Phytochemical Analysis
Phytochem. Anal. **19**: 218–228 (2008)
Published online 21 September 2007 in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/pca.1019

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Classification of Commercial Catuaba Samples by NMR, HPLC and Chemometrics

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Received 19 December 2007; Revised 12 June 2007; Accepted 14 June 2007

Abstract: For over a century, Catuaba has been used in Brazilian folk medicine as an aphrodisiac even though the identity of the plant material employed is often uncertain. The species recommended by the *Brazilian Pharmacopeia* is *Anemopaegma arvense* (Bignoniaceae), but many other plants, regionally known as Catuaba, are commercialised. Frequently, the quality control of such a complex system is based on chemical markers that do not supply a general idea of the system. With the advent of the metabolomics approach, a global analysis of samples becomes possible. It appears that ^{1}H-NMR is the most useful method for such application, since it can be used as a wide-spectrum chemical analysis technique. Unfortunately, the generated spectra is complex so a possible approach is to look at the metabolite profile as a whole using multivariate methods, for example, by application of principal component analysis (PCA). In the present paper, we describe for the first time a proton high-resolution magic angle spinning nuclear magnetic resonance (^{1}H-HR-MAS NMR) method coupled with PCA for the metabolomic analysis of some commercial Catuaba samples, which provided a reduction in the time required for such analysis. A comparative study of HPLC, HR-MAS and liquid-NMR techniques is also reported. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: NMR; HPLC; ^{1}H-HR-MAS; PCA; HCA; Catuaba.

INTRODUCTION

During the whole of human history there has been a great interest in the availability of aphrodisiacs and remedies for erectile dysfunction, and recently there has been a resurgence in the sales of exotic herbal preparations for use in these areas (Drewes *et al.*, 2003; Zanolari *et al.*, 2003a). For over a century, Catuaba has been the most popular herbal medicine in Brazilian folk medicine for use as an aphrodisiac and with tonic properties (Teixeira da Fonseca, 1922; De Almeida, 1993), even though no one seems to be able to state with certainty the correct species to use (Daly, 1990). Plants of different genera within the families Erythroxylaceae, Bignoniaceae, Sapotaceae, Euphorbiaceae, Myrtaceae, Meliaceae, Apocynaceae and Burseraceae have all been referred to as Catuaba (Zanolari *et al.*, 2003b). Nevertheless, the species recommended by the *Brazilian Pharmacopeia* is *Anemopaegma arvense* (Vell.) Stellfeld (Bignoniaceae), particularly its roots. A small number of studies of this species have been published and the alkaloid yohimbine has been attributed to this plant (Marques, 1998). The unregulated use of *A. arvense* over a long period has caused this plant to disappear from the market and other plants, regionally known as Catuaba, to be commercialised in its place. Thus, recent studies indicate that in herbal commerce today the species *Trichilia catigua* (Meliaceae) is the most consumed species. It is the bark of this species that is used for medicinal purposes, and the literature records some pharmacological studies with this plant (Manabe *et al.*, 1992; Vaz *et al.*, 1997; Antunes *et al.*, 2001; Pizzolati *et al.*, 2002).

In order to resolve this uncertain situation, the development of new analytical methods by which to determine the identity of herbal medicines became desirable. Nowadays, a great deal of effort is dedicated to characterising and quantifying metabolites of plants. In addition, it is important to develop methods that can provide rapid information about factors such as authenticity and adulteration. A possible approach is to use NMR as a rapid metabolite profiling technique that is able to detect a broad range of metabolites in a non-targeted way. The profile in question here is the NMR spectrum and it encompasses peaks that, for a given solvent, appear at characteristic frequencies. In crude biological extracts, however, there are many peaks, leading to overlapping signals, and this makes

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Contract/grant sposor: Fundação de Amparo à Pesquisa do Estado de São Paulo.
Contract/grant sposor: Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior.
Contract/grant sposor: Conselho Nacional de Desenvolvimento Científico e Tecnológico.

the profiles very complex: these are sometimes referred to as 'fingerprints' and a visual examination is usually insufficient to assess fully a whole series of such profiles. Alternatively, chromatographic methods may be employed in determining the metabolic profiles of herbal products (Gong *et al.*, 2003). The advantages of liquid chromatography include its high reproducibility, its ease of automation and its ability to analyse a large number of constituents in botanicals and in herbal preparations, although most of the time the generated data are also too complex to be interpreted visually (Belton *et al.*, 1998). In order to accomplish this task, multivariate analysis of the resulting data is a promising strategy.

Based on the conception of phytoequivalence, the chromatographic fingerprints of herbal medicines can be utilised to address the problem of quality control of herbal medicines as reviewed recently (Liang *et al.*, 2004). The use of hyphenated chromatographic techniques, combined with chemometric approaches, has also shown its value with herbal medicines such as *Rhizoma chuanxiong* and *Ginkgo biloba* (Gong *et al.*, 2003).

Chemometrics applied to NMR data has been used to address different issues such as food authenticity and origin. More recently, Sacchi *et al.* (1998) applied high-field ^{1}H-NMR to the characterisation of olive oil and to the determination of its geographical origin. The results obtained indicated that samples were correctly classified in 96% of cases and that the discrimination between classes was sufficient to obtain a good characterisation of the geographical origin of the oil. A second example is the identification of the nature and origin of beer samples. This approach has already proven to be of value for the detection and interpretation of spectral changes in apple juices (Mohler-Smith and Nakai, 1990). In the latest decade, this approach has become a tool for the interpretation and quality assessment of industrial and natural products (Kim *et al.*, 2005).

Exploratory data analysis consists mainly of principal component analysis (PCA), which provides a way to summarise the information contained in large sets of spectra. The initial variables (or measurements) are transformed into a much smaller set of variables or principle components (PC) (Defernez and Colquhoun, 2003), and the explanation of what each PC represents in relation to the original measurements lies in the loadings. Another formal method of exploring the patterns in data involves hierarchical component analysis (HCA) where the aim of the method is to detect similarities among samples (Brereton, 2003).

In the present study, the use of HPLC and NMR spectroscopic data together with multivariate analysis is reported for the classification of commercial samples of Catuaba. The described technique has allowed the discrimination of samples based on the kind of raw material employed in their manufacture.

EXPERIMENTAL

Solvents and chemicals. Analytical-grade methanol was purchased from J. T. Baker (Philipsburg, NJ, USA): chloroform and methanol were from Synth (Diadema, SP, Brazil). Methanol-d_4 (99.8% D) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA) and deuterium oxide (99.8% D) was purchased from Acros (New Jersey, NJ, USA). The silica gel used in chromatography was Hypersil® C_{18} from Thermo (Waltham, MA, USA; 5 μm particle size, 120 Å pore size).

Plant material. *Trichilia catigua* A. Juss bark was obtained in Maringá, Brazil, in October 2001. The plant was authenticated by Professor Ismar Sebastião Moscheta and a voucher specimen (Exsiccate HUM 9908) was deposited in the Herbarium of the Universidade Estadual de Maringá, Maringá-PR, Brazil. *Anemopaegma arvense* (Vell.) Stellf. root was obtained from Itirapina-SP, Brazil, in December, 2001. The plant was authenticated by Professor Marta Regina Barroto do Carmo and a voucher specimen (Exsiccate HRCB-3542) was deposited on the Herbarium of the Universidade Estadual de Rio Claro, Rio Claro-SP, Brazil. Commercial samples of Catuaba were purchased from several pharmaceutical stores and companies in the States of São Paulo, Paraná and Mato Grosso do Sul as shown in Table 1.

***Preparation of standard tinctures of* T. catigua *and* A. arvense.** The 20% hydroalcoholic standard tinctures of air-dried bark (40 g) of *T. catigua* and of air-dried root (70 g) of *A. arvense* were prepared in water:ethanol (34:66 v/v) at room temperature for 7 days. The ethanol was removed under vacuum at 40°C to provide aqueous extracts that were lyophilised to yield 6.26 and 6.57 g of residue, respectively.

Instrumentation and chromatographic columns. For HPLC analysis a Shimadzu (Kyoto, Japan) gradient HPLC system was employed composed of two LC-10AD pumps, a SUS mixer, an auto injector model SIL 10A and either an SPD 10D UV–vis detector or photodiode array detector (PAD) model SPD-10AVP. A CBM 10A interface was used for both detectors. Data acquisition was performed using CLASS LC10 software. The analytical column (150 × 4 mm i.d.) was packed with Hypersil C_{18} by the ascending slurry method using methanol for the preparation of the slurry (50 mL) and for the packing. These operations were carried out at a pressure of 7500 psi (Cass *et al.*, 2003). Rotation locular counter-current chromatography (RLCC) was performed using a model RLCC-100 apparatus (Tokyo Rikakikai, Tokyo, Japan), which consisted of 16 columns (450 × 11 mm i.d.) divided by centrally perforated PTFE (polytetrafluoroethylene) discs into 37

DOI: 10.1002/pca

Table 1 A summary of the tested samples

Sample code	Source	Origin (State)	Sampling part	Technique[b]
AA[a]	Standard	São Paulo	Roots	All
TC[a]	Standard	Paraná	Bark	All
TCl[a]	Standard	Paraná	Leaves	I
S1	Pharmacy	São Paulo	Chopped bark	All
S2	Pharmacy	São Paulo	Chopped bark	All
S3	Pharmacy	Paraná	Chopped bark	All
S4	Pharmacy	São Paulo	Chopped bark	All
S5	Central Market	Mato Grosso do Sul	Chopped bark	I, II
S6	Pharmacy	São Paulo	Chopped bark	I, III
S7	Pharmacy	São Paulo	Chopped bark	All
S8	Pharmacy	São Paulo	Chopped bark	All
S9	Pharmacy	São Paulo	Tincture	II, III
S10	Pharmacy	São Paulo	Fluid extract	III
S11	Pharmacy	São Paulo	Dried extract	III

[a]AA, *Anemopaegma arvense*; TC, *Trichilia catigua*; and TCl, *T. catigua* leaves.
[b]I, HR-MAS; II, liquid NMR; III, HPLC.

loculi each. Injections were performed manually by filling the RLCC loop (volume 3 mL).

Analysis of standard tinctures. The standard lyophilised extracts (500 mg) were submitted to RLCC using methanol:water:chloroform (17:33:50 v/v) as the solvent system. The methanol:water phase was used as the stationary phase, whilst the chloroform phase formed the mobile phase.

The solvents were prepared using a separator funnel (2 L), and the phases were allowed to equilibrate prior to their separation. RLCC was employed in the descending mode at 100 rpm and 0.5 psi of pressure at a flow-rate of 0.8 mL/min.

The chloroform fraction (the lower phase) was collected over a period of 12 h (as one fraction only) and after drying under vacuum it was cleaned using the conditions described below.

The chloroform fraction of *T. catigua* was analysed using the C_{18} column by gradient elution with methanol (B) in water (A) from 38 to 100% B in 35 min (Δ%B = 1.77). An isocratic period at 100% B was maintained for 10 min before the gradient was reversed to 38% B, also in 10 min. The chloroform fraction of *A. arvense* was also analysed using the C_{18} column by gradient elution. The conditions were methanol (B) in water (A) from 38 to 70% B in 21 min, then 70–100% B in 10 min prior to the reverse gradient to 38% B, also in 10 min.

Sample clean-up for HPLC analysis. The C_{18} cartridges (Varian, Walnut Creek, CA, USA; 100 mg) were conditioned by treatment with methanol (3 mL) followed by water (3 mL). After this, aliquots (100 µL) of the *T. catigua* or *A. arvense* chloroform fractions were passed through the cartridges, which were then washed with 3 mL of water:methanol (80:20 v/v) and dried prior to extraction with methanol (1 mL). For HPLC analysis, 800 µL of these solutions were placed in an autosampler vial and aliquots of 40 µL were injected into the chromatographic system.

NMR measurements. All of the proton high-resolution magic angle spinning (^{1}H-HR-MAS)-NMR and liquid NMR data were obtained using a Bruker Spectrospin (Karlsruhe, Germany) Avance DRX 400 instrument (operating at 400.13 MHz for ^{1}H-NMR) equipped with a 4 mm rotor with a Kel-F cap and a 5 mm inverse detection probe, respectively. In the ^{1}H HR-MAS NMR technique crude bark (*T. catigua*) and roots (*A. arvense*) were used without any pre-treatment, whilst for the liquid experiments a tincture was re-suspended in 600 µL of methanol-d_4.

^{1}H HR-MAS NMR. All spectra were obtained at a 5 kHz spinning speed without temperature regulation using the Carr–Purcell Meiboom–Gill (CPMG) spin-echo pulse sequence: D–(90–$t_{1/2}$–180–$t_{1/2}$)n–FID. Spectra were the result of summation of 256 free induction decays, with data collected into 32K data points using a spectral width of 6868.11 Hz and an acquisition time of 2.38 s. Prior to Fourier transformation, the FIDs were zero-filled and an exponential weighting factor corresponding to a line broadening of 1.00 Hz was applied. The spectra were referenced to TSP -d_4 at δ 0.00 ppm.

Liquid NMR. The ^{1}H-NMR data were the result of 256 free induction decays, with data collected into 64K data points, a spectral width of 6038.67 Hz and an acquisition time of 2.71 s. Prior to Fourier transformation, an exponential line broadening equivalent to 0.3 Hz was applied to the free induction decays. The spectra were referenced to tetramethylsilane

DOI: 10.1002.pca

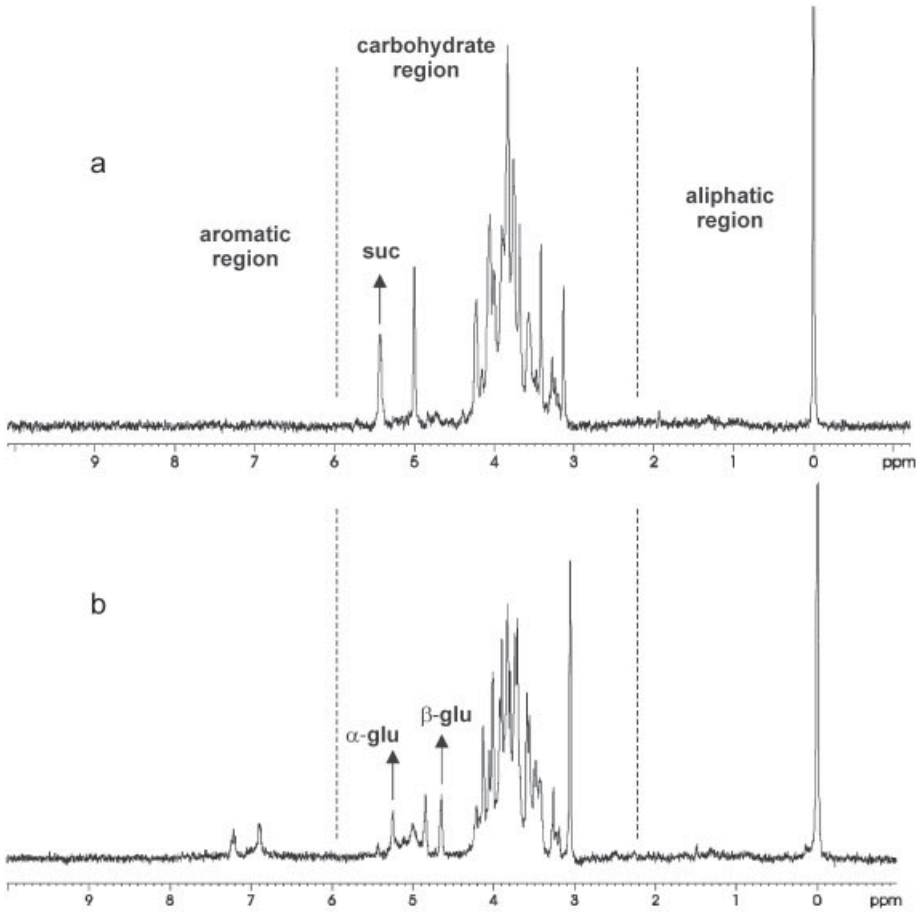

Figure 1 ^{1}CPMG NMR spectra for the samples of (a) *A. arvense* and (b) *T. catigua*.

at δ 0.00 ppm. The tincture used was prepared by macerating 300 mg of each sample in methanol (50 mL) at room temperature for 7 days. The methanol was completely removed under vacuum at 40°C and the dried extract was re-suspended in methanol-d_4.

Data analysis. The ^{1}H-NMR spectra (from both HR-MAS and liquid NMR) were reduced to ASCII files using the function TOJDX in the XWinNMR program (version 3.11) according to the manufacture's recommendations. This function converts a data-set into a JCAMP-DX ASCII format and the produced file includes the parameters followed by a table containing the *X* and *Y* coordinates for the data. The resulting data matrix was imported into Origin (version 5.0, Microcal, Northampton, MA, USA), and the residual water region (δ 4.6–5.8 ppm) was removed prior to statistical analyses thus eliminating any variability in the suppression of water in the sample. The residual proton signals corresponding TSP-d_4 and TMS (δ 0.00 ppm) were also removed at this stage. The generated data were imported into Pirouette® (version 2.7, Infometrix, Bothell, WA, USA) for PCA and HCA. Chromatographic data were also reduced to ASCII files using CLASS LC10 software. The resulting data matrix was imported into Microsoft Excel and multivariate analyses were performed with Pirouette software.

RESULTS AND DISCUSSION

Features of ^{1}H-NMR spectra and of the chromatographic fingerprint of the standard samples

In this study, samples classified botanically as *A. arvense* or *T. catigua*, together with some commercial samples, were analysed using ^{1}H HR-MAS NMR, liquid ^{1}H-NMR and HPLC (Table 1). Although an extensive assignment of the constituents of the samples is outside the scope of this study, a global compositional description for samples of *A. arvense* and *T. catigua* is given in Fig. 1. In general, the ^{1}H HR-MAS NMR spectra obtained showed a dominance of signals in the carbohydrate region of the spectrum deriving from the primary metabolism of the plant. In the aromatic region (δ 6.0–8.0 ppm) a few signals were present in the spectra of *T. catigua*. Some signals in the relatively clear trace areas could be assigned to particular carbohydrates (e.g. α- and β-glucose anomeric hydrogens

DOI: 10.1002/pca

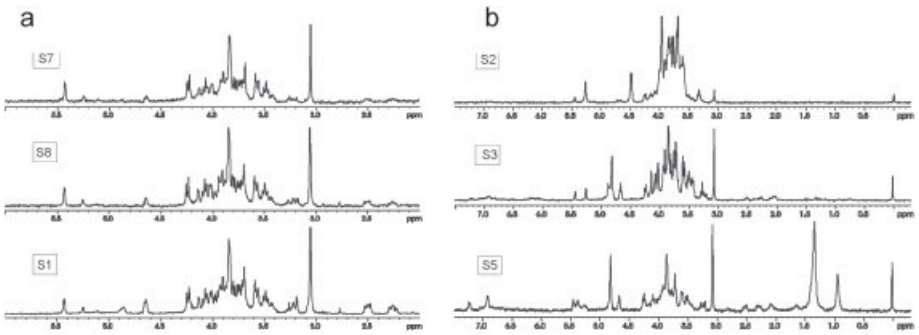

Figure 2 ^{1}H CPMG NMR spectra for commercial samples. (a) Samples with similar NMR profile and (b) with different NMR profile.

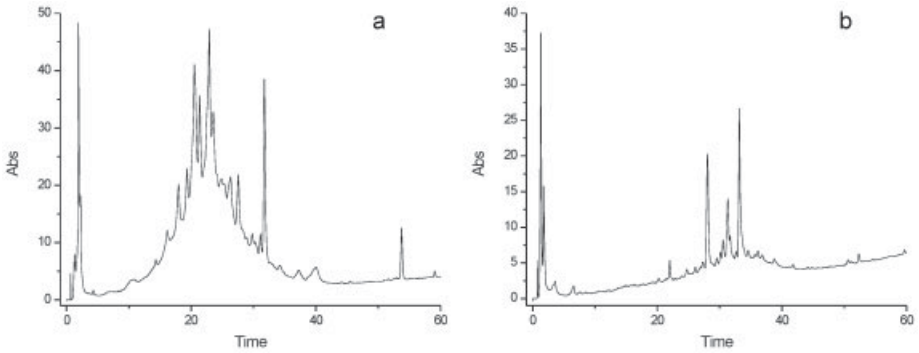

Figure 3 Chromatograms for the hydroalcoholic extracts of (a) *T. catigua* and (b) *A. arvense.*

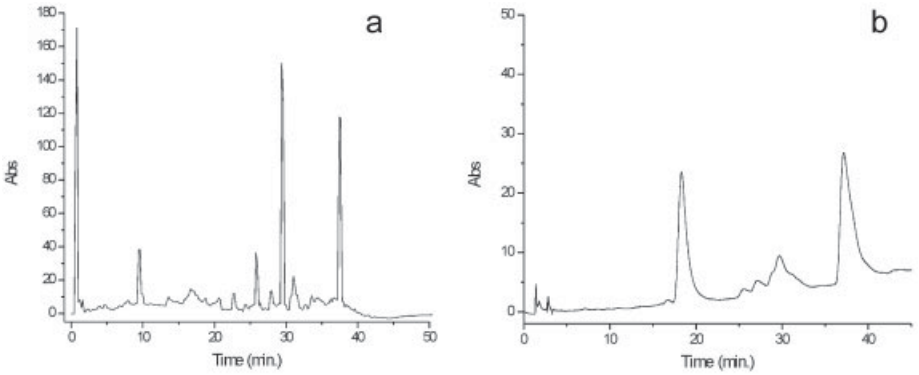

Figure 4 Chromatograms for the samples (a) *T. catigua* and (B) *A. arvense.*

at δ 5.20 and 4.60 ppm for *T. catigua* and sucrose anomeric hydrogen at δ 5.40 ppm for *A. arvense*).

From visual analysis of the spectra of commercial samples, clear differences were evident. For example, some samples had a similar NMR spectral profile while others had a totally different profile. In addition, other differences included a variation in the intensities of the same signals. The spectra are shown in Fig. 2. Using the liquid NMR technique, the spectra of the commercial samples showed the same pattern as mentioned before (data not shown).

In order to obtain initial information on the compounds present in the crude lyophilised extract of the standard samples of *A. arvense* and *T. catigua*, an HPLC-PAD (200–400 nm) analysis was carried out on an C_{18} silica column with gradient elution of methanol (B) in water (A) from 5 to 100% B in 60 min. Compounds of high polarity and absorption in the crude hydroalcoholic extracts interfered with the analysis (as shown in Fig. 3), and so samples were pre-purified by RLCC (Beltrame *et al.*, 2005). The lower phases obtained were submitted to a solid-phase extraction. In order to do this, C_{18} cartridges were used and a variety of solvent mixtures were tested under different conditions to remove the polar compounds. The methanolic fractions obtained were evaluated and the gradient elution conditions were optimised for the two standard samples. As in the chromatograms of the crude lyophilised extract of the standard samples of *T. catigua* and of *A. arvense*, the chromatograms after clean-up in Fig. 4 were also completely different from each other.

DOI: 10.1002.pca

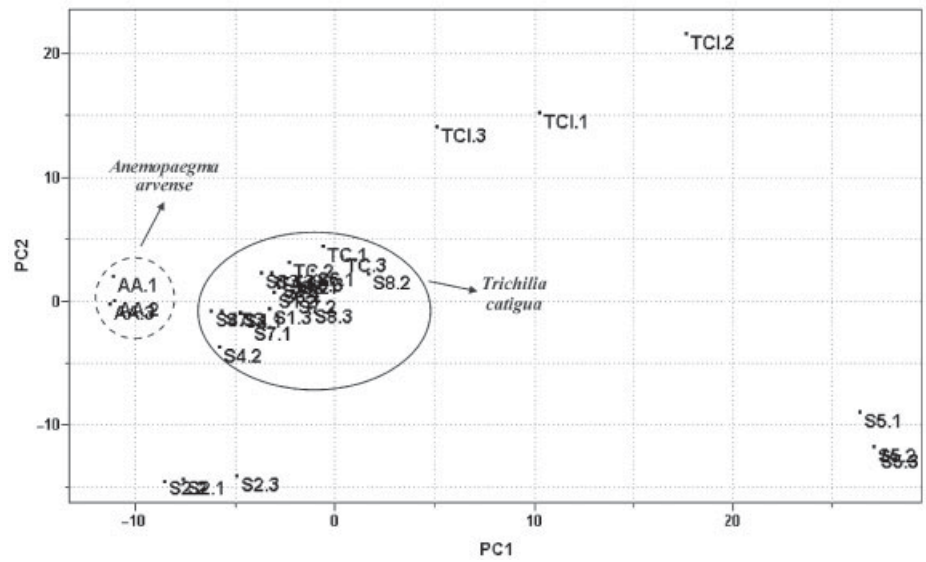

Figure 5 PCA scores plot of ^{1}H HR-MAS NMR data: PC1 (21.8%) vs (12.7%).

Multivariate statistical Analysis of ^{1}H HR-MAS NMR data sets

Prior to PCA, the spectra data were auto-scaled (pre-processing) and normalised. As displayed in Fig. 5, the PCA of the ^{1}H HR-MAS data showed, as expected, that the raw materials of the commercial samples were not composed of roots of *A. arvense*. In Fig. 5, it may be seen that the samples are displayed essentially in five groups. On the left of the plot it is possible to observe a distinct group formed by the sample from the *A. arvense* standard, while most of the commercial samples are very similar to the standard of *T. catigua* (the biggest group in the centre of the plot). It can also be observed that some commercial samples, namely, S2 and S5, are completely different from the two species analysed, but the behaviour of the sample S5 could be explained by the fact that this sample was bought in another state of Brazil (i.e. Mato Grosso do Sul) where, probably, a different species is commercialised as Catuaba. The replicates from the TCl sample (TCl.1, TCl.2 and TCl.3) were separated from the main group due to the fact that the raw material of these samples was of leaves instead of bark of *T. catigua*. It may also be noticed that the replicate analysis of these samples is spread out due to the fact that packaging of these samples in the rotor for HR-MAS NMR analysis was not reproducible.

HCA using the incremental linkage method was also performed for the same ^{1}H HR-MAS NMR spectral data as used above. It is important to note that, in the dendrogram, the objects are organised according to their similarities, indicated by the scale at the top of the plot. For the dendrogram in Fig. 6, with a similarity index of 0.36, the large group is formed mainly of the commercial samples and the standard sample of *T. catigua*. These results are in agreement with those previously found using PCA. With a similarity index of 0.45, the highlighted group of the analysed samples is divided into two smaller groups indicated by dotted lines in Fig. 6. The analysis of the results indicated that these two groups distinguish between the places where the commercial samples where bought. The majority of the commercial samples were bought in the State of São Paulo, but sample S3 was purchased in Paraná State (the same region where the standard sample of *T. catigua*, TC, was collected). These results demonstrate that the HCA was able not only to distinguish between the types of raw materials but also in grouping the samples in accordance to where the commercial samples were acquired.

Multivariate statistical analysis of liquid ^{1}H-NMR data sets

The analysis was repeated for liquid ^{1}H-NMR data, but the number of replicates employed was larger than for ^{1}H HR-MAS NMR (five instead of three). The results found were similar to those obtained with the HR-MAS data but the PCA scores plot showed a different distribution for the samples, probably due to contributions of the minor components. Thus, a more effective evaluation of minor metabolites might be achieved by considering the results of a particular spectral region and not the entire spectrum, as shown in Fig. 7.

PCA was performed separately on the three major regions of the liquid ^{1}H-NMR spectra, namely, the aliphatic, carbohydrate and aromatic regions. However satisfactory results were found only when PCA was applied on the aromatic region as shown in Fig. 8. The liquid ^{1}H-NMR data were auto-scaled and normalised,

DOI: 10.1002.pca

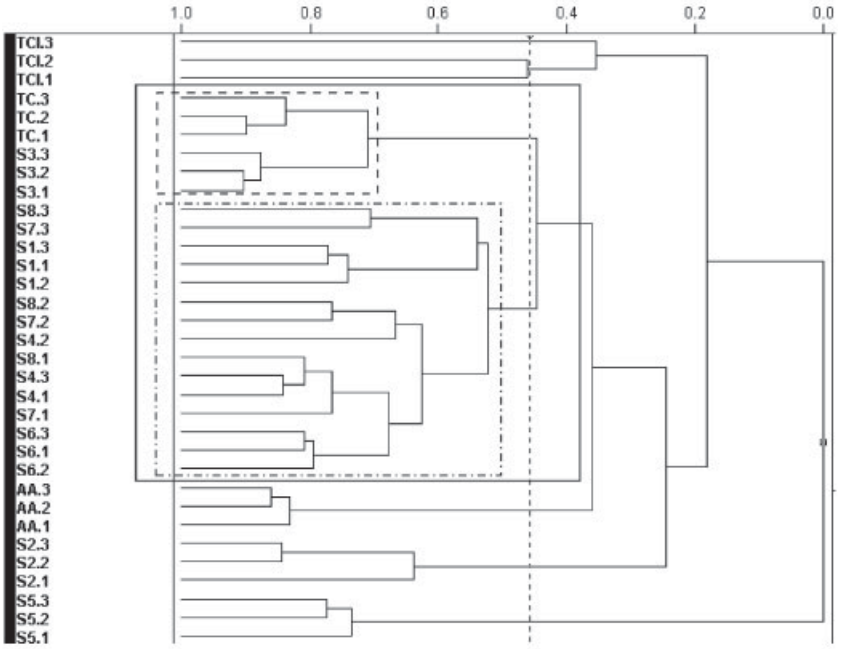

Figure 6 Dendogram of ^{1}H HR-MAS NMR data.

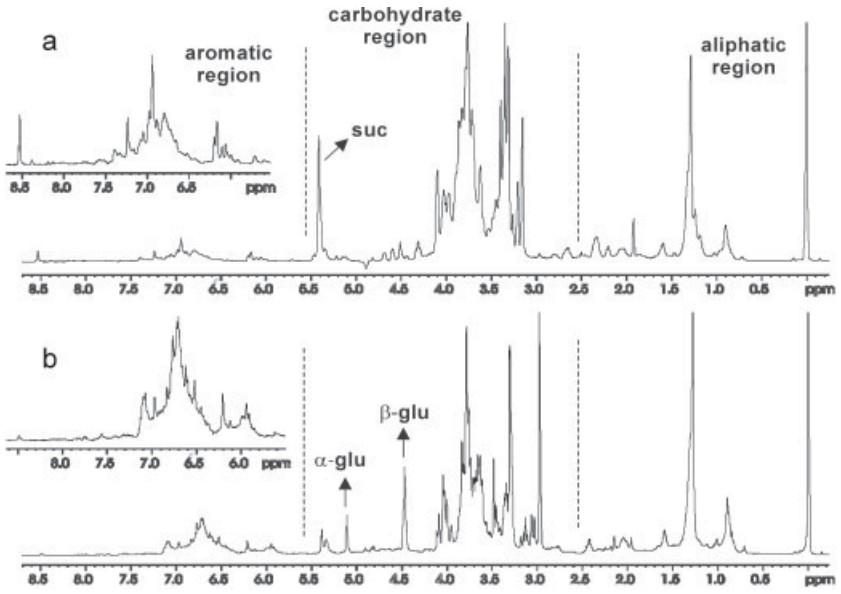

Figure 7 ^{1}H liquid NMR spectra for the samples of (a) *A. arvense* and (b) *T. catigua*.

and the new score plot for these data showed the replicate of *T. catigua* standard joined to sample S3 as found before on HR-MAS PCA. Again, the pattern of *A. arvense* was different from that of *T. catigua*. Unexpectedly all the commercial samples were separated according to their origin (São Paulo, Paraná, Mato Gosso do Sul States) and in addition sample S9 (on the bottom of the PCA scores plot) showed a different profile, probably due to the fact that this sample was a tincture instead of chopped bark like the others.

With respect to the HCA analysis shown in Fig. 9, the results are in agreement with those previously found in PCA. The biggest group was formed by the samples from São Paulo State, and the other samples were grouped together according to their origin on the dendrogram. The linkage method used was incremental. Probably the overlapping signals between δ 6.0 and 8.0 ppm are responsible for these results. When the ^{1}H spectra from both techniques (HR-MAS and liquid NMR) are compared it is possible to see that the profiles for the carbohydrate region are quite similar (e.g. α- and β-glucose for *T. catigua* and sucrose for *A. arvense*) and the PCA results found for the aliphatic regions were inconclusive.

DOI: 10.1002.pca

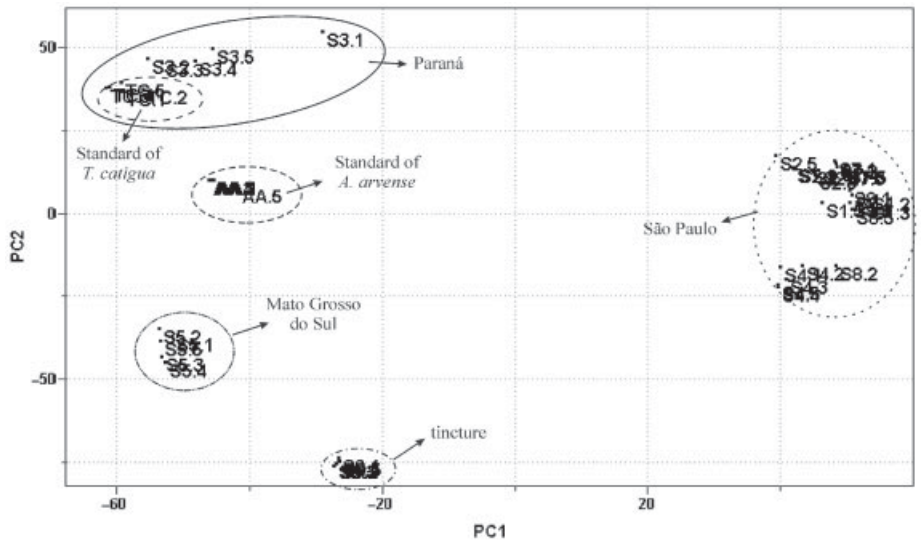

Figure 8 Score plot of ^{1}H liquid NMR data: PC1 (35.8%) vs PC2 (19.3%).

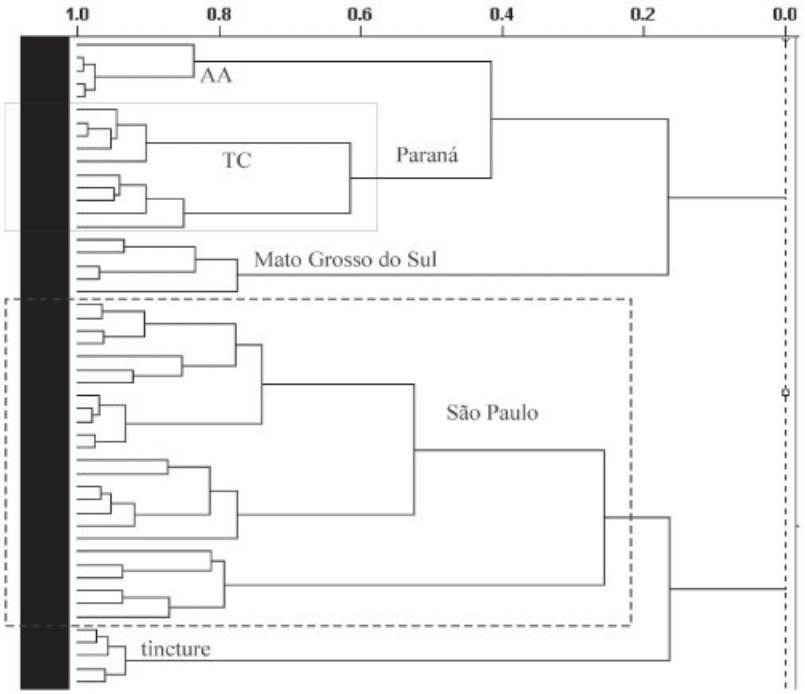

Figure 9 Dendogram of ^{1}H liquid NMR data.

Multivariate statistical analysis of HPLC data sets

The same commercial samples, together with some additional ones, were analysed using HPLC (Table 1), thus providing data not only for chopped bark but also for three further commercial phytomedicine products named as Catuaba (one tincture, one fluid extract and one dried extract). The standard samples of *T. catigua* and *A. arvense* were prepared and analysed in triplicate to certify the extraction procedure and the conditions of analysis. Having done that, each commercial sample was prepared only for a single chromatographic analysis. After transforming the chromatograms, PCA was applied to the data matrix. The scores plot depicted in Fig. 10 shows the presence of two distinct groups of commercial samples, one containing the chopped bark samples and the other with the evaluated phytomedicines. Analysing the PC scores plot carefully one can distinguish that the standard samples of *T. catigua* and *A. arvense* are on opposite sides while the majority of the commercial samples are closer to the *T. catigua* standard sample. The HPLC data were

DOI: 10.1002/pca

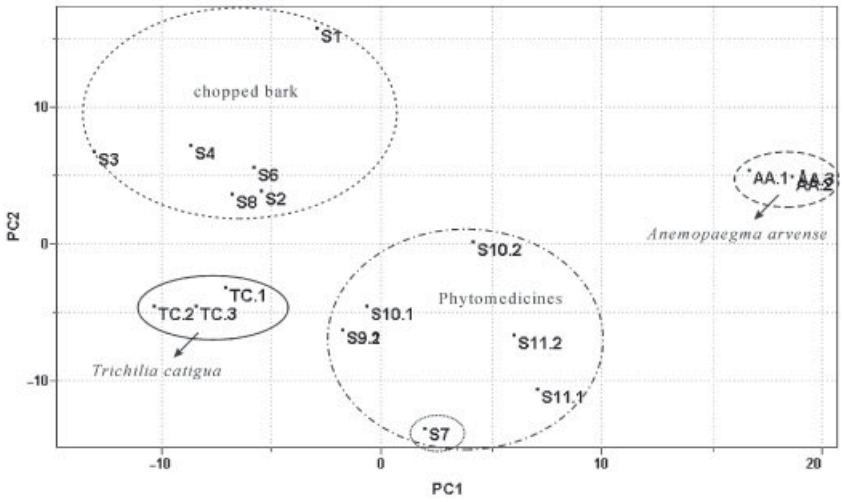

Figure 10 Score plot of principal component analysis of HPLC data: PC1 (35.0%) vs PC2 (20.0%)

auto-scaled and normalised and the entire chromatogram was employed.

In the above scores plot, sample S7, which appears at the bottom of the plot in the phytotherapic group, can be considered as an exception due to the fact that this sample was a chopped bark. Thus, this sample was expected to be in the top group of the PC scores plot.

The dendogram from HCA (using the incremental linkage method) for the HPLC data is shown in Fig. 11. One can see again that two groups are formed at a similarity index of 0.46. However, the replicate of sample S3 (from Paraná State) did not join with the *T. catigua* pattern as expected.

Multivariate statistical analysis of HR-MAS, liquid NMR and HPLC scores

In order to apply the PCA to the NMR and HPLC data, only those samples analysed by all three methods were considered. First of all, PCA was applied to each of the techniques separately (as shown before), three PCs were chosen for each generated data, and then the PCA was performed on this condensed data matrix such that a new PC scores plot was generated.

In Fig. 12 it is possible to see that the standard sample of *T. catigua* is very different from the standard sample of *A. arvense*, and that the commercial samples

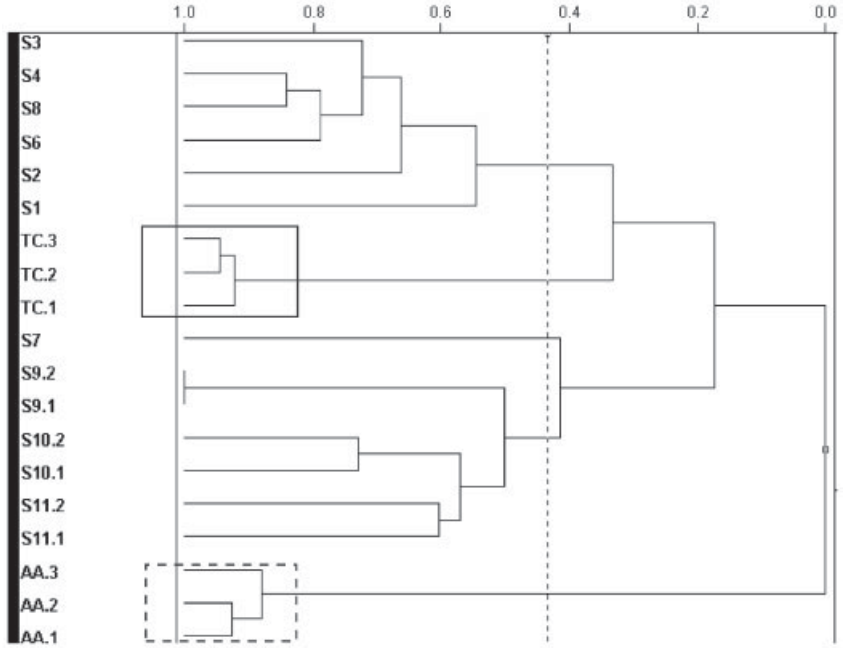

Figure 11 Dendogram of HPLC data.

DOI: 10.1002.pca

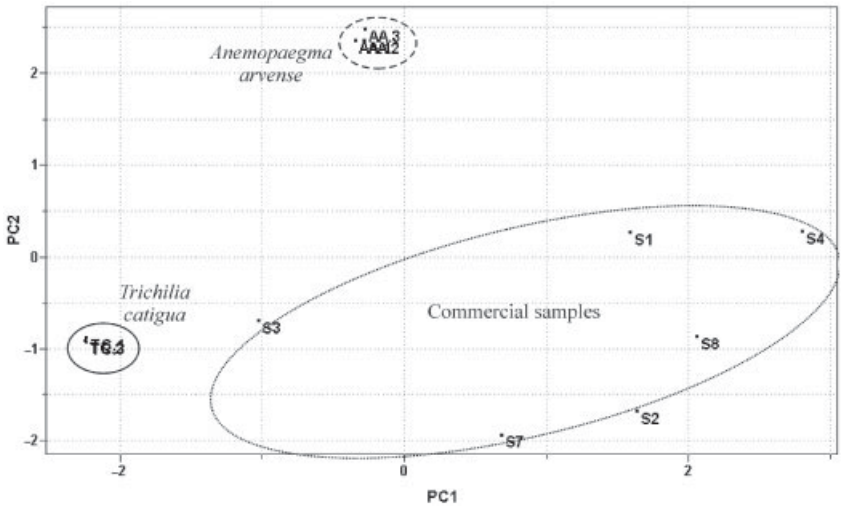

Figure 12 Score plot of principal component analysis of NMR and HPLC data: PC1 (34.4%) vs PC2 (28.0%).

are arranged on the same axis as the *T. catigua* sample (PC2).

This same behaviour was observed when each technique was used separately. Analysing the loadings plot one can observe a correspondence between the experimental technique and the profile of the PC scores plot. For example: the standard sample of *A. arvense* is on the top side of the scores plot when compared with the rest of the samples and the HPLC technique was responsible for this discrimination. These results were based on the loadings plot information shown in Fig. 13. Nevertheless, the NMR technique was effective in grouping all the commercial samples as from bark of *T. catigua*.

In the light of the above, HPLC and NMR spectroscopy are clearly valuable tools for unbiased metabolite fingerprinting of Catuaba. Principal component analysis highlighted genuine differences between commercial samples and the standards. Differences could be detected in both the carbohydrate region and the aromatic region, with sugars and other compounds contributing to the differences in the sample set. The present study has demonstrated how ^{1}H-NMR analyses may be used in the future as a first-pass screening to determine and characterise differences in molecular composition of plant samples in a very simple way.

All the techniques together with the multivariate analysis were shown to be efficient to discriminate the samples based on their raw material, and the HR-MAS technique with HC analysis showed that it is important to take into account the regionalism of the samples. Additionally, it is important to note that the HR-MAS technique is highly feasible because it does not require any pre-treatment of the samples, but it is insufficient alone for structural elucidation or quantification due to strong signal overlap in the ^{1}H-NMR spectra.

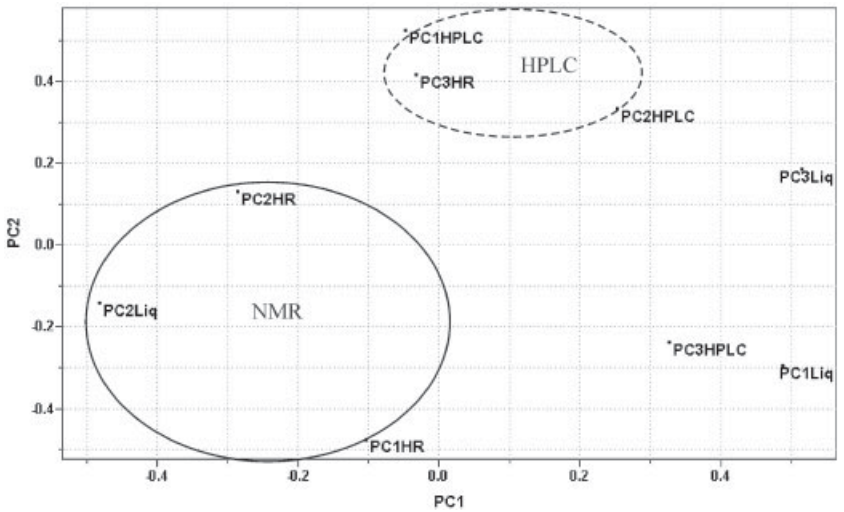

Figure 13 Loadings of principal component analysis of NMR and HPLC data.

DOI: 10.1002/pca

These initial results open up the possibility that NMR/HPLC/multivariate analysis might be a powerful combination, when applied to a suitably enlarged group of samples, as a first step screening to rapidly determine some compositional differences in plant samples, in a very simple way.

Based on results discussed in this work, it can also be concluded that the herbal medicine industries in Brazil do not employ the roots of *A. arvense* to manufacture the phytomedicine Catuaba, as recommended by the *Brazilian Pharmacopiea*, but instead use the bark of *T. catigua*.

Acknowledgements

The authors are grateful to Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support. We also thank Professor Marta Regina Barroto do Carmo for the identification of the *Anemopaegma arvense* specimen.

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