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Artigo

Indole monoterpene alkaloids from C*himarrhis turbinata* DC Prodr.: a contribution to the chemotaxonomic studies of the Rubiaceae family

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RESUMO: "Alcalóides indólicos monoterpênicos de *Chimarrhis turbinata* DC. Prodr.: uma contribuição para os estudos de quimiotaxonomia da família Rubiaceae". A utilização de parâmetros apenas morfológicos para posicionar taxonomicamente diversas espécies em sub-famílias e tribos na família Rubiaceae é bastante problemática devido à falta de informações sobre a distribuição geográfica e de características morfoanatômicas nos níveis hierárquicos mais baixos, como por exemplo, o gênero *Chimarrhis*. O perfil micromolecular de diferentes espécies pode auxiliar na delimitação de tribos indicando tendências filogenéticas mais completas entre as tribos das sub-famílias, já que os metabólitos secundários são expressões de adaptação, regulação e evolução de um determinado táxon. Nesse contexto, os alcalóides indólicos monoterpênicos isolados de *Chimarrhis turbinata* foram bastante úteis para embasar a classificação taxonômica feita por Robbrecht, em que posiciona *Chimarrhis* como um gênero da tribo Condamineae e subfamília Cinchonoideae.

Unitermos: *Chimarrhis turbinata*, Rubiaceae, alcalóides indólicos monoterpenos, quimiotaxonomia.

ABSTRACT: The morphological parameters used to establish close connections among species taxonomically different into the Rubiaceae family is complex, mainly due to the lack of information on habitat and morphoanatomical characters in the lower hierarchic groups, for example, *Chimarrhis* genus. The micromolecular profile of delimited species into determined taxa can be useful to establish the boundaries among close taxonomic groups, and to indicate evolutionary phylogenetic trends into the taxa. Several indole alkaloids isolated from *C. turbinata* showed to be a valuable tool to support the taxonomic classification performed by Robbrecht, who established the most recent taxonomy for Rubiaceae, based on morphological characters, and concluded that *Chimarrhis* belong to Condamineae, and subfamily Cinchonoideae.

Keywords: *Chimarrhis turbinata*, Rubiaceae, indole monotepene alkaloids, chemotaxonomy.

INTRODUCTION

Chimarrhis turbinata is a tree that grows from Caribbean to tropical South America, and occurs predominantly in the Amazonian region (Boom and Campos, 1991). It is popularly named "pau de remo", due to its light and yellow hardwood. It is also a resistant wood to insects and other predator attacks, and due to this characteristic it is used for sawmill, mainly in the craft and manufacture, of pawls (Delprete, 1996). In our previous study on leaves of *Chimarrhis turbinata* we have isolated strictosidine (**1**), a monoterpene indole alkaloid glycoside, well-known as the precursor of the monoterpene indole alkaloids and it was first isolated from *Rhazya stricta* (Smith, 1968). In addition, several corinantheane derivatives: 5α-carboxystrictosidine (**2**), isovallesiachotamine (**3**), vallesiachotamine (**4**) (Arbain et. Al., 1992; Bolzani et al., 2001), turbinatine (**5**) and 3,4-dehydro-strictosidine (**6**) (Cardoso et al., 2003; Bolzani et al., 2001, Cardoso et al., 2004) were also isolated. Turbinatine (**5**) was considered an important intermediate key in the biosynthesis of the corinanthean indole alkaloids (Cardoso et al., 2003). Aiming to determine the chemical profile from this plant species for further chemosystematics evaluation of this biogenetic group in Rubiaceae, we performed

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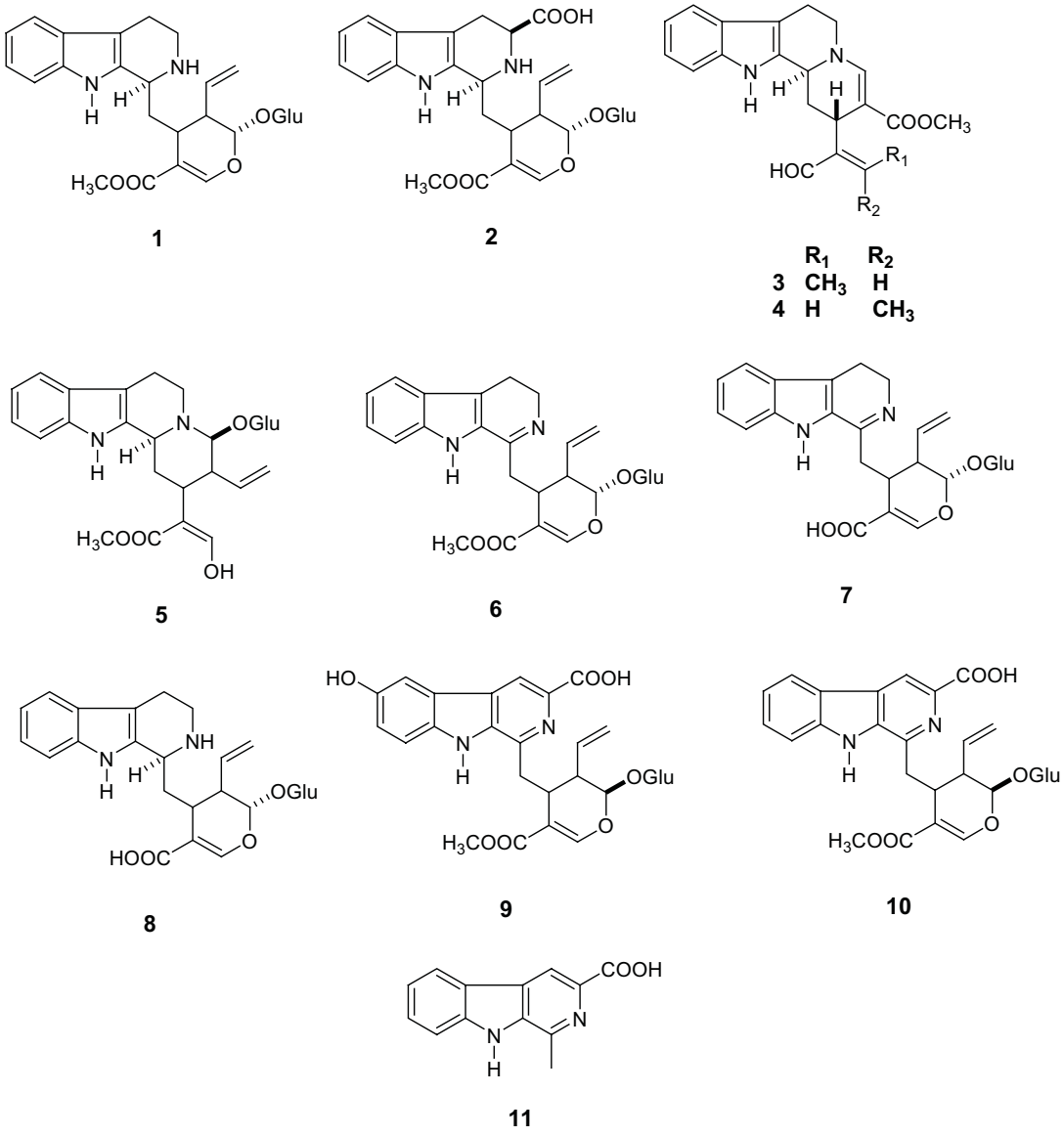

Figure 1. Indole terpene alkaloids profile from *Chimarrhis turbinata.*

the chemical studies of the EtOH extract obtained from barks of this species, which resulted in the isolation of the indole glucoalkaloids identified as 3,4-dehydro-strictosidine acid (**7**) (Cardoso et al., 2004), strictosidine acid (**8**), the β-carboline alkaloids: cordifoline (**9**), deoxycordifoline (**10**), and harman-3-carboxylic acid (**11**) previously isolated from *Adina* species (Brown and Warambwa, 1978; Blackstock et al., 1972), along with strictosidine (**1**), 5α-carboxistrictosidine (**2**) and turbinatine (**5**) already isolated from the leaves of *C. turbinata* (Cardoso et al., 2003) Figure 1.

Concerning the distribution of the main secondary metabolites in Rubiaceae (Robbrecht, 1988), indole alkaloids were the chemotaxonomic markers more intensely studied so far, aiming the establishment of phyllogenetic correlations between secondary metabolites and taxonomic data. Our chemical studies revealed several interesting correlations among tribes and subfamilies of Rubiaceae due to their structural variability and restrict distribution (Bolzani et al., 2001). In Rubiaceae, the occurrence and distribution of iridoids, indole alkaloids and anthraquinones has provided valuable chemosystematic clues (Young et al., 1996).

MATERIAL AND METHODS

Plant material

Chimarrhis turbianta (DC) Prodr. (Rubiaceae) was collected in the Reserva do Viro, Belém, PA, Brazil in February 2000 and identified by Dr. Marina Thereza V. de A. Campos. An air-dried voucher specimen was deposited in the Herbarium of the Botanic Garden, São Paulo and catalogued as Lopes-51.

Extraction and isolation

The bark (300 g) was extracted with EtOH and dissolved in *n*-BuOH: H_2O, followed by partition with CH_2Cl_2: hexane (1:1), $CHCl_3$ and EtOAc. The remaining aqueous layer after concentration resulted in a fraction WT (10 g). The *n*-BuOH:H_2O solution was concentrated and dissolved in MeOH:H_2O followed by partition with CH_2Cl_2:hexane (1:1), $CHCl_3$ and EtOAc. The MeOH:H_2O layer after concentration, resulted in fraction WM (12 g). Fraction WT was chromatographed by VLC (vacuum liquid chromatography) on reverse phase silica-gel, using H_2O, MeOH:H_2O in a gradient (25-100%), acetone (100%) and CH_2Cl_2 yielding 11 fractions (A-K). Fraction A was purified by HPLC (Phenomenex-Luna C18, 25.0 cm x 21.20 mm x 5 µm column; MeCN:H_2O + HOAc (0.05%) 15:85 λ = 237 nm, flow rate 12 mL.min^{-1}) affording 10 fractions. From these A-5, A-7 and A-9 were identified as strictosidine acid (**8**) (15 mg), strictosidine (**1**) (6 mg) and 5α-carboxystrictosidine (**2**) (25 mg) respectively. Fractions A-3, A-4 and A-10 were purified by HPLC using the following conditions (Phenomenex-Luna C18, 25.0 cm x 21.20 mm x 5 µm column; MeCN:H_2O + HOAc (0.05%) 15:85 λ = 237 nm, flow rate 7.5 mL.min^{-1}); (Phenomenex-Luna C18, 25.0 cm x 21.20 mm x 5 µm column; MeCN:H_2O + HOAc (0.05%) 13:87, λ = 237 nm, flow rate 12 mL.min^{-1}); (Phenomenex-Luna C18, 25.0 cm x 21.20 mm x 5 µm column; ACN:H_2O + HOAc (0.05%) 2:8, λ = 237 nm, flow rate 12 mL.min^{-1}), respectively. From fraction A-3 the iridoid sweroside (**19**) (2.5 mg) only representative of this class of secondary metabolite, isolated from this species.

From fractions A-4 and A-10 we isolated: harman-3-carboxylic acid (**11**) (20 mg), cordifoline (**9**) (16.2 mg), turbinatine (**5**) (14 mg) and deoxycordifoline (**10**) (20 mg) previously isolated from leaves of *C. turbinata* (Cardoso et al., 2003).

Fraction WM (12 g) was purified by HPLC (Phenomenex-Luna C-18, 25.0 cm x 21.20 mm x 5 µm, MeCN:H_2O + HOAc (0.05%) 15:85 λ = 254 nm, flow rate 12 mL.min^{-1}), affording 10 fractions. After recrystallization, fraction W-4 was identified as harman-3-carboxylic acid (**11**) (17 mg). Fractions W-5 and W-6 were identified as cordifoline (**9**) (7 mg), and 3,4-dehydro-strictosidine acid (**7**) (5 mg), respectively (Cardoso et al., 2004). Fractions W-9 and W-10 were identified as 5α-carboxystrictosidine (**2**) (154 mg) and deoxycordifoline (**10**) (148 mg), respectively.

RESULTS AND DISCUSSION

According to the new taxonomic classification currently adopted for the Rubiaceae family, *C. turbinata* is placed into the subfamily Cinchonoideae, which had its classification based on a series of morphological parameters such as placentation, fruit and seed morphology and anatomy (Robbrecht, 1988). The chemical profile for each subfamily, as expressed by occurrence of the major categories of secondary metabolites (indole alkaloids, iridoids, triterpenes and anthraquinones) is remarkably distinctive (Young et al., 1996). However, according to our studies, and classification adopted by Robbrechet (Robbrecht, 1988), the position of many species into the several Rubiaceae tribes, including Condamineae, remain problematic, mainly due to the lack of information on the geographic distribution and morphoanatomical characters of many taxa. So far, secondary metabolites profile can contribute to the taxonomic position of some tribes, which remain with a morphological controversy. Indole terpene alkaloids isolated in this study corroborated the

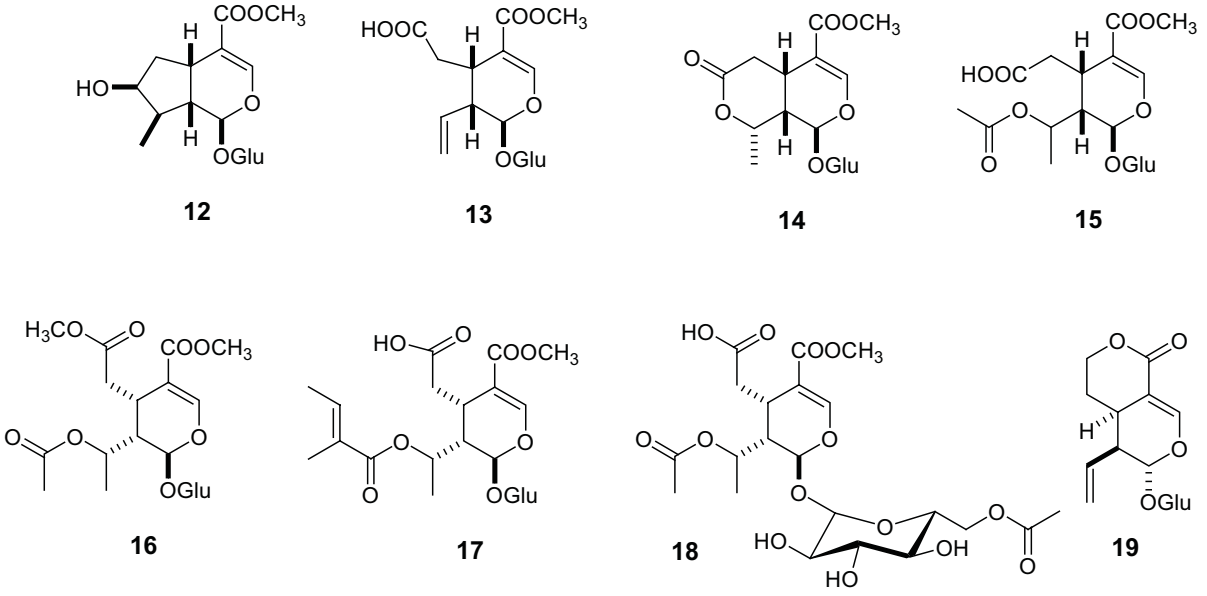

Figure 2. *Seco*-iridoids isolated from *Calycophylum spruceanum.*

evolutionary taxonomic distribution made by Robbrecht, who placed *C. turbinata* in the Condamineae contrary to that proposed by Verdcourt , who placed this genus into the Rondeletieae (Verdcourt, 1958).

The corynantheane indole alkaloids isolated from *C. turbinata* revealed its great affinity with Cinchonoideae, which was proved by the occurrence of several alkaloids with corynanthean skeleton. The morphoanatomical evaluation performed by Delprete on *Chimarrhis, Calicophyllum* and *Bathysa* indicated some parallelism among these genera, placing Chimarrhis at the basal clad position (Delprete, 1996). However, the chemical profile of some species from these genera evidences the occurrence of different biosynthetic pathways. For example, triterpenes (Benevides et. al., 2001) and paeonol (Weeks et al., 1977) have been isolated from *Bathysa meridionalis*. Triterpenes are frequent in several species of Rubiaceae, and so far are not considered taxonomic markers. *Calycoplyllum spruceanum* accumulates *seco*-iridoids (**12-18)** as major metabolites (Zuleta et. al., 2003) (Figure 2), whereas *Chimarrhis turbinata* indole-alkaloids (Cardoso et al., 2003, Cardoso et al., 2004) (Figure 1). The chemotaxonomical correlations found in this taxon, point out seco-iridoids as the precursor of all carboxy- or seco-iridoids, (ex. Ixoroideae subfamily). However, in Cinchonoideae, seco-iridoids are involved in the biosynthesis of indole terpene alkaloids and thus establishing two distinct chemotaxonomic branches (Nagakura et al., 1979).

Evaluation of the chemical data through Robbrecht parameters evidenced a good correlation between the biosynthetic pathways and morphological features for Rubiaceae subfamilies (Bolzani et al., 2001). In subfamily Cinchonoideae, indole alkaloids predominates, while iridoids are exclusively found in Ixoroideae (Young et al., 1996). Nevertheless, the micromolecular profile and the presence of indole alkaloid both in leaves and barks of *C. turbinata* can be helpful to the taxonomy and phylogeny and corroborating *Chimarrhis* classification according to Delprete as a species from the Cinchonoideae subfamily. However, the *Chimarrhis*'*s* position into the Condamineae is still difficult to support and a deep evaluation, and additional chemotaxonomic studies will be necessary in order to establish the boundaries along this genus.

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