás” or “sassafrás” in Brazil, where it is widespread in Rain Forest regions. Phytochemical analysis showed the species to contain benzylisoquinoline alkaloids.

Separation of these compounds from plant extracts by classical chromatographic techniques often involves irreversible adsorption phenomena on solid stationary phase that usually cause decomposition of the sample. Countercurrent chromatography (CCC) is a separation technique in which there is no solid matrix, thus eliminating the problem of sample adsorption observed in traditional adsorption chromatography. In the last years the pH-zone-refining countercurrent chromatography has been used to separate acid and base compounds due to the advantages as: increase in sample loading capacity, high purity and high concentration of fractions. This method uses a retainer base (or acid) in the stationary phase to retain the substance in the column and an eluter acid (or base) to elute the substances according to their pKa values and hydrophobicities.

In order to separate benzylisoquinoline alkaloids from methanol extract of *O. odorifera* bark, pH-zone-refining countercurrent chromatography was used.

Dried and ground bark from *O. odorifera* was submitted to a Soxhlet extraction successively with hexane and methanol. The methanol extract was fractionated by acid-base partition in order to concentrate all alkaloids in a unique fraction (OOMCB). This fraction was submitted to pH-zone-refining countercurrent chromatography using the solvent system MBE/ AcN/ H₂O (3/ 1.5/ 4) where to the mobile phase (organic phase) has been added 10 µM of TEA (eluter base) and to the stationary phase (aqueous phase) has been added 9.5 µM HCl (retainer acid).

This procedure yielded two alkaloids, norarmepavin and reticulin, that were confirmed by ¹H NMR and ¹³C NMR (COSY, DEPT, HSQC and HMBC) analyses and phytochemical comparison with the literature records.

Refs.
Phytochemical investigations of *Piper* species have led to the isolation of several classes of physiologically active compounds such as alkaloids, amides, pyrones, dihydrochalcones, flavonoids, phenylpropanoids, lignans and neolignans. A micellar electrokinetic chromatography (MEKC) method was developed and validated for the determination of two bioactive butenolides; 4,6-dimethoxy-5-\(E\)-phenylbutenolide (1) and 4,6-dimethoxy-5-\(Z\) phenylbutenolide (2) in extract from leaves of *P. malacophyllum*. Both compounds had presented antifungal activity against *Cladosporium cladosporioides* and *C. sphaerospermum*. The analysis was performed in a 75 \(\mu\)m i.d. uncoated fused-silica capillary with 40.2 cm length (effective length of 30.0 cm) using a micellar system containing 20 mmol/L sodium dodecyl sulfate, 20% (v/v) acetonitrile and 10 mmol/L sodium tetraborate aqueous buffer at pH 9.2. Samples were injected hydrodynamically by applying 0.5 psi pressure during 3 s. The applied voltage was +25 kV and direct UV detection at 308 nm. For quantitative determination, coumarin (3) was used as internal standard. Under optimized conditions, the migration times for butenolides and coumarin were approximately 4.3; 4.1 and 3.1 min, respectively. Analytical curve of peak area ratios versus concentration in the range of 10.0 to 50.0 \(\mu\)g/mL gave a coefficient of correlation of 0.999, establishing the method linearity. The limits of detection and quantitation were 1.14 \(\mu\)g/mL and 3.47 \(\mu\)g/mL for butenolide 2 and 2.08 \(\mu\)g/mL and 6.31 \(\mu\)g/mL for butenolide 1, respectively, with 0.12\% relative percentage standard deviation (%RSD) for retention factor and 1.0% RSD for peak area (\(n = 10\)). An average recovery of 100.1±1.3\% at three concentration levels was obtained. Based on the performance characteristics, the proposed methodology was found suitable for the inspection of butenolides in extracts of *P. malacophyllum*, presenting additional advantages inherent to the CE technology such as low consumption of reagents and column endurance.

CHEMICAL PROFILE OF THE INFUSION FROM *G. noxia* (NETTO) LUNDELL

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The medicinal use of plants is an ancient tradition, far older than the contemporary sciences of medicine, pharmacology and chemistry. An ethnopharmacological inventory made in the formation of the Brazilian Cerrado showed a high number of medicinal plants used to treat gastric disorders. The most popular form of use is by infusing the leaves of the plant. Based on this we selected *Guapira noxia* (Nyctaginaceae), which is popularly known in Brazil as “Capa-rosa” or “Maria–mole” and employed in Brazilian folk medicine against gastric disturbances. High-performance liquid chromatography (HPLC) coupled to photodiode array detection (PDA) is widely used to investigate secondary metabolites found in infusions because it allows the recognition of a large number of compounds from plant origin. Previous work on the methanolic extract of *G. noxia* leaves led to isolation of di- and tri-flavonol glycosides. Leaves of *G. noxia* were collected at Pratânia, São Paulo State, Brazil, dried and powered. Infusions of leaves were prepared by pouring 10.0 mL of boiling water onto 1.0 g of plant material, allowing the mixture to stand for 10 min, and then filtering and lyophilizing. The sample was dissolved in water and analyzed by on-line HPLC/UV/PDA. According to their retention times, co-injection with standards and UV spectra of the peaks, the results showed the presence of eight tri- and di-flavonoid glycosides, confirming their occurrence on *G. noxia* tea. There are many studies related to the antiulcerogenic properties of flavonoids and their glycosides because they also may be involved in the scavenging of the reactive oxygen species on the surface of gastric mucosa, thus protecting cells from gastric injury. The presence of these compounds may probably explain the antiulcerogenic effect of the infusion of *G. noxia* and their use in folk medicine.
**Biflavonoids from leaves of *Cupressus lusitanica* analyzed by NMR and LC-MS**

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*Cupressus lusitanica*, commonly known as cedar of Goa, Mexican cypress and Portuguese cypress, belong to the systematic family Cupressaceae. It is a tree attaining 25–30m in height growing in many countries as ornamental garden and in commercial forest plantations throughout the tropical and temperate world. The leaves of this plant are used in indigenous practice to treat catarrh and headache. As reported the main chemotaxonomic chemical components of the *Cupressus* genus are the biflavonoids. A wide variety of biological activity has been ascribed to these molecules, e.g., peripheral vasodilatation, hypoglycemic, antimicrobial, and antidiabetic effects, and inhibitory effects on lipid peroxidation.1 Recently, we found that some *Cupressus* trees growing in UFSCar campus are heavily infected by fungi. Then we started an investigation to check whether there is a parallelism between fungi infection and flavonoid biosynthesis by the host plant. We wish report now the chemical composition studies of ethanolic extract from the leaves of *Cupressus lusitanica* by LC-MS and NMR. Leaves of *Cupressus lusitanica* were collected at UFSCar, dried, pulverized and extracted with ethanol and then submitted to an acid/base partition with NH₄OH/HCl. The flavonoids were extracted with ethyl acetate and n-butanol respectively. Qualitative analyses were made using an HPLC coupled with triple quadrupole mass spectrometer in previously optimized conditions. It was verified the presence of the ions of *m/z* 537 and 551 in the negative mode ([M-H]) which are described in literature as biflavonoids amentoflavona, cupressuflavona (isomers) and their methyl ether derivatives respectively. To confirm the presence of biflavonoids, the extract was further chromatographed with C18 (H₂O:MeOH gradient) to furnish yellow powder which was analyzed by ¹H NMR. The characteristic chemical shifts of this class of compounds are shown bellow as δ 6.46 (s) δ 7.51 (dd, 2H). Through analysis of LC-MS experiments, it could be verified the presence of biflavonoids in leaves of *Cupressus lusitanica*, and this was confirmed by NMR experiment.

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1 A. Romani, C. Galardi; P. Pineli; N. Mulinacci; D. Heimler; *Chromatographia*, 2002, 56, 469-474
2 R. C. Swamy; O. Kunert; W. Schuhly; F. Bucar; D. Ferrerira; V. S. Rani; B. R. Kunar; A. V. N. A Rao; *Chemistry & Biodiversity*, 2006, 3(4), 405-413.
Brazil houses an enormous biological and cultural diversity, with several medicinal plants identified by local people as being useful for CNS disorders. *Ptychopetalum olacoides* Bentham (Olacaceae), known as “Marapuama”, is widely consumed throughout the Amazon region [1]. Local communities use alcoholic infusions as a “nerve tonic”, aphrodisiac, appetite modulator and as an anti-tremor agent [2]. In the present study we report the isolation and structural identification of vanillic acid and two other compounds present in *Ptychopetalum olacoides* and the use of vanillic acid as the phytochemical marker in a validated chromatography method to standardization of commercial extracts of *Ptychopetalum* species.

The isolation method was made using a fluid extract fractioned in a chromatographic column packed with C18 and eluted in gradient mode with different proportion of MeOH:H$_2$O. The final purification was made in a preparative HPLC using a phenyl-hexyl column and acetonitrile and acetic acid 0.1% in water as eluents. The structural identification of the compounds was made by NMR. For the analytical separation a HPLC-DAD method was developed and validated using a Phenomenex Luna® phenyl-hexyl column, acetic acid 0.1% in water and acetonitrile as mobile phase in a linear gradient, flow rate of 1.0 mL/min and wavelength at 260 nm.

The three compounds were isolated and identified as vanillic acid 1, protocatechuic acid 2 and theobromine 3. The vanillic acid, the majoritary compound present in plant material and in different types of commercial extracts studied was used as external standard to chromatographic method developed. This analytical method was validated presenting good response to selectivity/specificity, linearity, reproducibility, accuracy, detection and quantification limits and robustness assays.

The study concluded that vanillic acid is an efficient phytochemical marker to standardization analysis of commercialized extracts and herbal medicine sold as "marapuama" in Brazil.

Refs.
Analysis of Phyllantin, Hipophyllantin, and Nirantin in *Phyllanthus amarus* by GC-MS

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*Phyllanthus amarus* widely used plant in treatment of diverse illnesses, specially for hepatitis B, presents lignans as main components with pharmacological interest, with chief compounds phyllantin and hipophyllantin. *In vitro* anticancer studies have demonstrated nirant to inhibit cell proliferation. The objective of this work was to develop an analytical methodology to quantify simultaneously the three lignans (figure 1) in the methanolic extract.

![Figure 1: Structure of the lignans: Phyllantin (A), Hipophyllantin (B) and Nirantin (C).](image)

The samples were obtained from 200 mg dried and chopped leaves, by extraction with methanol by Polytron®. The analysis was performed on Agilent HP-6890/HP-5975 gas chromatography/mass selective detector, equipped with HP-5MS capillary column (30m x 0.25mm x 0.25µm). The operation temperatures were: injector: 280°C; detector: 300°C and column oven: 180°C, 5°C·min⁻¹ up to 220°C, 10°C·min⁻¹ up to 260°C, 3°C·min⁻¹ up to 300°C (4.7 min). Chromatography grade helium (1.0 ml·min⁻¹) was carrier gas. Split ratio: 20 ml·min⁻¹. The standards concentrations range used for analytical curves were 4 to 40 µg·ml⁻¹. The SIM mode (Single Ion Monitoring) was applied and the parameters are described in table 1.

![Table 1: Parameters of quantification of the lignans by GCMS.](table)

<table>
<thead>
<tr>
<th><em>t_R</em> (min)</th>
<th>Analite</th>
<th>Ions of quantification</th>
<th>Ions of confirmation</th>
<th>Time Window (min)</th>
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<tbody>
<tr>
<td>17.822</td>
<td>Phyllantin</td>
<td>151</td>
<td>203 e 418</td>
<td>3.0</td>
</tr>
<tr>
<td>19.896</td>
<td>Hipophyllantin</td>
<td>151</td>
<td>45, 353 e 430</td>
<td>18.5</td>
</tr>
<tr>
<td>19.992</td>
<td>Nirantin</td>
<td>166</td>
<td>151 e 432</td>
<td></td>
</tr>
</tbody>
</table>

The yields of Phyllantin, hipophyllantin and nirantin in the methanolic extract of the *P. amarus* were respectively: 0.53%, 0.061% and 0.27% (m/m). The results confirmed the methods efficiency and demonstrated an easier and quicker analysis. Method validation is under study.
COMPARISON OF TWO METHODS OF BIOACTIVE ALKALOIDS EXTRACTION FROM SENNA SPECTABILIS IRWIN & BARNEBY (FABACEAE)

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The standardization of the fractioning method directly interferes on separation, identification, quantitative and qualitative analysis of a plant extracts. In this way, the standardization of an efficient method is essential in the chemical study of plant species. This work aims to compare two extraction methods of bioactive piperidine alkaloids. The crude ethanolic extract from leaves of *Senna spectabilis* were purified using two different liquid-liquid partitioning techniques. The chromatographic profiles and anticholinesterase activities were determined with alkaloidic fractions. For the first method, 3.0 g of crude ethanolic extract were re-dissolved in 30 mL methanol:water solution (8:2) (v/v). The hydromethanolic (HM) solution was partitioned with hexane, dichloromethane and ethyl acetate. The fractions were dried and the acid-base extraction was made giving alkaloidic fractions (Hex 1 - 21.4 mg, DCM 1 - 52.3 mg, EtOAc 1 - 48.7 mg and HM 1 - 25.8 mg). In the second method, 3.0 g from the crude ethanolic extract were re-dissolved in 30 mL hydrochloric acid (0.01 M) and the insoluble portion was filtered off. The acid phase was partitioned with hexane (Hex 2 - 51.5 mg) to yield a non-alkaloidic portion. The residual acid solution was basified (pH 9.0) with ammonium hydroxide and then extracted with dichloromethane (DCM 2 - 186.4 mg), ethyl acetate (EtOAc - 51.1 mg), buthanolic alcohol (But 2 - 264.9 mg) remain an aqueous fraction (Aq 2 - 315.2 mg). The resulting extracts were dried over anhydrous sodium sulfate and concentrated furnishing the crude alkaloidic fraction from leaves. To detect the anticholinesterase activity the fractions were spotted on TLC and then sprayed with acetylcholinesterase enzyme (6.66 U/mL), thoroughly dried and incubated at 37°C for 20 min (moist atmosphere). Enzyme activity was detected by spraying a solution consisting of 0.25% of 1-naphthyl acetate in ethanol (5 mL) plus 0.25% aqueous solution of Fast Blue B salt (20 mL). Potential acetylcholinesterase inhibitors appeared as clear zones on a purple colored background ¹. In the first method of this work, the TLC showed two compounds with anticholinesterase activity in several fractions (Hex 1 Rf 0.48; DCM 1 Rfs 0.32 and 0.48; EtOAc 1 Rfs 0.32 and 0.48), whereas in the second method, four active compounds were detected (DCM 2 Rfs 0.12, 0.28, 0.32 and 0.48) in only one fraction. In conclusion, analyzes in gas chromatography-FID showed that the active compounds are cassine (RT = 30.62 min) present in all active fractions (Hex 1, DCM 1 EtOAc 1 and DCM 2) on Rf 0.48 and spectaline present in the fractions (DCM 1; EtOAc 1 and DCM 2) on Rf 0.32. The second method was more efficient to extract alkaloids because it yielded 22% more cassine and 65% more spectaline than the first method and maintaining all the bioactive piperidine alkaloids in one fraction.

Refs.
TRITERPENES AND STEROLS PROFILE IN HEXANE EXTRACTS FROM 
Casearia SPECIES USING GAS CHROMATOGRAPHY

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The increasing interest in the use of medicinal herbs requires consistent and fast methods for
the identification of the phytochemical constituents and for the quality control of
phytotherapics. In the case of non-polar extracts, the biological and pharmacological activities
were often believed to be due to a number of triterpenoid and steroid compounds, such as α- and β-amyrins, lupeol, betulin and betulinic acid\(^1,2\). As part of our ongoing research on
bioactive compounds from Brazilian plants for the treatment of tropical diseases, we have
investigated species of Casearia, a genus belonging to the Flacourtiaiceae family, with
approximately 1300 known species\(^3\). In this work, we have employed gas chromatography
(GC-FID) to investigate the hexane extracts composition of four Casearia species (C. gossypiosperma, C. obliquoa, C. decandra, and C. rupestris).

The analysis were carried out using the Crevelin et al\(^4\) modified method, which is rapid and
simple, and does not require pre-derivatization of the crude botanical extract. The extracts (10
mg – leaves and twig) were dissolved in chloroform (3 mL) and submitted to solid phase
extraction on silica gel (200 mg), eluted with chloroform (10 mL). The fraction obtained was
dried, dissolved again in chloroform (1mg/mL) and analyzed by GC-FID in duplicate. All the
hexane extracts were analyzed by GC-FID on a Varian model CP-3800 gas chromatograph,
with temperature of injector and of flame ionization detector adjusted to 260 °C and 290 °C,
respectively. The injected volume was 2.0 \(\mu\)L. SPB-50 (cross-linked 50% phenyl-methyl-
silicone, 30 m × 0.25 mm × 0.25 \(\mu\)m) capillary column was employed and the column
temperature was 280 °C (isotherm). Triterpenes and sterols were identified by comparison of
the relative retention (RR) of the samples with the RR of the standard sterols and triterpenes.
Cholesterol was used as the internal standard.

The results show that the steroid β-sitosterol is present in all the analyzed extracts.
Stigmasterol and campesterol were detected only in the leaves and twigs extracts of C. gossypiosperma. The triterpenes α-amyrin and β-amyrin were only detected in C. gossypiosperma (twigs) and C. rupestris (leaves), respectively, while the lupeol acetate was
detected in twigs of C. gossypiosperma. These results clearly demonstrate that the chemical
profiles of the four Casearia species concerning sterols are very similar, just the species C. gossypiosperma and C. rupestris presented small differences in the steroidal and triterpenic
composition. In summary, we have used a simple, rapid, and relatively cheap method to
identify triterpenes and sterols from hexane extracts of Casearia species using GC-FID.

Refs.
EXTRACTION METHODOLOGY OF *Casearia sylvestris* USING RESPONSE SURFACE

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*Casearia sylvestris* Swartz (Flacourtiaceae) is a tree that is widely distributed within various ecosystems of South America, such as the Cerrado and the Atlantic and Amazon forests. In the popular medicine of Brazil, the use of the plant is correlated with its pharmacological properties including anti-inflammatory, anti-ophidian and anti-ulcer activities. A number of phytochemical investigations of this species have revealed the occurrence of tricyclic clerodane diterpenes, the casearins, which exhibited both cytotoxic and anti-fungal activities. Considering the activity of casearins obtained from *C. sylvestris*, the aim of this work was to optimize the extraction condition of these compounds, by ultrasound, in order to substitute the chloride solvent (CH$_2$Cl$_2$) proposed by BANDEIRA (2006). The extractor solvent composition was studied using an experimental planning and the optimum composition determinate by response surface method. All solvents were saturated with NH$_4$OH as proposed by BANDEIRA (2006). After that, the response surface was used again to optimize the extraction time and the proportion between plant material/solvent (w:v). Yield and selectivity of the extraction were also available. The optimized extraction conditions was established as Hex/AcOEt/IPA (08:91:01), by one hour, using 115 mg of plant material by milliliter of solvent. Using these conditions was possible to obtain around 700 mg of crude extract with 73 % of total casearins, by gram of dried leaves.

Refs.
QUANTIFICATION OF BERGENIN IN *ENDOPLEURA UCHI* BARK BY HPLC

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*Endopleura uchi* (Huber) Cuatrec. is a Humiriaceae widely spread in the Amazon known as “uxi-amarelo” or “uxi-liso”. The bark tea of *E. uchi* is used in the traditional medicine as anti-inflammatory¹. Hereby the isolation and quantification of bergenin in bark of *E. uchi* are described. The methanolic extract of bark from *E. uchi* was dissolved in MeOH/H₂O (9:1) and partitioned in hexane, chloroform and ethyl acetate. The methanolic extract and its fractions have been previously evaluated for their antioxidant² and the ethyl acetate and hidroalcoholic fractions have been the most active in the assays of radical scavenger of DPPH and ferric reduction antioxidant power. Then, 1.0 g of the ethyl acetate fraction has been purified in Sephadex LH-20 column eluted with methanol and afforded a fraction that has been further purified over silica flash column eluted with a CHCl₃/MeOH gradient and yielded 37.0 mg of bergenin, identified by its NMR spectra. Since the anti-inflammatory activity of bergenin has previously been reported³,⁴, we have quantified it in aqueous extract prepared as prescribed in the traditional medicine, ie, decoction for 5 min of ca 30 g of bark in half liter. The quantification has also been determined by RP-HPLC, at 272 and 254 nm, using a LiChrospher 100 RP-18e (250 x 4; 5 µm particle size) column, in isocratic mode using 15 % of MeOH in aqueous TFA (pH 2.0) at flow of 1.0 mL/min, 50 µL injection (duplicate) and external standard method. The average retention time of bergenin has been 16 min and considered pure. Calibration curves of bergenin with five different concentrations have been constructed. All curves have shown excellent linearity and the coefficient of variance on different days were inferior to 3 %. The determined concentration of bergenin has been 3 % in bark of *E. uchi*. This result indicates a high concentration of bergenin in the teas and in the bark. The anti-inflammatory activity *in vivo* of aqueous extract has been investigated to confirm the popular use as anti-inflammatory.

ARRABIDAEA CHICA VERLOT RED DYE EXTRACTION BY BIOTECHNOLOGICAL TECHNIQUES

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Keywords: Arrabidaea chica, dye extraction, biotechnology

Folk medicine is important in biotechnology, since 75% of active compounds isolated from plants come from traditional knowledge (Azevedo, 2005). Arrabidaea chica (H&B) Verlot, popularly known as Pariri, is a native tropical American vine, with pink flowers. Amazon Indians use a red pigment extracted from this specie, carajurin (6,7-dihydroxy-5,4′-dimethoxy-flavilium), as tattooing agent, astringent and for treatment of various diseases. The use of natural dyes has decreased to a large extent due to the advent of synthetic products. Recently, dyes derived from natural sources have emerged as an important alternative to synthetic ones. A literature review of Arrabidaea chica indicated that this genus is a source of anthocyanins, flavonoids and tannins. The red color has been attributed to carajirun (6,7-dihydroxy-5,4′-dimethoxy-flavinium) and carajurone. Kim et al. (2005) proposed a method of enzymatic pigment production based on the introduction of hydrolytic enzymes prior to the usual extraction process, improving pigment extractable yields. Other studies undertaken by our group revealed that A. chica produces a high content of glycosylated anthocyanins. Nevertheless, the orange-red colors originate from the molecules aglycones. Therefore in accordance to Kim et. al (2005), we proposed to study the influence of the red color dye extraction with enzymatic incubation of plant material prior to extraction procedures. Initially the optimum enzyme incubation time was determined (Taffarello, 2006), establishing 2 hours treatment period as the enzyme activity condition that yielded the best carajirun and carajurone content providing desirable red color. Secondary metabolites are elaborated and accumulated by plants as a response to the environment encountered. Climate and regional factors have shown to significantly affect the production of chemical compounds. Hence we were prompted to study the seasonal anthocyanin production behavior, with enzyme treatment prior to extraction process, among seven different plant introductions from different Brazilian regions adapted at CPQBA’s experimental field under the same agronomical conditions. We evaluated the best time of year for enhancing dye extraction yields and verified if the proposed process was capable of producing a standard final product although the different plant introductions have different glycosilated anthocyanins content. Despite the final extraction yield without enzyme treatment were higher (24.28%) compared to the enzyme treated material (19.03%), the aglycones anthocyanin ratio was enhanced in the latter case as determined by HPLC-DAD (Shimatsu), Phenomenex Gemine C-18 colun (4,6 mm x 250mm i.d. 3 µm), flow rate 1 mL/min, mobile phase Methanol: water phosphoric acid pH 2.0 gradient elution. The best month for harvesting the plant species for dye extraction were March and April independently on the plant introduction processed. In conclusion, our results suggest a different approach involving enzymatic treatment for enhancement of natural dye yield production.

Refs.
TAFFARELLO, D. Anais of The 3rd Brazilian Symposium on Medicinal Chemistry (BrazMedChem) S2-158, São Pedro, Brazil (2006).
The search for bioactive compounds from medicinal plants plays an important role in contemporary biomedical research concerning therapeutics. The first step for bioactive substance attainment is the extraction process. Classical methods such as maceration, percolation and Soxhlet extraction require long time of extraction, high solvent amount, stirring and, in some cases, high temperatures. Many reports on effects of ultrasound-assisted (sonication) extractions have been published, and the main reported improvements prove to be enhanced efficiency and shortening of extraction time. The most important aspect for a successful ultrasonically assisted extraction is to establish appropriate values for extraction parameters relating to the biological properties of the plant material to be extracted. The objective of this work was the optimization of the ultrasound extraction of fruits from *Genipa americana* L. (“jenipapo”) and to compare with classical extraction techniques (Soxhlet and maceration). Approximately 10 g of dried and crushed fruits were extracted with 300 ml of methanol by maceration (6 days at room temperature), Soxhlet apparatus (refluxed by 240 min) and in ultrasonic bath (75 min at 30°C). The extracts were filtered and concentrated under reduced pressure until dryness. The mass yield (%) of each extraction and the qualitative chromatographic profile were used as parameters for comparison. Optimization of sonication procedure was firstly performed using two complete factorial drawing $2^2$ with two levels (+1 and -1) for each parameter. The proposed matrix provided 4 experiments to each procedure and the evaluated parameters were: $X_1$ (amount of sample) - 5 and 10 g and $X_2$ (cycles of extraction) - the effect was evaluated using 1 and 3 cycles. Extraction time and volume of solvent were investigated using 40 ml of fresh solvent in each cycle and setting time intervals of 25 min. The upper and lower values given to each variable were selected from the experience in preliminary experiments (different times were tested from 5 to 125 min and different volumes solvent ranging from 40 to 500 ml). The comparison results of extraction yields of maceration, Soxhlet and ultrasound extraction are 49,40%, 56,30% e 54,95%, respectively. Soxhlet extraction gives significantly higher values, while maceration yielded lower values. However, Soxhlet extraction requires long time of extraction and raised temperatures that can cause structure modification in thermally unstable substances present in the plant material. On extraction time, ultrasonic-assisted extraction was faster than maceration and Soxhlet. Optimization of the sonication extraction parameters of *G. americana* fruits indicated that the extraction time of 75 min (3 x 25 min) resulted in better mass yields having methanol as solvent. At extraction times longer than 75 min, a decrease in the mass yield was observed, which may be explained considering the possibility of decomposition of organic compounds by the effect of the sound waves. The extraction efficiency is the highest under sonication when the sample is extracted three times for 25 min using fresh solvent than when the extraction was performed once for 75 min. Comparison of these extraction methods revealed that they afford qualitatively similar extracts. The preliminary results show that the ultrasound is a simpler, faster and more effective method to extract plant material.
The Brazilian natural patrimony presents big worldwide relevance expressed by continental extension, through diversity and biologic species endemism and its genetic patrimony (according Nodari & Gerra (1999), it’s one of the countries with the biggest vegetal genetic diversity, counting with more than 55,000 of catalogued species), as well as the ecosystemic biomes variety. Among the richest savannas in the world, the Brazilian “cerrado” flora counts with 6,420 vascular species (Mendonça et al., 1998). It’s pointed in the total 6,671 native táxons, distributed in 170 families and 1,140 class (Ratter et al.,1997). As Ribeiro and Walter (1998) add, the biome “cerrado” is the second biggest in area in the country, occupy 23% of the national territory (two million Km²), being localized basically in the central plateau. The medicinal potential of “cerrado” is very valuable for the medicines research’s for human health. The Phytoterapy viability is dependent on the correct identification of species, on the identification of chemical markers, soil and weather adequate and it extends to the dryness, pounder, storage and extracts preparation. The aim of this work is to establish of banc of dados and valid the chemistry quality control of chemical markers of 65 medicinal plants from the “cerrado” of usage in Manipulation Pharmacies. The species were collected in areas of natural occurrence in the cities São João Batista do Gloria (Serra da Canastra region), Passos, Araxá, Uberaba and Ribeirão Preto, in two seasons of the year, georeferences, making exsiccatas. After the identification, the aerial parts and/or the roots systems were dried in greenhouse at 50ºC and ground for the preparation of the hidroalcholic extracts, using 200g of the powder in 800ml of hidroalcholic solution 80% (V/V), maceration static during 20 days. The chromatographic analysis of the extracts will be done through the classic methodologies of TLC and in Whatmann paper. The chromatography and the plates will be photographed and archived for later confection of the monograph.
EFFECT OF PSYCHOLLATINE, AN ALKALOID FROM Psychotria umbellata Vell. (Rubiaceae), ON AMINO ACIDS LEVELS IN HIPPOCAMPUS AND PREFRONTAL CORTEX OF RATS

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Psychollatine (I) is an indol monoterpene alkaloid, isolated from Psychotria umbellata Vell. (Rubiaceae) leaves. This compound has shown an interesting psychopharmacological profile, including mild analgesic activity against a number of algogenic stimuli¹, and anxiolytic, antidepressive and amnesic effects in mice models². These pharmacological data indicate that psychollatine (I) is able to modulate different neurotransmitter systems, including NMDA, opioid and 5-HT²/C receptors¹,². Moreover, in subsequent pharmacological investigations, the involvement of dopamine central receptors in the mode of action of I has been established³. As part of our research on this alkaloid, the present study was carried out in order to investigate the effects exerted by psychollatine (I) on amino acids neurotransmitter levels (aspartate, glutamate, glycine, glutamine and GABA) in hippocampus and prefrontal cortex of rats, brain areas implicated in the behavioral effects induced by NMDA ligands. Psychollatine (I) (7.5 mg/kg) or saline were administered i.p. in male Wistar rats (n=10). One hour after the treatment, brains were extracted and dissected out, and the hippocampus and prefrontal cortex were stored at –80 °C. The structures were extracted with perchloric acid 0.1 N and the homogenates centrifuged. After that, the supernatants were derivatized with PITC and, subsequently, analyzed by HPCL/UV at 254 nm as previously described by Steffen et al.⁴. The treatment with psychollatine (I) 7.5 mg/kg caused a significant decrease on the levels of Asp (10.64 ± 0.55; 8.23 ± 0.50 µmol/g, saline and psychollatine respectively) and a significant increase on the GABA levels (11.43 ± 0.43; 13.72 ± 0.53 µmol/g), both in hippocampus (P < 0.05). The levels of Glu (15.95 ± 0.58; 14.59 ± 0.64 µmol/g) and Gly (1.50 ± 0.18; 1.44 ± 0.058 µmol/g) did not show significant changes (P < 0.05) in this brain area. In prefrontal cortex, the treatment with I caused a significant increase on the levels of Gln (3.29 ± 0.30; 7.37 ± 0.85 µmol/g, P < 0.01). The levels of Asp (1.02 ± 0.11; 1.35 ± 0.27 µmol/g), Glu (8.91 ± 1.78; 11.92 ± 1.03 µmol/g), Gly (3.23 ± 0.95; 3.56 ± 0.55 µmol/g) and GABA (11.63 ± 2.17; 16.64 ± 1.79 µmol/g) did not show significant changes (P < 0.05). Our results indicate that animals treated with psychollatine showed an important increase on the levels of GABA (20.00 %), which is an inhibitory neurotransmitter, as well as a decrease on the aspartate levels (22.63 %), an excitatory neurotransmitter, in hippocampus. Moreover, a significant augment was observed for glutamine levels in the prefrontal cortex. These data could support the involvement of the glutamateregic system in the psychollatine’s mode of action, once aspartate acts as a NMDA agonist and glutamine is converted to glutamate in the astrocytes. However, other mechanisms could be involved in the changes on amino acids levels described by this work. This work was supported by CAPES, CNPq.

Refs.
Aims: *Passiflora edulis* variety *flavicarpa* (“passionfruit”) have been traditionally used to treat several conditions related to the central nervous system such as anxiety, convulsions and insomnia. Considering that the Brazilian production of this plant species is about 150 thousand ton/year and that the pericarp of the fruit represents about 65-75% of this amount, being considered a residue or industrial trash, the investigation of its central effects is a promising field due the putative economic impact, especially in developing countries such as Brazil. Therefore, this study investigated the effects of the oral treatment with the pericarp extract of *P. edulis* (PE) on the stress-induced hyperthermia paradigm (SIH), light-dark transition test (LDT) and ether-induced sleep test (EIS).

Methods and Results: Male Swiss mice (3 months old) were treated by oral route (0,1 ml/10 g) and all experiments were accompanied by the control and positive control groups (vehicle and diazepam at the doses of 3 mg/kg in SIH, 2.5 mg/kg in LDT and 1 mg/kg in EIS). In the SIH paradigm, animals were isolated in their home cages and, 24 h later, the basal rectal temperature (TB - °C ) was measured, followed by the treatment with different doses (100, 300, 600 and 1000 mg/kg) of the pericarp extract. After 30 min, T1, T2 and T3, respectively), the rectal temperature was measured again. In the LDT test, animals were treated (100 and 300 mg/kg) and, 1h after, they were individually placed in the light compartment of the apparatus; each animal were observed during 5 min to register the following parameters: number of transitions between the two compartments and total time (s) spent in the light compartment. Immediately after the LDT test, animals were placed in the open field apparatus to evaluate the influence of the extract on their motor activity, registering the number of rears and crossings. In the SIE paradigm, 1 h after the treatment (100 and 300 mg/kg), animals were placed in an ethyl ether saturated glass cage, registering the latency (s) and the duration (s) of the induced sleep. Results were expressed by means ± E.P.M.. The pericarp extract prevented the occurrence of SIH in T1 at the highest doses (600 mg/kg: 34.4±0.2; 1000 mg/kg: 34.2±0.4) and at all doses in T2 (100 mg/kg: 34.5±0.2; 300mg/kg: 34.6±0.3; 600mg/kg: 34.5±0.2; 1000mg/kg: 34±0.2) and T3 (100mg/kg: 34.2±0.2; 300mg/kg: 34.1±0.1; 600mg/kg: 34.2±0.2; e 1000mg/kg: 34.2±0.1). In the LDT test, the pericarp extract increased the time spent in the light compartment at both tested doses (100 mg/kg: 113.7±17 and 300 mg/kg: 123.4±5.3) whereas the transitions between the compartments were not affected. No changes were observed in the open field parameters. In the SIH paradigm, the two tested doses reduced the latency to induce sleep (100 mg/kg: 27.3±1.2 and 300 mg/kg: 31.9±1.8) and increased its duration (100 mg/kg: 138.7±16.6 and 300 mg/kg: 144.3±13.7).

Conclusions: These results suggest the existence of pronounced central effects of the pericarp extract of PE, effects represented by the protection against the HIE, by the increasing of the time spent in the light compartment (suggesting an anxiolytic-like effect) and by the reducing of the latency and the increasing of the total time of the ether-induced sleep (indicating an hypnotic effect), similar to the effects observed with the diazepam treatment. These results encourage further investigations about the neurobiologic activity and the possible underlying mechanisms of action of the extract and/or fractions of the PE pericarp on specific neurotransmitter systems.

Financial support: CNPq
PHYTOCHEMICAL STUDY OF RHEEDIA BRASIILENSIS AND ANTIOXIDANT ACTIVITY EVALUATION

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*Rheedia brasiliensis*, a tree known popularly as bacupari, is used in folk medicine in urinary tract diseases, arthritis and to relieve pain. Have already been reported the presence of biflavonoids, xanthones, proanthocyanins, poliprenilated benzophenones and pentacyclic triterpenes from *Rheedia* species.

The leaves were dried at room temperature and extracted with ethanol-water (50%), followed by liquid-liquid partition with hexane, chloroform and ethyl-acetate. The last one was chromatographed in column, and was isolated fukugetin. After this, the leaves were dried and submitted to a dichloromethane extraction and after that the extract was submitted to chromatography in column, where was isolated epiclesianone. These structures were identified by UV, IV and RMN methods. According to the literature, this is the first time that these substances are isolated from leaves of *Rheedia brasiliensis*.

The antioxidant activity of the extracts and isolated substances were tested by scavenging of free radicals DPPH and reducing power. The results are shown in the figures below:

According to these methods, fukugetin and ethyl-acetate extract presented a high antioxidant activity, comparing to the standard, ascorbic acid. In the future new tests will be made to verify this activity by others mechanisms.

Refs.
ANTILEISHMANIAL ACTIVITY OF COUMARIN FROM *Calophyllum brasiliense* LEAVES AND DERIVATIVES OF THIS AGAINST *Leishmania amazonensis*

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In the present study were evaluated the in vitro antileishmanial activity of (-) mammea A/BB from leaves of *Calophyllum brasiliense* Camb. (Clusiaceae) and derivatives of this against promastigote forms of *Leishmania amazonensis*. In our previous studies, *Calophyllum brasiliense* displayed activity against *Leishmania amazonensis*¹ and *Leishmania braziliensis*.² The derivatives were obtained from (-) mammea A/BB by methoxilation, hydrogenation and reductions reactions. The compound (-) mammea A/BB showed significant activity against promastigote of *L. amazonensis*, with IC₅₀ at a concentration of 3.0 µg/ml and the derivatives 1, 2, 3 and 4 showed IC₅₀ at concentrations of 0.37; 0.88; 6.57 and 1.02 µg/ml respectively. The derivatives 1, 2 and 4 showed greater inhibitory effect than did the (-) mammea A/BB.

This study has contributed for study of structure activity of (-) mammea A/BB and it is a part of a continued search for new drugs with high activity and low side effects against diseases associated with protozoan parasites, such as leishmaniasis.

![Chemical structures](image)

INSECTICIDAL ACTIVITY OF NATURAL AND SYNTHETIC AMIDES ON
SPODOPTERA FRUGIPERDA

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The fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) presents an ample distribution in the American continent, with occurrence since Mexico to South America. It is the most important pest of maize in Brazil, and can cause reduction of up to 34% in its production.1 Usually, the control of this insect is carried out with conventional insecticides, but the abusive use of them can have negative effects. Investigation for other methods of control includes development of substances less toxic and aggressive to the environment, and more selective.

The phytochemistry of genus *Piper* (Piperaceae) has been widely studied, due to biological properties of amides from these plants. Recently, we reported the insecticidal activity of natural and synthetic amides against *S. frugiperda*.2 The most active natural piperamides were N-[3-(3’,4’-methylenedioxyphenyl)-2-(E)-propenoyl]piperidine (2), found in roots of *P. piresii*, and piperine (1), from *P. nigrum*, with LD$_{50}$ of 1.07 and 41.79 µg/mg larvae, respectively. In order to screen a large number of compounds, we prepared an indexed solution phase combinatorial library of amides.3 This methodology is a very simple approach to readily identify bioactive compounds from mixture screenings. Thus, a 100 member library of amides was prepared employing 10 amines and 10 acyl chlorides. The library was fully analyzed by GC/MS and evaluated against *S. frugiperda* larvae in the second instar (5 day old).

The library deconvolution through cross-analysis of the results among the mixtures showed that the most active compound would be 1-[4-(trifluorometoxy)benzyl] piperidine (3), which was then prepared in its pure form and evaluated, exhibiting an LD$_{50}$ of 18.29µg/ mg larvae. From these results we concluded that double bonds in the side chain and methylenedioxi group in aromatic ring may not be essential for insecticidal activity. By the other hand, piperidine in the amide moiety always showed the best results.


Acknowledgments: FAPESP, CNPq, CAPES
The objective of this work was to carry out phytochemical screening of hydroalcoholic extracts of *Achillea millefolium* L. and *Artemisia vulgaris* L., both belonging to the Asteraceae family. *A. millefolium*, is a perennial herb that has been used for hundreds of years in folk medicine as an analgesic agent in several countries and is called popularly by anador®, novalgina® or atroveran®. *A. vulgaris* is a perennial weed growing wild and abundantly in temperate and cold-temperature zones of the world and known popularly by anador® and cibalena®. This plant has been known not only as an edible plant but also as a folk medicine resource. In Oriental medicine, this plant has been employed as an analgesic agent and in conjunction with acupuncture therapy. The two extracts were evaluated by HPLC/DAD, which were carried out using a HP system, SERIES II 1090 and UV diode array detector working at 270 and 360 nm, equipped with a Zorbax – 5B - RP-18 (Hewlett Packard) column (4.6×250 mm, 5 µm). The solvent system for the separation of the samples was an elution gradient from methanol (B) : water (A) - 0 min. – 20%B; 10 min. – 30%B, 20 min. – 50%B; 30 min. – 70%B; 40 min. – 90%B; 45 min. – 40%B and 50 min. – 20% de B. The constituents of extracts are indicated by retention time (RT) and were characterized by UV/diode array detection (220-400 nm) that took into account their spectral characteristics. In the HPLC analyses “fingerprint”, both hydroalcoholic extracts exhibited the same phenolic compounds as principal constituents, which showed the same RT and UV/DAD spectra. The peaks at 17.6 (3.2 and 4.5%) and 26.7 (10.0 and 13.3%) for *A. vulgaris* and *A. millefolium*, respectively showed UV band at 300, 330 nm, being caffeic acid derivatives. Two caffeic acid derivatives, 3,5-dicaffeoylquinic acid and chlorogenic acid were previously found in *A. millefolium*1. Small quantities of apigenin and apigenin derivatives were also found in both extracts. The principal constituent at 28.3 (21.6 and 45.2%) for *A. vulgaris* and *A. millefolium* respectively, showed UV band at 260, 310,350, being a flavonoid glycoside, probably rutin. Recently, phytochemical investigations with *A. millefolium* led to the isolation of many flavonoid derivatives, such as, apigenin, luteolin, luteolin-7-O-β-d-glucopyranoside and rutin1. Many flavonoids were also found in *Artemisia vulgaris*, such as apigenin, luteolin, luteolin 7-glucoside, kaempferol 3-O-rutinoside, quercetin and rutin2. Luteolin 7-glucoside, apigenin and rutin are common for both plants. This similar chemical profile was reflected in analgesic activity since both extracts exhibited antinociceptive peripheral effect in the same dosage3.

Refs.

Financial support: AFIP and CNPq
PHYTOCHEMICAL CONSTITUENTS OF HYDROALCOHOLIC EXTRACTS OF
ALTERNANTHERA DENTATA, OCIMUM SELLOI, AND PLECTRANTHUS
BARBATUS, PLANTS POPULARLY KNOWN BY BRAND NAMES OF ANALGESIC
DRUGS.

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In the last decades, many studies have investigated the analgesic effect produced by
plants. Despite of progress in the development of pain therapies, it still necessary to discover
new analgesic agents with confirmed efficacy and devoid of the side effects observed with
the current available analgesic drugs, mainly for the treatment of chronic pain conditions. The
objective of this work was to carry out phytochemical screening of hydroalcoholic extracts of
Alternanthera dentata, popularly known in Brazil as AAS®, anador® and doril®; Ocimum
selloi popularly known by atroveran® and elixir paregórico® and Plectranthus barbatus called
by anador®, cibalena® and melhoral®. These extracts were evaluated by HPLC/DAD, which
were carried out using a HP system, SERIES II 1090 and UV diode array detector working at
270 and 360 nm, equipped with a Zorbax - 5B - RP-18 (Hewlett Packard) column
(4.6×250 mm, 5 µm). The solvent system for the separation of the samples was an elution
gradient from methanol (B) : water (A) - 0 min. – 20%B; 10 min. – 30%B, 20 min. – 50%B;
30 min. – 70%B; 40 min. – 90%B; 45 min. – 40%B and 50 min. – 20% de B. The constituents
of extracts are indicated by retention time (RT) and were characterized by UV/diode array
detection (220-400 nm) that took into account their spectral characteristics. For Alternanthera
dentata, the principal constituents at 5.7 and 6.1 with UV band 220, 260 nm are gallic acid
derivatives, the compound at 22.4 with UV band 310, 330 nm is a caffeic acid derivative and
at 26.7 with UV band 267, 349 nm is a flavonoid. For Ocimum selloi, the principal constituent
at 29.3 with UV band 310, 330 nm is a caffeic acid derivative, the compound at 28.7 with UV
band 258, 290, 335 nm is a flavonoid and the compound at 25.0 with UV band 280 nm is a
benzoic acid derivative. For Plectranthus barbatus, the principal constituents found at 27.6
with UV band 260, 360 nm, at 30.0 with UV band 270, 340 nm and at 35.2 with UV band
265, 340 nm are flavonoids and was also found two non-identified compounds at 37.3 with
UV band 230 nm and 43.81 with UV band 280 – 450 nm. There are few data about the
chemical composition of these three plants. Flavonoids were found in some species of
Alternanthera1 and in Ocimum gratissimum2. P. barbatus showed the highest concentrations
of flavonoids and also the best analgesic activity in pharmacological tests3. Some flavonoids
exhibited analgesic activity4. The presence of alkaloid boldine, which possess analgesic
activity, was detected in P. barbatus. In conclusion, the phytochemical screening showed that
flavonoids could contribute for the biological activity of the plants.

Refs.

Financial support: AFIP and CNPq
Acidic Triterpenes From the Roots of *Chiococca alba*

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Oleanane and Ursane triterpenes (including their derivatives) are widely distributed in plants. They occur in the form of free acids or as aglycones in saponins. Both oleanolic and ursolic acids are of great importance for human diet due to their wide occurrence in foods like teas, grains and other important food sources¹. Owing to this importance, more than 700 research papers were published in the last decades dealing with isolation and purification, chemical modifications, pharmacology, toxicology and other medical utilizations²,³,⁴.

As part of our ongoing phytochemical studies of the roots of *Chiococca alba*³,⁴ we report in this communication the characterization of several triterpenes following the application of an efficient methodology to obtain an acidic triterpene rich fraction. The characterization of two other pentacyclic triterpenes (filic-7-en-3-one and fern-7-en-3-one) from the same botanical sample, was already reported by the group⁵. In addition, α and β-amirin, oleanolic acid, ursolic acid and 3β-hydroxyolean-12,15-dien-28-oic acid were identified in the roots of *C. alba*⁶.

Ultrasound assisted extraction of the dried and powdered roots with CH₃Cl₂ provided the main extract for the study. After precipitation of a polymeric resin with CH₃OH, a SPE was carried out with KOH impregnated silica gel for obtaining the acid rich fraction. The SPE column was washed with hexane and CH₃Cl₂ and then eluted with CH₃OH and CH₃OH/CH₃PO₄ 20%. The CH₃OH fraction was evaporated and the residue was methylated with diazomethane/ethyl ether. The methylated fraction was then subjected to vacuum distillation on a Kugelrohr apparatus. The bottom fraction was filtered through activated charcoal. Top and bottom fractions were analyzed by GC. The bottom fraction was analyzed by GC/MS and displayed seven distinct acid triterpenes. Quick analysis of the mass spectra obtained allowed us to classify these acid triterpenes as oleanane and ursane derivatives. Characteristics values of *m/z* were identified for all the given compounds. The M⁺ ion at *m/z* 470 (r.t.: 21.42 and 22.54 min.), 468 (r.t.: 20.33, 20.49 and 21.65) and 466 (r.t.: 19.54 min.) were identified as the M⁺ of methyl ester pentacyclic triterpene acids with increasing number of double bonds. Fragment ions at *m/z* 260 and 262 are very indicative of RDA (π-bonding at C₁₂-₁₃) at the C ring of methyl ester of pentacyclic triterpenes structures. The fragment ions at *m/z* 205 and 204 were identified as derived by cleavage of the C₈-₁₄ and C₁₁-₁₂ bonds. The fragment ions at *m/z* 201 and 203 were derived by the loss of 59 unities from the fragment ion at *m/z* 260 and 262. The fragment ions at *m/z* 131 and 133 were derived by the cleavage of the C₁₈-₁₉ and C₁₇-₂₂ bonds from the fragment ions at *m/z* 201 and 203. Other fragment ions characteristic of pentacyclic triterpene structures were identified such as *m/z* 245, 247, 249, 233, 231, 215, 189, 187, 161, 157, 147 and 145. In addition, the intensity of the fragment ion *m/z* 133 (and 131) compared to *m/z* 203 (and 201) or to *m/z* 262 (and 260) can be used to distinguish the ursolic structure from the oleanolic one. I(19.54 min.) *m/z*: 466, 407, 260, 245, 231, 205, 201, 187, 157, 145 and 131. II(20.33 min.; methyl ester of 3β-hydroxyolean-12,15-dien-28-oic acid) *m/z*: 468, 260, 245, 231, 207, 201, 187, 157, 145 and 131. III(20.49 min.) *m/z*: 468, 408, 262, 249, 233, 203, 189, 161, 147 and 133. IV(21.42 min.; methyl ester of oleanolic acid) *m/z*: 470, 411, 262, 247, 233, 220, 203, 189, 161, 147 and 133. V(21.65 min.) *m/z*: 468, 408, 393, 262, 249, 203, 189, 159, 145 and 133. VI(22.54 min.; methyl ester of ursolic acid) *m/z*: 470, 410, 262, 249, 233, 215, 205, 203, 189, 161, 147 and 133. VII(23.60 min.) *m/z*: 452, 262, 249, 233, 215, 203, 189, 175, 161, 147 and 133.

IDENTIFICATION OF MAJOR CONSTITUENTS FROM LEAVES CRUDE EXTRACTS OF *Chiococca alba* AND *C. brachiata* (RUBIACEAE) BY LC/DAD-UV AND LC/MS.

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*Chiococca alba* Hitch., known in Brazil as “Cipó Cruz”, has been reported to be a source of quinoline alkaloids, lignans, coumarins, ketoalcohols, triterpenes, diterpenes, iridoids, and seco-iridoids\(^1\). On the other hand, *C. brachiata* evidenced flavonoids and terpenoids\(^2\). These species belong to Rubiaceae and are shrubs endemic to Americas. Roots of *Chiococa* have been used in folk medicine as tonic for ganglion inflammation, diuretic, antiviral, antieodema, and as aphrodisiac\(^4,5\). Coupling of LC with spectroscopic techniques such as UV and MS provides a useful tool for rapid data collection and structure elucidation\(^6\). Thus, crude extracts from leaves of *C. alba* and *C. brachiata* were analyzed by high performance liquid chromatography (HPLC) coupled to UV photodiode array detection (LC/DAD-UV) and high resolution mass spectrometry (LC/HRMS). The LC/HRMS spectra were recorded during the same run and TIC were obtained and processed using Bruker Daltonics DataAnalysis software, with molecular formulae generated for the most abundant [M - H\(^-\)] ions with a tolerance of 50 ppm. Taking into account that one mass unit was added to [M-H\(^-\)] ions due to the selected detection mode, a search was performed in the site http://dnp.chemnetbase.com/ (DNP 16.1, 2007, Taylor & Francis Group) aiming to establish possible chemical structure and molecular formulae for each ion. Although some ions showed more than one possible molecular formulae, those which best fitted the observed UV data, previously knowledge on the species chemical profile, and MS/MS fragmentation patterns were selected. Eight compounds were identified in the analyzed extracts from eleven chromatographic bands, six of which were flavonoids. Additionally, as there are no reporters of flavonoids from *C. alba*, compounds 1 and 2 were isolated and identified by NMR data analysis as kaempferitrin (1), and lepidoside (2).

Refs.
COMPARATIVE STUDY OF THE TOXICITY OF DIFFERENT ETHANOLIC EXTRACTS OF Ocimum gratissimum L.

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Ocimum gratissimum L. (Lamiaceae) is widely distributed in tropical and warm temperature regions (1). It has been used as topical antiseptic medication (2, 3) and also as a treatment for cough, fever, conjunctivitis (4). This work aims to determine if the extraction method (maceration and soxhlet) changes the toxicity of the ethanolic extract of O. gratissimum L. by brine shrimp (Artemia salina L.) lethality test (BSL). The dried aerial parts of O. gratissimum were extracted with ethanol 95% by maceration (three times, 8 days) and by soxhlet (for about 28 h). The BSL was performed according to standard protocols (5) adapted to our laboratory conditions and the extracts were tested at concentrations of 0.4, 4, 40 µg/mL. The values obtained of LC_{50} were 2.24 (1.38-3.42) µg/mL and 2.58 (1.65-3.82) µg/mL for maceration and soxhlet extraction, respectively. These results suggest that the ethanolic extract of O. gratissimum displays toxicity (LC_{50} < 1000 µg/mL) in BSL which is not influenced by the extraction method.

Refs.
**ISOLATION AND STRUCTURAL IDENTIFICATION OF DITERPENES FROM DODONAEA VISCOSA**

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*Dodonaea viscosa* (Sapindaceae) has a wide distribution in the Brazilian coast. In Southern of Brazil it is known as “vassoura-vermelha” and the leaves are used in folk medicine for the treatment of rheumatism. The literature reports for this species showed investigations concerning anti-inflammatory, antiulcerogenic and antimicrobial properties and the presence of secondary metabolites as diterpenes (dodonic acid, hautriwaic acid) and flavonoids (quercetin, isorhamnetin). The aim of this study was to isolate and identify diterpenes from *Dodonaea viscosa* leaves. The plant material was collected in November 2006, in Florianópolis, SC and was identified by the botanist Prof. Dr. Daniel Falkenberg (Department of Botany, Federal University of Santa Catarina). The air-dried leaves of *Dodonaea viscosa* were pulverized and macerated with EtOH at room temperature for 7 days. The extract was filtrated and evaporated under reduced pressure at temperature below 60º C, resulting in a lipophilic fraction and a hydrophilic fraction. The hydrophilic fraction was dissolved in alkaline medium (NaOH 1%) and extracted with n-butanol to afford the butanolic fraction. This fraction was chromatographed on silica gel column (CHCl₃: EtOH: H₂O) yielding a pure compound codified as DF2. The lipophilic fraction was submitted to a vacuum column (Petrol: EtOAc gradient) to give 10 sub-fractions. The sub-fraction 3 was submitted to successive chromatographic columns affording the compounds codified as DF3 and DF4. The NMR spectra of these three compounds allowed the proposal of partial structures of labdane diterpenes; the compound DF2 was identified as a glycosylated diterpene. The complete structures of the compounds are currently under analysis.

Refs.
Natural products have been utilized by humans since ancient times and the assistance and cure of their diseases was the first purpose for using natural products in medicine. In the last 30 years, the development of new bioassay techniques, biotechnology methods, bio-guided phytochemical studies, automated high throughput screening and high performance analytical methods, have introduced new concepts and possibilities of rational drug design and drug discovery, but the base of studies starting with a preliminary phytochemical screening, that directs all studies and techniques. Polygonum acre belongs to the family Angiospermae and is commonly found in Central and South America, mainly in Brazil and Argentina. Aerial parts are used as folk remedies for hemorrhage and infectious diseases. The leaves were dried at room temperature and powered using mortar and pestle. To phytochemical screening, the flavones were tested by Shinoda, Taubock, Pew, ferric chloride and aluminum chloride reactions. To anthraquinones were used two tests, one to glycosylated anthraquinones and other to free species. The cardiotonic glycosides were characterized by unsaturated lactone rings using Legal and Kedde reactions, and for deoxyl sugars characterization, Pesez, Keller-Kiliani and Liebermann Burchard reactions. Qualitative tannin tests used were gelatin, iron salts and lead acetate reactions. For saponins was used a test to verify the formation of foam. The preliminary phytochemical screening of crude ethanol extract of P. acre showed the presence of flavones in 60% of the tests, suggesting the possible potential as antiinflammatory, antialergic, antiviral and anti-cancer. The presence of hydrolyzable tannins and condensed tannins groups, could present effects against microorganisms and hemorrhage. The presence of saponins, a polycyclic aglycone that is a choline steroid, could be indicative of use as anticholesterolemia, anti-inflammatory and antiviral agents.
CHEMICAL PROFILE OF *Murraya paniculata*
STUDIED BY HPLC-UV-MS

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*Murraya paniculata* (Rutaceae), also popularly known in Brazil as “murta-do-campo”, has long been studied by our research group because it exhibits a differentiated behavior, compared to other plants, since it is asymptotically infested by many microorganisms. *M. paniculata* coexists with the bacteria *Candidatus liberibacter* sp, responsible for “Greening”, a sickness present in many citriculture orchards¹, without any sickness manifestation. Our early studies on this system indicate that probably the chemical contents in organs of the plant are used to control growth of microorganisms. In this sense, the aim of the present work includes investigations on the qualitative chemical profile of *M. paniculata*, through hyphenated techniques, such as high performance liquid chromatography coupled with ultraviolet and mass spectroscopy (HPLC-UV-MS). Figure 1 represents a flow diagram that expresses some of the studies of this project, which includes sample preparation tests - extraction of leaf compounds with different solvents and enrichment of samples using solid phase extraction technique (SPE) - and development of liquid chromatography methods, first using UV detection. The ultraviolet spectrum (Figure 2), relative to each chromatographic band, was analyzed and compared with model substances like hesperidin, rutin, naringin and others. Then, it was concluded that the fraction is enriched of flavonoids. Many of these substances show bactericidal characteristics and many other biological activities already studied².

There are a great number of problems associated with the microbiological contamination of topical drug products, nasal solutions and inhalation products. The significance of microorganisms in non-sterile pharmaceutical products should be evaluated in terms of the use of the product, the nature of the product, and the potential hazard to the consumer. The USP recommends that certain categories be routinely tested with specified indicator microbial contaminants\(^1\). For example plant, animal and some mineral products are tested for \textit{Salmonella} \textit{sp}, oral liquids for \textit{Escherichia coli}, topicals for \textit{Pseudomonas aeruginosa} and \textit{Staphylococcus aureus}, and articles intended for rectal, urethral, or vaginal administration for yeasts and molds. The increasing consumption of natural drugs has becoming their use a Public Health issue, due to the possibility of accessing products without quality. The concern about quality is mainly due to the potential microbiological contamination of the products, by their natural origin. The situation in Brasil showed that great number of samples of the herbal drugs failed in to fulfill the pharmacopoeia parameters of acceptance and therefore, regulatory and educational measures are needed in order to guarantee the quality of these products. The objective of this work was evaluate the microbial contamination of \textit{Polygonum acre} extract. The plant was collected at “Horto de Plantas Medicinais e Tóxicas da Faculdade de Ciências Farmacêuticas de Araraquara – UNESP” and the extract was obtained by percolation with ethanol 70\%, dried in rotavapor and lyophilized until the total dryness. To microbiological quality control of \textit{P. acre} were used the determinations of the total number of microorganisms and search of \textit{Salmonella} \textit{sp}, \textit{E.coli}, \textit{P. aeruginosa} and \textit{S. aureus}. The analysis were done in agreement of Brazilian Pharmacopoeia\(^3\) and British Pharmacopoeia\(^4\). The tests of microbiological quality control showed that all the developed extracts do not present contaminations microorganisms. This fact may be resulted of good practice of collection and manipulation. Is important to point that in this species there is the presence of condensed tannins, wich could protect the plant against attack of herbivorous, microorganisms, beyond presenting enzymatic inhibition in human beings.\(^2\)

\textbf{Refs.}
\begin{enumerate}
\item K.F. Migliato \textit{et al.}, \textit{Rev. bras. Farmacogn}, \textbf{17}, 1 (2007)
\item Farmacopéia Brasileira, \textbf{4}, (1988)
\item British Pharmacopoeia, (2001).
\end{enumerate}
DETERMINATION OF THE PERCENTAGE OF WATER AND TOTAL ASHES FROM LEAVES OF *Cissus verticillata* (L.) Nicolson & C.E. Jarvis subsp. *verticillata*

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The specie *Cissus verticillata* (L.) Nicolson & C.E. Jarvis subsp. *verticillata* (Vitaceae) is known popularly as vegetable insulin, cipó-pucá⁵, cortina japonesa, uva-brava e anil trepador² and is used under the tea’s form in the diabetes’s treatment⁵, as anti-inflammatory, anti-epileptic, anti-hypertension, anti-thermic, anti-rheumatic², influenza and against respiratory infections⁴.

Considering that the effectiveness and safety of vegetable species used for therapeutic depends on the quality, it is important to mind of the ideal conditions of cultivation, crop, dried, stabilization, conservation and armazenation³.

Rare are the existent information on the process of dried of *C. verticillata*, once, if accomplished in an inadequate way they alter the quality of the vegetable species, because the presence of excessive amount of water in vegetable drugs propitiates the development of mushrooms, bacteria, insects and it can cause hydrolyze of its chemical constituents¹.

The determination of the percentage of total ashes establishes the amount of substance no-volatile residual resultant of the incineration process¹. That methodology is so fast, simple and it can be easily applied in the plant’s control quality, becoming possible to detect adulterations in order to avoid the exhibition of the consumer to the real risk of the employment of inappropriate vegetable material to the consume³.

Seeking improvement of the dried conditions, storage, dispensation and use for the population of leaves of *C. verticillata*, the percentage of water was calculation and total ashes through artificial dry. Thus the leaves of *C. verticillata* collected in the neighborhood Antônio Dias, Ouro Preto, Minas Gerais, were selected, washed, distributed in six repetitions and droughts with ventilation forced at 45ºC for period of 96 hours. In the rehearsal determination of total ashes (methodology V.4.2.4., Farm. Bras. IV, 1988), 3g of dry powder of leaves, with four repetitions, were placed in porcelain melting-pot in muffle to 450ºC, for approximately 30 minutes, even total formation of ashes.

In leaves of *C. verticillata* the percentage of water is of 85.9830 ± 1.68% and of ashes totals of 17.9933 ± 0.84%, attesting the effectiveness of the dried process.

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