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Artigo

Four Brazilian *Maytenus salicifolia* Reissek (Celastraceae) groups studied by TLC and UV/Vis spectrophotometry

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RESUMO: "Estudo de quatro populações de *Maytenus salicifolia* Reissek (Celastraceae) por CCD e espectrometria na região do UV/Vis". A grande variedade de angiospermas apontou a necessidade do desenvolvimento de sistemas de classificação botânica apoiada pela fitoquímica, bioquímica e outras. Recentemente, técnicas de análise utilizadas para o isolamento e caracterização de metabólitos secundários vêm sendo empregadas como métodos auxiliares rápidos e eficientes para identificação e classificação de espécies vegetais. *M. salicifolia* é popularmente conhecida no Brasil, como "cafezinho". O chá obtido a partir de folhas frescas é usado topicamente para aliviar pruridos e sintomas alergiformes. Este trabalho apresenta a utilização do CCD em sílica gel e espectrofotometria no UV / Vis como métodos auxiliares na identificação botânica de *M. salicifolia*. Os resultados demonstraram que este processo pode ser usado na diferenciação de plantas do mesmo gênero, assim como detectar variações químicas entre indivíduos de uma mesma espécie.

Unitermos: *Maytenus salicifolia,* Celastraceae, triterpenos pentacíclicos, UV/Vis, CCD.

ABSTRACT: The great variety of angiosperms shows the need to development of botanical classification systems supported by phytochemistry, biochemistry and others. Recently, techniques of analysis used for the isolation and characterization of secondary metabolites have been employed as auxiliary quick and efficient methods for the identification and classification of plant species. *M. salicifolia* is popularly known in Brazil, as "small coffee" and decoct obtained from its fresh leaves is topically used to alleviate itches and other skins allergic symptoms. This work presents the use of TLC and UV/Vis spectrophotomety processes to be applied like an auxiliary method in botanical taxonomy. The results demonstrate that this process can be used in differentiation of the same genera species, and in the selection of chemical variations between individuals of the same species.

Keywords: *Maytenus salicifolia,* Celastraceae, pentacyclic triterpenes, UV/Vis, TLC.

INTRODUCTION

The angiosperms or flowering plants are one of the major groups of plants. This clade are the largest group of embryophytes with at least 260 000 living species classified in 453 families. They are characterized by the great power of adaptation to different environmental conditions (Raven et al., 2001; Barroso, 2002; APG II, 2003; Soltis & Soltiz, 2004).

During the past 130 million years, flowering plants have colonized practically every available *habitat* on Earth except the highest mountain tops, the regions surrounding the poles, and the deepest oceans. These plants occur as epiphytes, floating and rooted aquatics in both freshwater

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and marine habitats, and terrestrial plants that vary greatly in size, longevity, and overall form. Furthermore, the diversity in chemistry, reproductive morphology, and genome size and organization is unparalleled in the Plant Kingdom (Raven et al., 2001; Barroso 2002; APG II, 2003; Soltis & Soltis, 2004).

The great variety of angiosperms showed the need to development of diverse systems of botanical classification systems supported by specific areas of science, such as, biochemistry, phytochemistry, morphology, genetics, cytology, paleobotany, geological history, and others. These knowledges are applied to appropriate classification of the members of these plants group. Prosperous information about flowering plants is the profile of secondary metabolites distribution. In certain cases, the chemical fingerprint is very similar to specify clusters of plant from those members of a particular group. This discovery justifies the adoption of chemical markers in vegetal taxonomy in order to assist in a correct botanical identification (Falkenberg, 2001; Nascimento et al., 2008).

Many compounds or secondary metabolites are used in quality control of medicinal herbs. For example, friedelin and β-friedelanol isolated from *Maytenus* species are particularly used for the *M. ilicifolia* quality control (Alberton, 2002; Brasil, 2008). On the other hand, some secondary compounds are found in species of distinct families due to convergent evolution, where genes that encode a specific biosynthetic route remaining in diverse branches of the angiosperms evolutionary tree (Falkenberg, 2001).

Despite the increase of researches involving natural products, the utilization of the phytochemical and classification data in botanical taxonomy is limited by the number of species chemically investigated, which are yet still very small, leading to an insufficient data available in the scientific literature. There is also a tendency to look for constituents more likely to be isolated and purified, and thus leaving to investigate the occurrence of some minority constituents, but with great taxonomic relevance in many families. The phenomenon of chemical convergence can occur often in plants not related; consequently species that are not directly linked can produce the same substances, demonstrating that some compounds shouldn't be used as chemical markers (Falkenberg, 2001).

The soil and weather conditions, the stage of growth and biological interactions with other organisms can induce changes in metabolic routes. Therefore this fact shows that some kind of interactions can stimulate variations between populations of the same species with consequent alteration in the plant chemical constitution (Valter et al., 2008). The cost of fractionation processes, isolation and structural elucidation, as well as the difficulty to obtaining pure compounds that only are present in small concentrations in the plant, make these procedures virtually impossible. Even with limitations, the knowledge of the chemical profile of particular specie represents a great value for the restructuring of taxonomic classification systems (Falkenberg, 2001).

Recently, techniques of analysis used for the isolation and characterization of secondary metabolites have been employed directly in the species identification. Chromatographic (Scora, 1966; Rogers, 2000; Hillig, 2004; Ferrante et al., 2007; Reis et al., 2008), spectroscopic (Schrader et al., 1999; Zodrow et al., 2003) and spectrometric (Smedsgaard & Frisvad, 1996) analyses of plants extracts have been used as auxiliary, quick and efficient methods for the identification and classification of species. Despite the need to deeper researches in order to validate these methods and prove its efficiency, there is no doubt about the contribution of these techniques in the vast and complex field of plant taxonomy process.

The need to develop a method to help the "identification of species" becomes even more important when it comes to plants used for therapeutic purposes. In this context, *Maytenus* genus presents various species that are used in popular medicine (Grandi, 1989; Falkenberg, 2001; Agra et al., 2007; 2008; Marliére et al., 2008). *Maytenus acanthophylla, M. truncata, M. ilicifolia*, among others, have great potential to chemotaxonomy studies as a result of morphological similarities observed between the different species of this clade (Carvalho-Okano, 1992; Carvalho-Okano, 2005).

Maytenus salicifolia is a polymorphical tree, found in different habitats of Minas Gerais state, Brazil. Aiming to the select of Celastraceae species used in traditional medicine, by ethnopharmacological studies was possible to known that "small coffee" mainly its leaves is used, in the decoct form to alleviate itches and other allergy symptoms (Grandi, 1989; Carvalho-Okano, 1992). The great anatomical similarity of this specie with other belonging to the Celastraceae family and also with species of the other families awakened the interest of our group to search and develop an analytical technique that permit to identify and differentiate species. Especially to study specimens that form groups of the same specie, but that live in different *habitats.*

This paper shows to the development of a chemotaxonomic analysis method using silica gel thin layer chromatography (SG-TLC), together with UV/Vis absorption spectroscopy applied to comparison of similar *M. salicifolia* populations, with the aim of mapping out a chemical profile, to help plant taxonomy studies of *Maytenus* species.

MATERIAL AND METHODS

Leaf samples of *Maytenus salicifolia* specimen located in Ouro Preto (OP) region were collected in the months of March (3), April (4), September (9) and November (11) in 2004. Others distinct *Maytenus salicifolia* specimens were also collected in November of

the same year, in other three different Minas Gerais regions: Ouro Branco (OB), Nova Lima (NL) and Belo Horizonte (BH). Collected specimens were botanically identified by comparison with a voucher (No NBHCB 22856) of *Maytenus salicifolia* collected in the region of Belo Horizonte city, Minas Gerais state (Brazil), and deposited at the Herbarium of Instituto de Ciências Biológicas da Universidade Federal de Minas Gerais.

The fresh leaves collected from each of these M. salicifolia populations were immediately dried at room temperature (r.t) and powdered in a mill. Two grams of each powder material were submitted to hexane (50 mL) extraction, at r.t., under magnetic stirring during two hours. The hexane solution obtained was filtered and the volume adjusted to 50,0 mL in a volumetric flask. The resulted standardized hexane extract solution was immediately analyzed by UV/Vis spectrophotometry technique using scanner method (200 to 700 nm). The analyses were carried out on Hitachi model U-2010 double-beam spectrophotometer, equipped with 10 mm path length quartz cells. The repeatability of the analytical protocol was evaluated using three preparation of the same leave sample (Figure 1 to 3).

The concentrated extract resulted after partial hexane withdraw was also analyzed by silica gel G 60 (0.25 mm) thin layer chromatography (SG-TLC) using chloroform as eluent. The chromatoplate spots (R_f) (Figure 4 and 5) were obtained with spray of vanillin/perchloric acid solution, as suggested by Wagner (1996). Friedelin, 3β-friedelanol, 3α-friedelanol, α-amyrin, β-amyrin and 3β-sitosterol previously isolated from *Maytenus* species and chemically identified by spectrometric techniques (Silva et al., 2002; Miranda et al., 2006) were used as standard compounds in TLC experiments.

RESULTS

The spectrophotometric analyses realized to determinate the repeatability of the analytical protocol showed a considerable similarity in relation to chemical fingerprints and the peak bands in the same length wave. On the contrary, the absorption bands (Abs) showed differences in the relation of the absorbance value.

The UV/Vis spectra of standardized hexane extract solution of *M. salicifolia* OP leaves showed a significant repeatability of experimental results, which demonstrate the protocol efficacy (Figure 1). The spectra of OP hexane extract obtained in different months reveled different fingerprints, mainly in the absorption bands around 400 to 500 nm associated to pigments (Figure 2). In Minas Gerais state, the months of September and November are characterized by absence of rain and higher solar light intensity. The enhancement of endogenous pigment concentration protects the plant against these climate adversities. Then, is easy to conclude the necessity to obtain a minimum of two UV/Vis spectra for the same specimens, collected in a moment with same specific climate characteristics. The spectrum of leaves hexane extracts obtained from the four groups at the same season indicate similarities between the populations OB, OP-11 and NL, mainly in relation to the pigment absorption bands (400 to 500 nm). On the other hand, this result demonstrates the existence of chemical differences in the population BH (Figure 3). Nevertheless, there aren't botanical significant differences under the viewpoint of botanical taxonomy. The chromatogram of the four *M. salicifolia* specimen groups (Figure 5) showed that the leaves hexane extract obtained from BH population showed chemical fingerprint distinct from the others.

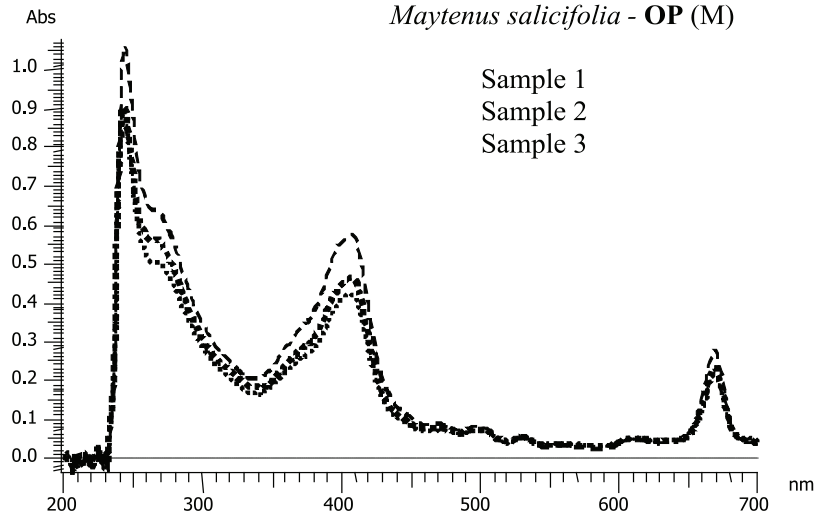

Figure 1. UV/Vis spectra of the hexane solution from *Maytenus salicifolia* (OP) leaves collected in November (2004).

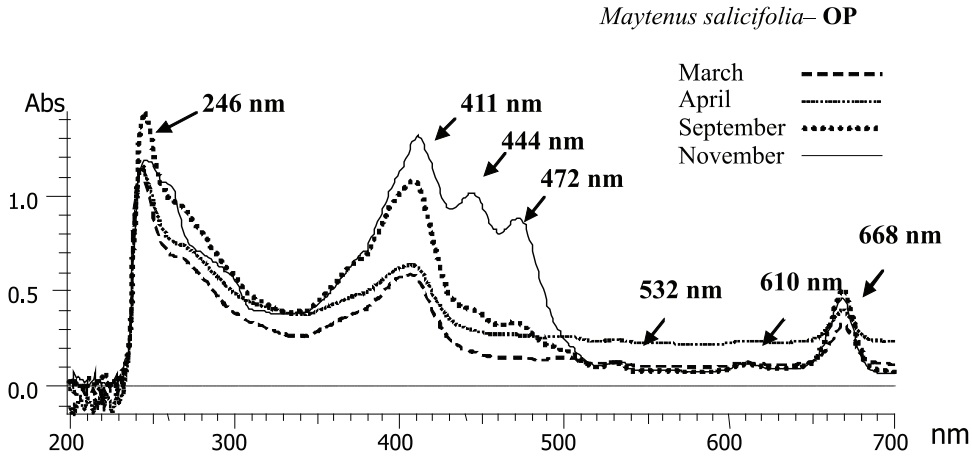

Figure 2. UV/Vis spectra of hexane solution from *Maytenus salicifolia* (OP) leaves collected in March, April, September and November (2004).

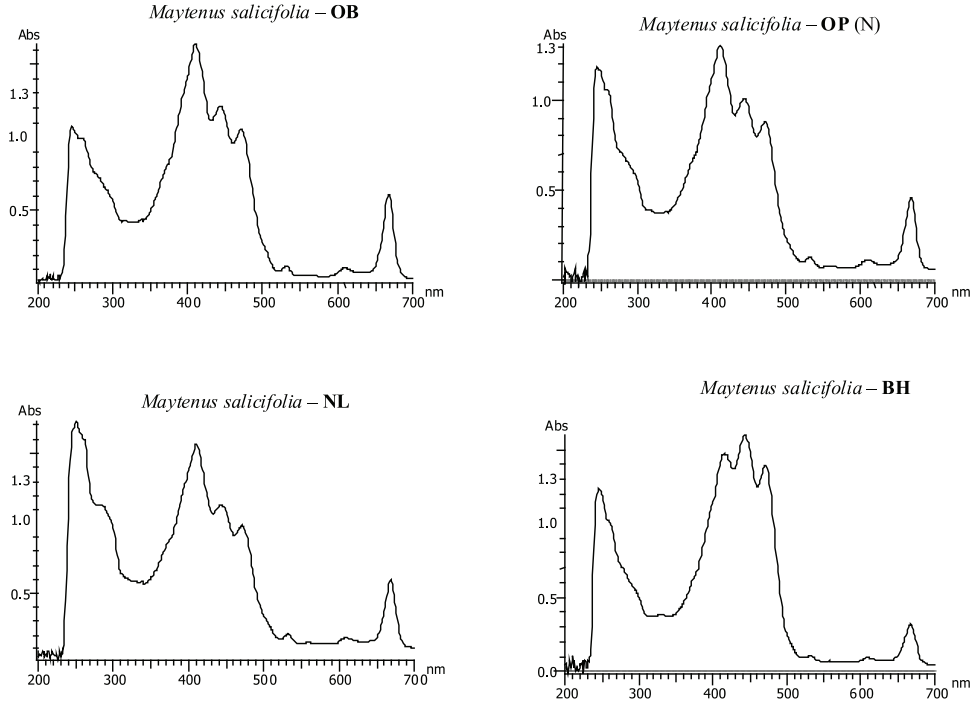

Figure 3. UV/Vis spectrum of hexane solution from *Maytenus salicifolia* leaves collect in the region of Ouro Branco (OB), Ouro Preto (OP), Nova Lima (NL) and Belo Horizonte (BH), in November (2004).

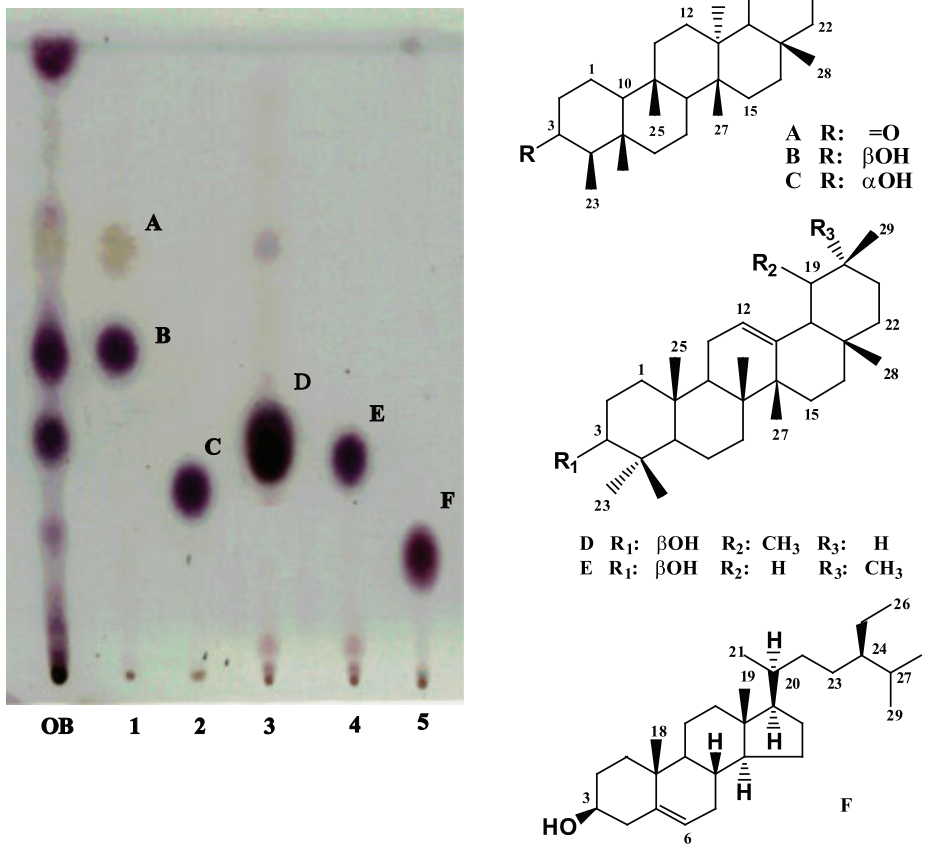

Figure 4. Chromatogram obtained by TLC of *Maytenus salicifolia* (OB) leave hexane extract, together with the organic compounds friedelin (A), 3β-friedelanol (B), 3α-friedelanol (C), α-amyrin (D), β-amyrin (E) and β-sitosterol (F).

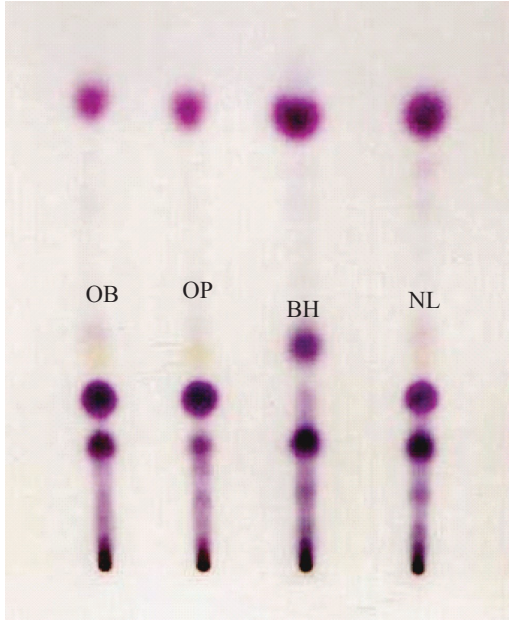

Figure 5. Chromatogram obtained by TLC for different specimens of *Maytenus salicifolia.* Sample collected in distinct regions of Minas Gerais, Brazil: OB, Ouro Branco; OP, Ouro Preto; BH, Belo Horizonte; NL, Nova Lima.

DISCUSSION

The UV/Vis spectrophotometric analysis of the standardized hexane extract solution of *M. salicifolia* leaves obtained from specific population groups were systematical and carefully developed. The analysis realized using hexane solution of the plant material collected in the months of March and April didn't show significant absorptions bands at 430-490 nm (Figure 2). In accordance with Schoefs (2002) this absorption bands are associated mainly to the presence of carotenoids. The analysis of standardized hexane extract solution obtained from similar sample of the same group, collected in September showed bands in the spectral regions at 430-490 nm, but with medium intensity. On the other hand, the standardized hexane solution of the leaves collected in November showed a broadband absorption band at 400-500 nm, corresponding to the presence of the pigments (Schoefs, 2002). The results indicates that OP populations modify its metabolism during the spring and summer in Minas Gerais state, Brazil, when occur low water stress and hot period, that intensify the biosynthesis of carotenes, xanthophylls, phenols and others derivative compounds that absorbs light

in 400-500 nm spectral region. This fact indicate that the biosynthesis of carotenoids and others pigments is seasonal, and is stimulated by the low water availability during September and November. In this period occurs enzyme activation induced by environmental light and temperature conditions and the energy consumption caused by the enhancement of photosynthesis. A similar hypothesis was also proposed by Lafta & Lorenzen (1995) and Machado et al. (2002). Maximum absorption band at 411, 444, 472 and 668 nm are associated to absorptions of carotenes, chlorophylls and other similar pigments (Schoefs, 2002). In this work, a fine spectral profile similarity in the visible region of the spectra of *M. salicifolia* populations OB, OP-11 and NL was observed. However, some fingerprint differences were observed in the spectral region of 200-350 nm. The spectral profile of the *M. salicifolia* NL population (Figure 3) showed an absorption band at 280-300 nm more evident than the same absorption band of OB and OP-11 populations. The spectral data of *M. salicifolia* BH population revealed differences in relation to NL, OP and OB plant groups at 230-300 nm and the lack of a band at 280-300 nm.

The chromatogram obtained for the concentrated hexane extracts of the leaves (Figure 5) from the four *M. salicifolia* groups suggested the presence of similar constituents in NL, OP-11 and OB populations, like the TTPC, friedelin, 3β-friedelanol, α-amyrin, β-amyrin and 3β-sitosterol (Figure 4). The samples of *M. salicifolia* BH population exhibited a distinct chemical profile that differs from the others. The chromatogram of BH group didn't show the spot corresponding to friedelin, but the presence of an unknown constituent with retention factor (R_f) around 0,45 (Figure 5).

CONCLUSION

The SG-TLC and UV/VIS spectrophotometry showed to be a good approach to establish differences between these four groups of *Maytenus salicifolia*. By this processes was possible to identify two distinct chemical plant varieties, being one NL, OP-11 and OB *M. salicifolia* and the other, BH *M. salicifolia*. The data obtained showed that populations of the same plant specie living near in the same geographical region and in similar biome could present variances in leave chemical composition and constitution. The results demonstrate that the use of SG-TLC and UV/VIS spectrophotometry may be applied in chemotaxonomy and phytochemical studies.

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