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 Petunioideae. Schizanthoideae appeared somewhat similar to these subfamilies while Cestroideae appeared dissimilar to the others.

Keywords: Solanaceae; Simple pyrrolidines; Nicotinoids; Tropane alkaloids; Calystegines

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, Euasterideae) and are phylogenetically related to the Convolvulaceae (sister to the Solanaceae), Hydrocleaeae, Montiniaceae, and Sphenocleaceae [1]. The Solanaceae are considered a subcosmopolitan taxon, with the greatest biodiversity found in the Western hemisphere [2]. According to Hunziker [3], 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 [3] and based on morphological and chemical criteria, comprises approximately 2300 species in 92 genera distributed across the subfamilies Solanoideae, Cestroideae, Juanuloidae, Salpiglossoideae, Schizanthoideae, and Anthocercidoideae. The second, more recent proposal was presented by Olmstead et al. [2] in a molecular study conducted on a sample of 89 genera and 190 species. The authors propose seven subfamilies: Solanoideae, Cestroideae, Nicotianoideae, Petunioideae, Schizanthoideae, Goetzoeideae, and Schwencinioideae.

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 (Figure 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 [4]. The calystegines are also an important class of Solanaceae metabolites. According to Dräger [5], 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 micro molecular and morphological data [6] and can be used to test for correlation between morphological and chemical diversity [7]. 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 [8]. 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 phytosystematic 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.

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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. [9] and updated by Santos et al. [8]. 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

Figure 1: Simplified schematic diagram of the ornithine-derived alkaloid biosynthesis pathway. After Bachi [15] and Grynkiewicz and Gadzikowska [16].
used the structural classes proposed by Eich [10]. Calystegines were divided into trihydroxynortropanes, tetrahydroxynortropanes and pentahydroxynortropanes (Figure 2).

The absolute frequency of occurrences (NO) was calculated as described by Santos et al. [8], and the relative frequency of occurrences (NO%) was obtained following: occurrence’s number of each structural class by 100. The value obtained 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 ornithinederived 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) (Figure 3). Table 1 provides occurrence data of each of the four classes of ornithinederived alkaloid, stratified by structural type and by genus, classified according to the subfamily scheme proposed by Olmstead et al. [2]. Of the seven Solanaceae subfamilies proposed by Olmstead et al. [11] 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 (Figure 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, Lycium, Mandragora, Physoclinia, Przewalska, Salphichroa, 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. Ester 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, Physoclinia, and Przewalska. 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 metelodine (Figure 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 scopalinone (NO=55), which has been isolated from Anisodus, Atropa, Atropanthe, Brugmansia, Datura, Hyoscyamus, Lycium, Mandragora, Physoclinia, Przewalska, 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 (Figure 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-scopadonine, and β- scopadonine. The first two were found in Atropa, Hyoscyamus, Mandragora and Physoclinia, 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 (Figure 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, Physoclinia, Salphichroa 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 and 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 (Figure 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
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 (Figure 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 (Figure 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 above mentioned subfamilies were.

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 (Figure 2).

Table 1: Occurrence of ornithine-derived alkaloids in genera of the five subfamily Solanaceae Juss.
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 Solanoidae and Nicotianoidae, 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 Solanoidae. The only class not reported in the Nicotianoidae 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 [9], 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 [3] used chemical data to define subfamilies, noting the occurrence of nicotinoids and tropane alkaloids in the subfamilies Cestroideae and Solanoidae 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, Brunnfelsia, 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 [2] 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 [3] to the subfamily Salpiglossoideae), changing the chemical profile of the Cestroideae, as these three genera were only found to contain nicotinoids. Olmstead et al. [2] place Nierembergia and Brunfelsia among the Petunioideae; Latua was attached to the subfamily Solanoidae; and Nicotiana, alongside the Australian genera Anthocercis, Anthothroche, Crenidum, Cyphanthera, Duboisia, Grammosolen and Symonanthus, of the tribe Anthocercideae (assigned by Hunziker to the subfamily Anthocercidoideae), were assigned to the subfamily Nicotianoidae.

The subfamily Solanoidae 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 [13]. It contains approximately 47 genera and approximately 1800 widely distributed species [3]. 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 [4]. The calystegines were also representative in the Solanoidae. 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 [12]. 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.

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 [14]. 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. [2,11]. As early as 1992, Olmstead and Palmer [12] noted the potential relationship between the subfamily Solanoidae, 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 Nicotianoidae, which included the tribes Nicotianae (Nicotiana) and Anthocercideae (Anthocercis, Anthothroche, 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 [2,11,13].

The Petunioideae subfamily was also proposed on the basis of molecular studies carried out by Olmstead et al. [11]. The taxa of this subfamily were moved from the Cestroideae and include those assigned by Hunziker [3] to the tribe Nicotianae (Petunia and Fabiana), except...
Figure 5: Dendrogram constructed from analysis of Dice's coefficient for comparison of chemical similarity among Solanaceae subfamilies.

Figure 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: (a) Nicotianoideae - tribe Nicotianaeae, and, (b) Nicotianoideae - tribe Anthocercideae.
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. [2].

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 [2,3]. 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.

References


