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Análise quimiométrica usando RMN de ^{13}C como ferramenta para diferenciar duas espécies de *Maytenus*

Chemometric analysis using ^{13}C NMR as a tool to differentiate two species of *Maytenus*

DOI 10.5935/2446-4775.20170003

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Abstract

Maytenus robusta was recently synonymized with *Maytenus gonoclada* due to overlapping of morphological characters used in the botanical classification. The present study investigates the chemical differences between *Maytenus gonoclada* and *Maytenus robusta* using chemometric analysis. The ^{13}C NMR data of different triterpenes obtained from these species were evaluated with Principal Component Analysis (PCA). Friedelanes were the main metabolites that contributed to the recognition of differences among these two taxa. Chemometric analysis seems to be an important tool in Celastraceae taxonomy.

Keywords: *Maytenus gonoclada*. *Maytenus robusta*. Metabolomics. Pentacyclic Triterpenes. Chemotaxonomy.

Resumo

Maytenus robusta foi recentemente sinonimizada com *Maytenus gonoclada* devido a sobreposição de caracteres morfológicos utilizados na classificação botânica. No presente trabalho foram investigadas as diferenças químicas entre *Maytenus gonoclada* e *Maytenus robusta* usando análise quimiométrica. Os dados de RMN de ^{13}C de triterpenos pentacíclicos obtidos a partir dessas espécies foram avaliados por meio da Análise de Componentes Principais (PCA). Os friedelanos foram os principais metabólitos que contribuíram para o reconhecimento de diferenças entre os táxons. Análises quimiométricas demonstram ser uma importante ferramenta em taxonomia de Celastraceae.

Palavras-chave: *Maytenus gonoclada*. *Maytenus robusta*. Metabolômica. Triterpenos Pentacíclicos. Quimiotaxonomia.

Introduction

Maytenus Molina sl. is the largest genus in the family Celastraceae, comprising about 300 species, widely distributed in the tropics and subtropics with 49 of them occurring in Brazil (MCKENNA et al., 2011). In several countries *Maytenus* species are routinely used in traditional medicine to treat a variety of illnesses, such as gastric disorders, rheumatism and diarrhoea (LEITE et al., 2001; SOSA et al., 2007). Phytochemical studies show that many of these species present bioactive metabolites, especially pentacyclic triterpenes (NIERO, ANDRADE and CECHINEL FILHO, 2011). The *Maytenus* genus has been chemically characterized by the production of terpenoids, especially dihydroagarofuran sesquiterpenes, triterpenes and some unusual constituents, such as dimers and trimers of sesquiterpenes, diterpenes and triterpenes (NIERO, ANDRADE and CECHINEL FILHO, 2011). Pentacyclic triterpenes such as friedelan-3-one and friedelan-3*β*-ol are considered taxonomic markers of *Maytenus* (OLIVEIRA et al., 2007).

Maytenus is a polyphyletic genus and additional research is important to better understand its taxonomy (JOFFILY and VIEIRA, 2005). For example, some species were synonymized with *M. gonoclada*, such as *M. robusta* Reissek (GROPPO and ERBERT, 2015) and *M. salicifolia* Reissek (GROPPO, 2009) because of their morphological diagnostic characteristics were overlapping, mainly about stem apex form, fruit shape and size. Biral and Lombardi (2012) considered *M. gonoclada* and *M. robusta* as synonymous because of the taxonomic distinction problems, but highlighted the need for more precise characteristics to establish this definition. On the other hand, Niero, Andrade and Cechinel Filho (2011) together with Carvalho-Okano (1992) pointed out that these species present relevant differences. *M. gonoclada* fruits are orbicular, smaller and its branches have quadrangular shape whereas *M. robusta* fruits are elliptical, bigger and its branches have a flattened-cylindrical shape. In addition, *M. gonoclada* is typical from highlands, occurring in *campos rupestres* or mountain forests, while *M. robusta* occurs in the Atlantic Lowland Rainforest (CARVALHO-OKANO, 1992; CARVALHO-OKANO and LEITÃO-FILHO, 2004).

Metabolomics represent an important tool to taxonomic classification. This technique is based on a qualitative and quantitative evaluation of a number of compounds in plants or other organisms. This tool utilizes chemical data from NMR, IR, and/or MS spectrometry in combination with multivariate analysis, to study the metabolome of live beings (MAULIDIANI et al., 2012). The Principal Component Analysis (PCA) is one of the chemometric methods routinely used. Its purpose is the data reduction from linear combinations of the original variables, what provides a better visualization of the results (MASSART, 1998).

PCA has been recognized as an important statistical tool and has been used to differentiate samples according to their chemical composition (MAULIDIANI et al., 2012). Cruz and coworkers (2008) used PCA to investigate the relationships between the families Celastraceae and Hippocrateaceae, using a large number of compounds together with botanical information. The results contributed to the inclusion of Hippocrateaceae in Celastraceae family. Maulidiani and coworkers (2012) studied the metabolites of three species of Apiaceae utilizing NMR spectral data and PCA. They differentiated the species *Centella asiatica*, *Hydrocotyle bonariensis* and *H. ibthorpioides*, showing the potential of multivariate data analysis. In this

context, the combination of NMR spectroscopy and PCA can provide more information to clarify the taxonomy of *Maytenus* species.

This study investigated the chemical differences between *M. gonoclada* Martius and *M. robusta* Reissek (synonymized with *M. gonoclada* Martius) (GROPPO, 2009) using combination of ^{13}C NMR spectral data with PCA analysis.

Materials and Methods

A literature review was performed in order to obtain the ^{13}C NMR chemical shifts of the triterpenes from leaves and branches of *M. gonoclada* and *M. robusta* (OLIVEIRA et al., 2007; SILVA et al., 2011a; SILVA et al., 2011b; SILVA et al., 2013, NIERO et al., 2006; SOUSA et al., 2012a; SOUSA et al., 2012b). The compounds (13 triterpenes for each species) were analyzed based on the chemical shifts of the carbon atoms C1 to C30, generating a matrix. These data were subjected to PCA using Matlab R2009b program (Math Works, Natick, MA, USA) together with PLS Toolbox 6.2 (Eigenvector Research, Wenatchee, WA, USA) for the chemometric treatment.

In order to obtain more accurate results, the matrix was re-evaluated and only the chemical shifts of triterpenes with the friedelane skeleton (9 of *M. gonoclada* and 10 of *M. robusta*) were selected. This new analysis was performed because the friedelanes provided more consistent data since their occurrence is more reported in both species than triterpenes with other skeletons. Then, the matrix was re-subjected to PCA.

Results and Discussion

To provide more information to support differences or similarities among the two species of *Maytenus*, a study was performed applying PCA to the ^{13}C NMR data of triterpenes from leaves and branches of *M. gonoclada* and *M. robusta*. A number of 26 triterpenes were selected, 13 of each species (**TABLE 1**).

TABLE 1: Triterpenes isolated from *M. gonoclada* and *M. robusta*, grouped in accordance with skeleton.

Triterpenes Pentacyclic	Skeleton	Species	
		MG	***MR***
friedelan-3-one	Friedelane	+	+
friedelan-3*β*-ol		+	+
friedelane-3,16-dione		+	+
29-hydroxyfriedelan-3-one		+	+
friedelane-3,11-dione		+	-
12*α*-hydroxyfriedelane-3,16-dione		+	-
friedelane-3,12-dione		+	-
12*α*-hydroxyfriedelan-3-one		+	-
12*α*,29-dihydroxyfriedelan-3-one		+	-
11*β*-hydroxyfriedelan-3-one		-	+
friedelane-3*β*,11*β*-diol		-	+
21*α*-hydroxyfriedelane-3,15-dione		-	+
friedelane-3,15-dione		-	+
3,4-*seco*-friedelan-3,11*β*-olide		-	+
3,4-*seco*-friedelan-3-oic acid		-	+

21*β*-H-hop-22(29)-en-3*β*-ol	Hopane	-	+
3,4-*seco*-21*β*-H-hop-22(29)-en-3-oic acid		-	+
21*β*-H-hop-22(29)-en-3-one		-	+
Lupeol	Lupane	+	-
α-amirine	Ursane	+	-
β-amirine	Oleanane	+	-
Taraxerol	Taraxerane	+	-

MG = *M. gonoclada*; *MR* = *M. robusta*; (+) Isolated; (-) Not yet found in this species of *Maytenus*.

The triterpenes ^{13}C NMR spectral data of both *Maytenus* species were obtained from the literature. Although the collected spectra were obtained with various solvents and NMR equipments, the differences did not invalidate the study because the chemical shift assignments of the analyzed triterpenes are well known. Firstly, the PCA analysis considered the data of the 26 triterpenes using the ^{13}C NMR chemical shifts as variables. However, this analysis was unproductive due to the high number of scores that demonstrated inconsistencies in the results. This may be justified by the high skeleton variety of the compounds, as well as the low number of examples for each triterpene class except the friedelanes. Therefore, the PCA analysis was repeated employing the ^{13}C NMR chemical shifts of the 19 friedelanes. Each carbon atom was considered a variable and projected in smaller dimensions through linear combinations. According to the results, only three major components comprised 92.0% of the total variance of the model after analysis of the 30 original variables. In other words, three components are sufficient to explain the difference between *M. gonoclada* and *M. robusta*. The principal components PC1, PC2 and PC3 showed a variance of 83.48%, 5.19% and 3.29%, respectively (**FIGURE 1**).

FIGURE 1: Principal Component Analysis (PCA) highlighting PC1, PC2 and PC3 to differentiate *Maytenus gonoclada* (A) and *Maytenus robusta* (B) according to the friedelanes ^{13}C NMR data.

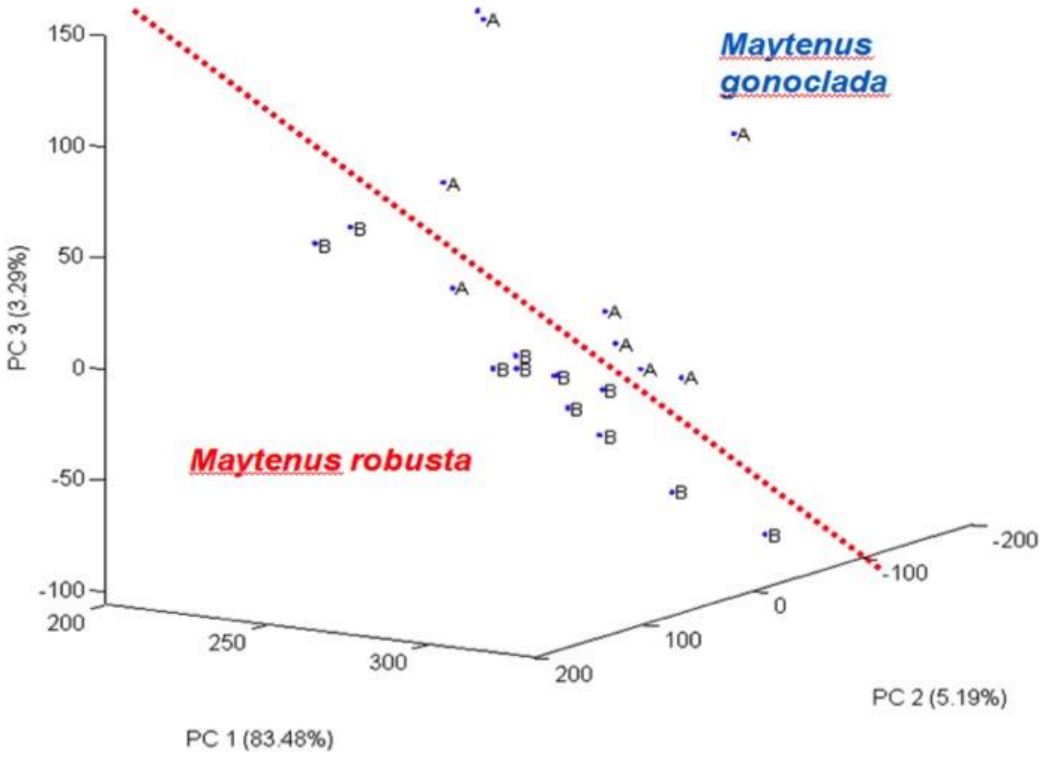
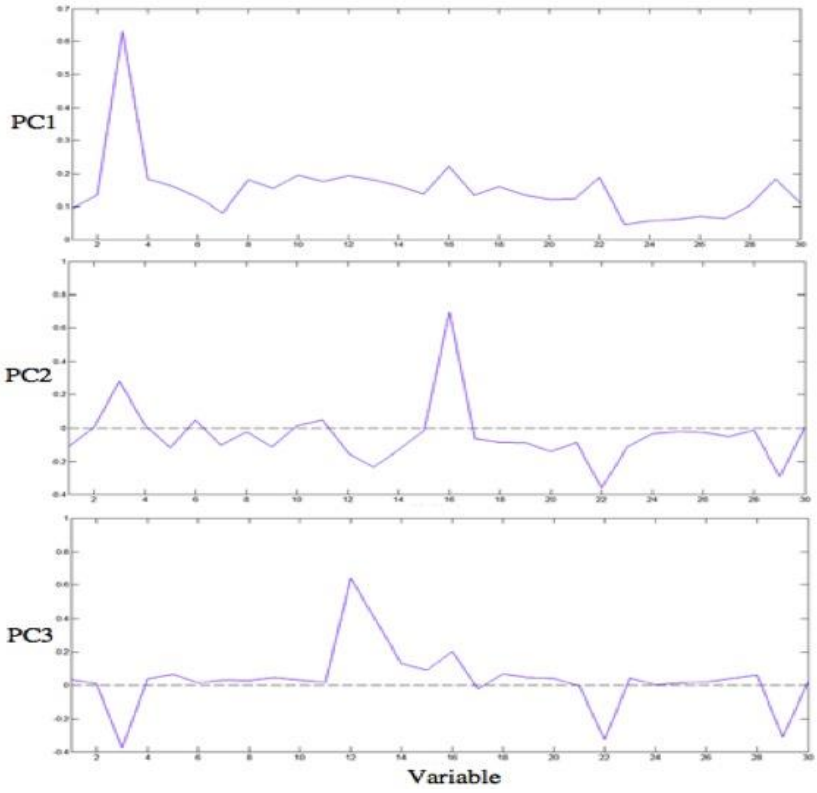


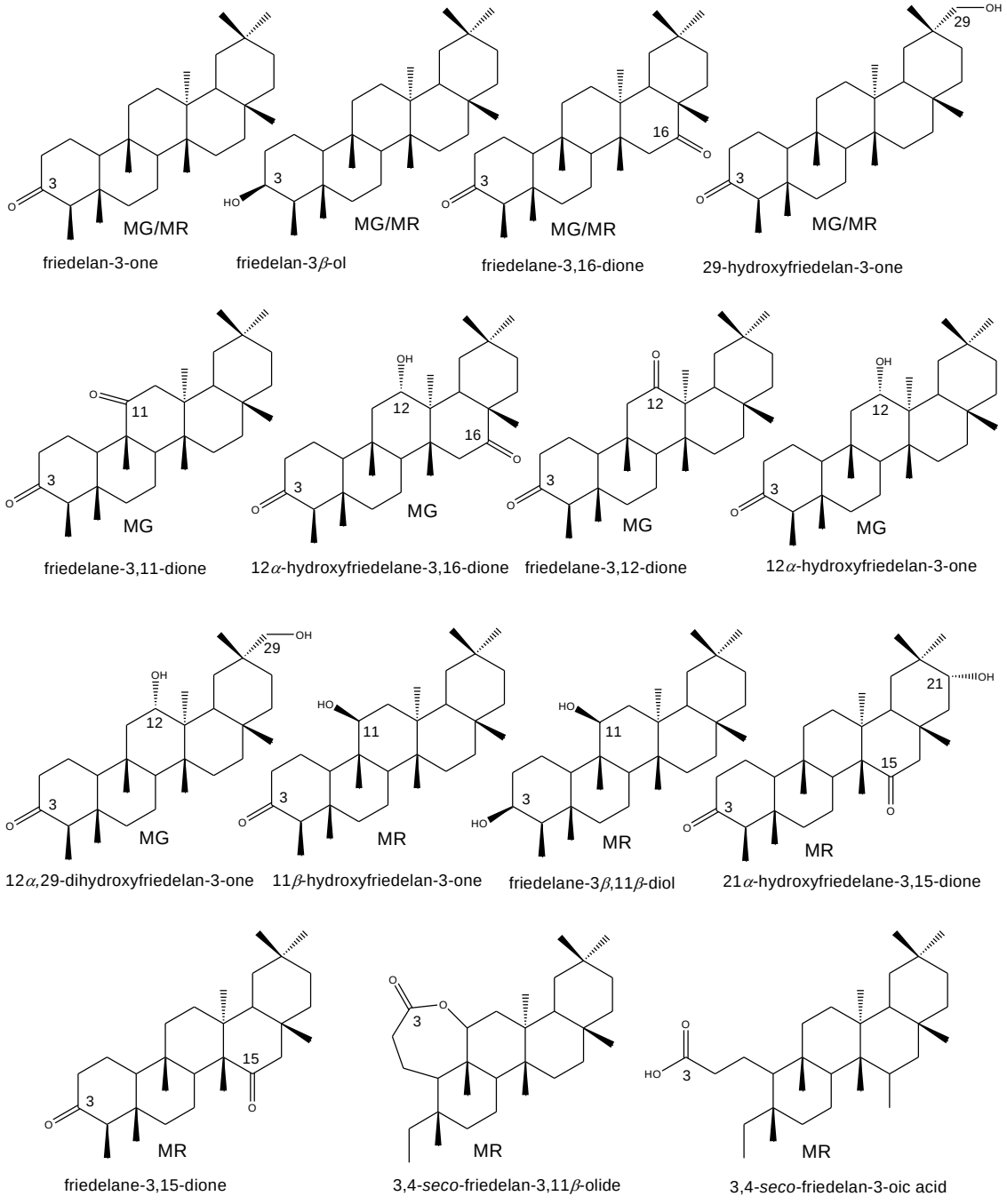
FIGURE 2: Loading plot of PC1, PC2 and PC3 of friedelanes from *Maytenus gonoclada* and *Maytenus robusta*.



The most important variables in the construction of PC1, PC2 and PC3 are connected to the carbon atoms 3, 16 and 12, respectively. Indeed, the friedelanes from *M. gonoclada* tend to present carbonyl groups at C3, C12 and C16 and hydroxyl groups at C3 and C16. On the other hand, *M. robusta* friedelanes tend to have predominantly carbonyl group at C15, hydroxyl groups at C11 and carbonyl, carboxyl and lactone groups at C3 (**FIGURE 3**).

Despite the morphological similarities and difficulties for the taxonomic distinction between *M. gonoclada* and *M. robusta*, differences in their chemical profile corroborate the separation of both species. Besides the friedelanes, the hopanes mark a distinction since this class of compounds is uncommon for *Maytenus* genus. Several *Maytenus* species are commonly used in folk medicine especially to treat gastric disorders and the imparity in their chemical constitutions should affect their therapeutic properties. The differences in *Maytenus* species chemical compounds can be exploited in the quality control of vegetable raw material intended for medicinal use.

FIGURE 3: Chemical structures of friedelanes from *Maytenus gonoclada* (MG) and *Maytenus robusta* (MR).



Conclusion

This study indicated chemical differences between *M. gonoclada* and *M. robusta* based on ^{13}C NMR chemical shifts of friedelanes through PCA analysis. The obtained results corroborate the classification of *M. gonoclada* and *M. robusta* as being different species as proposed by Niero, Andrade and Cechinel Filho (2011) and Carvalho-Okano (1992).

Acknowledgments

The authors thank to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

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Conflito de interesses: O presente artigo não apresenta conflitos de interesse.
Histórico do artigo: Submissão: 10/10/2016 | Aceite: 12/07/2017 | Publicação: 22/09/2017
Como citar este artigo: SOUSA, G. F.; SILVA, F. C.; ALEME, H. G.; MESSIAS, M. C. T. B. ; VIEIRA-FILHO, S. A.; DUARTE, L. P. Análise quimiométrica usando RMN de ^{13}C como ferramenta para diferenciar duas espécies de *Maytenus*. *Revista Fitos*. v.11, n.1. p. 24-32. Rio de Janeiro. 2017. e-ISSN 2446.4775. Disponível em: <http://revistafitos.far.fiocruz.br/index.php/revista-fitos/article/view/474>. Acesso em: 11 maio 2017.