UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
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ALINE GROHE SCHIRMER PIGATTO

TESE DE DOUTORADO

ANÁLISE QUIMIOTAXONÔMICA DE SOLANACEAE Juss. COM BASE EM ALCALOIDES DERIVADOS DA ORNITINA E VITANOLÍDEOS

Orientador: Prof. Dr. Geraldo Luiz Gonçalves Soares
Co-orientadora: Profª Drª Lilian Auler Mentz

PORTO ALEGRE – RS
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DEDICATÓRIA

Dedico este trabalho:

Aos meus pais Odone e Maria, pela vida, pelo amor, pela nobreza de seus corações, pelo exemplo de dedicação à família...

"Cabelos de pratas os dois, mateando a sombra do rancho, recordando primaveras, e antigos sonhos caranchos, sempre com o olhar na estrada, a espera dos passarinhos, porque os filhos batem asas, e acabam deixando o ninho."

(Clovis Mendes)

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"Eu te bendigo no silêncio desses campos, quando a luz do teu encanto, enfeitar os sonhos meus, eu te bendigo por ser teu o meu apego, e se um dia achar sossego à de ser nos braços teus."

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RESUMO

O status atual do conhecimento taxonômico e fitoquímico de Solanaceae torna essa importante família de eudicotiledôneas um ótimo modelo para estudos quimiotaxonômicos, os quais se constituem em uma valiosa ferramenta para a compreensão da diversidade biológica, além de favorecer o esclarecimento de dúvidas existentes no arranjo taxonômico dessa família em diferentes níveis hierárquicos. Desse modo, o presente trabalho avaliou as polarizações quimio-evolutivas em Solanaceae, por meio da análise do padrão de ocorrência dos seus marcadores no emprego da abordagem metodológica da quimiossistemática micromolecular. Para isso, um banco de dados foi elaborado a partir de um amplo levantamento de ocorrências de micromoléculas, de modo especial alcaloides derivados da ornitina e vitanolídeos, em espécies de Solanaceae. Em relação à caracterização quimiotaxonômica das subfamílias de Solanaceae com base na ocorrência de alcaloides derivados da ornitina observou-se que o maior número de ocorrências foi registrado para os alcaloides tropânicos (NO = 927), seguido pelos nicotinóides (NO = 353), pirrolidinas simples (NO = 133), e, finalmente, calisteginas (NO = 100). O maior número de ocorrências dessas substâncias foi registrado em Solanoideae e Nicotianoideae, que juntas somaram 95% das ocorrências dos quatro grupos de alcaloides estudados. Em Petunioideae foi observada a presença de alcaloides tropânicos, pirrolidinas simples e calisteginas, em Schizanthoideae foram registradas ocorrências de pirrolidinas simples e alcaloides tropânicos e, em Cestroideae, somente a ocorrência de nicotinóides foi observada. A análise de similaridade baseada nos alcaloides derivados da ornitina sugere que o perfil químico das subfamílias está, de modo geral, correlacionado com a atual classificação de Solanaceae. O dendrograma de similaridade evidenciou a proximidade de Solanoideae e Nicotianoideae e dessas com Petunioideae. Schizanthoideae mostrou-se pouco similar às subfamílias citadas acima, e Cestroideae, mostrou-se dissimilar às demais. A análise realizada com o conjunto de informações de alcaloides tropânicos e calisteginas, separadamente, evidenciou que as calisteginas foram mais relevantes do que os alcaloides tropânicos para a caracterização de grupos distintos de gêneros de Solanaceae. Isto corrobora a tendência de uma dicotomia química observada pela análise qualitativa do banco de dados e, de certa forma, confirma a correlação entre a distribuição geográfica e ocorrência de metabólitos secundários, uma vez que a presença exclusiva de calisteginas foi relatada apenas em gêneros que têm a América do Sul como centro de diversidade. E, finalmente, a análise do banco de dados com a ocorrência de vitanolídeos na subfamília Solanoideae, elaborado para caracterizar o perfil químico e verificar a semelhança química entre suas tribos/gêneros, revelou a importância deste grupo de substâncias como marcadores quiomiotaxonômicos, especialmente, para Physaleae, *Deprea* e *Jaborosa* ratificando a importância da quimiotaxonomia como uma ferramenta para elucidar problemas taxonômicos, por exemplo, as relações incertas de alguns gêneros que ainda não estão incluídos em tribos na subfamília.

ABSTRACT

The current state of knowledge on the taxonomy and phytochemistry of the Solanaceae makes this important family of eudicots an excellent model for chemotaxonomic studies, which are a valuable tool for advancing our understanding of biological diversity and can help elucidate uncertainties as to the taxonomic arrangement of this family at several hierarchical levels. Within this context, the present study assessed the evolutionary polarities of secondary metabolites in the Solanaceae by means of an analysis of the pattern of occurrence of small-molecule chemotaxonomic markers. Toward this end, a database was compiled with the results of a wide-ranging survey of small-molecule occurrences in Solanaceae species, with particular emphasis on ornithine-derived alkaloids and withanolides. Regarding ornithine derivatives, the tropane alkaloids accounted for the greatest frequency of occurrence (NO = 927), followed by nicotinoids (NO = 353), simple pyrrolidines (NO = 133) and, finally, calystegines (NO = 100). The greatest frequency of occurrence of these substances was recorded in the Solanoideae and Nicotianoideae, which together accounted for 95% of all occurrences of these four alkaloid classes. The Petunioideae were characterized by the presence of tropane alkaloids, simple pyrrolidines, and calystegines, and the Schizanthoideae, by simple pyrrolidines and tropane alkaloids, whereas the Cestroideae were found to contain nicotinoids exclusively. Analysis of similarity on the basis of ornithine-derived alkaloid occurrence suggests that the chemical profile of these subfamilies largely correlates with the current classification of the Solanaceae. A similarity dendrogram revealed close relationships between the Solanoideae and the Nicotianoideae, and between these subfamilies and the Petunioideae. The Schizanthoideae had only minor similarity to the aforementioned subfamilies, and the Cestroideae were dissimilar to all others. Separate analysis of data on the occurrence of tropane alkaloids and of calystegines showed that the latter were more relevant than the tropane alkaloids as chemotaxonomic markers for characterization of distinct clusters of Solanaceae genera. This corroborates the trend toward a chemical dichotomy as shown by qualitative analysis of the database, and somewhat confirms the correlation between geographic range and occurrence of secondary metabolites, as the exclusive presence of calystegines was reported solely in genera that have South America as their center of diversity. Finally, analysis of the database of withanolide occurrences in the Solanoideae subfamily, compiled to characterize the chemical profile and ascertain the chemical similarity of the tribes/genera of this subfamily, revealed the importance of this class of substances as chemotaxonomic markers, especially in the Physaleae and the genera *Deprea* and *Jaborosa*, corroborating the relevance of chemotaxonomy as a means of elucidating taxonomic issues, such as the uncertain relationships of genera that are not yet included in tribes of this subfamily.

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SUMÁRIO

INTRODUÇÃO GERAL

A quimiotaxonomia é um ramo da Sistemática Vegetal que se constitui em uma valiosa ferramenta para a compreensão da diversidade biológica, além de possibilitar a inferência de tendências evolutivas para um determinado táxon, por meio da análise de dados micromoleculares e morfológicos (GOTTLIEB, 1982). Para Soares e Kaplan (2001) a busca por correlações entre diversidade morfológica e química nas plantas é o grande desafio dessa área de conhecimento.

Poser e Mentz (2002) comentam que embora trabalhos de taxonomia molecular estejam se tornando cada vez mais numerosos, os produtos do metabolismo secundário devem ser utilizados em estudos visando o estabelecimento de relações filogenéticas. A presença de certos grupos de metabólitos secundários pode indicar afinidades entre as plantas que os produzem. Dahlgren (1980, 1985), por exemplo, considerou em seus sistemas de classificação o padrão de distribuição de vários produtos do metabolismo vegetal como, por exemplo, alcaloides provenientes de diferentes rotas biossintéticas, iridoides, poliacetilenos, entre outros, evidenciando a grande importância dos metabólitos secundários.

Sabe-se que tanto a morfologia quanto a química micromolecular constituem o nível fenotípico de manifestação da informação genética, assim, o metabolismo vegetal deve refletir não somente as relações filogenéticas de grupos de plantas, como também o seu caminho evolutivo (GOTTLIEB, 1982). Para Castro et al. (2004) a pesquisa biológica moderna, relacionada à interação dos organismos com o meio ambiente, não deve contemplar apenas a descrição macroscópica dos fenômenos em nível de espécies, populações ou indivíduos, mas buscar explicações científicas com maior profundidade em nível químico, uma vez que a utilização de um sistema filogenético considerando este nível, pode fornecer subsídios para a previsão da ocorrência de determinada classe de substâncias em um dado grupo vegetal. Essa característica é de alta relevância para a racionalização de estudos fitoquímicos.

O status atual do conhecimento taxonômico e fitoquímico de Solanaceae Juss. torna essa grande família das angiospermas um ótimo modelo para estudos quimiotaxonômicos. Há diversas tendências de dicotomias químicas, relacionadas à produção de tipos alcaloídicos e terpenoídicos apenas conhecidos de maneira superficial (EICH, 2008). A avaliação dessas tendências em diversos níveis hierárquicos e a verificação de sua congruência com estudos filogenéticos atuais favoreceriam a construção de um esquema de classificação com base química cujo valor científico é inquestionável.

Solanaceae é uma das maiores e mais importantes famílias das eudicotiledôneas. Espécies alimentícias, como por exemplo, *Solanum tuberosum* L. (batata-inglesa), *Solanum* *lycopersicum* L. (tomate), *Solanum melongena* L. (berinjela), além de diversas espécies de *Capsicum* L. (pimentas e pimentões), pertencem a essa família de plantas floríferas. Diversas espécies têm interesse devido a riqueza de sua química micromolecular que origina metabólitos secundários com acentuada atividade biológica, como por exemplo, *Atropa belladonna* L. (beladona), *Hyoscyamus niger* L. (meimendro) e espécies de *Datura* L. Desse táxon também fazem parte espécies de relevância econômica, como *Nicotiana tabacum* L. (tabaco) e espécies tóxicas como *Nicotiana glauca* Graham (fumo-bravo). Solanaceae é considerada um grupo vegetal subcosmopolita, cuja maior diversidade de espécies é encontrada no continente americano (Olmstead et al., 2008). Segundo Hunziker (2001) seu centro de diversidade é a América do Sul.

Do ponto de vista sistemático, Solanaceae situa-se na ordem Solanales, sendo filogeneticamente relacionada às famílias Convolvulaceae, Hydroleaceae, Montiniaceae e Sphenocleaceae (APG III, 2009). Convolvulaceae é considerada o grupo irmão de Solanaceae, compartilhando características, como por exemplo, similaridades anatômicas e morfológicas, e distribuição geográfica semelhante. Pode-se dizer que, em relação ao metabolismo secundário, ambas as famílias apresentam algumas similaridades, como a presença de alcaloides tropânicos e calisteginas. Entretanto, apresentam também, algumas importantes e significativas diferenças. Em Solanaceae, por exemplo, ocorrem capsaicinoídeos, saponinas esteroidais, alcaloides esteroidais e vitanolídeos, que não ocorrem em Convolvulaceae (EICH, 2008).

Historicamente a família foi dividida em diferentes números de subfamílias e tribos. A primeira classificação para Solanaceae data do início da segunda metade do século XIX e foi proposta por Dunal, que dividiu os 61 gêneros conhecidos na época em duas tribos. Duas décadas depois, em 1873, Bentham e Hooker propuseram uma nova classificação considerando 67 gêneros divididos em cinco tribos. No final daquele século, Wettstein, pela primeira vez, dividiu a família em subfamílias: Solanoideae, com três tribos e Cestroideae, com duas tribos. No século XX, mais duas classificações foram propostas. D´Arcy em 1979 e posteriormente em 1991, acrescentou à família a subfamília Nolanoideae, além das outras duas subfamílias já propostas anteriormente. Em 1987, Tétényi apresentou uma classificação baseada em informações químicas. Nela, o autor dividiu a família em três subfamílias com base principalmente na ocorrência de alcaloides e esteroides.

Atualmente são aceitas duas propostas de circunscrição. A primeira, apresentada por Hunziker (2001), baseada em critérios morfológicos e químicos, contempla 92 gêneros e, aproximadamente, 2300 espécies distribuídos nas subfamílias Solanoideae, Cestroideae, Juanulloideae, Salpiglossoideae, Schizanthoideae e Anthocercidoideae. A segunda, mais recente, foi proposta por Olmstead et al. (2008) em um estudo molecular que reuniu uma amostragem de 89 gêneros e 190 espécies. Nela os autores admitem sete subfamílias, Solanoideae, Cestroideae, Nicotianoideae, Petunioideae, Schizanthoideae, Goetzeoideae e Schwenckioideae. Em ambos os trabalhos os autores concordam com o status de Solanoideae como subfamília mais derivada em relação à Cestroideae. Gêneros que pertencem a Cestroideae, segundo Hunziker (2001), neste sistema são encontrados em três distintas subfamílias (Cestroideae, Nicotianoideae e Petunioideae), e gêneros tradicionalmente aceitos em Salpiglossideae passam a Solanoideae, entre outras alterações significativas.

Segundo Hunziker (2001), no Brasil há representantes das subfamílias Solanoideae, Cestroideae e Juanulloideae, totalizando 31 gêneros e cerca de 500 espécies nativas e cerca de seis gêneros com espécies consideradas introduzidas, cultivadas ou assilvestradas. A maior riqueza é atribuída ao gênero *Solanum* L., cujo número estimado de espécies nativas no país deve chegar a 300 (Mentz, L. A., comunicação pessoal).

Segundo Nee (1999, 2001), o tratamento sistemático da família Solanaceae precisa ser aprimorado. Alguns gêneros ou partes de gêneros têm sido e continuam sendo extensivamente investigados utilizando métodos tradicionais e modernos, como por exemplo, o gênero *Solanum*. Por outro lado, há gêneros que embora possuam um grande número de espécies necessitam de mais estudos, como por exemplo, *Cestrum* L. O autor reconhece a necessidade de aliar esses estudos mais tradicionais com os mais modernos, como por exemplo, a cladística para resolver problemas “taxonômicos”.

Do ponto de vista químico cinco subfamílias foram, pelo menos em parte, investigadas: Solanoidaeae, Cestroideae, Anthocercidoideae, Salpiglossoideae e Schizanthoideae (HUNZIKER, 2001). O autor fala da extraordinária riqueza de informações disponíveis sobre a fitoquímica nesta família e que estas poderiam ser úteis para estudos taxonômicos em nível específico, infraespecífico ou mesmo genérico, porém elas ainda são pouco utilizadas. Alcaloides e esteroides constituem os principais grupos de metabólitos secundários encontrados nesta família. Dentre os alcaloides, Hunziker (2001) comenta serem os tropânicos os mais comuns e ocorrem em cinco das seis subfamílias, sendo que na subfamília Solanoideae há o registro desses alcaloides em 17 gêneros.

Apesar da riqueza química das espécies de Solanaceae, pode se considerar que há poucos estudos mais profundos de sua química que vão além da abordagem fitoquímica clássica, que tem por objetivo o isolamento e identificação dos metabólitos secundários de uma determinada espécie (NASCIMENTO, 2006). Por outro lado esses estudos fitoquímicos fornecem dados de valor inestimável para a execução de estudos quimiotaxonômicos (GOTTLIEB et al., 1996).

O conhecimento quimiotaxonômico atual das Solanaceae é restrito, porém sugere alguns marcadores químicos cujo estudo seria promissor, como por exemplo, os alcaloides, derivados predominantemente das vias do acetato e mevalonato. Griffin e Lin (2000) publicaram uma revisão sobre a distribuição de alcalóides tropânicos na família Solanaceae mostrando que esta família é o “lugar” desses alcalóides e que eles ocorrem caracteristicamente nos gêneros *Datura* L., *Brugmansia* Persoon e *Duboisia* R.Br. Dentre os alcalóides tropânicos encontrados destacam-se a escopolamina e a hiosciamina.

Espécies do gênero *Solanum* produzem uma grande variedade de glicoalcalóides esteroidais o que, segundo Silva et al. (2005), justifica a atividade moluscicida de algumas espécies. De acordo com Roddick (1996) os alcalóides esteroidais ocorrem como glicosídeos nos gêneros *Solanum* e *Cestrum* e já naquela data haviam sido isolados de aproximadamente 350 espécies. A análise da distribuição dos tipos alcaloídicos, que pertencem a rotas biossintéticas distintas, em Solanaceae pode revelar dicotomias químicas com grande importância evolutiva.

Outro marcador quimiotaxonômico interessante para Solanaceae são as lactonas esteroidais, entre as quais se destacam os vitanolídeos. Mais de 300 esqueletos diferentes de vitanolídeos já foram isolados até agora, considerando-se apenas a subfamília Solanoideae. Esses esteróides peculiares já foram isolados nos gêneros *Withania* Pauq., *Physalis* L., *Acnistus* Schott, entre outros. Estes compostos são metabólitos interessantes do ponto de vista farmacológico, devido à atividade biológica que possuem, como por exemplo, propriedades citotóxicas, antimicrobianas, anti-inflamatórias e hepatoprotetora (HUNZIKER, 2001). Almeida-Lafetá (2003) isolou vitanolídeos de espécies brasileiras de *Aureliana*.

Estudos de sistemática em evolução são importantes para elucidar problemas taxonômicos e podem servir como ótima ferramenta para estudos filogenéticos. A quimiossistemática vegetal micromolecular constitui uma ferramenta valiosa para esclarecer dúvidas existentes no arranjo taxonômico atual de Solanaceae.

Esse trabalho está organizado em três capítulos. O primeiro capítulo enfoca a caracterização quimiotaxonômica das subfamílias de Solanaceae com base em alcaloides derivados da ornitina e avalia a similaridade química dessas subfamílias. No segundo capítulo, a ocorrência de alcaloides tropânicos e calisteginas em Solanaceae é discutida, de modo a verificar padrões de distribuição e tendências evolutivas nessa família. E, o terceiro capítulo apresenta a caracterização quimiotaxonômica de tribos e gêneros da subfamília Solanoideae, bem como a similaridade química entre eles, com base na ocorrência de vitanolídeos. Nos três capítulos, as discussões acerca da similaridade química, dicotomias químicas, tendências de produção dos metabólitos estudados, entre outros aspectos, levam em consideração a atual classificação para Solanaceae baseada nos estudos moleculares de Olmstead et al. (2008).

1. HIPÓTESE

A análise do padrão de ocorrência de marcadores químicos em Solanaceae, de modo especial os derivados da ornitina e os vitanolídeos, pode elucidar polarizações evolutivas e auxiliar na compreensão das relações filogenéticas nessa família em diferentes níveis hierárquicos.

2. OBJETIVO

2.1 OBJETIVO GERAL

Elucidar polarizações quimio-evolutivas em Solanaceae, por meio da análise do padrão de ocorrência dos seus marcadores no emprego da abordagem metodológica da quimiossistemátíca micromolecular, auxiliando na melhor compreensão das relações filogenéticas nesse importante táxon angiospérmico.

2.2 OBJETIVOS ESPECÍFICOS

- Caracterizar o perfil químico das subfamilias de Solanaceae com base na ocorrência de alcaloides derivados da ornitina.
- Avaliar a similaridade química das subfamílias de Solanaceae com base na ocorrência de alcaloides derivados da ornitina.
- Verificar o padrão de distribuição de alcaloides tropânicos e calisteginas, além de tendências evolutivas em Solanaceae.
- Caracterizar o perfil químico das tribos e gêneros da subfamília Solanoideae com base na ocorrência de vitanolídeos.
- Avaliar a similaridade química das tribos e gêneros da subfamília Solanoideae com base na ocorrência de vitanolídeos.

3. METODOLOGIA

3.1 BANCO DE DADOS

Um amplo levantamento de ocorrências de micromoléculas em espécies da família Solanaceae foi realizado a partir da consulta de banco de dados especializados disponíveis na rede de computadores (ISI Web of Science e Chemical Abstracts) e pela consulta direta em periódicos especializados de acordo com a metodologia proposta por Gottlieb et al. (1996) e modificada por Santos et al. (2010). Para esse levantamento foram utilizadas como palavraschave os termos Solanaceae, “simple pyrrolidines”, “nicotinoids”, “calystegines”, “tropane alkaloids” e “withanolides”. O levantamento não incluiu restrição na data das publicações e os artigos foram consultados na íntegra.

3.2 NÚMERO DE OCORRÊNCIAS E NÚMERO DE OCORRÊNCIAS PERCENTUAL

O número de ocorrências (NO) de um determinado metabólito secundário em um dado táxon é um parâmetro extremamente útil em quimiotaxonomia a despeito de sua simplicidade matemática. Essa variável é calculada levando em conta a soma do número de diferentes metabólitos secundários citados para cada táxon pertencentes a uma determinada classe de compostos ou rota metabólica. Eventualmente o NO pode ser convertido em um percentual relativo ao total de ocorrências de um metabólito secundário, sendo então denominado de número percentual de ocorrências (NO%) (SANTOS et al. 2010).

3.4 ANÁLISE DE DADOS

Nos artigos 1 e 3 foram realizadas análises de similaridade química. Para essa análise foram criadas matrizes de dados com as informações de ocorrências dos derivados da ornitina (Artigo 1) e da ocorrência de vitanolídeos (Artigo 3) e tabulados de acordo com os tipos estruturais. O NO foi transformado em NO% e as matrizes foram analisadas utilizando o PAST® software. Dados quantitativos foram escolhidos para a análise e o coeficiente de Dice foi utilizado para o agrupamento das subfamílias (Artigo 1) ou tribos/gêneros (Artigo 3) conforme sua similaridade química. Os agrupamentos foram obtidos pelo método UPGMA.

No artigo 2 foram realizadas análises de agrupamento e análise de componente principal (PCA), ambas utilizando o MULTIV v.2.90b software (PILAR, 2011). Para a análise de agrupamento foi avaliada a dissimilaridade entre unidades amostrais utilizando a distância euclidiana e o método de agrupamento de variância mínima. Para a análise de componentes principais (PCA), foi utilizada a correlação entre as variáveis para a definição dos padrões de agrupamentos.

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ARTIGO 1

CHEMOTAXONOMIC CHARACTERIZATION AND CHEMICAL SIMILARITY OF SOLANACEAE SUBFAMILIES BASED ON ORNITHINE DERIVATIVES

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Chemotaxonomic characterization and chemical similarity of Solanaceae subfamilies based on ornithine derivatives

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Abstract. In the present study, the database on ornithine-derived alkaloids was elaborated in order to verify the chemical profile and the chemical similarity of the subfamilies of Solanaceae. Overall, 1513 occurrences were recorded at five subfamilies. The most commonly occurring compounds were tropane alkaloids (NO = 927), followed by nicotinoids (NO = 353); simple pyrrolidines (NO = 133), and, finally, calystegines (NO = 100). The greatest number of occurrences of these substances was recorded in Solanoideae and Nicotianoideae, which together accounted for 95% of occurrences of the four groups of substances studied. Petunioideae characterized by the presence of tropane alkaloids, simple pyrrolidines and calystegines in Schizanthoideae were observed instances of simple pyrrolidines and tropane alkaloids and in the presence of only Cestroideae nicotinoids. Similarity analysis based on the Dice coefficient suggests that the chemical profile of subfamilies Solanaceae correlated with the current classification. The similarity dendrogram showed great similarity between chemical and Solanoideae Nicotianoideae and those with Petunioide. Schizanthoideae appeared somewhat similar to these subfamilies while Cestroideae appeared dissimilar to the others.

INTRODUCTION

Solanaceae Juss. is one of the largest and most important families of eudicots. Several crop plants, such as *Solanum tuberosum* L. (potato), *Solanum lycopersicum* L. (tomato), *Solanum melongena* L. (eggplant/aubergine), and *Capsicum* L. species (chili peppers/bell peppers), belong to this family. Several other species are of interest due to their pharmacologically active secondary metabolites, such as *Atropa belladonna* L. (nightshade), *Hyoscyamus niger* L. (henbane), and the *Datura* L. species. This taxon also includes economically relevant species, such as *Nicotiana tabacum* L. (tobacco) and toxic species such as *Nicotiana glauca* Graham (tree tobacco).

From a systematic standpoint, the Solanaceae belong to the order Solanales (Eudicotyledoneae, Euasteridae) and are phylogenetically related to the Convolvulaceae (sister to the Solanaceae), Hydroleaceae, Montiniaceae, and Sphenocleaceae (APG III 2009). The Solanaceae are considered a subcosmopolitan taxon, with the greatest biodiversity found in the Western hemisphere (Olmstead *et al.* 2008). According to Hunziker (2001), South America is their center of diversity.

Historically, the Solanaceae were divided into a different number of subfamilies and tribes. Two proposed classifications are currently accepted. The first, proposed by Hunziker (2001) and based on morphological and chemical criteria, comprises approximately 2300 species in 92 genera distributed across the subfamilies Solanoideae, Cestroideae, Juanulloideae, Salpiglossoideae, Schizanthoideae, and Anthocercidoideae. The second, more recent proposal was presented by Olmstead *et al*. (2008) in a molecular study conducted on a sample of 89 genera and 190 species. The authors propose seven subfamilies: Solanoideae, Cestroideae, Nicotianoideae, Petunioideae, Schizanthoideae, Goetzeoideae, and Schwenckioideae.

Current chemotaxonomic knowledge of the Solanaceae suggests that study of certain chemical markers might play an important role in the elucidation of evolutionary polarity, thus providing a better understanding of the phylogenetic relationships within this taxon. Alkaloids and steroid derivatives (including steroidal alkaloids) are known to be the most important classes of these secondary metabolites. Four groups of ornithine-derived alkaloids that share a common biosynthetic pathway (Fig. 1) are found in the Solanaceae: simple pyrrolidines, nicotinoids, tropane alkaloids, and calystegines. The tropane alkaloids are particularly typical of the Solanaceae, and some genera, such as *Datura*, *Brugmansia*, and *Duboisia*, are characterized by their occurrence (Griffin and Lin 2000). The calystegines are also an important class of Solanaceae metabolites. According to Dräger (2004), the center of occurrence of tropane alkaloids appears to be the Solanaceae, and calystegines are also basically restricted to this family and to the Convolvulaceae and were originally discovered.

Chemotaxonomic studies are an important tool for assessment of biological diversity, as they enable drawing of evolutionary inferences for a given taxon by analysis of micromolecular and morphological data (Gottlieb 1982) and can be used to test for correlation between morphological and chemical diversity (Soares and Kaplan 2001). These studies are based on databases of occurrence of chemical compounds constructed after a review of the literature. Analysis of these databases enables selection of chemotaxonomic markers, which are classes of compounds characterized by widespread occurrence in the taxon of interest and structural diversity (Santos *et al.* 2010). Another important contribution of chemotaxonomic studies is in the prediction of whether specific compounds or classes of secondary metabolites will occur in a given taxon, thus enabling rationalization of phytochemical studies and aiding research in the field of natural products chemistry.

In the present study, we used a database of ornithine-derived alkaloids to assess the chemical profile of the subfamilies of Solanaceae according to these compounds. Our research hypothesis presupposes that ornithine-derived alkaloids are of chemotaxonomic importance to the Solanaceae and will corroborate the latest classification proposed for this family.

MATERIALS AND METHODS

Database

A database of the occurrence of ornithine-derived alkaloids (simple pyrrolidines, nicotinoids, tropane alkaloids and calystegines) in the Solanaceae was constructed using the method proposed by Gottlieb *et al.* (1996) and updated by Santos *et al*. (2010). A wide-ranging review of the literature was carried out by means of a search of online databases (ISI Web of Science and Chemical Abstracts) and a hand search of relevant journals. The keywords “Solanaceae”, “simple pyrrolidines”, “nicotinoids”, “tropane alkaloids”, and “calystegines” were used as search terms. The full text of all articles was analyzed and the search was unfiltered by date of publication.

Search results were entered into a Microsoft Excel® spreadsheet, where rows indicated species cited in the literature and columns indicated which ornithine-derived alkaloids have been related from each species. Data were tabulated and analyzed by structural class of alkaloid. For simple pyrrolidines, nicotinoids and tropane alkaloids, we used the structural classes proposed by Eich (2008). Calystegines were divided into trihydroxynortropanes, tetrahydroxynortropanes and pentahydroxynortropanes (Fig. 2).

Absolute and relative frequency of occurrences

The absolute frequency of occurrences (NO) was calculated as described by Santos *et al*. (2010), and the relative frequency of occurrences (NO%) was obtained following: occurrence’s number of each structural class by 100. The value obtnained were divided by the total occurrence’s number.

Cluster analysis

For analysis of similarity and principal components analysis, we constructed a data matrix with information on all occurrences of ornithine-derived alkaloids tabulated by genus and structural type. The NO was converted to NO% and the data matrix analyzed in the PAST® software environment. Quantitative data were chosen as the input for cluster analysis, and Dice’s coefficient was used to group subfamilies according to their chemical similarity. Clustering was conducted using the unweighted pair group method with arithmetic averaging (UPGMA).

RESULTS

Chemotaxonomic profile of Solanaceae subfamilies according to occurrence of ornithine-derived alkaloids

Overall, 1513 occurrences of ornithine-derived alkaloids were recorded. The most commonly occurring compounds were tropane alkaloids (NO=927), followed by nicotinoids, also known as tobacco alkaloids (NO=353); simple pyrrolidines (NO=133); and, finally, calystegines (NO=100) (Fig. 3). Table 1 provides occurrence data of each of the four classes of ornithine-derived alkaloid, stratified by structural type and by genus, classified according to the subfamily scheme proposed by Olmstead *et al*. (2008).

Of the seven Solanaceae subfamilies proposed by Olmstead *et al*. (1999) on the basis of molecular research, five have been reported to contain ornithine-derived alkaloids (Table 1): Solanoideae, Nicotianoideae, Petunioideae, Cestroideae, and Schizanthoideae. No occurrences of ornithine-derived alkaloids have been reported in the Goetzeoideae and Schwenckioideae.

Both tropane alkaloids and simple pyrrolidines occurred in the Solanoideae, Nicotianoideae, Petunioideae, and Schizanthoideae. Calystegines were only observed in the Solanoideae and Nicotianoideae. Nicotinoids were detected in Solanoideae, Nicotianoideae, and Cestroideae (Fig. 4).

The chemical profile of the Solanoideae subfamily in terms of ornithine-derived alkaloids is characterized by presence of tropane alkaloids (NO=665), calystegines (NO=89), and simple pyrrolidines (NO=110), as well as—far less frequently—nicotinoids (NO=7).

Tropane alkaloids were found in *Anisodus*, *Atropa*, *Atropanthe*, *Brugmansia*, *Datura*, *Hyoscyamus*, *Latua*, *Mandragora*, *Physochlaina*, *Przewaslkia*, *Salpichroa*, *Solandra*, *Physalis*, *Scopolia*, and *Withania*. Of these genera, *Brugmansia* and *Datura* had the highest absolute frequency of occurrences (Table 1).

Group 1 tropane alkaloids (TA1) are structurally characterized as esters of 3α-hydroxytropane with aliphatic acids and were found in 12 genera (Table 1). Among these alkaloids, 3α-tigloyloxytropane was the most common (NO=30), occurring in *Brugmansia*, *Datura*, *Hyoscyamus*, *Mandragora*, *Physalis*, *Solandra* and *Withania* species. Esters of 3α,6β- or 3α,7β-dihydroxytropanes (TA2) were the most diverse group of compounds (24 overall). Anisodamine, 3α-tigloyloxy-6β-hydroxytropane, 3α-hydroxy-6β-tigloyloxytropane, and 3α,6β-ditigloyloxytropane were the most commonly occurring compounds (NO=54), and were isolated from *Anisodus*, *Atropa*, *Brugmansia*, *Datura*, *Hyoscyamus*, *Mandragora*, *Physochlaina*, and *Przewalskia*. Esters of 3α,6β,7β-trihydroxytropanes (TA3) have only been reported in *Brugmansia* and *Datura*, and may thus be suggested as chemotaxonomic markers for these two genera. Of the seven substances in this class, the most common were those containing a tigloyl ester group, such as meteloidine (Fig. 2). Fifteen esters of 3α-hydroxytropane with phenylpropanoid acids (TA4), including hyoscyamine, atropine, noratropine, 3α-apotropoyloxytropane, and littorine, were reported, and were particularly common, accounting for 149 (78.4%) of the 190 occurrences of compounds in this class. Furthermore, these compounds have been found in most Solanoideae genera except *Lycium*, *Physalis*, and *Withania*. Alkaloids in group TA5 (esters of 6β,7β-epoxy-3α-hydroxytropane) were also quite common (NO=121). This group of eight compounds includes scopolamine (Figure 8B) (NO=55), which has been isolated from *Anisodus*, *Atropa*, *Atropanthe*, *Brugmansia*, *Datura*, *Hyoscyamus*, *Latua*, *Mandragora*, *Physochlaina*, *Przewalskia*, *Scopolia*, and *Solandra*. Sixteen esters of 3β-hydroxytropane (AT6) were detected for a total NO of 66. Of these occurrences, 17 (25.7%) were of tigloidine (Fig. 2), which was detected in *Brugmansia*, *Datura*, *Hyoscyamus*, *Physalis*, and *Solandra*. The presence of tigloyl esters in other compounds of this class is quite peculiar, although the absolute frequency of occurrence of these substances (nine, excepting tigloidine) in the genera analyzed was low (NO=17). Dimeric and trimeric tropanes, which are considered rare compounds, accounted for 14 occurrences of four different substances: α-belladonnine, β-belladonnine, α-scopadonnine, and β-scopadonnine. The first two were found in *Atropa*, *Hyoscyamus*, *Mandragora* and *Physochlaina*, and the latter two, in *Datura*.

The calystegines are also of chemotaxonomic importance to the Solanaceae. In the Solanoideae subfamily, these compounds have been found (NO=89) in the genera *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycium*, *Mandragora*, *Nicandra*, *Physalis*, *Scopolia*, *Solanum*, and *Withania*. Of the 89 occurrences reported, 68 (71.5%) were recorded in *Hyoscyamus*, *Lycium*, *Physalis*, *Scopolia*, and *Solanum*. Tetrahydroxylated calystegines (Table 1) were the most common compounds of this class (54 occurrences, 56.8%) and were found in all 11 genera of this subfamily ever found to contain calystegines. Simple pyrrolidine alkaloids are characterized by the presence of one or two isolated pyrrolidine rings, with no other heterocyclic chains. The main compounds of this class are hygrine and cuscohygrine (Fig. 2). Among the simple pyrrolidines, cuscohygrine was the most common, with 51 occurrences (45.9%) in 16 genera (Table 1). Hygrine was isolated in the genera *Atropa*, *Brugmansia*, *Datura*, *Hyoscyamus, Nicandra*, *Physalis*, *Physochlaina*, *Salpichroa*, and *Withania*, with 25 occurrences overall.

Among the nicotinoids, only seven occurrences of nicotine were recorded in the Solanoideae, specifically in the genera *Brugmansia*, *Capsicum*, *Datura*, *Solanum*, and *Withania*.

The chemical profile of the Nicotianoideae, just as that of the Solanoideae, is characterized by the presence of all four classes of ornithine-derived alkaloids studied in the present investigation. However, in this subfamily, the nicotinoids are the leading class (NO=315), followed by the tropane alkaloids (NO=208). Calystegines were detected only six times, in the genus *Duboisia*, and only five occurrences of simple pyrrolidines were reported:

two (cuscohygrine) in *Anthocercis* and three (hygrine [NO=2] and cuscohygrine [NO=1]) in *Duboisia*. The presence of nicotinoids was highly representative of this subfamily, particularly of the genus *Nicotiana*, in which 315 occurrences (94%) were recorded. Nicotine and nornicotine (NI1) and anabasine e anatabine (NI2) were the most common compounds, with 84, 75, 74, and 66 occurrences respectively. Nicotine was found in all species included in the study. *Duboisia* (NO=11), *Cyphanthera* (NO=8), and *Crenidium* (NO=1) were also found to contain nicotinoids. Tropane alkaloids have been detected in the genera *Anthocercis*, *Anthotroche*, *Crenidium*, *Cyphanthera*, *Duboisia*, *Grammosolen*, and *Symonanthus*. Among these, *Anthocercis* and *Cyphanthera* had the highest frequency of occurrences (84 and 51 respectively). In both genera, esters of 3α-hydroxytropane with phenylpropanoid acids (TA4) and esters of 6β,7β-epoxy-3α-hydroxytropane (TA5) were more representative (Fig. 2).

Analysis of the chemical profile of the Petunioideae revealed few occurrences of the compounds of interest. Simple pyrrolidines were detected six times: four occurrences in *Brunfelsia* (cuscohygrine) and two in *Nierembergia* (hygrine and norhygrine). Calystegines (NO=5) were reported only in *Brunfelsia*, and tropane alkaloids (NO=2), in *Nierembergia*. The presence of nicotinoids was not reported in this subfamily.

Only nicotinoids were detected in the Cestroideae (NO=11): six reported occurrences in *Streptosolen* and three in *Salpiglossis*.

The subfamily Schizanthoideae, represented by the genus *Schizanthus*, was found to contain simple pyrrolidines (NO=12) and tropane alkaloids (NO=43). Simple pyrrolidines were represented by hygrine, cuscohygrine, and hygrolines A and B. Tropane alkaloids were represented by esters of 3α-hydroxytropane with aliphatic acids (TA1), esters of 3α,6β- or 3α,7β-dihydroxytropanes (TA2), and dimeric and trimeric tropanes (TA7), such as schizanthine A and grahamine (Fig. 2).

Chemical similarity among Solanaceae subfamilies

The dendrogram constructed from analysis of Dice's coefficient to demonstrate chemical similarity among the Solanaceae subfamilies found to contain ornithine-derived alkaloids identified two well-defined groups (Fig. 5). The first comprises the subfamilies Schizanthoideae, Petunioideae, Nicotianoideae, and Solanoideae, and the second contains the subfamily Cestroideae, which was chemically dissimilar from all others.

Analysis of group 1 showed substantial chemical similarity between the subfamilies Solanoideae and Nicotianoideae, with a similarity coefficient of nearly 0.95 (Fig. 5). The subfamily Petunioideae was somewhat similar to the Solanoideae and Nicotianoideae, with a coefficient of similarity near 0.7. Similarity coefficients between the Schizanthoideae and all of the aforementioned subfamilies were <0.6, demonstrating little chemical similarity.

DISCUSSION

A review of the literature on the occurrence of ornithine-derived alkaloids in the Solanaceae proved the importance of nitrogenous secondary metabolites in this taxon, particularly in the subfamilies Solanoideae and Nicotianoideae, which together accounted for 95% of occurrences. Furthermore, there was great structural diversity of ornithine-derived alkaloids in both subfamilies. All classes of ornithine-derived alkaloids of interest to this study were found in the Solanoideae. The only class not reported in the Nicotianoideae were simple pyrrolidines of structural group SP3 (Table 1). The number of occurrences is an index that denotes the importance of a category of metabolites to a certain taxon (Gottlieb *et al*. 1996), and thus provides evidence of trends in their production. It is also an important index for definition of chemotaxonomic markers: small molecules, usually secondary metabolites, that enable characterization of differences between individuals at any rank. Therefore, secondary metabolites found to be characteristic of a group of plants can be used for chemotaxonomic identification.

In his taxonomy of the Solanaceae, Hunziker (2001) used chemical data to define subfamilies, noting the occurrence of nicotinoids and tropane alkaloids in the subfamilies Cestroideae and Solanoideae and, likewise, reporting the occurrence of tropane alkaloids alone in the Schizanthoideae. The Hunziker classification included 23 genera in the Cestroideae, of which *Cestrum*, *Latua*, *Nicotiana*, *Nierembergia*, *Brunfelsia*, *Streptosolen*, and *Schwenckia* are included in the present study, as only these have been found to contain ornithine-derived alkaloids. However, the most recent, molecular-based classification of the Solanaceae (Olmstead *et al*. 2008), that adopted in our study, changes assignment of the Cestroideae subfamily. Therefore, data on ornithine-derived alkaloids were only obtained for the genera *Cestrum*, *Streptosolen*, and *Salpiglossis* (the latter was assigned by Hunziker (2001) to the subfamily Salpiglossoideae), changing the chemical profile of the Cestroideae, as these three genera were only found to contain nicotinoids. Olmstead *et al*. (2008) place *Nierembergia* and *Brunfelsia* among the Petunioideae; *Latua* was attached to the subfamily Solanoideae; and *Nicotiana*, alongside the Australian genera *Anthocercis*, *Anthotroche*, *Crenidum*, *Cyphanthera*, *Duboisia*, *Grammosolen* and *Symonanthus*, of the tribe Anthocercideae (assigned by Hunziker to the subfamily Anthocercidoideae), were assigned to the subfamily Nicotianoideae.

The subfamily Solanoideae is believed to be the most derived of the Solanaceae, and has been described as monophyletic since the earliest molecular studies of Olmstead and Palmer (1994). It contains approximately 47 genera and approximately 1800 widely distributed species (Hunziker 2001). In our study, 22 genera (approximately 47%) were found to contain ornithine-derived alkaloids. Tropane alkaloids were the most representative structural class of this subfamily, characteristic of such genera as *Brugmansia*, *Datura*, *Hyoscyamus*, and *Solandra*. Overall, the tropane alkaloids are regarded as chemical markers of major importance to the Solanaceae family as a whole (Griffin and Lin 2000). The calystegines were also representative in the Solanoideae. Seven genera were found to contain both tropane alkaloids and calystegines, and 12 genera were found to contain tropane alkaloids and simple pyrrolidines alike. The simultaneous occurrence of cuscohygrine, a simple pyrrolidine, and tropane alkaloids in the same taxa is quite common, as both compounds share the same biosynthetic pathway (Evans 1972). Indeed, this finding was corroborated by the present study, as, of the 17 genera found to contain cuscohygrine, 15 have also been reported to contain tropane alkaloids.

The subfamily Nicotianoideae was proposed by Olmstead and Palmer (1994) on the basis of molecular studies. This monophyletic subfamily includes the genus *Nicotiana* (tribe Nicotianeae) and the Australian genera of the tribe Anthocercideae. There is a clear divergence between the chemical profiles of these two tribes. Whereas the Nicotianeae contain nicotinoids, the Anthocercideae predominantly contain tropane alkaloids. According to Olmstead and Palmer (1994), the fact that both tribes share a range in Australia is not indicative of a common origin; colonization of Australia by a group of *Nicotiana* species is likely to represent a recent event. One may thus infer that this genus experienced geographic divergence and chemical convergence, as, despite distribution in another continent, it preserved its chemical profile, which is closer to that of the Cestroideae than to that of the Nicotianoideae. This finding can be proved by conducting a similarity analysis of the Solanaceae subfamilies while separating the Nicotianeae and Anthocercideae from the Nicotianoideae into two groups instead (Fig. 6). The dendrogram of such an analysis shows the greater chemical similarity between the Nicotianeae (Fig. 6a) and the Cestroideae and the similarity between the tribe Anthocercideae and the Solanoideae (Fig. 6b), particularly due to the high frequency of occurrence of tropane alkaloids in the latter tribe.

Despite a predominance of tropane alkaloids, the tribe Anthocercideae has also been found to contain nicotinoids. The chemotaxonomic significance of the presence of tropane alkaloids and nicotinoids in genera of this tribe has been reported elsewhere (Evans and Ramsey 1983). Furthermore, the authors highlighted the importance of the tropane alkaloids as chemotaxonomic markers, as all classes of tropane alkaloids other than the dimeric bases found in *Schizanthus* and *Atropa* have been reported in the Anthocercideae.

Similarity analysis based on the Dice coefficient suggests that the chemical profile of Solanaceae subfamilies correlated with the current Solanaceae classification proposed by Olmstead *et al*. (1999; 2008). As early as 1992, Olmstead and Palmer noted the potential relationship between the subfamily Solanoideae, the tribe Anthocercideae and the genus *Nicotiana* in view of their chromosome counts (x=12). Improved molecular studies of the Solanaceae later confirmed the affinity between *Nicotiana* and the tribe Anthocercideae, thus leading to the proposed subfamily Nicotianoideae, which included the tribes Nicotianeae (*Nicotiana*) and Anthocercideae (*Anthocercis*, *Anthotroche*, *Crenidum*, *Cyphanthera*, *Duboisia*, *Grammosolen,* and *Symonanthus*, all genera endemic to Australia). These two subfamilies were included in the clade x=12, and the chromosome count is considered a synapomorphic character for this group (Olmstead *et al*. 1992; Olmstead *et al*. 1999; Olmstead *et al*. 2008).

The Petunioideae subfamily was also proposed on the basis of molecular studies carried out by Olmstead *et al*. (1999). The taxa of this subfamily were moved from the Cestroideae and include those assigned by Hunziker (2001) to the tribe Nicotianeae (*Petunia* and *Fabiana*), except for *Nicotiana* and *Brunfelsia*. Therefore, we may infer that the results of chemical similarity analysis that show a close relationship between the Petunioideae and the Solanoideae and Nicotianoideae on the basis of ornithine-derived alkaloid occurrence is consistent with the current classification of the Solanaceae proposed by Olmstead *et al.* (2008).

The similarity dendrogram constructed on the basis of our analysis also showed the Schizanthoideae to be closer to the aforementioned subfamilies than previously thought. The Schizanthoideae subfamily, which is endemic to Chile and restricted to South America, is believed to be the most basal of the Solanaceae subfamilies. The presence of tropane alkaloids alone, including some with unique structural features, such as esters of senecioic, angelic, mesaconic, and itaconic acid, is characteristic of the Schizanthoideae. The relative chemical similarity of the Schizanthoideae to the Petunioideae, Nicotianoideae, and Solanoideae appears to be more related to the occurrence of tropane alkaloids in the Schizanthoideae than to any taxonomic aspects, since this family, as mentioned above, is regarded as the most basal group of the Solanaceae (Hunziker 2001; Olmstead *et al*. 2008). Regarding the Cestroideae, which were not similar to any other subfamily in our analysis, one must bear in mind that the number of ornithine-derived alkaloid occurrences reported for this subfamily is very low (Table 1) and comprises only one of the chemical marker classes of interest to this study; hence, we conclude that the ornithine-derived alkaloids are not good chemotaxonomic markers for the Cestroideae.

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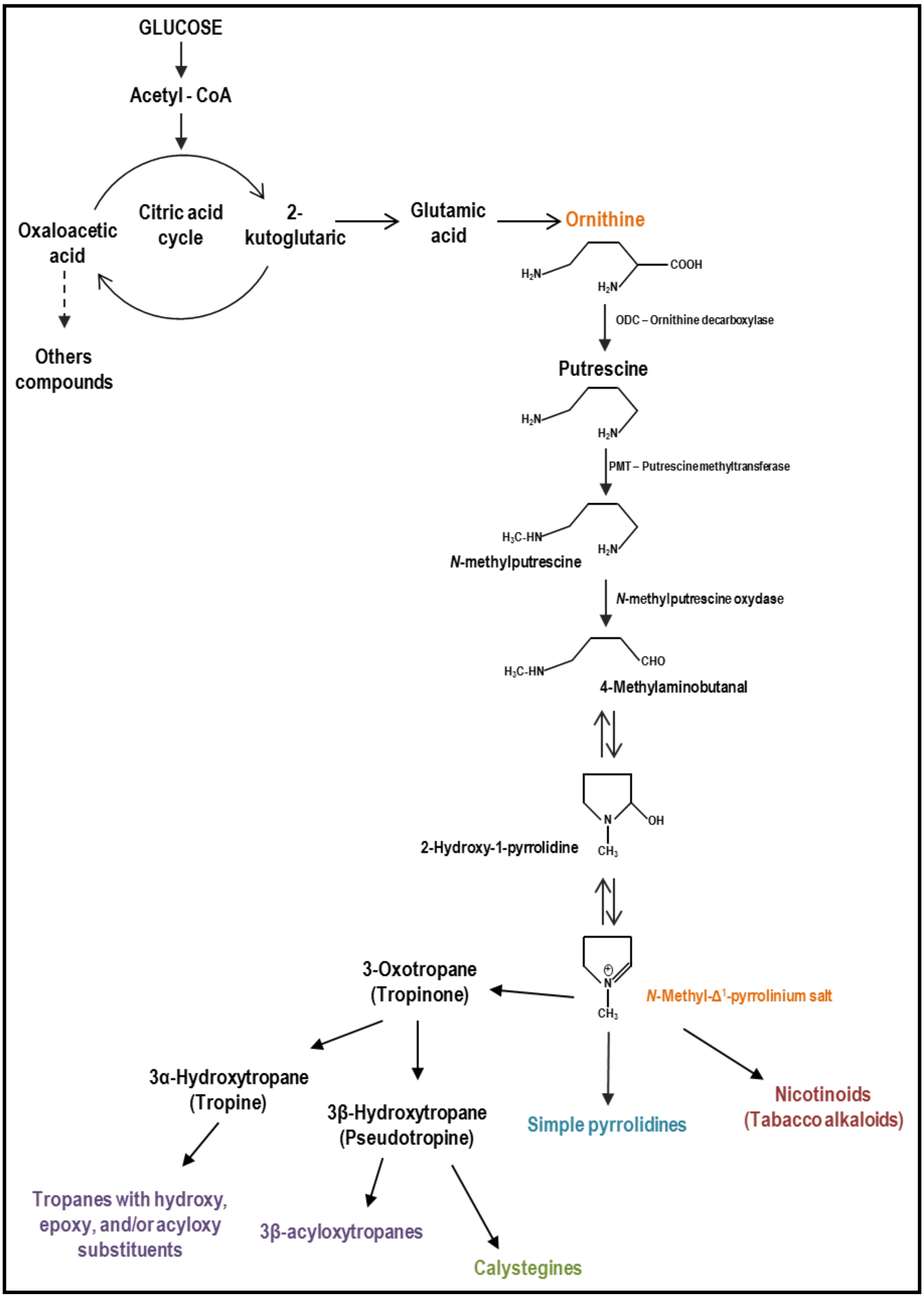
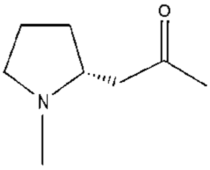


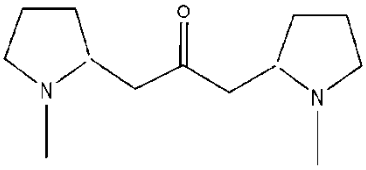
Fig. 1. Simplified schematic diagram of the ornithine-derived alkaloid biosynthesis pathway. After Bachi (2010) and Grynkiewicz and Gadzikowska (2008).

Ornithine-derived alkaloids

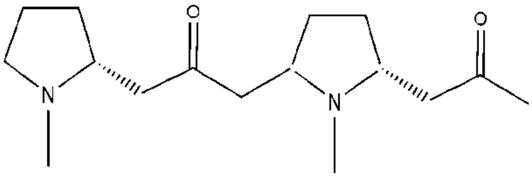
Simple pyrrolidines



Hygrine

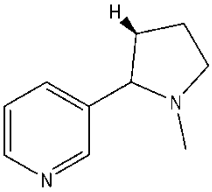


Cuscohygrine

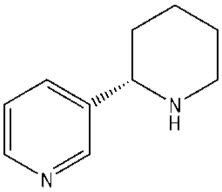


Phygrine

Nicotinoids



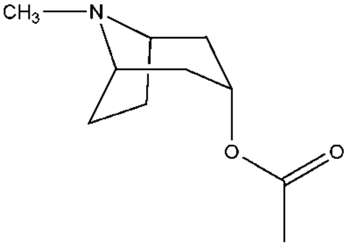
Pyridylpyrrolidines



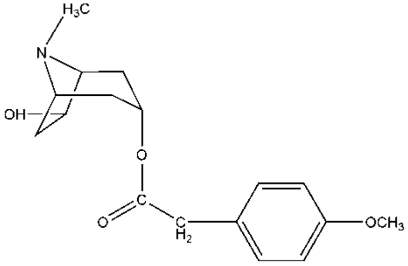
Pyridylpiperidines

Tropane alcaloides

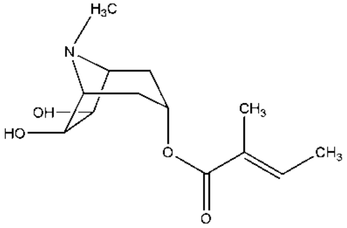
Aliphatic esters of 3α-hydroxytropanes



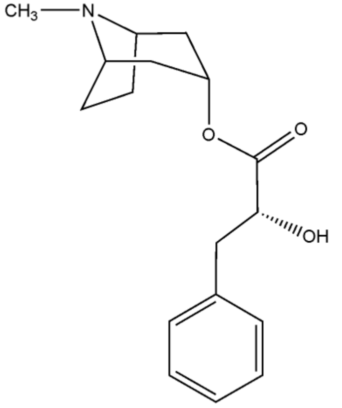
Esters of 3α,6β-dihydroxytropane or Esters of 3α,7β-dihydroxytropane



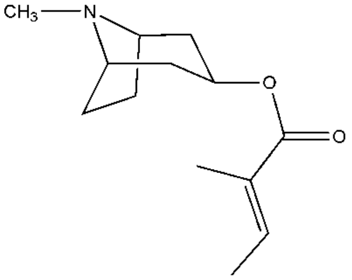
Esters of 3α,6β,7β-trihydroxytropane



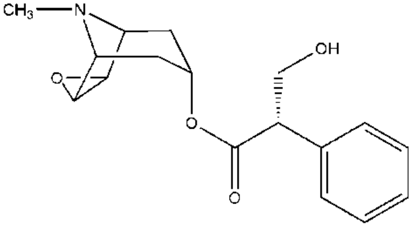
Esters of 3α-hydroxytropane/-nortropane with phenylpropanoid acids



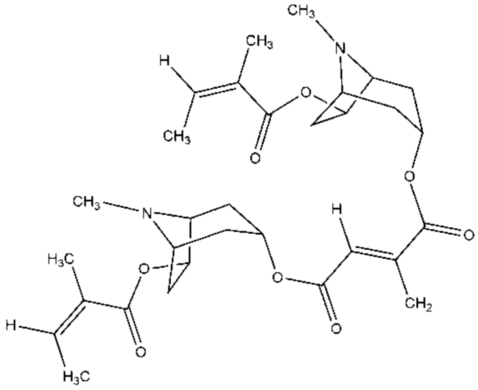
3β-hydroxytropanes



Esters of 6β,7β-epoxy-3α-hydroxytropane with phenylpropanoid acids

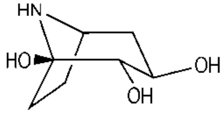


Dimeric and trimeric forms

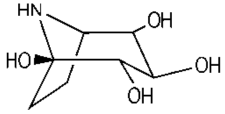


Calystegines

Trihydroxynortropane



Tetrahydroxynortropane



Pentahydroxynortropane

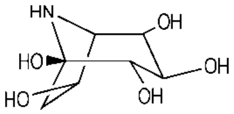


Fig. 2. Ornithine-derived alkaloids

Table 1. Occurrence of ornithine-derived alkaloids in genera of the five subfamily Solanaceae Juss.

Subfamily	Simple pyrrolidines			Nicotinoids		Calystegines			Tropane alkaloids							**Total**
	SP1	SP2	SP3	NI1	NI2	CA1	CA2	CA3	TA1	TA2	TA3	TA4	TA5	TA6	TA7	
Solanoideae																
Anisodus Link	0	2	0	0	0	0	0	0	1	0	0	1	1	1	0	**6**
Atropa L.	2	2	3	0	0	1	3	0	1	3	0	16	8	1	2	**42**
Atropanthe Pascher	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
Brugmansia Persoon	2	5	1	1	0	0	0	0	12	33	18	19	16	15	0	**122**
Capsicum L.	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	**3**
Chamaesaracha (A. Gray) Benth. & Hook.	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	**2**
Datura L.	5	9	3	1	0	1	3	0	27	63	27	61	38	26	4	**268**
Hyoscyamus L.	7	8	11	0	0	7	7	0	14	5	0	34	27	8	4	**132**
Iochroma Benth.	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
Latua Phil.	0	0	0	0	0	0	0	0	0	0	0	6	2	0	0	**8**
Leucophysalis Rydberg	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	**1**
Lycium L.	0	0	0	0	0	4	6	3	0	0	0	0	0	0	0	**13**
Mandragora L.	0	3	0	0	0	2	5	0	0	1	0	4	3	0	2	**20**
Nicandra Adans.	1	1	3	0	0	0	1	0	0	0	0	0	0	0	0	**6**
Physalis L.	5	3	12	0	0	3	6	1	4	0	0	0	0	3	0	**37**
Physochlaina G.Don	1	4	0	0	0	0	0	0	2	9	0	9	8	0	2	**35**
Przewalskia Maxim.	0	1	0	0	0	0	0	0	0	2	0	2	4	0	0	**9**
Salpichroa Miers	1	1	0	0	0	0	0	0	1	0	0	1	0	1	0	**5**
Scopolia Jacq.	0	0	0	0	0	3	6	1	7	5	0	18	17	2	0	**59**
Solandra Swartz	0	4	0	0	0	0	0	0	20	0	0	25	6	10	0	**65**
Solanum L.	0	2	0	3	0	5	12	0	0	0	0	0	0	0	0	**22**
Withania Pauq.	1	1	1	1	0	1	4	2	2	0	0	0	0	1	0	**14**
Nicotianoideae																
Anthocercis Labill.	0	2	0	0	0	0	0	0	6	16	8	30	17	7	0	**86**
Anthotroche Endl.	0	0	0	0	0	0	0	0	4	0	0	13	3	0	0	**20**
Crenidum Haegi	0	0	0	0	1	0	0	0	1	1	0	4	3	0	0	**10**
Cyphanthera Miers	0	0	0	5	3	0	0	0	1	1	0	4	3	0	0	**17**
Duboisia R. Br.	2	1	0	7	4	1	3	2	10	10	2	15	13	1	0	**71**
Grammosolen Haegi	0	0	0	0	0	0	0	0	9	1	0	9	6	1	2	**28**
Symonanthus Haegi	0	0	0	0	0	0	0	0	2	2	0	2	2	0	0	**8**
Nicotiana L.	0	0	0	185	130	0	0	0	1	3	1	0	2	1	0	**323**
Petunioideae																
Brunfelsia L.	0	4	0	0	0	1	3	1	0	0	0	0	0	0	0	**9**
Nierembergia Ruiz & Pav.	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	**4**
Cestroideae																
Cestrum L.	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	**5**
Streptosolen Miers	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	**3**
Salpiglossis Ruiz & Pav.	0	0	0	2	1	0	0	0	0	0	0	0	0	0	0	**3**
Schizanthoideae																
Schizanthus Ruiz & Pav.	1	2	9	0	0	0	0	0	5	24	0	0	0	0	14	**55**
TOTAL	**29**	**58**	**46**	**213**	**140**	**30**	**60**	**10**	**131**	**179**	**56**	**273**	**179**	**79**	**30**	**1513**

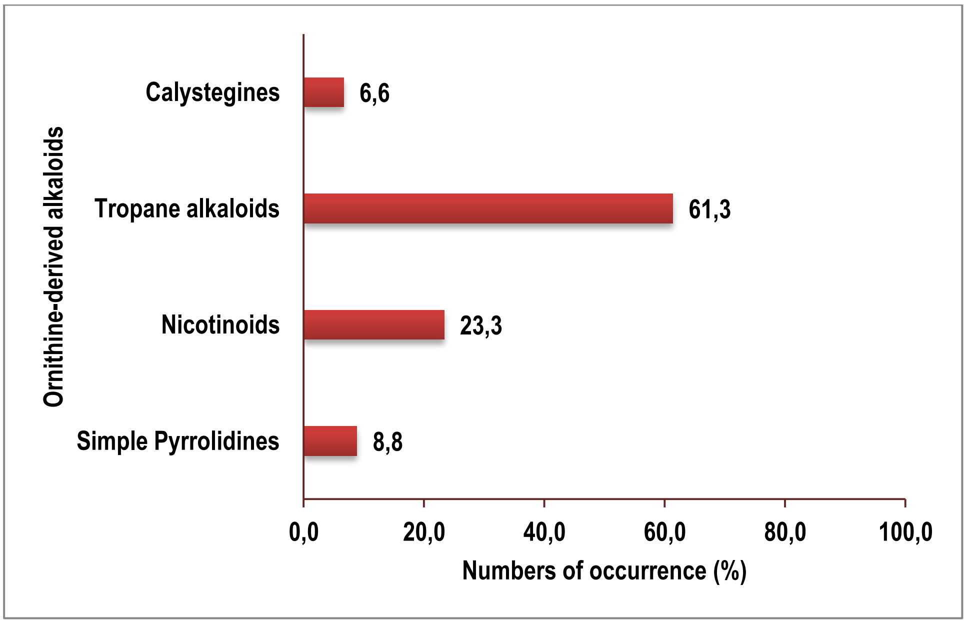


Fig. 3. Relative frequency of occurrence of ornithine-derived alkaloids found in the Solanaceae.

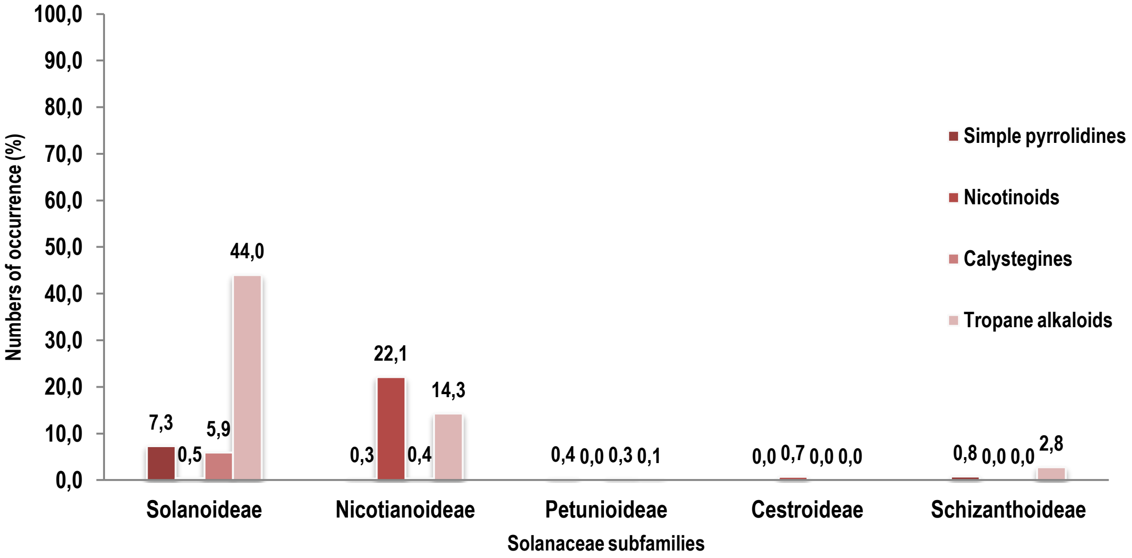


Fig. 4. Relative frequency of occurrence of ornithine-derived secondary metabolites in Solanaceae subfamilies according to the Olmstead *et al.* (2008) classification.

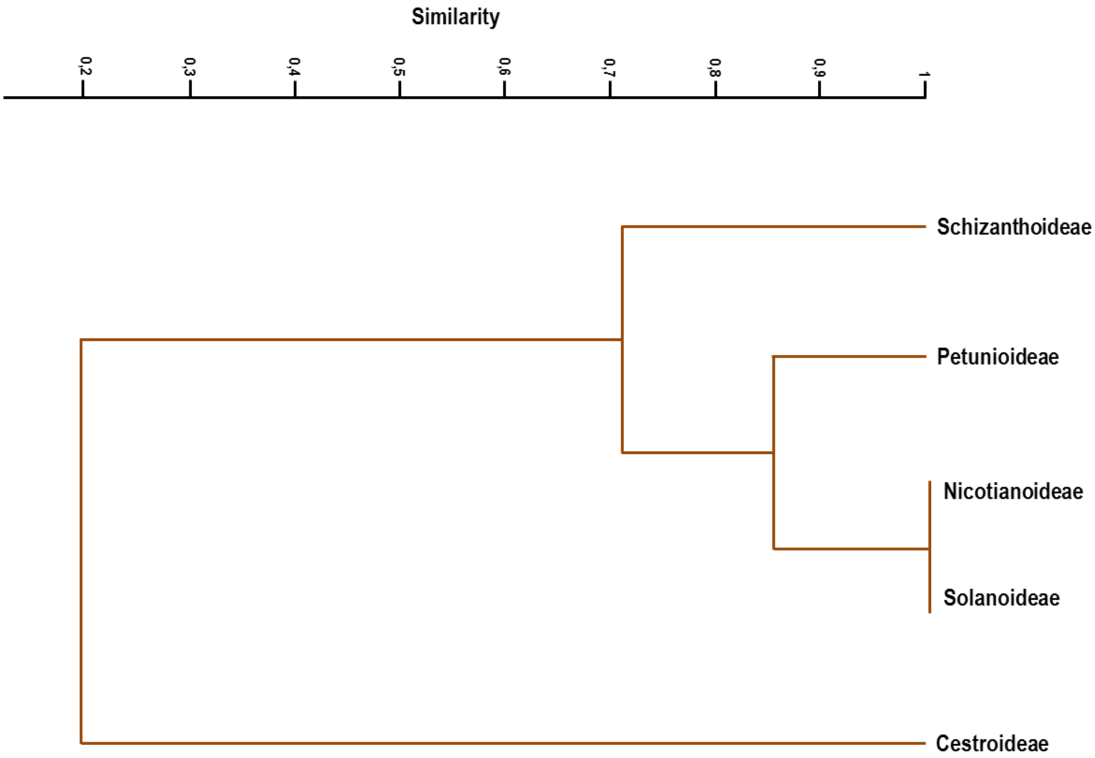


Fig. 5. Dendrogram constructed from analysis of Dice's coefficient for comparison of chemical similarity among Solanaceae subfamilies.

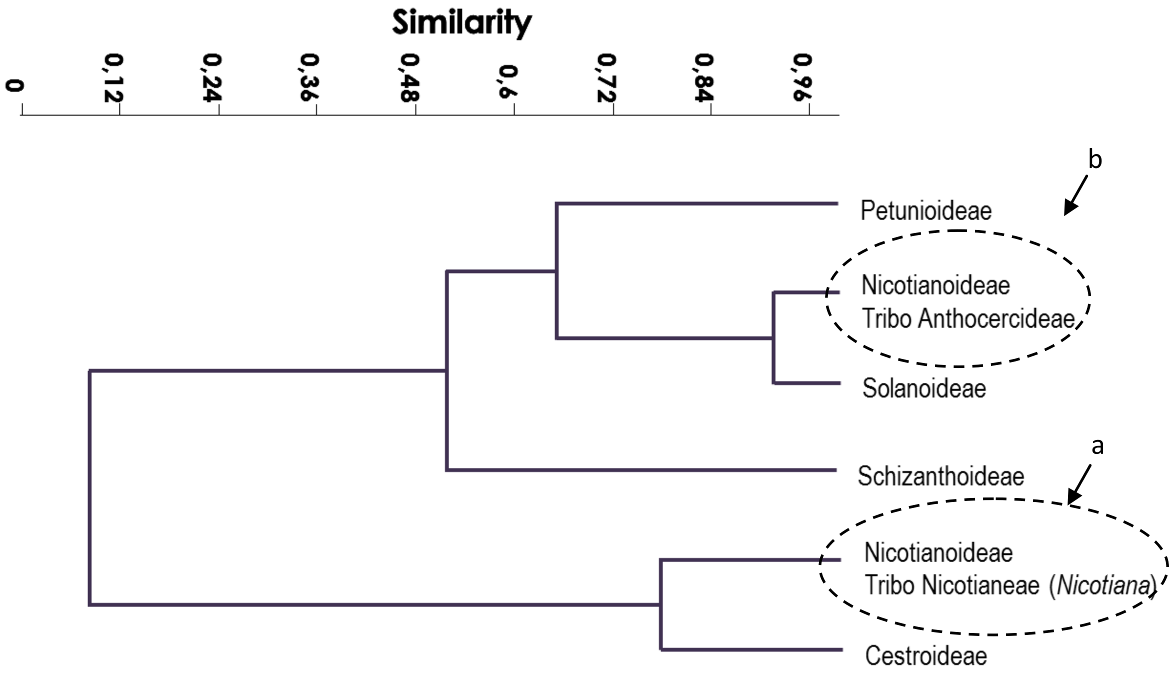


Fig. 6. Dendrogram obtained from analysis of Dice's coefficient for comparison of chemical similarity among the Solanaceae subfamilies, while breaking the subfamily Nicotianoideae down into two groups (Nicotianoideae, tribe Anthocercideae and Nicotianoideae, tribe Nicotianeae).

ARTIGO 2

TROPANE ALKALOIDS AND CALYSTEGINES AS CHEMOTAXONOMIC MARKERS FOR SOLANACEAE JUSS.

A ser submetido para *Plant Systematics and Evolution*

Tropane alkaloids and calystegines as chemotaxonomic markers in the Solanaceae

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Abstract

This study assessed the occurrence and distribution of tropane alkaloids (TAs) and calystegines (CAs) in genera of the family Solanaceae to identify patterns of distribution and make evolutionary inferences. A database of TA and CA occurrences was constructed from the results of a search of scientific websites and a hand search of periodicals. The terms “Solanaceae”, “tropane alkaloids”, and “calystegines” were used as index terms for a full-text literature search unrestricted by date of publication. The frequency of occurrence and chemical diversity indices were calculated and cluster analysis and principal components analysis (PCA) performed. Overall, 996 occurrences were reported: 879 of TAs (88.3%) and 115 of CAs (11.7%). The CAs were significantly more relevant than TAs for characterization of distinct groups of genera, both on cluster analysis and PCA. This corroborates the trend toward a chemical dichotomy observed on database analysis and somewhat reinforces the correlation between geographic distribution and occurrence of secondary metabolites, as the presence of CAs alone (without TAs) was only reported in genera that have South America as their center of diversity.

Key words: Solanaceae, chemical diversity, frequency of occurrence, multivariate analysis, geographic distribution.

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Introduction

Solanaceae Juss. is one of the largest and most important families of flowering plants and major crop plants species such as *Solanum tuberosum* L., *Solanum lycopersicum* L., *Solanum melongena* L., and *Capsicum annum* L. belong to this taxa. Several species with of pharmaceutical interest due to their secondary metabolites (i.e. *Atropa belladonna* L., *Hyoscyamus niger* L., and *Datura stramonium* L.), species of economic relevance (i.e. *Nicotiana tabacum* L.) and toxic species (i.e. *Nicotiana glauca* Graham) are also classified in this cosmopolitan family. The greatest species diversity of Solanaceae is observed in the Americas (Olmstead et al. 2008) and according to Hunziker (2001) the center of diversity of this taxa is in South America.

The first systematic classification of the Solanaceae was proposed by Dunal in the mid-19th century and consisted of a division of the 61 genera known at the time into two tribes. Two decades later, in 1873, Bentham and Hooker proposed a new division of 67 genera into five tribes. In the late 19th century, Wettstein was the first to divide the Solanaceae into subfamilies: the Solanoideae and Cestroideae, comprising three and two tribes respectively. A further two classifications were proposed in the 20th century. In 1979 and 1991, D'Arcy added the Nolanoideae subfamily to the two subfamilies proposed before. In 1987, Tétényi (1987) proposed a classification based on chemical characteristics, dividing the family into three subfamilies based on the occurrence of alkaloids and steroids. The author stressed that the validity of the chemical pattern is based on the biosynthetic pathways of these substances, rather than their isolated occurrences.

Two proposed classifications are currently accepted. The first, proposed by Hunziker (2001) and based on morphological and chemical criteria, comprises approximately 2300 species in 92 genera distributed across the subfamilies Solanoideae, Cestroideae, Juanulloideae, Salpiglossoideae, Schizanthoideae, and Anthocercidoideae. The second, more recent proposal was presented by Olmstead et al. (2008) in a molecular study conducted on a sample of 89 genera and 190 species. The authors propose seven subfamilies: Solanoideae, Cestroideae, Nicotianoideae, Petunioideae, Schizanthoideae, Goetzeoideae, and Schwenckioideae. Both proposals agree that Solanoideae is the most derived subfamily in relation to the Cestroideae.

The wealth of information available on the secondary metabolites produced by Solanaceae species may be used to elucidate taxonomic issues at the subspecies, species, or even genus level. Alkaloids and steroid derivatives (including steroidal alkaloids) are known to be the main secondary metabolite classes in the Solanaceae. Among the nitrogen-containing secondary metabolites, the tropane alkaloids (TAs) and calystegines (CAs) exhibit a pattern of distribution and frequency of occurrence that establish them as chemotaxonomic markers in the Solanaceae (Schimming et al. 1998; Hunziker 2001; Griffin and Lin 2000).

TAs are among the earliest active pharmaceutical ingredients used by man: the first scientific studies of a TA - namely atropine, isolated from *Atropa belladonna* L. - were published in 1809 (Eich 2008). The broad pharmacological effect profile of this class of compounds includes mydriatic, antiemetic, antispasmodic, and bronchodilator activity (Grynkiewicz and Gadzikowska 2008). The calystegines, in turn, were only discovered in the 1980s, when a group of French researchers isolated calystegine A_3, B_1, and B_2 from the roots of *Calystegia sepium* R.Br. (Convolvulaceae) (Tepfer et al. 1988). Research interest in the CAs is on the rise, particularly in view of their potential antiviral, anticancer, and antidiabetic effects (Drager 2004).

The TAs and CAs are ornithine-derived alkaloids and share the bicyclic tropane skeleton (8-methyl-8-azabicyclo[3.2.1]octane). The tropane ring consists of a pyrrolidine and a piperidine ring fused to form a bridged bicyclic structure. Hydroxyl substitution of tropane at the C3 position yields one of two stereoisomers - tropine or pseudotropine - depending on the orientation (α or β) of the hydroxyl group (Bacchi 2002). Tropane alkaloids with a 3α-hydroxyl substituent are divided into several different groups according to structural type (Eich, 2008), including esters of 3α-hydroxytropane with aliphatic acids, esters of 3α,6β- or 3α,7β-dihydroxytropane, esters of 3α-hydroxytropane with phenylpropanoid acids, and esters of 6β,7β-epoxy-3α-hydroxytropane with *S*-(–)-tropic acid. Conversely, the 3β-hydroxyl-substituted tropane alkaloids constitute a rather small group of compounds, including 3β-acetoxytropane and 3β-tigloyloxytropane. It bears stressing that the biosynthetic pathway that leads to the formation of these compounds also leads to the formation of CAs, which are nonesterified polyhydroxylated nortropane alkaloids whose structure consists solely of the tropane ring and a varying number of hydroxyl substituents (three, four, or five) (Drager 2004).

It is believed that elucidation of the pattern of TA and CA distribution in the Solanaceae may aid understanding of the subdivisions of this family and its geographic distribution patterns. Within this context, the present study sought to construct a database of the occurrences of TAs and CAs in Solanaceae species and as certain whether the patterns of distribution of these compounds corroborate the phylogenetic classification proposed by Olmstead et al. (2008) and are associated with geographic distribution. Furthermore, evolutionary inferences shall be made whenever possible.

Methods

Database

A database containing information on the occurrence of TAs and CAs in Solanaceae was constructed using the method proposed by Gottlieb et al. (1996) and updated by Sampaio-Santos et al. (2010). A wide-ranging review of the literature was carried out by means of a search of online databases (ISI Web of Science and Chemical Abstracts) and a hand search of relevant journals. The keywords "Solanaceae", "tropane alkaloids", and "calystegines" were used as search terms. The full text of all articles was analyzed, and the search was unfiltered by date of publication. The Lounasmaa and Tamminen (1993) review was used as a basis for pre-1992 research.

Occurrences were tabulated by structural class. TAs were defined as esters of 3α-hydroxytropane with aliphatic acids, esters of 3α,6β- or 3α,7β-dihydroxytropane, esters of 3α,6β- or 3α,7β-trihydroxytropane, esters of 3α-hydroxytropane with arylpropionic acids, esters of 6β,7β-epoxy-3α-hydroxytropane, esters of 3β-hydroxytropane, and their rare dimeric and trimeric forms. The CAs of interest were trihydroxynortropanes, tetrahydroxynortropanes and pentahydroxynortropanes.

Number of occurrences (NO) and diversity index (DI)

The number of occurrences (NO) and the diversity index (DI) were calculated as reported by Sampaio-Santos et al. (2010). The NO was defined as the sum of all TA and CA structural types found in each of the studied species. The number of occurrences is an indicator of the degree of importance of a certain category of metabolites within a given taxon (Gottlieb et al. 1996) thus providing a snapshot of the trend toward production of these compounds.

The DI, a rate that expresses the frequency of distribution of a biosynthetic class (Silva et al. 1988), was obtained by multiplying the NO by the number of structural types and dividing the result by the number of species studied. The NO and DI were calculated first for the sampled genera and then for the tribes as proposed in the Olmstead et al. (2008) classification, and, finally, plotted onto the Solanaceae phylogeny proposed by the same author. These indexes were also plotted onto the Solanaceae phylogeny proposed by the same author.

Geographic distribution

Data on the core center of diversity for TA- and/or CA-producing Solanaceae genera were obtained from Hunziker (2001). Genera were organized jointly by geographic distribution and division into subfamilies, according to the classification scheme proposed by Olmstead et al. (2008). Depending on their centers of diversity, genera were categorized as cosmopolitan, South American, North American, Eurasian, Asian, and Oceanic.

Statistical analysis

Cluster analysis and principal components analysis (PCA) were carried out in the MULTIV v.2.90b software environment (Pillar 2011). For cluster analysis, we assessed the dissimilarity between genera (sampling units) in terms of the most frequently occurring TAs and CAs (variables), using the Euclidean distance between sampling units and the minimum variance clustering method. Cluster robustness was then assessed by the bootstrap method (Pillar 1999). For principal components analysis (PCA), we used the correlation between variables to assess the relative contributions of TAs and CAs towards a definition of the genus clustering patterns in terms of the frequency of TA and CA occurrence.

Results and Discussion

Database, number of occurrences and diversity index

The occurrence of TAs and CAs in 29 genera of Solanaceae is shown in Table 1. The total number of occurrences was 996, 879 instances of TAs (88.3%) and 115 of CAs (11.7%). TAs were found in 24 genera, with *Anthocercis* Labill., *Brugmansia* Pers., *Datura* L., *Hyoscyamus* L., and *Solandra* Swartz accounting for 65.4% of occurrences. CAs were found in 14 genera, with *Hyoscyamus* L., *Lycium* L., *Solanum* L., *Physalis* L., and *Scopolia* Jacq. accounting for 62.6% of occurrences.

We identified occurrences of 107 compounds in the TA class. The esters of 3α,6β- or 3α,7β-dihydroxytropane (group TA2 in Table 1) were the most diverse group (29 compounds), whereas esters of 3α-hydroxytropane with phenylpropanoid acids (group TA4 in Table 1), comprising 16 different compounds, were the most commonly occurring group (261 occurrences). Compounds in this group, particularly hyoscyamine and atropine, were found in 70 species of 17 genera. Scopolamine, an ester of 6β,7β-epoxy-3α-hydroxytropane (group TA5 in Table 1), was also quite common, with 80 occurrences in 18 genera.

Overall, 17 CAs were identified: five trihydroxynortropanes, nine tetrahydroxynortropanes, and three pentahydroxynortropanes.

Table 1 shows a broad range of frequencies of occurrence of TAs and CAs among the various Solanaceae genera: some had extremely high frequencies of occurrence, such as *Datura* L., *Brugmansia* Pers., *Hyoscyamus* L., and *Anthocercis* Labill., with 246, 95, 91, and 84 occurrences of TAs respectively, whereas other genera had very low frequencies, such as *Nicandra* Adans. and *Capsicum* L. (with only two and three occurrences of CAs respectively).

It bears stressing that the widespread use and medicinal relevance of some species have prompted more in-depth studies of these species, which, in turn, leads to isolation and knowledge of a greater number of active metabolites. Examples include *Datura stramonium* L., which is one of the most widely studied plant species ever from a phytochemical standpoint (Berkov et al. 2005) and from which over 60 different TAs have been isolated (El Bazaoui et al. 2011). In an attempt to mitigate any discrepancies brought about by calculation of the number of occurrences alone, we calculated the diversity index of ornithine-derived alkaloids (TAs and CAs) (Table 1). This index expresses the frequency of occurrence of a biosynthetic class (Silva et al. 1988) and, unlike the number of occurrence, takes into account the number of species studied, thus somewhat obviating the bias caused by the lack of standardization of the frequency of occurrence. However, the DI must still be interpreted with caution, as it may reflect aspects other than chemical diversity proper. The genera of the tribe Datureae provide a good example of this phenomenon.

Datura L. and *Brugmansia* had the highest frequencies of occurrence of TAs: 246 and 95 respectively. However, despite a nearly threefold higher occurrence count for the *Datura* genus as compared with the *Brugmansia* genus, analysis of the chemical diversity index (DI) yielded nearly identical values: 144 and 143 for *Datura* and *Brugmansia* respectively. These values represent the highest diversity indices found in the present study. There is unquestionably a great diversity of TAs in these two genera. However, in this particular case, the DI also reflects the research effort put into these two genera, both of which are major sources of pharmacologically relevant active substances.

The DI of the subfamily Nicotianoideae, represented in this study by the tribe Anthocercideae, can also be called into question. Analysis of the frequency of TA occurrence shows a broad range of values: from eight occurrences of TAs each in the *Grammosolen* and *Symonanthus* genera to 84 occurrences in the *Anthocercis* genus (Table 1). However, analysis of the DI yielded values of 32 and 56 respectively, which demonstrates the importance of this index.

The diversity index of CAs observed in this tribe – 18 - also merit analysis. Although this value represents the DI for the tribe as a whole, it only concerns the genus *Duboisia*, the only one of the seven Anthocercideae genera in which CAs were detected.

Another important aspect that merits discussion is the trend toward correlation between TA and/or CA synthesis pathways and the centers of diversity of each genus. Overall, the broader the latitudinal range of a cluster (with South America as the starting point), the greater diversity of secondary metabolite classes. Therefore, South American genera preferably produce CAs or CAs and TAs. South American genera with a more restricted range, such as *Nicandra* and *Capsicum* produce CAs alone. *Brunfelsia*, a genus from South and Central America, also contain

CAs alone. Conversely, species of *Datura*, a genus from Central and North America, contain TAs and CAs alike.

Eurasian genera also exhibit production of TAs and CAs alike, just as *Datura,* whereas exclusively Asian genera product TAs alone, as do all Oceanic genera analyzed other than *Duboisia*.

The genera *Schizanthus* and *Withania* did not follow the trends toward TA and CA occurrence found in other genera with a similar geographic distribution. However, it should be noted that these two genera are distributed over more restricted ranges. *Schizanthus* is endemic to the Chile region, whereas *Withania* is an Old World genus, found mostly by the Mediterranean.

Based on our comparison of the patterns of occurrence of these ornithine-derived alkaloids and the phylogenetic scheme proposed by Olmstead et al. (2008), we may suggest that the TA synthesis pathway is more basal than the CA pathway, as the occurrence of tropane alkaloids has been reported from the most basal genus of the Solanaceae, *Schizanthus*, to the most derived Solanoideae genera. Conversely, in the Solanoideae family, and particularly in the Solaneae tribe, which is considered to be more derived, only calystegines were detected.

Chemical data can contribute to the elucidation of relationships among genera. Two examples were observed during the course of this study: *Nicandra* and *Latua*, both of which are monotypic genera with a highly restricted range. The former is native to South America, most specifically from the Peruvian Andes to Argentina, and the latter is endemic to Southern Chile.

In the phylogeny proposed by Olmstead et al. (Olmstead et al. 2008) (2008), *Nicandra* is placed into an as-yet unresolved branch, near genera that mainly produce TAs of different structural classes. However, as other South American genera, *Nicandra* has only been reported to contain CAs. According to D'Arcy (1979) the *Nicandra* genus is related to *Solanum* and *Physalis*, two genera remarkable for their frequency of CA occurrence and that are located on more derived branches of the Olmstead phylogeny.

According to Olmstead et al. (2008) the genus *Latua* was moved to the subfamily Solanoideae, more specifically to the Atropina clade, which is consistent with the chemical data reported for this genus. The earliest classifications proposed for the Solanaceae included *Latua* in the tribe Solaneae. The classification proposed by Tetényi (1987) postulated that this genus should be moved to the Jaboroseae tribe of the Solanoideae subfamily, due to the presence of tropane alkaloids, among other characteristics. In the Olmstead phylogeny, the genus *Jaborosa* lies close to *Latua*, supporting these previous findings.

Cluster analysis and principal components analysis

On qualitative analysis of the database, we noticed a clear trend toward correlation between the geographic distribution of genera and the joint distribution of TAs and CAs. To corroborate these potential patterns, we conducted multivariate analysis, that is, exploratory analysis that provide summary insight into the complexity of the observed information so as to enable visualization and ratification of suggested patterns (Valentin, 2000).

We also tested the significance of the patterns observed on multivariate analysis by means of the bootstrap method, which enables testing of group sharpness in cluster analysis and assessment of the significance of principal components in PCA (Pillar 1999).

Cluster analysis suggested the formation of two distinct groups of genera ($P_{(G^{\circ} \leq G^{*})} = 0.381$ – probability after bootstrap resampling with 10,000 iterations; (Pillar 1999)) in terms of the most frequently occurring TAs and CAs (Fig. 1).

Group A comprises the New World genera *Lycium*, *Solanum*, *Nicandra*, *Capsicum*, *Physalis*, and *Nierembergia* and the Eurasian genus *Withania*. All are in the subfamily Solanoideae, except for *Nierembergia,* which is in the subfamily Petunioideae. *Solanum* and *Lycium*, despite their cosmopolitan distribution, have America - particularly South America for the genus *Solanum* - as their center of diversity and the center of their range. The genera *Capsicum, Lycium, Nicandra* and *Solanum* have in common the exclusive presence of CAs, whereas species in the genera *Nierembergia, Physalis* and *Withania* also produce TAs of the type TA1 and TA6 (Table 1).

It bears stressing that CAs were isolated from species of the subfamilies Petunioideae, Nicotianoideae, and Solanoideae. Furthermore, the greatest frequency of occurrence was found in the more derived genera, which tend to specialize in production of these compounds.

Group B shows evidence of three distinct clusters: B1 comprises the Eurasian genera of the Solanoideae subfamily - *Scopolia, Mandragora, Hyoscyamus* and *Atropa* - and the Australian genus *Duboisia*, of the family Nicotianoideae. Cluster B2 comprises the Solanoideae genera *Solandra, Salpichroa* and *Latua* (South American), the genus *Atropanthe* (exclusively Asian), and the Australian genera *Anthotroche* and *Crenidium,* of the Nicotianoideae.

In cluster B3, *Schizanthus* (Schizanthoideae) lies relatively far from the remaining genera, probably due to the occurrence of dimeric and trimeric forms of the compounds of interest, which are exclusive to this genus; *Symonanthus* belongs to the subfamily Nicotianoideae, whereas *Datura* and *Brugmansia* belong to the Solanoideae. This cluster also includes the Australian genera of the Nicotianoideae subfamily, *Anthocercis*, *Physochlaina*, *Gramnosolen* and *Cyphanthera,* and the Asian genera of the Solanoideae, *Anisodus* and *Przewalskia*.

The use and interpretation of data on secondary metabolites to elucidate taxonomic issues is beset by two major challenges. The first is construction of a reliable database; the second is use of statistical methods that can prove and validate data (Alvarenga et al., 2001).

Indeed, statistical analysis has been used in previous chemotaxonomy studies as a valuable tool for analysis of the correlation between chemical and taxonomic data; such studies include

Depege et al. (2006), Alvarenga et al. (2001), Halinski et al. (2011), and Silva et al. (2011). In the present study, multivariate analysis at least partly elucidated correlations between the occurrence of secondary metabolites and taxonomic and phytogeographical aspects.

The diagram generated by PCA (Fig. 2) represents 57.8% of total variation and shows that the pattern of occurrence of two sharply distinct groups of genera (defined by the first principal component – axis 1) correlates highly with the presence of CAs (represented by number 8) ($r = -0.93$) (Group A) and esters of 6β,7β-epoxy-3α-hydroxytropane (represented by number 5) ($r = 0.73$) (Group B). Furthermore, within Group B, there was a clear trend toward the formation of two subgroups (defined by the second principal component – axis 2) with respect to the presence of 3α,6β,7β-trihydroxytropanes (represented by number 3) and esters of 3α-hydroxytropane with phenylpropanoid acids (represented by number 4), with $r=-0.56$ and $r = 0.56$ respectively.

Several authors have provided evidence of the importance of TAs as chemotaxonomic markers for the Solanaceae (Hunziker 2001; Griffin and Lin 2000), the Datureae tribe (Doncheva et al. 2006), the genus *Schizanthus* (Eich 2008), and the Convolvulaceae genus *Merremia* Dennst. ex Endl. (Jenett-Siems et al. 2005). Likewise, the CAs have been described as chemotaxonomic markers in the Convolvulaceae and Solanaceae (Schimming et al. 1998).

Analysis of the frequency of occurrence of TAs and CAs may ratify the hypothesis that these compounds are good chemotaxonomic markers for the Solanaceae. On the other hand, we could not ascertain the applicability of these markers for subdivisions of the family, as the groups defined by cluster analysis (Fig. 1) do not reflect any current taxonomical classification of the Solanaceae, including those proposed by Hunziker (2001) and Olmstead et al. (2008), although some trends toward distribution of these compounds can be observed, as noted in section 3.1.

In a 2003 study designed to reconstruct phylogenies and map and interpret the distribution of secondary metabolites, including TAs in the Solanaceae, Wink found that TAs are produced in a number of apparently unrelated taxa, that the occurrence of these compounds does not represent a consistent characteristic and that isolated use of this criterion might lead to misguided clustering. Wink argues that different biosynthetic pathways for these compounds evolved independently and that their occurrence in unrelated taxa may be regarded as a result of convergent evolution (Wink 2003).

In the present study, we sought to carry out a joint analysis of TAs and CAs to ascertain the extent to which these compounds contribute to the chemotaxonomy of the Solanaceae. We found that the CAs were significantly more relevant as a variable for characterization of groups of genera, as shown both by cluster analysis and principal components analysis (Fig. 1 and Fig. 2). This is consistent with the trend toward a chemical dichotomy observed during data analysis, and reinforces, to a certain extent, the association between geographic distribution and occurrence of chemical compounds, as CAs were found to occur only in genera whose center of diversity is South America.

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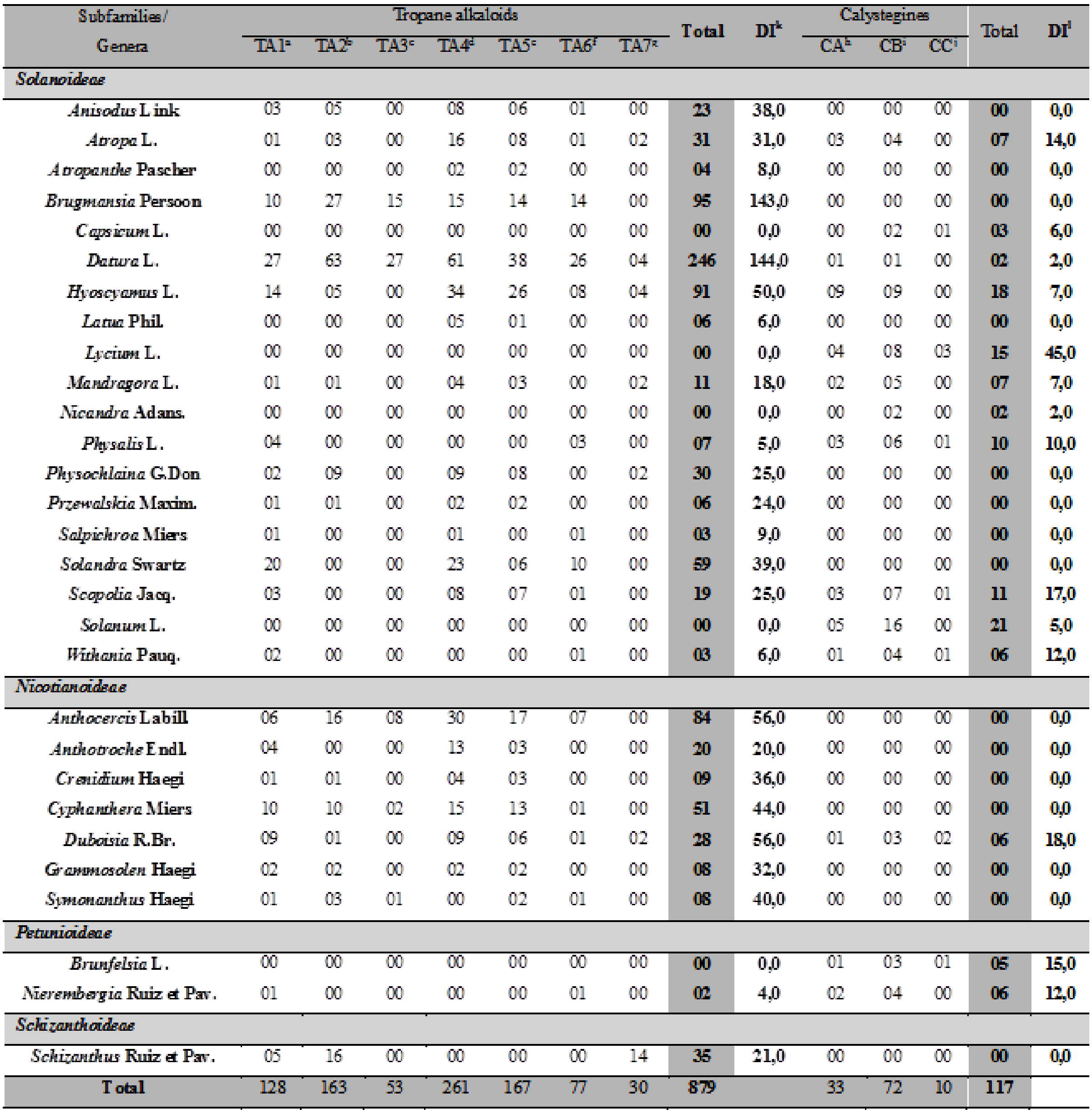
Table 1
Number of occurrence and diversity index (DI) of tropane alkaloids and calystegines of the Solanaceae subfamilies and genera (based on Olmstead et al., 2008), according to structural types.



Subfamilies/ Genera	Tropane alkaloids							Total	DI[k]	Calystegines			Total	DI[l]
	TA1[a]	TA2[b]	TA3[c]	TA4[d]	TA5[e]	TA6[f]	TA7[g]			CA[h]	CB[i]	CC[j]		
Solanoideae														
Anisodus Link	03	05	00	08	06	01	00	**23**	38,0	00	00	00	**00**	0,0
Atropa L.	01	03	00	16	08	01	02	**31**	31,0	03	04	00	**07**	14,0
Atropanthe Pascher	00	00	00	02	02	00	00	**04**	8,0	00	00	00	**00**	0,0
Brugmansia Persoon	10	27	15	15	14	14	00	**95**	143,0	00	00	00	**00**	0,0
Capsicum L.	00	00	00	00	00	00	00	**00**	0,0	00	02	01	**03**	6,0
Datura L.	27	63	27	61	38	26	04	**246**	144,0	01	01	00	**02**	2,0
Hyoscyamus L.	14	05	00	34	26	08	04	**91**	50,0	09	09	00	**18**	7,0
Latua Phil.	00	00	00	05	01	00	00	**06**	6,0	00	00	00	**00**	0,0
Lycium L.	00	00	00	00	00	00	00	**00**	0,0	04	08	03	**15**	45,0
Mandragora L.	01	01	00	04	03	00	02	**11**	18,0	02	05	00	**07**	7,0
Nicandra Adans.	00	00	00	00	00	00	00	**00**	0,0	00	02	00	**02**	2,0
Physalis L.	04	00	00	00	00	03	00	**07**	5,0	03	06	01	**10**	10,0
Physochlaina G.Don	02	09	00	09	08	00	02	**30**	25,0	00	00	00	**00**	0,0
Przewalskia Maxim.	01	01	00	02	02	00	00	**06**	24,0	00	00	00	**00**	0,0
Salpichroa Miers	01	00	00	01	00	01	00	**03**	9,0	00	00	00	**00**	0,0
Solandra Swartz	20	00	00	23	06	10	00	**59**	39,0	00	00	00	**00**	0,0
Scopolia Jacq.	03	00	00	08	07	01	00	**19**	25,0	03	07	01	**11**	17,0
Solanum L.	00	00	00	00	00	00	00	**00**	0,0	05	16	00	**21**	5,0
Withania Pauq.	02	00	00	00	00	01	00	**03**	6,0	01	04	01	**06**	12,0
Nicotianoideae														
Anthocercis Labill	06	16	08	30	17	07	00	**84**	56,0	00	00	00	**00**	0,0
Anthotroche Endl.	04	00	00	13	03	00	00	**20**	20,0	00	00	00	**00**	0,0
Crenidium Haegi	01	01	00	04	03	00	00	**09**	36,0	00	00	00	**00**	0,0
Cyphanthera Miers	10	10	02	15	13	01	00	**51**	44,0	00	00	00	**00**	0,0
Duboisia R.Br.	09	01	00	09	06	01	02	**28**	56,0	01	03	02	**06**	18,0
Grammosolen Haegi	02	02	00	02	02	00	00	**08**	32,0	00	00	00	**00**	0,0
Symonanthus Haegi	01	03	01	00	02	01	00	**08**	40,0	00	00	00	**00**	0,0
Petunioideae														
Brunfelsia L.	00	00	00	00	00	00	00	**00**	0,0	01	03	01	**05**	15,0
Nierembergia Ruiz et Pav.	01	00	00	00	00	01	00	**02**	4,0	02	04	00	**06**	12,0
Schizanthoideae														
Schizanthus Ruiz et Pav.	05	16	00	00	00	00	14	**35**	21,0	00	00	00	**00**	0,0
Total	128	163	53	261	167	77	30	879		33	72	10	117	

[a] Esters of 3α-hydroxytropane with aliphatic acids
[b] Esters of 3α, 6β- or 3α, 7β-dihydroxytropanes
[c] Esters de 3α, 6β, 7β-trihydroxytropanes
[d] Esters de 3α-hydroxytropane with phenylpropanoid acids
[e] Esters de 6β, 7β-epoxy-3α-hydroxytropane
[f] Esters de 3β-hydroxytropane
[g] Dimeric and trimeric forms
[h] Trihydroxynortropane
[i] Tetrahydroxynortropanes
[j] Pentahydroxynortropanes
[k] Diversity index of tropane alkaloids
[l] Diversity index of calystegines.

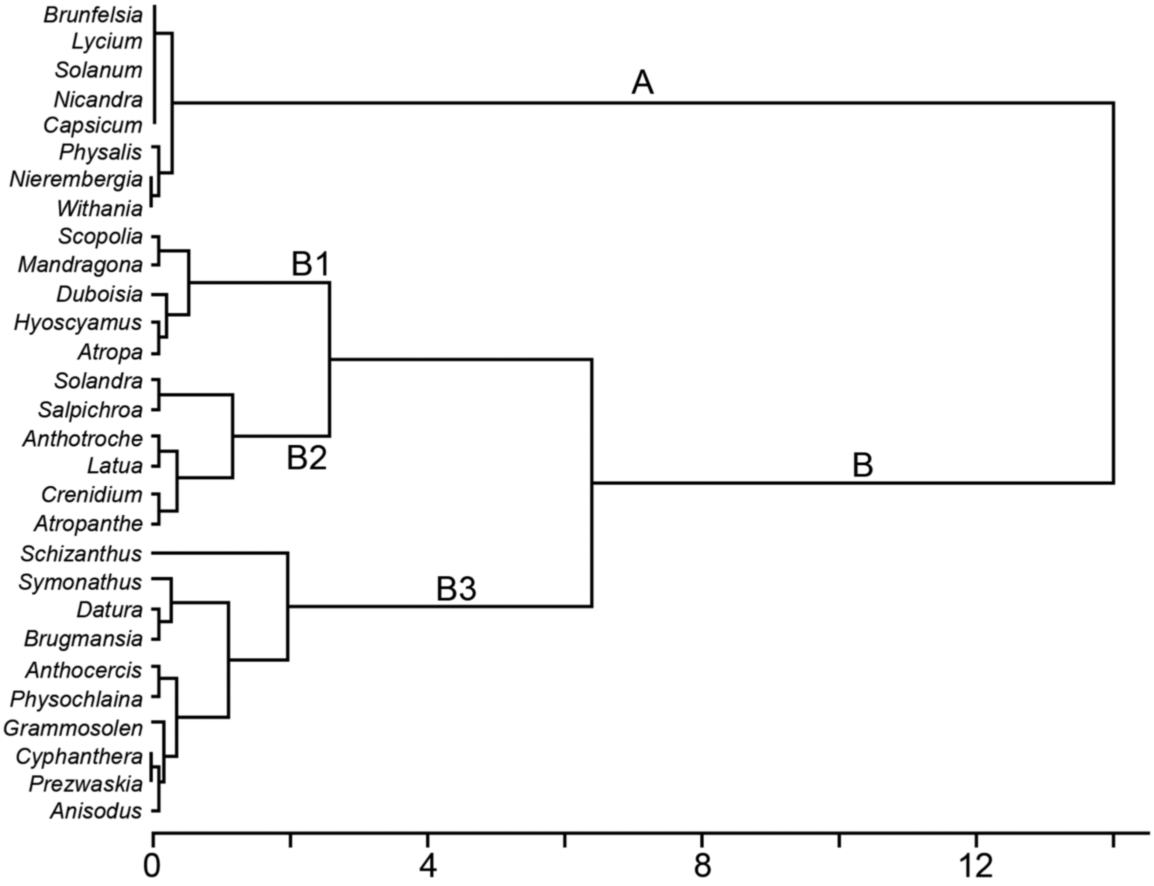


Fig. 1. Dendrogram generated by Cluster Analysis (Ward's method) from 29 species in relation to tropane alkaloids and calistegines, indicating the formation of two cluster (A and B) ($P_{(G^{\circ} \leq G^{*})}$ = 0.05 – bootstrap probability estimated by 10.000 replicates. The similarity measure used was Euclidean distance

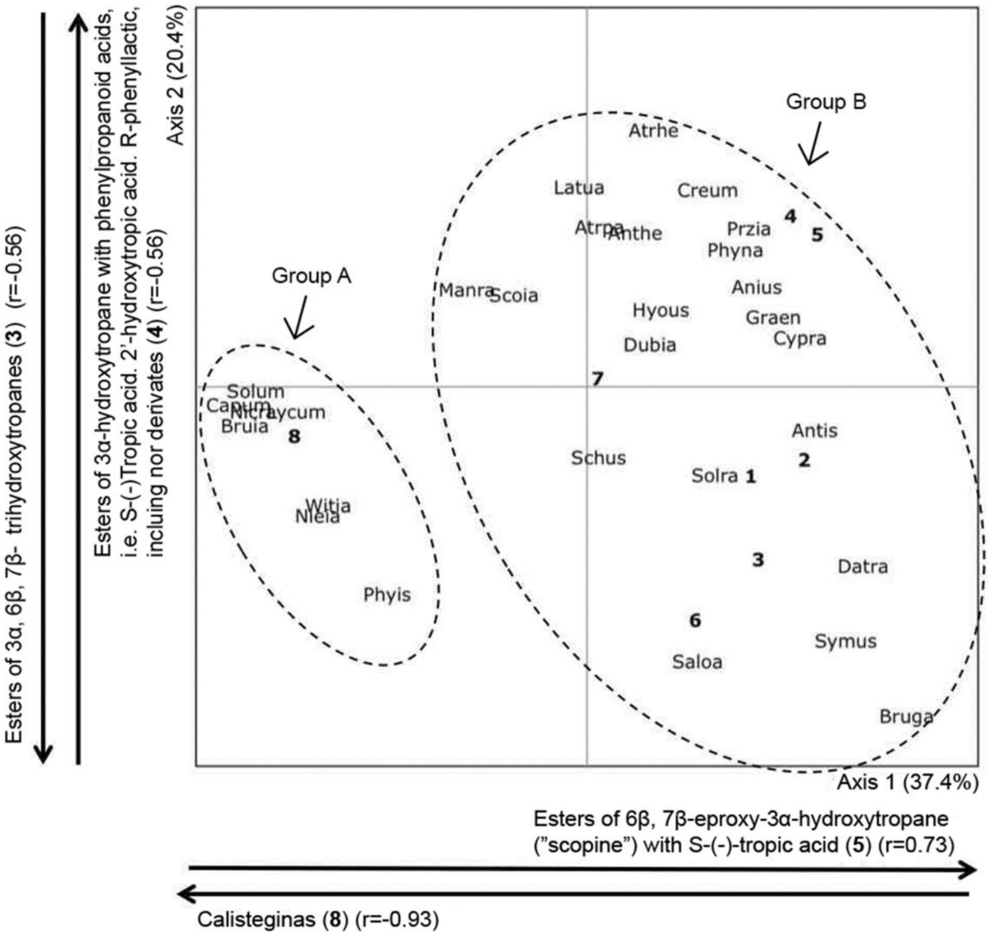


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Fig. 2. Ordination diagram of the tropane alkaloids and calystegines occurences of the Solanaceae genera. Principal coordinates analysis with Euclidean distance between sample units was used. 1 = Esters of 3α-hydroxytropane with aliphatic acids, 2 = Esters of 3α, 6β- or 3α, 7β-dihydroxytropanes, 3 = Esters de 3α, 6β, 7β-trihydroxytropanes, 4 = Esters de 3α-hydroxytropane with phenylpropanoid acids, 5 = Esters de 6β, 7β-epoxy-3α-hydroxytropane, 6 = Esters de 3β-hydroxytropane, 7=- Dimeric and trimeric forms, 8 = Calystegines

ARTIGO 3

CHEMOTAXONOMIC CHARACTERIZATION AND CHEMICAL SIMILARITY OF TRIBES/GENERA OF THE SOLANOIDEAE SUBFAMILY (SOLANACEAE) BASED ON OCCURRENCE OF WITHANOLIDES

A ser submetido para a *Biochemical Systematic and Ecology*

CHEMOTAXONOMIC CHARACTERIZATION AND CHEMICAL SIMILARITY OF TRIBES/GENERA OF THE SOLANOIDEAE SUBFAMILY (SOLANACEAE) BASED ON OCCURRENCE OF WITHANOLIDES

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ABSTRACT

Withanolides are well established as chemotaxonomic markers in the Solanaceae. A large number of withanolides have been isolated from the subfamily Solanoideae (Solanaceae). A database of the occurrences of withanolides in Solanoideae was compiled to help characterize the chemical profile and verify the chemical similarity of the tribes/genera of this subfamily. The study revealed the importance of this group of compounds as chemotaxonomic markers, especially for Physaleae, *Deprea*, and *Jaborosa*, ratifying the importance of chemotaxonomy as a tool for elucidation of taxonomic problems, such as undefined relationships of genera that are not yet included in tribes of Solanoideae.

Keywords: Solanaceae; chemotaxonomic markers; withanolides.

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1. Introduction

Withanolides are a group of natural compounds that may be defined as substituted steroidal lactones (Glotter, 1991). The core withanolide skeleton or backbone consists of an ergostane ring in which C22 and C26 or C23 and C26 may be oxidized to form a δ- or ϒ-lactone or lactol. Depending on the side-chain substituents at C17, withanolides may be subdivided into two classes, Type A and Type B. The difference between these types is the position of the side-chain lactone or lactol: δ-lactones or δ-lactols constitute Type A, whereas a ϒ-lactone or ϒ-lactol constitutes Type B (Figure 1) (Chen et al., 2011).

Type A withanolides may be further subdivided into two subgroups: those with an unmodified backbone (Group I) and those with modifications to the withanolide skeleton (Group II). Group I withanolides are most common, and include those with a C5–C6 epoxide group, whether in beta orientation (5β,6β-epoxides) or alpha orientation (5α,6α-epoxides); those with a C6–C7 epoxide, also in beta (6β,7β-epoxides) or alpha orientation (6α,7α-epoxides); those with a C5 double bond (5-ene withanolides); and so-called intermediate withanolides (Figure 2). Group II comprises the physalins, neophysalins, withaphysalins, acnistins/epiacnistins, withajardins, withametelins, norbornane-type withanolides, sativolides, spiranoid-δ-lactones, 14α,20α-epoxides, subtriflora-δ-lactones, withanolides with an aromatic D ring, and withanolides with an aromatic A ring. Type B withanolides include the ixocarpalactones, spiranoid-ϒ-lactones, trechonolides, subtriflora-ϒ-lactones, and perulactones (Anjaneyulu et al., 1997; Chen et al., 2011) Some withanolides have structural characteristics not readily classifiable into any of the above types; these have been grouped into an umbrella “other” classification (Figure 2) (Chen et al., 2011).

A characteristic common to all withanolides is oxidation at C1, C22, and C26. In addition to oxidative capacity, plants that synthesize withanolides have an extraordinary ability to produce hydrogenated functional groups—e.g. hydroxyl, epoxide, and acetoxy—at several positions, both on the carbon skeleton and on the side chain (Anjaneyulu et al., 1997)

Since their discovery, withanolides have been a subject of research interest in view of their potential medical uses, which include antitumor, trypanocidal, leishmanicidal, antimicrobial, anti-inflammatory, and immunoregulatory activities; neuritic regeneration and synaptic reconstruction; and cholinesterase inhibition, as well as effects of environmental interest, such as antifeedant, insecticidal, and phytotoxic activity (Chen et al., 2011). Species in the genera *Withania* Pauq. and *Physalis* L. have seemed particularly promising in natural product studies as antitumor and cancer chemopreventive agents.

The first withanolide reported in the literature, withaferin A, was discovered in 1962 in the leaves of *Withania somnifera* (L.) Dunal (Yarden and Lavie, 1962). In 1969, withaferin A was isolated from the leaves of *Acnistus arborescens* (L.) Schltdl (Kupchan et al., 1969). Both species are in the Solanaceae, from which the vast majority of withanolides have been isolated to date, making this class of natural products of particular importance to the nightshade family.

Withanolides have been recorded in 22 genera of Solanaceae: *Acnistus* Schott*, Brachistus* Miers*, Browallia* L.*, Datura* L.*, Deprea* Raf.*, Discopodium* Hochst.*, Dunalia* Kunth*, Eriolarynx* (Hunz.) Hunz.*, Exodeconus* Raf.*, Hyoscyamus* L.*, Iochroma* Benth.*, Jaborosa* Juss.*, Leucophysalis* Rydberg*, Lycium* L.*, Nicandra* Adans.*, Physalis* L.*, Salpichroa* Miers*, Solanum* L.*, Tubocapsicum* (Wettst.) Makino*, Vassobia* Rusby*, Withania* Pauq., and *Witheringia* L'Heritier. In 2010, Suleiman *et al*. (Suleiman et al., 2010) and Almeida-Lafetá *et al*. (Almeida-Lafetá et al., 2010) isolated withanolides from *Mandragora* L. and *Aureliana* Sendtn. respectively. Furthermore, withanolides have also been isolated from Lamiaceae (Khan et al., 1999), Taccaceae (Yokosuka et al., 2002), Myrtaceae (Vankar et al., 2009), Fabaceae (Chen et al., 2011), and Dioscoreaceae (Kim et al., 2011) species. In the Solanaceae, occurrence of withanolides is essentially confined to the subfamily Solanoideae. The sole exception recorded to date is the genus *Browallia*, of subfamily Cestroideae.

The subfamily Solanoideae is characterized by the presence of fleshy fruits (Knapp, 2002) and flattened seeds with curved embryos—characteristics established as derived traits in the Solanaceae. From a phytochemical standpoint, withanolides and alkaloids—particularly tropane, steroidal, indole, pyrrolidine, and imidazole alkaloids—are the most frequently occurring metabolites (Hunziker, 2001). Although the current molecular-based classification corroborates placement of the Solanoideae as the most derived subfamily and its circumscription, the phylogenetic relationships of some genera are still uncertain. In the phylogenetic classification proposed by Olmstead *et al*. (Olmstead et al., 2008), the subfamily Solanoideae is divided into seven well-circumscribed tribes and 11 genera not covered by these tribes. Nevertheless, the current classification proposes a subdivision of the Solanoideae into four clades with well-defined phylogenetic relationships: Atropina, which comprises the tribes Hyoscyameae and Lycieae and the genera *Jaborosa* Juss., *Latua* Phil., *Nolana*, and *Sclerophylax*; Juanulloeae, which comprises the tribes *Solaneae*, *Capsiceae*, *Physaleae*, and *Datureae*; and Salpichroina, which comprises the genera *Salpichroa* and *Nectouxia* Kunth.

In the present study, a database of withanolides occurrences in the subfamily Solanoideae was constructed so as to characterize the chemical profile of the tribes and genera in this subfamily and assess their chemical similarities as indicated by the occurrence of withanolide compounds. The study hypothesis assumes that withanolides can serve as chemotaxonomic markers in the Solanaceae, particularly in the subfamily Solanoideae, and will corroborate the currently proposed classification for this botanical family.

2. Material and methods

2.1 Database

A database of withanolides occurrences in the Solanoideae (Solanaceae) was constructed using the method proposed by Gottlieb et al. (Gottlieb et al., 1996) and updated by Sampaio-Santos *et al*. (Sampaio-Santos et al., 2010). A wide-ranging review of the literature was carried out by means of a search of online databases (ISI Web of Science and Chemical Abstracts) and a hand search of relevant journals. The keywords “Solanaceae”, “Solanoideae” and “withanolides” were used as search terms. The full text of all articles was analyzed and the search was restricted to post-1960 publications.

Search results were entered into a Microsoft Excel® spreadsheet, where rows indicated species cited in the literature and columns indicated which withanolides have been isolated from each species. Data were tabulated and analyzed by structural class (Figure 2).

2.2 Absolute and relative frequency of occurrences

The absolute frequency of occurrences (NO) was calculated as described by Sampaio-Santos *et al*. (Sampaio-Santos et al., 2010)), and the relative frequency of occurrences (NO%) was obtained by multiplication of the number of occurrences of each structural class by 100 and division of the product by the total number of occurrences.

2.3 Chemical similarity

For analysis of similarity, we constructed a data matrix with information on all occurrences of withanolides, tabulated by genus/tribe and structural type. The NO was converted to NO% and the data matrix analyzed in the PAST® software environment. Quantitative data were chosen as inputs for analysis, and Dice's coefficient was used to group tribes/genera by chemical similarity. Clustering was conducted using the unweighted pair group method with arithmetic averaging (UPGMA).

3. Results

Overall, 800 occurrences of withanolides were recorded in 24 genera of Solanoideae. Type A withanolides were most common (NO=718; Group I=554, Group II=164). Type B withanolides were reported 70 times, and other (ungrouped) withanolides, only 12 times. Figure 3 provides data on the relative frequency of occurrence of withanolides, and Table 1 has occurrences stratified by structural type and by genus, classified according to the current classification scheme.

The chemical profile of the tribes Datureae, Hyoscyameae, Lycieae, and Solaneae was characterized by the presence of Type A withanolides. Both Group I and Group II withanolides were recorded in the Datureae, whereas only Group II withanolides occurred in the three remaining tribes. “Other” withanolides were also found in species of the Solaneae tribe. In the Datureae, withanolides were found in five species of the genus *Datura* (NO=75). Group I withanolides of the 5-ene and 6α,7α-epoxide classes were the most common, accounting for 22 and 19 occurrences respectively. The sole representatives of Group II were the withametelins (NO=18), with 12 occurrences in *Datura metel* L. and seven occurrences in *D. inoxia* Mill.. Only one species in the tribe Hyoscyameae had reported withanolides occurrences, *Hyoscyamus niger* L., from which three 6α,7α-epoxide withanolides have been isolated.

Lycium barbarum L. and *L. chinense* Mill. Were the only two species in the genus *Lycium* (tribe Lycieae) reported to contain withanolides, which, as in the genus *Hyoscyamus*, were of the 6α,7α-epoxide class (NO=2). In the Solaneae, 12 occurrences of 5-ene withanolides, one of intermediate withanolides, and four of other, ungrouped withanolides were reported, all in the genus *Solanum*.

The chemical profile of the Physaleae (Table 1) was characterized by the occurrence of Type A withanolides (Groups I and II), Type B withanolides, and other substances. Withanolides were most common in the tribe Physaleae (NO=582), specifically in the genera *Withania* and *Physalis* (NO=236 and 203 respectively), which are, indeed, the most prominent sources of this group of metabolites. Only Type A withanolides were reported in the genera *Acnistus*, *Aureliana*, *Brachistus*, *Discopodium*, *Dunalia*, *Eriolarynx*, *Iochroma*, *Larnax*, *Leucophysalis*, *Vassobia*, and *Witheringia*.

Six different structural classes of withanolides have been reported in the genus *Withania* (NO=219): 5β,6β-epoxides (NO=53); 5-ene withanolides (NO=84); 6α,7α-epoxides (NO=48); intermediate withanolides (NO=27); 5α,6α-epoxides (NO=4); and 6β,7β-epoxides (NO=3). The

5β,6β-epoxides, 5-ene withanolides, and 6α,7α-epoxides accounted for 78% of all occurrences (NO=185), comprising at least 150 different compounds. These findings corroborate the current literature, in which the genus *Withania*, and the species *W. somnifera* in particular, are reported as one of the leading sources of withanolides. In 2012, Zhang et al. estimated that over 130 Type A withanolides, including several functional groups, had been isolated from different parts of this species (whole plant, aerial parts, flowers, fruit, leaves, roots, stem), and that it contains the largest number of these substances recorded to date (Zhang et al., 2012).

Withania somnifera is a highly valuable species in traditional Indian medicine, and it has been ascribed antimicrobial, antitumor, radiosensitizing, antioxidant, anti-stress, and immunomodulatory properties. These therapeutic effects are largely attributable to the presence of withanolides (Budhiraja et al., 2000; Prakash et al., 2001). Regarding Group II withanolides, 12 occurrences of withametelins have been reported in the species.

The genus *Physalis* accounts not only for a large portion of occurrences, but also for the greatest diversity of structural classes of withanolides (Zhang et al., 2012). Group I withanolides isolated from *Physalis* included 5β,6β-epoxides (NO=61), 5-ene withanolides (NO=26), 6β,7β-epoxides (NO=3), intermediate withanolides (NO=35), and 5α,6α-epoxides (NO=1). Group II withanolides included physalins (NO=23), neophysalins (NO=7), withaphysalins (NO=13), 14α,20α-epoxides (NO=2), and subtriflora-δ-lactones (NO=5). Type B withanolides included ixocarpalactones (NO=9), trechonolides (NO=1), subtriflora-ϒ-lactones (NO=5), and perulactones (NO=23). Furthermore, three occurrences of other types of withanolides not grouped elsewhere were reported.

Physalis species are widely used as medicinal plants. The sap of *P. angulata* L. is used in folk medicine as a sedative, depurative, and in rheumatic conditions, and *P. pubescens* L. is regarded as a stimulant and indicated in the treatment of cystitis and otitis, for instance (Mors et al., 2000). Recent studies suggest that *Physalis* species may have antitumor or cancer chemopreventive effects, and these effects are attributed to the presence of withanolides (Hseu et al., 2011; Ma et al., 2007; Maldonado et al., 2011; Su et al., 2002).

Only Group I withanolides have been found in the genera *Aureliana*, *Iochroma*, *Larnax*, and *Vassobia*. In *Aureliana*, *Iochroma*, and *Vassobia*, these comprised 5β,6β-epoxides and 5-ene withanolides. *Vassobia* has also been reported to produce intermediate withanolides. The genus *Larnax* has only two reported occurrences of withanolides, both 6α,7α-epoxides. Physalins (Group II) were the only withanolides isolated from the genera *Brachistus*, *Leucophysalis*, and *Witheringia*. *Acnistus*, *Discopodium*, *Dunalia*, *Eriolarynx*, and *Tubocapsicum* have all had Group I and II withanolides occurrences reported, mainly 5β,6β-epoxides (Group II). Acnistins/epiacnistins (Group II) have also been reported in all of the above genera except *Eriolarynx*. *Acnistus* has been reported to contain 5-ene withanolides (Group I) and withaphysalins (Group II). The latter structural class has also been described in *Eriolarynx*. *Discopodium* has been reported to contain 6α,7α-epoxides (Group I), 5-ene withanolides have been isolated from *Dunalia*, and intermediate withanolides (Group I) and withajardins (Group II) have been recorded in *Tubocapsicum*.

As noted in the Introduction, within the current classification of the Solanaceae, some genera of the subfamily Solanoideae are not grouped into any tribes because their phylogenetic relationships are unclear . Six of these genera have been reported to contain withanolides. The structural class of 6α,7α-epoxides (Type A, Group I) has been recorded in *Exodeconus*, *Mandragora*, and *Nicandra* (NO=6, 5, and 14 respectively). *Deprea* and *Jaborosa* have been reported to contain Type A (Groups I and II) and Type B withanolides. In both genera, Group I withanolides included 5β,6β-epoxides and 5-ene withanolides. Conversely, the distribution of Group II withanolides was highly distinctive in each of the two genera: *Deprea* has been found to contain withajardins and subtriflora-ϒ-lactones, whereas *Jaborosa* has been reported to contain norbornane-type withanolides, sativolides, spiranoid-δ-lactones, and aromatic withanolides. As for Type B withanolides, *Deprea* has been found to contain subtriflora-ϒ-lactones, and *Jaborosa*, spiranoid-δ-lactones and trechonolides. The high absolute frequency of occurrences in *Jaborosa* (NO=69) bears stressing. The genus *Salpichroa* has been found to contain Type A (Group I) and

Type B withanolides. Group I withanolides were represented by the 5α,6α-epoxides (NO=2), and Type B, by the aromatic withanolides (NO=6).

4. Discussion

The withanolides are a peculiar group of chemical compounds. From a chemical standpoint, they are highly diverse substances, comprising a variety of structural types and functional groups, which account for their wide range of biological activities. From a taxonomic standpoint, they are particularly characteristic of the Solanoideae subfamily, in view of their frequency of occurrence in this taxon. The frequency of occurrence is a marker of the relevance of a given category of metabolites to the taxon under study,[15] and can thus be used to glean information on trends in the production of the class of compounds. It is also an important statistic for the definition of chemotaxonomic markers, i.e. small molecules—usually secondary metabolites—that are indicative of differences among individuals at a certain taxonomic level. Therefore, metabolites that are typical of a group of plants can be used in the chemotaxonomic characterization thereof.

The distribution of withanolides within the Solanoideae is quite heterogeneous. While some structural types (such as Group I, Type A) are prevalent across several genera, others are highly restricted, sometimes to a single genus. By way of example, 5β,6β-epoxides (Type A, Group I) have been reported in 13 of the 24 genera covered in this study (54%); 5-ene withanolides (Type A, Group I) in 11 genera (46%); and 6α,7α-epoxides (Type A, Group I), in 10 genera (41%). Conversely, norbornane-type withanolides, sativolides, and spiranoid-δ-lactones have only ever been isolated in the genus *Jaborosa*. Furthermore, some genera may share the same structural classes, but individual compounds often differ. The withaphysalins found in *Acnistus,* for instance, differ from those found in *Physalis*.

This study sought out to analyze the chemical similarity among Solanoideae tribes and genera. A similarity dendrogram constructed from analysis of Dice's coefficient yielded four clusters, with a chemical similarity cutoff of 70% (Figure 4).

The tribe Datureae and the genus *Salpichroa* (Figure 4, branch 1) had 100% chemical similarity. According to molecular studies conducted by Olmstead *et al*.,[14] the genus *Salpichroa* is not currently included in any tribe, but it is closely related to genera of the tribe Datureae—so much so that they occupy the same clade, which also includes the tribe Physaleae. This tribe, alongside the genera *Deprea* and *Jaborosa*, constituted the second cluster (Figure 4, branch 2). The tribe Physaleae shared over 80% chemical similarity with the aforementioned genera, which, in turn, had 100% chemical similarity between them. The genus *Deprea* is related to the genus *Larnax,* which is currently included in the tribe Physaleae. Morphological evidence suggests that *Deprea* and *Larnax* are closely related to the genus *Physalis*; all three share a woody habit, for instance (Estrada and Martinez, 1999) However, another study (Whitson and Manos, 2005) proposed that *Deprea* might be related to *Witheringia* and *Larnax* as a sister genus to most of the Physaleae. Olmstead *et al*.(Olmstead et al., 2008) admit that further molecular studies are required to elucidate these relationships. This limitation notwithstanding, our chemical similarity study found *Deprea* to be related to the Physaleae, corroborating the coherence in formation of branch 2 of the similarity dendrogram (Figure 4). *Jaborosa* is a South American genus that, according to Hunziker (Hunziker, 2001), is closely related to *Salpichroa* and *Nectouxia* (not sampled in this study) and less closely related to *Deprea*. In the phylogeny proposed by Olmstead et al. (Olmstead et al., 2008), this genus is basal among the Solanoides, closely related to the tribes Lycieae and Hyoscyameae to constitute the Atropina clade. Our chemical similarity study revealed a close relationship between *Jaborosa* and the tribe Physaleae and genus *Deprea* (branch 2), contradicting the findings of previous molecular studies.

The third and fourth clusters were dissimilar from the other clusters and from each other. The third cluster comprised the tribes Hyoscyameae and Lycieae and the genera *Exodeconus*, *Mandragora*, and *Nicandra,* all of which had 100% chemical similarity among one another. The fourth group comprised the tribe Solaneae.

In the current classification proposed for the Solanaceae, the tribes Hyoscyameae and Lycieae are part of the clade Atropina. Within this clade, the Hyoscyameae are characterized by a very strong presence of tropane alkaloids; indeed, some genera of this tribe, such as *Hyoscyamus*, are major sources of these compounds. There is no record of tropane alkaloids occurring in the Lycieae (and the genus *Lycium* in particular), although they have been reported to contain calystegines, compounds that contain a tropane ring, share a common biosynthetic pathway with the tropane alkaloids and are thus closely related. Tétényi grouped *Mandragora* with the genus *Atropa,* in the Hyoscyameae (Tétényi, 1987). According to Hunziker (Hunziker, 2001), this genus is closely related to *Nicandra*, which, in our analysis of chemical similarity, was also placed in cluster 3. However, these relationships were not corroborated by the molecular studies used to construct the current phylogeny of the Solanaceae. Nevertheless, it bears noting that, from a chemical standpoint, the tribes and genera in this cluster (Figure 4, branch 3) are characterized by the presence of tropane alkaloids and share the occurrence of a select few withanolides (Figure 4). Likewise, few withanolides have been reported to occur in the Solaneae. This tribe, and the genus *Solanum* in particular (covered by this study), is characterized by the occurrence of steroidal alkaloids.

In addition to highlighting similarity-based clusters, the dendrogram (Figure 4) also shows the relative frequency of occurrence of withanolides in each of these clusters. Bearing in mind that frequency of occurrence is a marker of the relevance of a given category of metabolites to the taxon under study (Gottlieb et al., 1996) and thus provides information on the production of these metabolites, we may infer that the withanolides are important chemotaxonomic markers, particularly for the tribe Physaleae and the genera *Deprea* and *Jaborosa*. The remaining clusters were characterized by a relatively lower frequency of withanolides occurrence. Branches 1 and 3 are characterized by the presence of tropane alkaloids, whereas steroidal alkaloids are of greater relevance in branch 4. This shows a chemical divergence in the production of metabolites whose biosynthesis follows different routes, as withanolides are produced via the mevalonate pathway, while the tropane alkaloids are produced via the citric acid pathway.

5. Conclusions

This study set out to outline the chemical profile of the tribes and genera of the Solanoideae (Solanaceae) and analyze their similarity on the basis of withanolide occurrence. We found that the various tribes and genera of the Solanoideae subfamily are quite heterogeneous in their production of the different structural types and groups of withanolides, as well as in the occurrence of certain structural classes, such as the sativolides and norbornane-type withanolides, which are exclusive to certain genera. We also found that the tribes Datureae and Physaleae and the genera *Salpichroa*, *Deprea*, and *Jaborosa* were chemically similar, as were the tribe Physaleae and the genera *Deprea* and *Jaborosa*. Overall, analysis of chemical similarity corroborated the current classification of the Solanaceae, as exemplified by the similarity between the tribed Hyoscyameae and Lycieae, between the tribe Datureae and the genus *Salpichroa,* and between the tribe Physaleae and the genus *Deprea*.

These findings suggest a chemical divergence in relation to the production of secondary metabolites by different biosynthetic pathways. Therefore, we believe that studies focusing on joint analysis of different classes of compounds produced by different metabolic pathways—such as tropane alkaloids and withanolides—are warranted.

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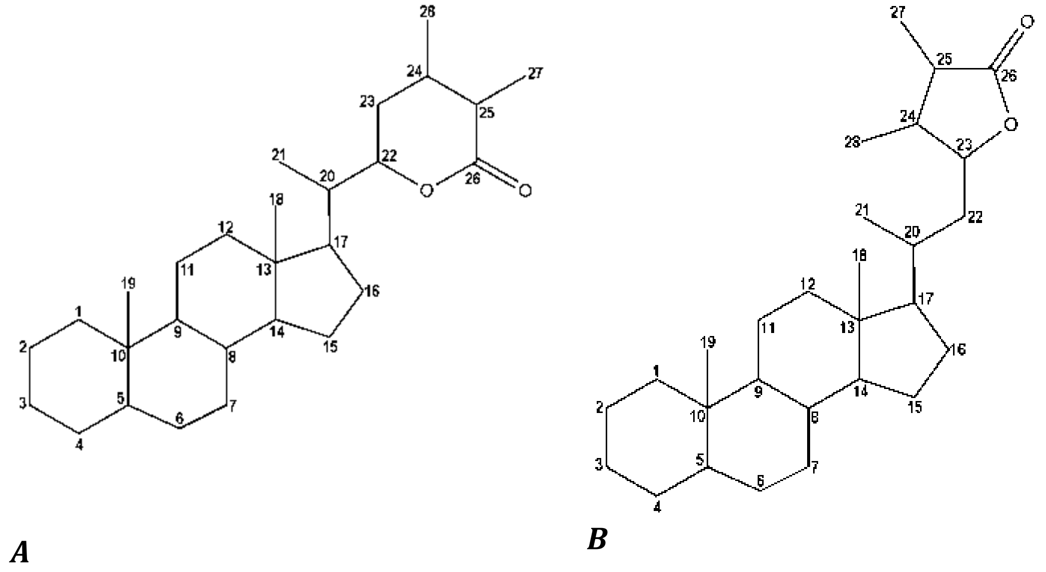
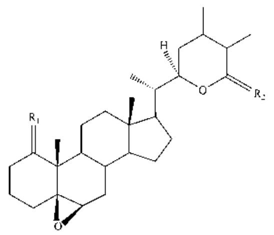


Figure 1. Core structure (backbone) of the withanolides. Type A withanolides (A). Type B withanolides (B).

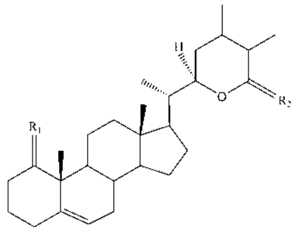
Classification of withanolides by structural type

Type A – Withanolides containing a δ-lactone or δ-lactol side chain

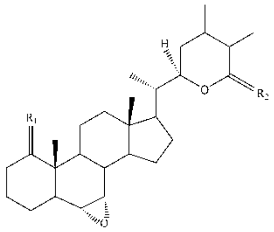
Group I – Withanolides with unmodified backbone



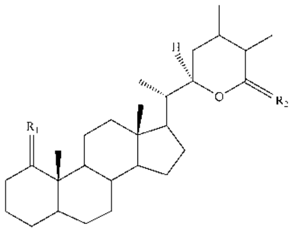
5β,6β-epoxides



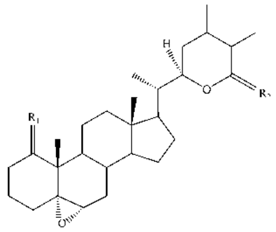
5-ene withanolides



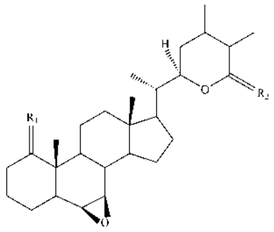
6α,7α-epoxide



Intermediate withanolides

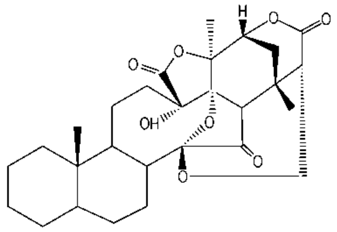


5α,6α-epoxides

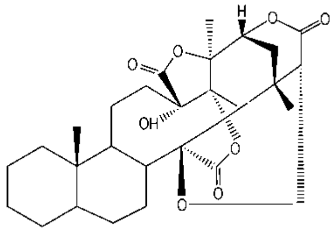


6β,7β-epoxides

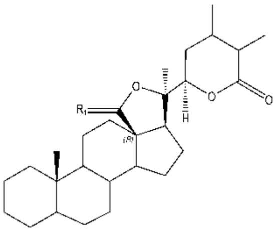
Group II – Withanolides with a modified backbone



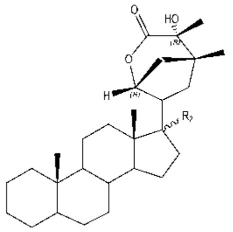
Physalins



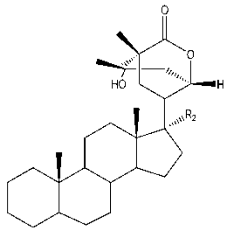
Neophysalins



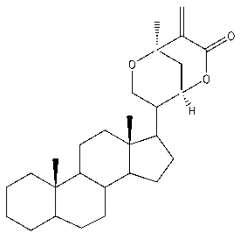
Withaphysalins



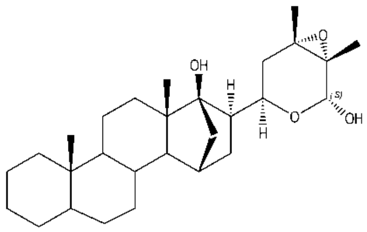
Acnistins/epiacnistins



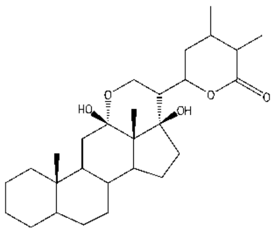
Withajardins



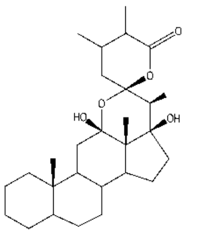
Withametelins



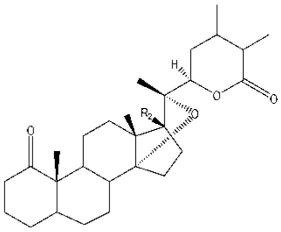
Norbornane-type withanolides



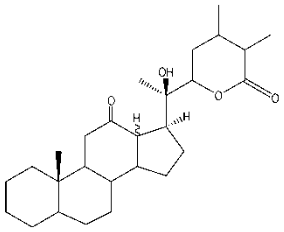
Sativolides



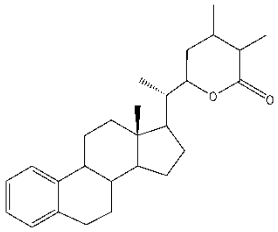
Spiranoid-δ-lactones



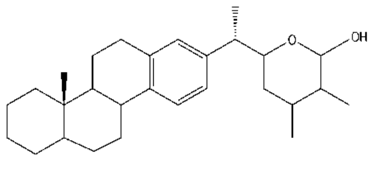
14α,20α-epoxide



Subtriflore-δ-lactones

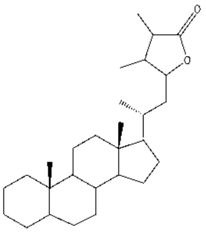


Ring-A aromatic withanolides

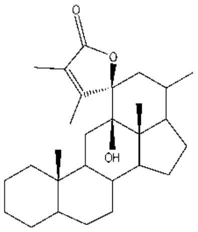


Ring-D aromatic withanolides

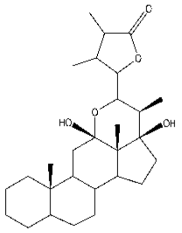
Type B – Withanolides with a ϒ-lactone or ϒ-lactol side chain



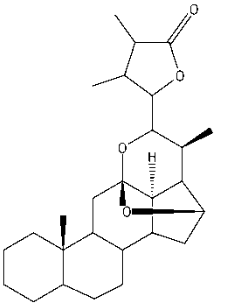
Ixocarpalactones



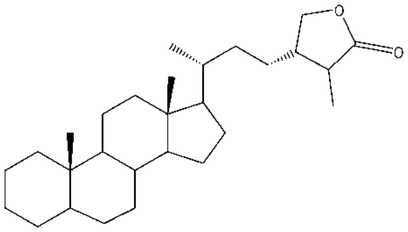
Spiranoid-ϒ-lactones



Trechonolides

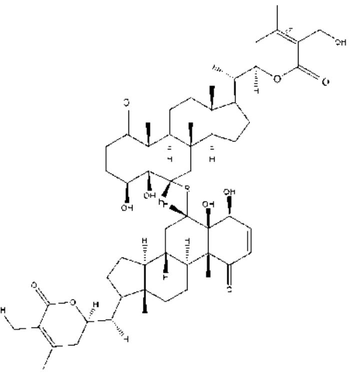


Subtriflora-ϒ-lactones



Perulactones

Other types of withanolides



Ashawagandhanolide

Figure 2. Classification of withanolides according to Chen et al. (2011)

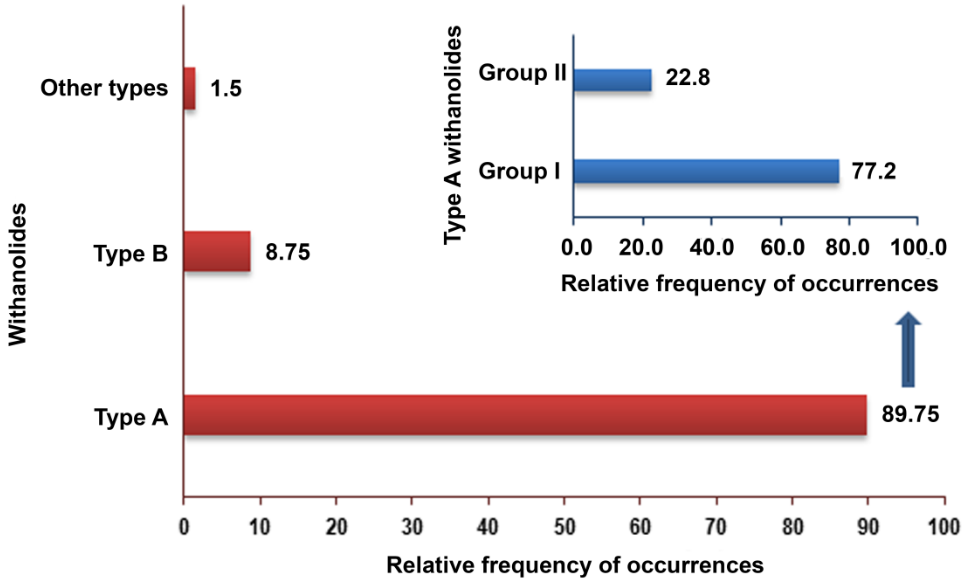


Figure 3. Relative frequency of occurrences of Type A, Type B, and other withanolides. Inset: relative frequency of occurrences of Group I and II Type A withanolides in the Solanoideae.

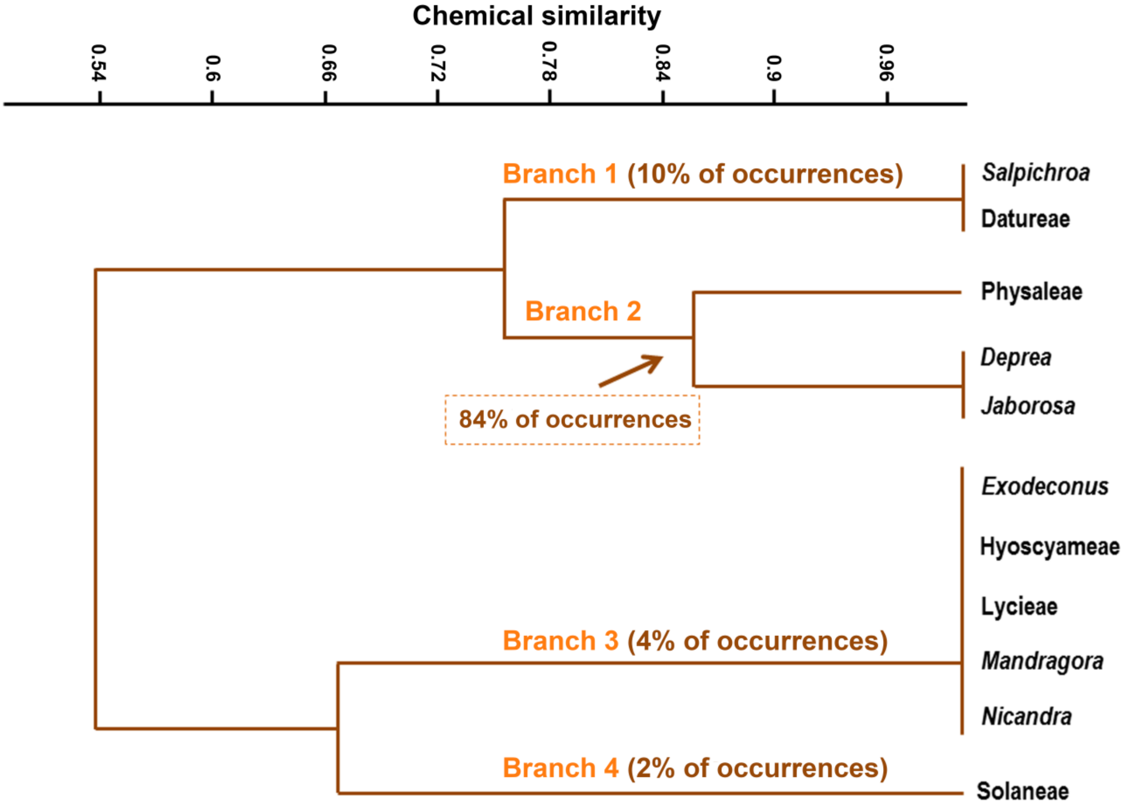


Figure 4. Dendrogram constructed from analysis of Dice's coefficient for comparison of chemical similarity among tribes/genera in the subfamily Solanoideae (Solanaceae). The relative frequency of occurrence of each type of withanolide in each of the established groups is shown in parentheses.

Table 1. Frequency of occurrence of withanolides by type (A, B, or other), group (I or II), and structural class (denoted by letters AX) in tribes/genera of the subfamily Solanoideae (Solanaceae), according to the classification proposed by Olmstead et al. (2008)

Tribes/Genera	Type A																		Type B					Other types	Total
	Group I						Group II																		
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	X		
Datureae																									
Datura	4	22	19	11	1							18													75
Hyoscyameae																									
Hyoscyamus			3																						3
Lycieae																									
Lycium			2																						2
Physaleae																									
Acnistus	10	1							4	3															18
Aureliana	10	1																							2
Brachistus							3																		3
Discopodium	4		6							1															11
Dunalia	6	3								8															17
Eriolarynx	1								13																14
Iochroma	23	8																							31
Larnax			2																						2
Leucophysalis							3																		3
Physalis	61	26	3	35	1		23	7	13							2	5		9		1	5	9	3	203
Tubocapsicum	7			3						9	3														22
Vassobia	12	1		6																					19
Withania	53	84	48	27	4	3						12													236
Witheringia							1																		1
Solaneae																									
Solanum		12		1																				4	17
Genera																									
Deprea	1	1									5						5		2			5			19
Exodeconus			6																						6

A=5β,6βepoxides; B=5ene withanolides; C=6α,7αepoxides; D=intermediate withanolides; E=5α,6αepoxides; F=6β,7βepoxides; G=physalins; H=neophysalins; I=withaphysalins; J=acnistins/epiacnistins; K=withajardins; L=withametelins; M= norbornanetype withanolides; N=sativolides; O=spiranoidδlactones; P=14α,20αepoxide withanolides; Q=subtrifloraδlactones; R=aromatic withanolides; S=ixocarpalactones; T=spiranoidϒlactonre; U=trechonolides; V=subtrifloraϒlactones; X=perulactones.

CONSIDERAÇÕES FINAIS

O presente estudo confirma o status dos alcaloides tropânicos, das calisteginas, dos nicotinóides e dos vitanolídeos como marcadores quimiotaxonômicos para Solanaceae e, evidenciou a importância e a aplicabilidade da abordagem da quimiossistemática micromolecular como ferramenta para a compreensão da diversidade biológica. Além da possibilidade do seu uso na inferência de tendências evolutivas e para o esclarecimento de problemas taxonômicos.

A análise de similaridade baseada nos alcaloides derivados da ornitina sugere que o perfil químico das subfamílias está, de modo geral, correlacionado com a atual classificação de Solanaceae evidenciando a proximidade de Solanoideae e Nicotianoideae e, dessas subfamílias com Petunioideae. Além disso, evidenciou uma convergência química no gênero *Nicotiana* (Tribo Nicotianeae, Nicotianoideae) que, embora tenha uma ampla distribuição geográfica, mantém seu perfil químico independente do continente que habite. Além disso, existe uma divergência química muito evidente no perfil químico das duas tribos de Nicotianoideae, pois enquanto Nicotianeae caracteriza-se pela ocorrência de nicotinóides, a tribo australiana Anthocercideae caracteriza-se pela ocorrência de alcaloides tropânicos.

A análise de agrupamento apontou uma dicotomia química na produção de alcaloides tropânicos e calisteginas confirmando a tendência de produção exclusiva de calisteginas em gêneros sul-americanos.

No que diz respeito à análise de similaridade baseada nos vitanolídeos, foi possível verificar que o perfil químico das tribos/gêneros tem uma boa concordância com a atual classificação para a subfamília Solanoideae. De modo especial, os vitanolídeos são importantes marcadores quimiotaxonômicos para a tribo Physaleae e para os gêneros *Deprea* e *Jaborosa*.

Derivados da ornitina estão mais amplamente distribuídos em Solanaceae, estando presentes desde os grupos mais basais até os grupos mais derivados. Por outro lado, os vitanolídeos ocorrem, sobretudo, na subfamília mais derivada em Solanaceae. É possível que este fato esteja relacionado com uma divergência, uma tendência de dicotomia na ocorrência de substâncias produzidas via metabolismo do acetato – no caso os derivados da ornitina – e do mevalonato – no caso os vitanolídeos – porém a comprovação dessa observação requer a análise conjunta dos dados de ocorrências das substâncias derivadas de ambas as rotas metabólicas.