Universidade Federal do Rio Grande do Sul
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Dissertação de Mestrado

Tendência evolutiva de Flavonoides em Linhagens de Mutisieae *sensu* Cabrera (Asteraceae)

Adriana Winter

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Dissertação apresentada como requisito parcial para obtenção do título de Mestre no Programa de Pós-Graduação em Botânica, Instituto de Biociências, Universidade Federal do Rio Grande do Sul.

Porto Alegre, 2019

“Só se põe a vida a perder quando ela para de evoluir”

Oscar Wilde

Para todas as pessoas que me inspiraram a seguir este caminho,

principalmente à Marie Curie que

me fez sonhar com moléculas.

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Sumário

Resumo

Flavonoides são metabólitos secundários de ampla ocorrência no reino vegetal. Os diversos derivados flavonoídicos apresentam distribuição heterogênea nos táxons vegetais, o que confere a essas substâncias valor como marcadores taxonômicos. Inclusive, alguns estudos já citaram a validade do uso de flavonoides como marcadores para Asteraceae. Essa família possui um grande número de espécies com grande diversidade fenotípica, tanto em termos morfológicos quanto químicos. Apesar de ter sempre sido considerada monofilética já passou por várias alterações taxonômicas internas. A tribo Mutisieae é um exemplo disso, comparada com a sua descrição de 1977 ela foi dividida em nove subfamílias e 13 tribos em cerca de 40 anos. Quatro destas subfamílias (Barnadesioideae, Mutisioideae, Wunderlichioideae e Gochnatioideae) foram estudadas neste trabalho. Inicialmente, foi feita uma revisão de literatura com publicações sobre a presença flavonoides nas subfamílias estudadas e, com base nos flavonoides encontrados foram criado bancos de dados sobre a ocorrência de flavonoides e sobre informações estruturais dos flavonoides. O banco de dados de ocorrência passou por análise qualitativa e o banco de dados de estrutura foi submetido à análise de cluster e de componente principal através do uso dos pacotes do Software R PVClust e FactoMine, respectivamente. O banco de dados consistiu em 88 espécies de quatro subfamílias de Asteraceae e 10 espécies da família Calyceraceae, que foi utilizada como grupo externo. Cada tribo apresentou um padrão químico de tipos flavonoídicos e de substituição no flavonoide característico e pode-se observar umacerta tendência neste padrão. Barnadesioideae é a subfamília quimicamente mais próxima de Calyceraceae do que das demais subfamílias com flavonóis glicosilados em C_3. Cada tribo de Mutisioideae apresentou seu próprio padrão químico e os caracteres não apresentaram uma evolução linear. Mutisieae apresentou antocianinas bisglicosiladas. Nassauvieae se caracterizou pela O-metilação e a produção de dihidroflavonois. Wunderlichioideae apresentou somente flavonas substituídas com O-acilglicosídeos, sendo relacionada tanto no nível de subfamília quanto de tribo com Gochnatioideae, que possui muitas flavonas com oxigrupos em C_6, metilada em mais de uma posição. Pode-se observar um padrão de flavonoides mais simples glicosilados em C_3 tendendo à moléculas mais complexas metiladas em várias posições. Flavonoides mostraram-se bons marcadores taxonômicos para essas linhagens.

Abstract

Flavonoids are secondary compounds with broad occurrence in the plant kingdom. Their derivatives have heterogeneous distribution in plant taxa, giving these substances value as taxonomic markers. Some studies already validated flavonoids as chemical markers for Asteraceae. The family is very large and the species very different phenotypically, both morphologically and chemically. Though it has always been considered monophyletic, the family has had several internal taxonomic changes. The Mutisieae is an example; compared to its description in 1977 it was divided tribe into nine subfamilies and 13 tribes, in about 40 years. Four of these subfamilies (Barnadesioideae, Mutisioideae, Wunderlichioideae e Gochnatioideae) were studied in this work. Initially a review of the literature was made with publications about the occurrence of flavonoids in the studied subfamilies; based on that, datasets with flavonoidic occurrence and with structural information of flavonoids were created. The occurrence dataset was qualitative analyzed and the structural dataset went through a cluster analysis and a principal component analysis, with the R packages PVClust and FactoMine, respectively. The dataset consists in 88 species within four subfamilies of Asteraceae and 10 species. from family Calyceraceae, used as an out-group in this work. Each tribe had its own chemical pattern of flavonoidic classes and of flavonoidic substitution, a certain tendency in these patterns was observed. Subfamily Barnadesioideae was chemically closer to Calyceraceae than the other subfamilies with flavonols glycosylated in C_3. Every tribe in Mutisioideae has its own pattern and did not show a linear evolution of character, Mutisieae had a biglycosylated anthocyanin. Nassauvieae is characterized by O-Methylation and the production of dihydroflavonols. Wunderlichioideae had only acyl glycosylated flavones, and is related both in the subfamilial and in tribal level with Gochnatioideae. The latter has flavones with oxigroups in C_6, methylated in more than one position. A patter was observed with simpler flavonoids glycosilated in C_3 tending to more complex molecules methylated in several positions. Flavonoids were good taxonomic markers for these lineages.

Introdução

Flavonoides são derivados fenólicos e desempenham várias funções em plantas, desde proteção contra patógenos, sinalização hormonal e até atração de polinizadores e dispersores de frutos (GOULD; LISTER, 2006).

São metabólitos com 15 átomos de carbono formando um sistema de três anéis, denominado anel flavânico (Figura 1). O nível de oxidação do anel C determina o tipo (subclasse) de flavonoide e a substituição dos anéis A, B e do carbono 3 do anel C determina a substância individual, por exemplo kaempferol (cujo nome técnico é 3,5,7,4'-tetrahidroxiflavona) (ZHENG et al., 2019). Os flavonoides são sintetizados por uma rota biossintética mista que envolve as rotas malonato/acetato e do chiquimato (Figura 2). Durante o processo de síntese algumas posições do anel são hidroxiladas formando o núcleo polifenólico característico. Essas hidroxilas comumente passam por reações de proteção (eterificações), havendo então a ocorrência de substituições, sendo as mais comuns a glicosilação e a metilação.

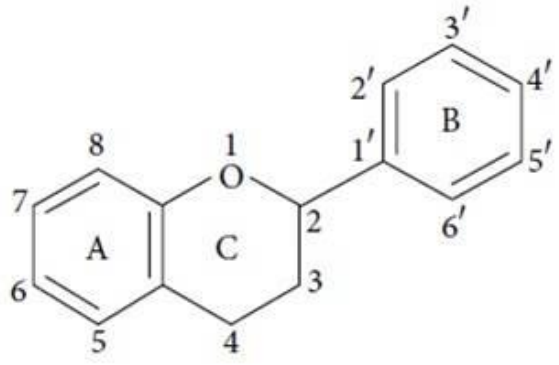

Figura 1: Esqueleto básico de flavonoides – anel flavânico, numerado segundo a IUPAC (BERIM; GANG, 2016). Na maioria dos flavonoides a posição 4 do anel se encontra oxidada como um grupo oxo de cetona.

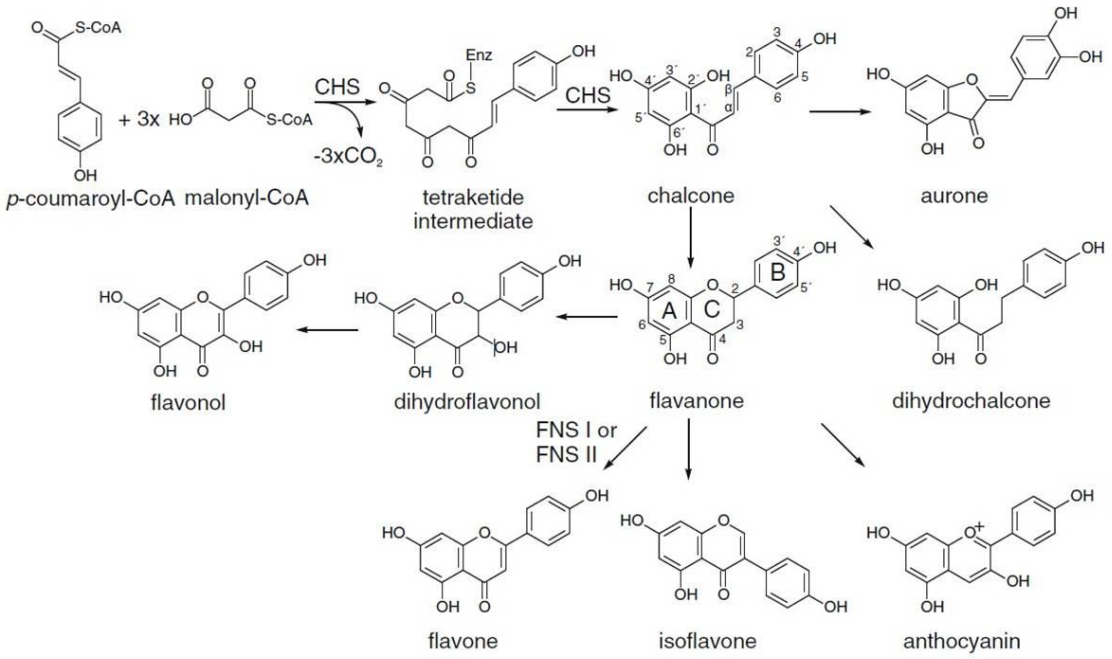

Figura 2: Esquema da rota de síntese de vários tipos de flavonoides (Berim e Gang, 2016).

A estrutura dos flavonoides, como as posições hidroxiladas, insaturações e cadeiras laterais afetam a função (SZEJK et al., 2017). Metilações, por exemplo, diminuem a solubilidade da molécula (BERIM; GANG, 2016).

Há cerca de 9000 flavonoides descritos (WANG; LI; BI, 2018) em todo o reino vegetal. Essas substâncias são encontradas em algas carofíceas (SAAD et al., 2017) e em todas as linhagens de plantas terrestres (RAUSHER, 2006).

A ampla ocorrência de flavonoides nas plantas, a restrição de certos tipos a alguns táxons, características da estrutura relativas à estabilidade química (CHAABAN et al., 2017), bem como, a persistência em plantas secas (MENDIONDO et al., 2011) faz com que essa classe química possa ser utilizada como marcadores quimiotaxonômicos (JUÁREZ; MENDIONDO, 2003).

Asteraceae (Bercht. and J. Presl) é maior família de angiospermas conhecida atualmente. Alguns fósseis colocam sua origem na América do Sul há cerca de 69,5 Mi de anos

(PANERO; CROZIER, 2016) e está relacionada à Calyceraceae e Goodeniaceae em Asterales (LUNDBERG, 2009)Possui em torno de 25 mil a 30 mil espécies descritas e 1600-1700 gêneros (FUNK et al., 2009).

Na família são encontradas espécies com grande variedade de hábitos, habitats e características. Algumas características morfológicas da família são anteras sinanteras, inflorescência do tipo capítulo com flores do raio e flores do disco, frutos do tipo cipsela com papus estigmas bifurcados (FUNK et al., 2009; KATINAS et al., 2016). Toda essa diversidade também se reflete em uma grande diversidade química de vários metabólitos, inclusive os flavonoides.

Asteraceae é monofilética e desde sua descrição passou por várias alterações taxonômicas, da classificação de Bentham até Funk et al. (2009), sendo a última a principal publicação da família.

A tribo Mutisieae passou por várias alterações, um dos grandes trabalhos do grupo (CABRERA, 1977)fez sua descrição química e a circunscrição. Os limites desse grupo foram alterados pelos trabalhos de vários autores (PANERO; FUNK, 2002, 2007, 2008; PANERO et al., 2014) e atualmente se encontram em cerca de nove subfamílias e 13 tribos.

Um dos primeiros grupos que foi criado a partir da tribo foi a subfamília Barnadesioideae (BREMER; JANSEN, 1992), que é a subfamília mais basal do grupo (JANSEN; PALMER, 1987). As subfamílias que em 1977 pertenciam a Mutisieae também são consideradas basais, estudar estes grupos e sua relação com outras famílias pode dar grandes pistas de como características evoluíram na família. Com base nisso foram escolhidas as subfamílias Barnadesioideae; Mutisioideae; Wunderlichioideae e Gochnatioideae de Asteraceae e a família Calyceraceae para este trabalho.

Existem mais de 20 mil registros da ocorrência de flavonoides em Asteraceae (CALABRIA et al., 2009), e alguns trabalhos discorrem sobre a utilização de flavonoides como marcadores taxonômicos para a família (DE OLIVEIRA et al., 2017; EMERENCIANO et al., 2001).

Para estudar a origem química em Asteraceae e observar como ela se comportaria ao longo das linhagens foi estudado no presente trabalho o padrão de ocorrências de flavonóides nas subfamílias Barnadesioideae; Mutisioideae; Wunderlichioideae e Gochnatioideae, comparando-as com Calyceraceae.

Original Article

Title: Flavonoidic profile of some lineages of Mutisieae *sensu* Cabrera

Journal: Acta Botanica Brasilica

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Abstract

Asteraceae is a very complex family in which the relations between levels of taxa are still being studied. The use of different taxonomic markers such as secondary metabolites might help understanding how the taxa are related. Focusing in a metabolite chemically diverse and common in the Plant Kingdom such as flavonoids might be a good choice for this purpose. Considering the flavonoidic diversity within five subfamilies of Asteraceae (Barnadesioideae; Mutisioideae; Stifftioideae; Wunderlichioideae and Gochnatioideae) a dataset was created and analyzed. The dataset consisted in articles published from 1950 to 2017, and information on sp, local of collection, compound, organ of extraction, method of extraction, flavonoidic class, hydroxylated positions, chemical substituent and the position it substitutes was tabled. Quantitative and qualitative analyses of the table were made. The whole dataset consists in 88 especies from 4 subfamilies with 78 different flavonoids, no flavonoidic data of Stifftioideae was found. Some patterns were observed in every taxa, Barnadesioideae has mainly flavonols glycosilated in C_3. Each tribe from Mutisioideae had its own pattern, Mutisieae was the only one with anthocyanidin and anthocyanin. Nassauvieae has dihydroflavonols, the tribes' flavonoids were mostly methylated in C_7 or both in $C_{4'}$ and C_7. In Wunderlichioideae only flavones with acyl glucosides were extracted. Gochnatioideae had flavones with additional oxygroups in C_6 and the most common substituent is the methoxyl (OMe) in various positions within the molecule, di and trimethylation are quite common in this lineage.

Keywords: Compositae; Flavonoidic Profile; Mutisieae *sensu* Cabrera, Flavonoid tendencies, chemical profile, Barnadesieae, Mutisieae, Nassauvieae, Onoserideae, Wunderlichieae, Gochnatieae

Introduction

Asteraceae Bercht. and J. Presl is a large family; therefore, a complicated one. Throughout the literature many changes in taxa circumscription can be observed (Katinas *et al.*, 2008; Ortiz *et al.*, 2009). Mutisieae is the tribe that had many circunscriptional changes since it's description by Cassini (1819), most of the changes occurred from 1977(Cabrera) to 2014 (Roque & Funk, 2013; Panero *et al.*, 2014), in which the tribe was segregated into nine subfamilies with 13 tribes using morphological or genetic markers; nevertheless their limits are not very precise.

Some works studied the evolution of secondary metabolites (Harborne, 1977) with some of those using them as taxonomic markers (Dahlgren, 1980), and recent works are using them again as markers in clustering techniques for different taxonomic levels (Haliński, Samuels, & Stepnowski, 2017). Considering flavonoids' universal presence in plants (Rausher, 2006; Berim & Gang, 2016) and some molecular peculiarities restricted to certain taxa, their validity as a taxonomic markers can be found in the literature (de Oliveira *et al.*, 2017).

The basic structure of flavonoids and the possible molecular alterations (Berim & Gang, 2016) reflect in their stability and the function they play in the plant (Gould & Lister, 2006; Brunetti *et al.*, 2013; Chaaban *et al.*, 2017). All these modifications make it possible to use them as markers since some molecular structures are present solely in certain groups.

One of the first descriptions of the Chemistry of Compositae , Mutisieae's chemical profile was compiled by Cabrera (1977). After 30 years, a new description for the family was made (Calabria *et al.*, 2009), but all the works cited were made before Mutisieae's division starting in 2002 until 2016, therefore different chemical patterns per taxa might be found and information of their evolution and ecology added. The value of flavonoids as taxonomic

markers for Asteraceae was already introduced (Emerenciano *et al.*, 2001), though the effectiveness of this sort of data in clustering techniques is still untested for many taxa.

Thus in this work we will describe the flavonoidic profile of five subfamilies of Asteraceae that were from Mutisieae *sensu* Cabrera and characterize their chemical patterns. The subfamilies in this study are Barnadesioideae; Mutisioideae; Wunderlichioideae; Stifftioideae; Gochnatioideae and their latest circumscriptions.

Material and methods

Dataset

A dataset with the flavonoidic profile of the studied taxa was created based in articles published from the 1950`s until november of 2017 found in the SCOPUS and PubMed databases. The research focused in flavonoidic profiles of different taxonomic levels from subfamilies Barnadesioideae; Mutisioideae; Wunderlichioideae; Stifftioideae and Gochnatioideae to more specific levels. The circumscription of these subfamilies was determined according to publications after 2009 and the species' status was checked in Tropicos and IPNI databases, only current names were used in this study.

The information gathered was divided into seven categories: species; location of collection; flavonoids found; method of extraction; organ of extraction; observation and reference.

Additional information of flavonoid structure and molecular alteration was obtained from the PubChem database and transcribed into the dataset. The categories are: flavonoid class; hydroxylated positions, type of substituent and position of substituent.

Data analysis

The tabled information of flavonoid occurrence was transcribed as seen in Table 2 in Supplementary Material. The extracted flavonoids are named with their common names or

simplified structure due to the long list of synonyms. In this work we use the current circumscription of the studied subfamilies and their current species.

The chemical patterns of the flavonoidic structure found per taxa are in Table 1, the patterns are described with information of the molecule cited in "Dataset creation" and are represented in percentage for each structure on the total of possibilities found.

Results

We found chemical information from 88 species, considering subspecies and varieties levels there are 95 taxa from the subfamilies, Barnadesioideae; Mutisioideae; Wunderlichioideae and Gochnatioideae. The new circumscription of these taxa was considered, therefore there are 24 genera from six tribes in the dataset. No information about the flavonoid chemistry of Stifftioideae was found.

The extracted flavonoids were mainly from dry aerial vegetative parts with methanol or ethanol extracts. The substance's characterization was by chromatography, either HPLC or paper chromatography ensued by spectrophotometry.

In table 1 we have a synopsis of the profile of flavonoids found. Table 2 is in Suplementary Material and has a complete description of the flavonoids found per taxa. Some molecules were not accurately described, such as kaempferol-3-O-glucoronide and/or quercetin-3-O-glucoronide (Bohm & Stuessy, 1995), the flavonoid found could be either one, thus it was not considered for the hydroxylated positions category but flavonoid class, substituent and substituents position is the same and therefore these informations were considered.

Some flavonoids have more than one substituent, like isorhamnetin-3-O-glucoside that is methylated (-OMe) position (3') and glycosylated (-OGluc) in position (3). Both substituents are accounted for in substituents and substituents' position in Table 1, leading to

a total higher than total flavonoids. Isorhamnetin-3-O-glucosides was extracted from *Barnadesia parviflora* Spruce ex Benth. & Hook. f. (Bohm & Stuessy, 1995), isorhamnetin-3-glucoronide from *Mutisia acuminata* Ruiz & Pav. (Juárez & Mendiondo, 2003) and isorhamnetin-3-O-rutinoside from *M. friesiana* Cabrera (Viturro, Molina, & Schmeda-Hirschmann, 1999).

The genera *Nassauvia* and *Triptilion* from the Nassauvieae tribe had some C-glycosil flavonoids detected, though the molecules were not characterized (Maraner *et al.*, 2012). In the same study there is only information about flavonoids' classes in 19 species of genus *Nassauvia*, *Calopappus acerosus* Meyen and *Triptilion spinosum* Ruiz & Pav., each class was counted once per species and are in Table 1.

Chemical patterns or tendencies were found in every taxa studied, some are more representative than others due to better representation throughout the literature.

Barnadesioideae consists mainly of flavonols hydroxylated in positions 3' and 4', and glycosylated in C_3.

The current three tribes of Mutisioideae were found in the literature, each with its own chemical pattern. Mutisieae is the only one to have anthocyanidin and anthocyanin documented. Most of the substituents are glycosides such as glucose, rutin and malonyl glucoside inserted mostly in C_3 and C_7, though in some other positions as well. Nassauvieae is the only analyzed group with dihydroflavonols, the most common substituent is O-Me (methoxyl group) found mostly at C_7 or both at $C_{4'}$ and C_7, for Onoserideae methoxyl was also the common substituent in the same positions as Nassauvieae.

Subfamily (N)	Barnadesioideae (43)	Mutisioideae(37)			Wunderlichioideae (2)	Gochnatioideae (11)
Tribe	Barnadesieae	Mutisieae (6)	Nassauvieae (28)	Onoserideae (3)	Wunderlichieae	Gochnatieae
Flavonoid class [N]	Flavonol [207] 98%	Flavonol [18] 65.4%	Flavonol [9] 49%	Flavonol [4] 40%	Flavone [5]100%	Flavonol [32] 65,3%
	Flavanone [4]2%	Flavone [11] 26,9%	Flavone [6] 39%	Flavone [3] 30%		Flavone [12] 24,5%
		Anthocyanidin [1] 4%	Flavanone [5] 7%	Flavanone [3] 30%		Flavanone [5] 10,2%
		Anthocyanin [1] 4%	Dihydroflavonol [3] 4%			
Totalflavonoids	211-100%	31-100%	23-100%	10-100%	5-100%	49-100%
Hidroxylated Positions [N]	3. 5. 7/ 4' [90] 42,6%	3, 5, 7/ 4' [6] 19,3%	3, 5, 7/ 4' [6] 26%	3, 5, 7/ 4' [2] 20%	5, 7/ 3', 4' [2] 40%	3, 5, 7/ 4' [11] 22%
	3. 5. 7/3'. 4' [98] 46,4%	3, 5, 7/ 3', 4' [13] 41,9%	3, 5,7/ 3', 4' [5] 21,7%	3, 5, 7/ 3', 4' [2] 20%	5, 7/ 4' [3] 60%	3, 5, 7/ 3', 4' [21] 38.8%
	5. 7/3'. 4' [4] 1,9%	5,7/ 4' [5] 16,1%	3, 5, 6, 7/ 3', 4' [1] 4,3%	5,7/ 4' [6] 60%		5, 7/ 4' [8] 18.5%
		5,7/ 3', 4' [2] 6,4%	5, 6, 7/ 4' [1] 4,3%			5,7/ 3', 4' [6] 14.8%
			5, 6, 7/ 3', 4' [1] 4,3%			5, 6,7/ 4' [2] 4%
			5, 7/ 4' [8] 34,8%			5, 6, 7/ 3', 4' [1] 2%
			5, 7 [1] 4,3%			
Subistituent [N]	O-Gluc [88] 41.7%	O-Gluc [11] 35,5%	O-CH3 [17] 74%	O-CH3 [4] 40%	O-Acilglucoside [2] 40%	O-CH3 [36] 73,5%
	O-Rut [76] 36%	O-Rut [4] 12,9%	O-Gluc [1] 4,3%	O-Gluc [2] 20%	O-CH3 [2] 40%	O-Acilgluc [3] 6,1%
	O-Rham [14] 6.6%	O-Malonilgluc [3] 9,7%	O-Rham [1] 14,3%	O-Rut [2] 20%	No [1] 20%	O-Rut [1] 2%
	O-Ac. Gluc [21] 9.9%	O-Ac.Gluc [4] 12,9%	No [4] 17,4%	No [2] 20%		O-Galac [1] 2%
	O-CH3 [2] 0.9%	O-CH3 [2] 6,4%				O-Ac.Gluc [1] 2%
	No [11] 5.2%	O-Glucopiran [1] 3,2%				No [7] 14,3%
		Bismanoside [1] 3,2%				
		No [7] 22,6%				
Position of Substituent	O-Gluc C_3	O-Gluc C_3; (C_3,C_5); C_7; C_4'	O-CH3 C_3; C_6; C_7; (C_7, C_3'); (C_7,C_4'); C_4'	O-CH3 C_7,C_4'	O-Acilglucoside C_7	O-CH3 C_3; (C_3, C_7); (C_3, C_7, C_4'); (C_3, C_3'), (C_3, C_4'); C_6; (C_6, C_4'); C_7; (C_7, C_3'); (C_7,C_4'); C_3'
	O-Rut C_3	O-Rut C_3; C_7	O-Gluc C_3	O-Gluc C_3	O-CH3 C_7; (C_7,C_4')	O-Acilgluc C_3
	O-Rham C_3	O-Malonilgluc C_3; C_7	O-Rham C_3	O-Rut C_3		O-Rut C_3
	O-Ac. Gluc C_3	O-Ac.Gluc C_3				O-Galac C_3
	O-CH3 C_3'	O-CH3 C_3'				O-Ac.Gluc C_7
		O-Glucopiran C_7				
		Bismanoside C_3, C_7				

Table 1- Flavonoidic tendencies per lineage. [total of flavonoids found per taxa] the % of flavonoids from total flavonoids per taxa. Position of substituent is according to flavonoid numbering system.

The flavonoids extracted in Wunderlichioideae were mainly acilglycosilated flavones. Gochnatioideae and Nassauvieae were the only groups to have carbon 6 hydroxylated, the first had mostly methylated flavonols in various positions within the molecule, di and trimethylation were also common in this group.

Table 2, found in supplementary material is a description of the flavonoids found per species in the literature in both the four subfamilies of Asteraceae and Calyceraceae. Individuals described with cf., varieties or forms are all included in the current species. With more than 80 different flavonoids for 98 species.

Discussion

The analyzed group consists in about a 1000 species overall, though throughout the literature there is information about 9% of it. Therefore the patterns found in this study might not represent the reality of these groups, though it is a good indication of possible patterns.

Finding these patterns it's an important method for many areas in pharmacological studies (Casado *et al.*, 2011), having probable taxa for certain molecular types helps narrow the search for active principles to certain plant groups.

Some chemical patterns (e.g. flavone/flavonol ratio) observed in plant groups can be correlated with evolutionary parameters calculated on basis of habit, lignification (Soares & Kaplan, 2001), thus knowing the proportion of flavonoids and their characteristics per taxa might indicate several characteristics of the studied taxa.

Flavonoids' molecular structure is responsible for its identity as it is for its location and activity. The characteristics in C-ring are the ones responsible for flavonoids' class

(Berim & Gang, 2016) and the hydroxylated positions in B-ring change the flavonoids' antioxidant activity and their location in plant tissues (Brunetti *et al.*, 2013).

The number of individuals per specie and the representation of these individuals for the studied taxa might not have been enough for us to be more certain if the patterns found are representative enough for the taxa, plants chemistry change due to environmental differences, populational differences and interaction with various factors.

Understanding these differences might help comprehend the biology and the relation of environmental factors and the plant´s physiology (Grignon-Dubois & Rezzonico, 2018). Also the role developed by these metabolites in the plant, how and why they are produced (Brunetti *et al.*, 2013; Chaaban *et al.*, 2017).

Knowing the chemical profiles assist in the search for certain patterns and substances, including their structural differences, that alter their stability. For example glucosilated phenols and their aglycones already make a difference in molecular stability against oxidative environments (Szejk *et al.*, 2017).

All things considered flavonoids have molecular and behavioral alterations that enable its use as taxonomic markers in Asteraceae, at least within the studied lineages. Thus studies using clustering analysis are required to confirm this statement.

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Original Article

Title : Flavonoids as chemical markers for Mutisieae *sensu* Cabrera

Journal : Botanical Journal of the Linnean Society

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Abstract

Flavonoids encompass a large variety of molecules. There are more than 9.000 known flavonoids. This diversity found in flavonoids makes it possible for them to be used as taxonomic markers. The Asteraceae has a record of around 20.000 flavonoids, and, for many taxonomic groups, only specific types of molecules occur. The Mutisieae since Cabrera has been segregated into 9 subfamilies and 13 tribes whose limits are still being determined. Therefore, in this work we tested how flavonoids would work as taxonomic markers and what characteristics are important to differentiate the studied lineages. We studied how Barnadesioideae, Mutisioideae, Wunderlichioideae and Gochnatioideae, their tribes and genera are related. Calyceraceae was used as an outgroup. We transformed an existing dataset of flavonoid profile into a binary matrix of presence and absence and analysed for Clustering (HCA), and principal components (PCA). We constructed dendrograms for subfamily, tribe and genera, Barnadesioideae is chemically closer to Calyceraceae, Mutisioideae tribes are chemically different and did not form a chemical monophyletic group and Wunderlichioideae and Gochnatioideae are more related to each other than the others. The PCA results also showed there are 5 groups within the studied taxa. Thus, we conclude that with the appropriate amount of chemical information flavonoids are good taxonomic markers, and could be used together with other taxonomic tools to reinforce phylogenetic trees and help segregate taxa.

Key-words: Chemotaxonomy, Flavonoids, Barnadesieae, Gochnatieae, Wunderlichieae, Nassauvieae, Onoserideae

Introduction

Flavonoids are phenolic derivatives with three rings in their structure A, B and C. Ring Cs structure determine the flavonoids class, and A and Bs determine the specific flavonoid. A and B are hydroxylated (-OH) in certain positions, due to the condensation of a coumayl unit with acetate units (Koirala *et al.*, 2016; Wang, Li, & Bi, 2018). The known number of flavonoid molecules is over 9.000 (Mierziak, Kostyn, & Kulma, 2014), they are told apart mainly by the hydroxylation pattern, a total of eight possible positions in the molecule, and the presence of substituent in one or more of the hydroxylated positions and its position.

Asteraceae have over 20.000 flavonoid occurrences (Calabria *et al.*, 2009), some taxa have specific flavonoidic structures (Emerenciano *et al.*, 2001). The extensive appearance in the family and exclusive structure might make them good taxonomic markers.

Mutisieae is a taxon that has undergone many changes. In 1977 (Cabrera) considered it a late divergent tribe was segregated into nine subfamilies and 13 tribes (Panero *et al.*, 2014) that are part of the basal nodes, all these changes made monophyletic taxa though the limits between them are still a bit uncertain.

The use of flavonoids as taxonomic markers for Asteraceae was already stated in other works (Emerenciano *et al.*, 2001; de Oliveira *et al.*, 2017), and so was the possibility for Mutisieae *sensu* Cabrera (Mendiondo *et al.*, 2011). Therefore our goal in this work was to test the potential of flavonoids as taxonomic markers for the basal nodes of Asteraceae and to see how the groups are different chemically.

Material and methods

The Studied Taxa

Even though Mutisieae *sensu* Cabrera currently consists of about nine subfamilies only four are in this study, Barnadesioideae, Mutisioideae, Wunderlichioideae and Gochnatioideae. They were chosen because there is chemical information available in the literature, they are phylogenetically close have the same original continent, and, since they are early diverging lineages, it is possible to infer how Asteraceae started chemically then how it evolved.

Dataset

To test if flavonoids can be used as taxonomic markers and how the studied lineages of Asteraceae are chemically related we used a dataset (Winter & Soares, unpublish) of flavonoidic profile of the four subfamilies. Calyceraceae was used as an outgroup. The dataset consisted of 88 species of Asteraceae and 10 species of Calyceraceae with over 330 occurrences of flavonoids.

The dataset was transformed in a binary table of presence (1) and absence (0) having information of flavonoids found per subfamily, tribe and genera. The chemical information was divided into four categories (flavonoids class, hydroxylated position, substituent and substituent position). For example, kaempferol-3-O-glucoside is a flavonol with hydroxyl groups (-OH) in C_3, C_5, C_7 and $C_{4'}$ positions with an -OGlucosyl substitution in C_3.

Statistic tests

For the clustering method we used HCA (High Clustering Analysis) with bootstrap resampling (n=10.000) to test the relation between lineages and examine how a tree with only chemical markers would present, for that we used the PVClust package in software R (Suzuki & Shimodaira, 2006).

For the analysis of the main chemical differences between the subfamilies, we ran a Principal Component Analysis (PCA) in the FactoMine Package (Lê, Josse, & Husson, 2008).

Results

There are three dendrograms (figures 1, 2 and 3), one for each taxonomic level. Eventhough all the trees are very similar to the known phylogenies the support is still a bit shallow, probably due to the amount of species studied per taxa.

Barnadesioideae and Calyceraceae are very close chemically (Figure 1), Mutisioideae is then close to both of them. Wunderlichioideae and Gochnatioideae are closser to each other but quite different to the other two lineages. Though it is very similar to the known phylogenies the support is still a bit shallow.

Mutisioideae's tribes are in various locations in the tree (figure 2), Onoserideae was placed closer to Barnadesieae and these two to Mutisieae. Nassauvieae is a group apart from all the other tribes. Wunderlichieae and Gochnatieae are also more related to each other then the rest of the groups.

The Genera of Barnadesioideae were all grouped together (figure 3), and except for Gypothamnium are a monophyletic taxon as seen in edge 9. As seen in the other trees the genera in Mutisioideae are all over the place, the Mutisieae in edge 18 are very close but maintain a polyphyletic relation with the other tribes within the subfamily. Nassauvieae's are in different branches, making it a paraphiletic tribe and are very close to the gendera of Gochnatieae and Wunderlichieae.

There are five main chemical groups (Figure 5) considering the four subfamilies and Calyceraceae, and their similarities reflect the results seen in figure 1, with Calyceracea and Barnadesioideae being very close, in the same quadrant Mutisioideae as Wunderlichioideae and Gochnatioideae are closer to each other. Acyl glucosides were the only flavonoidic characteristic to be divided per subfamily, the others characteristic form 5 groups that do not reflect the studied taxa.

In table 1 we can see how many spp. per subfamily were studied, and we can easily see that Barnadesioideae is the best represented taxon, with almost 50% of it being chemically studied, the other subfamilies were no more than 10%.

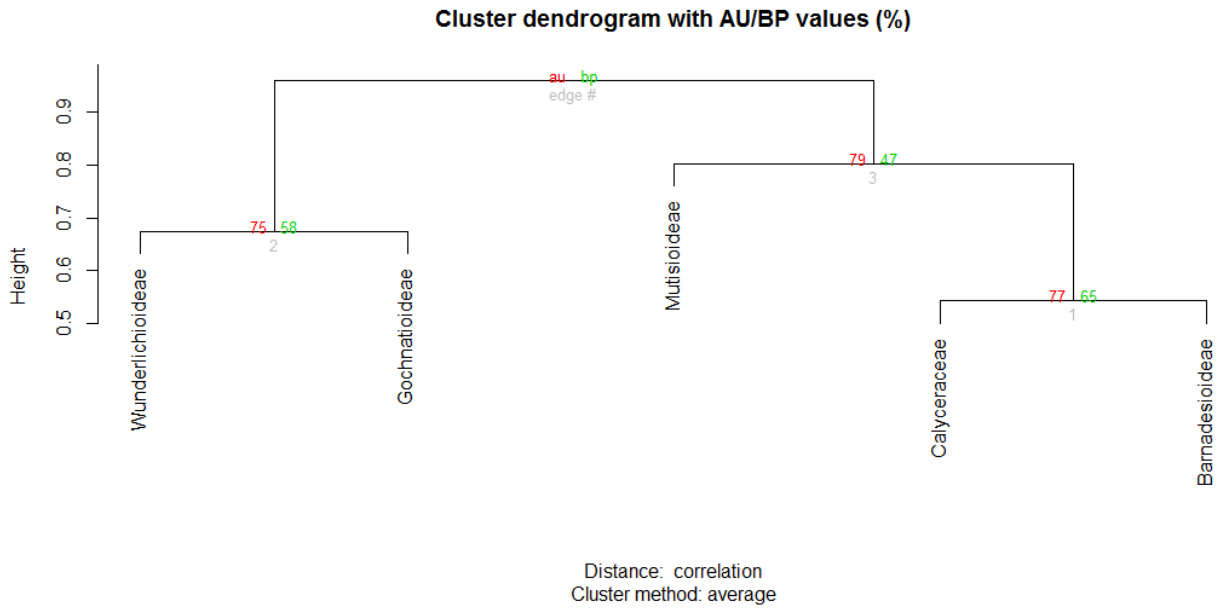

Figure 1: Hierarchical clustering analysis with bootstrap resampling of four Asteraceae subfamilies and Calyceraceae as an outgroup. Approximately unbiased (AU) probability values (left), Bootstrap probability (BP) values (right) and cluster labels (bottom).

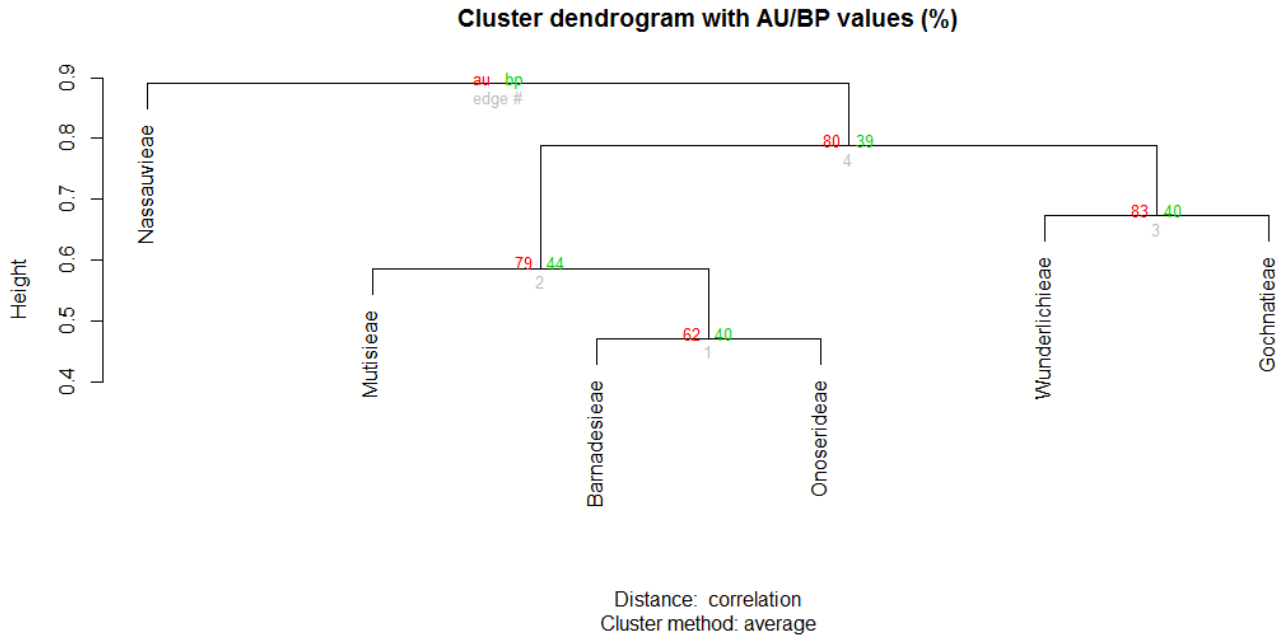

Figure 2: Hierarchical clustering analysis with bootstrap resampling of tribes of subfamilies Barnadesioideae; Mutisioideae; Wunderlichioideae and Gochnatioideae. Approximately unbiased (AU) probability values (left), Bootstrap probability (BP) values (right) and cluster labels (bottom).

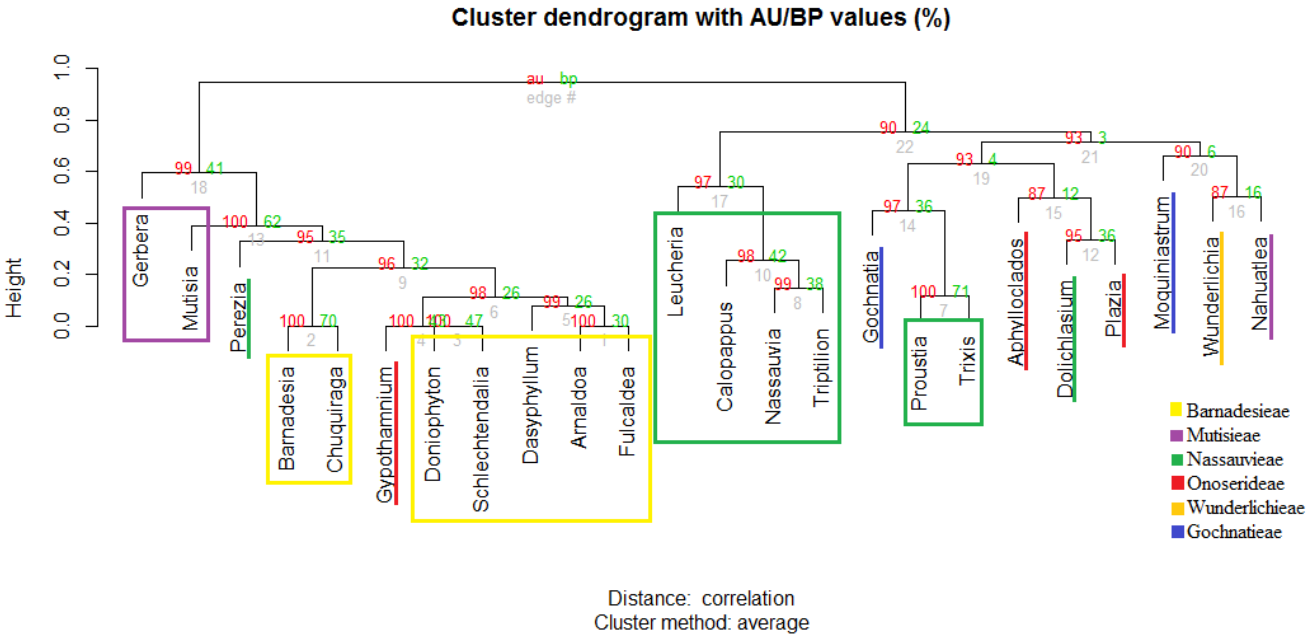

Figure 3: Hierarchical clustering analysis with bootstrap resampling of genera from subfamilies Barnadesioideae; Mutisioideae; Wunderlichioideae and Gochnatioideae. Approximately unbiased (AU) probability values (left), Bootstrap probability (BP) values (right) and cluster labels (bottom).

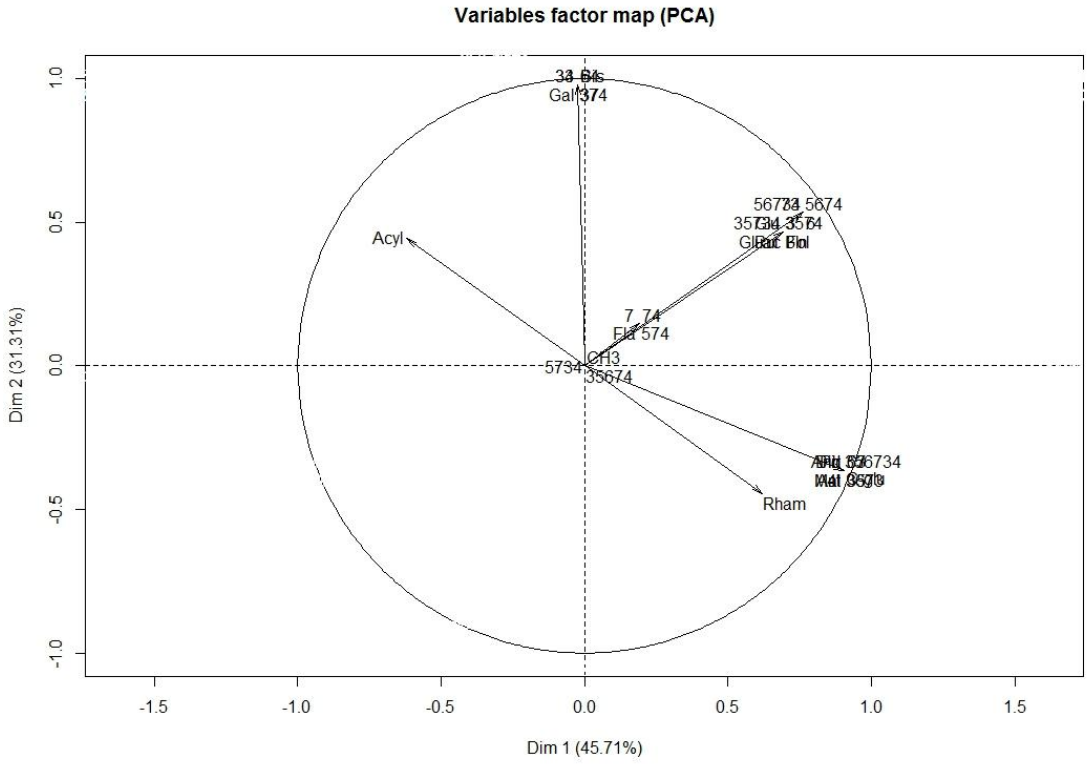

Figure 4: PCA result of the flavonoidic characters of subfamilies Barnadesioideae; Mutisioideae; Wunderlichioideae and Gochnatioideae and family Calyceraceae.

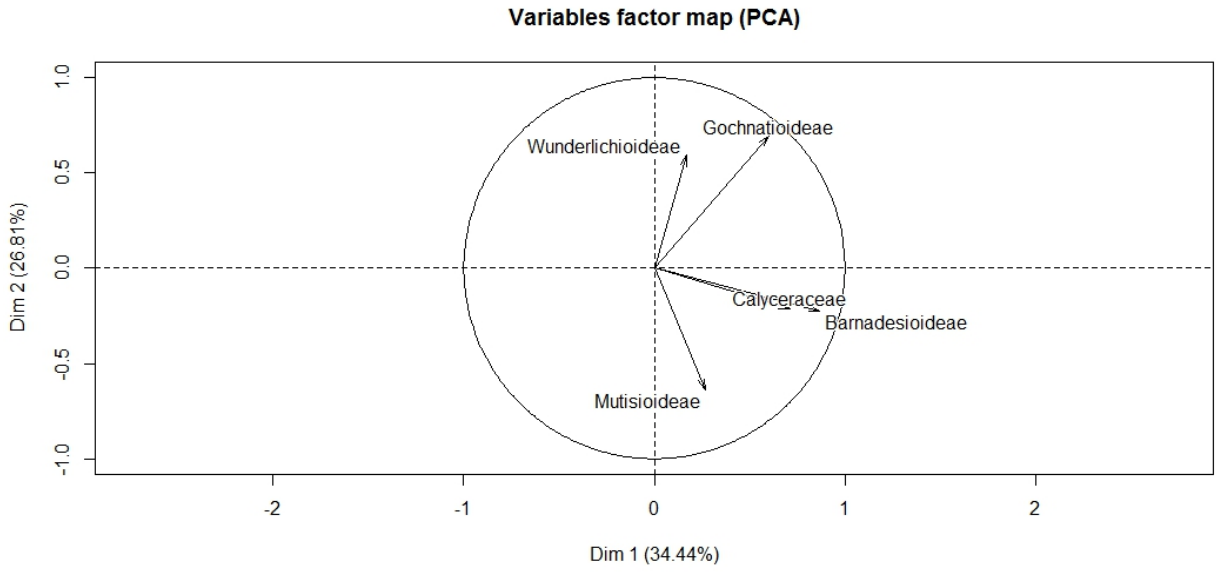

Figure 5: PCA result for the flavonoidic analysis of subfamilies Barnadesioideae; Mutisioideae; Wunderlichioideae and Gochnatioideae and family Calyceraceae.

Table 1: Number of taxa studies per group in this work

Subfamily (Tribe)		N of Genera/ Spp.		Studied Genera/ Spp.	
Barnadesioideae		9/91		7/43	
Mutisioideae	(Mutisieae)	44/≈630	M 16/≈200	13/37	M 2/6
	(Nassauvieae)	N 25/≈300		N 8/28	
	(Onoserideae)		O 7/ 52		O 3/3
Wunderlichioideae		8/≈50		1/2	
Gochnatioideae		8/≈90		3/9	

Discussion

Flavonoids are ubiquitous compounds in Asteraceae (Calabria *et al.*, 2009), structural analysis of these molecules showed that certain flavonoid characteristics might be related to the evolution of the family (Emerenciano *et al.*, 2001). As seen in some works (Mendiondo *et al.*, 2011; de Oliveira *et al.*, 2017) flavonoids are potential chemotaxonomic marker for certain taxa in the family, probably to the whole family.

Other metabolites were used as markers for Asteraceae (Calabria *et al.*, 2007; Padilla-González *et al.*, 2018). Their behaviour, and not all of them have taxonomic value.

This is the first work to use only flavonoids as markers in the construction of dendrograms for the origin and early evolution of Asteraceae.

Comparing the dendrograms of this work with the phylogeny in Panero *et al.(*2014) a resemblance can be seen in the subfamily level, Barnadesioideae as the subfamily closest to the out group (Calyceraceae). Both of them related to Mutisioideae, Wunderlichioideae and Gochnatioideae closer to each other than the other three taxa. This result was endorsed by the PCA, with the presence of Barnadesioideae, Calycerace and Mutisioideae in a quadrant and Wunderlichioideae and Gochnatioideae in the other.

While the tribal and generic dendrograms were different than expected especially because some groups weren't grouped together, this peculiarities observed in the chemical evolution of groups show us how different the evolutionary history of a group is when compared with other types of markers.

Conclusions

This is the first study using only flavonoids as markers for Asteraceae, and though there were some differences in the created dendrograms their similarities with the phylogeny is visible.

Seeing these similarities and the support the groups had with just chemical characters lead us to believe flavonoids are good taxonomic markers for Asteraceae, at least for subfamilies Barnadesioideae, Mutisioideae, Wunderlichioideae and Gochnatioideae. Further studies are still necessary to test them for the whole family.

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Conclusão

Esta é a primeira publicação a trabalhar com o padrão químico de Mutisieae *sensu lato* em mais de 40 anos, a única a considerar o perfil químico após a segregação desta tribo. Há agora um banco de dados com o perfil químico flavonoídico com a circunscrição atual das subfamílias Barnadesioideae, Mutisioideae, Wunderlichioideae e Gochnatioideae.

Além disso finalmente foi testado o potencial de Flavonoides como marcadores quimiotaxonômicos para esta porção da família e tal potencial foi confirmado utilizando métodos atualmente empregados em quimiotaxonômia. Mais estudos serão necessários para confirmar a validade para toda a família.

Fazendo uma análise de subfamília vimos que no grupo estudado há cinco grupos químicos, que são caracterizados principalmente pelo tipo de substituinte e a posição onde este é inserido na molécula. Um padrão de diferenciação foi observado ao longo das linhagens e tendeu à substituição em posições além de C_3 e à metilação de flavonoides, moléculas que costumam ser mais estáveis e produzidas com menor gasto energético.

Apesar da baixa utilização da quimiotaxonomia como ferramenta e da quantidade de informação química ser baixa para certos grupos, ela se mostrou uma ferramenta eficiente e pode acrescentar muitas informações evolutivas e da biologia das espécies.

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Considerações finais

O potencial de flavonoides como marcadores taxonômicos de Asteraceae finalmente foi testado, pelo menos para a porção basal da família. Uma redescrição de flavonoides encontrados por espécie foi feita, considerando as circunscrições atuais das subfamílias Barnadesioideae; Mutisioideae; Wundelichioideae e Gochnatioideae.

Essa tabela foi analisada qualitativamente e quantitativamente. A análise qualitativa foi quanto ao tipo de esqueleto flavonoídico encontrado por tribo, sendo possível observar um padrão de ocorrência de certos tipos de flavonoides para cada tribo estudada, e há uma tendência evolutiva entre elas. As análises quantitativas mostraram como essas subfamílias se relacionam quimicamente e que essa história é bem similar a da filogenia aceita.

Observamos que os flavonoides tendem de esqueletos mais simples glicosilados em C3 à esqueletos mais complexos metilados em várias posições, inclusive bi a trimetilações. A subfamília Barnadesioideae se mostrou mais relacionada com a família Calyceraceae, sendo a subfamília mais basal de Asteraceae o que foi observado faz bastante sentido. Esses dois clados são próximos à Mutisioideae, que está localizada no mesmo quadrante da PCA. Wunderlichioideae e Gochnatioideae são mais próximas entre si que dos outros clados estudados, tendo mostrado esse comportamento tanto nos dendrogramas por análise de cluster quanto pelo PCA.

Anexos

Supplementary Material

Table 2- Flavonoid Compounds in Barnadesioideae/Mutisioideae/Wunderlichioideae/Gochnatioideae (BMWG)

Specie	Flavonoid	Ref
Barnadesioideae		
Arnaldoa weberbaueri (Muschl.) Ferreyra	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
Barnadesia aculeata (Benth.) I.C.Chung	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Quercetin-3-O-rutinoside Eriodictyol	(Bohm & Stuessy, 1995)
B. arborea Kunth	Kaempferol-3-O-glucoside Quercetin-3-O-glucoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside Eriodictyol	(Bohm & Stuessy, 1995)
B. dombeyana Less.	Kaempferol-3-O-glucoside Quercetin-3-O-glucoside Quercetin-3-O-rhamnoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
B. hutchinsoniana Ferreyra	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)

	Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside	
B. jelskii Hieron.	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
B. kingii H.Rob.	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Quercetin-3-O-rhamnoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
B. lehmannii Hieron.	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-glucoside Quercetin-3-O-rhamnoside Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
B. odorata Griseb.	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995; Mendiondo, Juárez, & Seeligmann, 1997)
B. parviflora Spruce ex	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)

Benth. & Hook.f.	Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside Isorhamnetin-3-O-glucoside Eriodictyol	
B. spinosa L.f.	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Quercetin-3-O-rhamnoside Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
Chuquiraga atacamensis Kuntze	Quercetin-3-O-glucoside Quercetin-3-O-rutinoside Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside	(Juárez & Mendiondo, 2002)
C. aurea Skottsb.	Kaempferol-3-O-glucoside Quercetin-3-O-glucoside Quercetin-3-O-rhamnoside	(Bohm & Stuessy, 1995)
C. avellanedae Lorentz	Quercetin-3-O-glucoside Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside	(Mendiondo, Juárez, & Seeligmann, 2000)
C. calchaquina Cabrera	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol	(Mendiondo *et al.*, 1997)
C. erinacea D. Don	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Quercetin-3-O-rhamnoside Kaempferol and/or quercetin-3-O-	(Bohm & Stuessy, 1995; Mendiondo *et al.*, 2000)

	glucuronide Quercetin-3-O-rutinoside Kaempferol	
C. incana D.Don	Kaempferol-3-O-glucoside Quercetin-3-O-glucoside	(Mendiondo *et al.*, 2000)
C. jussieui J.F.Gmel.	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-glucoside Quercetin-3-O-rhamnoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-rutinoside Eriodictyol	(Bohm & Stuessy, 1995)
C. longiflora (Griseb.) Hieron.	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol Quercetin-3-O-glucoside	(Mendiondo *et al.*, 1997)
C. morenonis (Kuntze) C. Ezcurra	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-glucoside Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
C. oblongifolia Sagást. & Sánchez Vega	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-glucoside	(Bohm & Stuessy, 1995)

	Quercetin-3-O-rutinoside	
C. oppositifolia D.Don	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol and/or quercetin-3-O-glucuronide Kaempferol Quercetin-3-O-glucoside Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995; Mendiondo *et al.*, 2000)
C. parviflora (Griseb.) Hieron	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-rutinoside Quercetin	(Juárez & Mendiondo, 2002)
C. rosulata Gaspar	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol Quercetin-3-O-glucoside Quercetin-3-O-rutinoside	(Mendiondo *et al.*, 2000)
C. spinosa (Ruiz & Pav.) D.Don	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Quercetin-3-O-rutinoside	(Senatore *et al.*, 1999)
C. spinosa subsp. rotundifolia (= C. rotundifolia) Wedd	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside Kaempferol and/or quercetin-3-O-glucuronide Quercetin-3-O-glucoside Quercetin-3-O-rutinoside	(Bohm & Stuessy, 1995)
C. straminea Sandwith	Kaempferol-3-O-glucoside Kaempferol-3-O-rutinoside	(Mendiondo *et al.*, 1997, 2011; Juárez & Mendiondo,

	Kaempferol	2002)
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
	Quercetin	
C. ulicinia Hook.	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rhamnoside	
	Quercetin-3-O-rutinoside	
C. weberbaueri Tovar	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Kaempferol and/or quercetin-3-O-glucuronide	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
Dasyphyllum argenteum Kunth	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Kaempferol and/or quercetin-3-O-glucuronide	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rhamnoside	
	Quercetin-3-O-rutinoside	
D. brevispinum Sagást & M. O. Dillon	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Kaempferol and/or quercetin-3-O-glucuronide	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
D. diacanthoides (Less.) Cabrera	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	

	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
D. excelsum (D. Don) Cabrera	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Kaempferol and/or quercetin-3-O-glucuronide	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rhamnoside	
D. popayanense (Hieron.) Cabrera	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Kaempferol and/or quercetin-3-O-glucuronide	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rhamnoside	
	Quercetin-3-O-rutinoside	
D. spinescens (Less.) Cabrera	Kaempferol-3-O-glucoside	(Mendiondo *et al.*, 1997)
	Kaempferol-3-O-rutinoside	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
D. synacanthum(Baker) Cabrera	Kaempferol-3-O-glucoside	(Mendiondo *et al.*, 1997)
	Kaempferol-3-O-rutinoside	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
D. weberbaueri (Tovar) Cabrera	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rhamnoside	
	Quercetin-3-O-rutinoside	
Doniophyton anomalum Kuntze	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995; Juárez & Mendiondo, 2002)
	Kaempferol-3-O-rutinoside	

	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
D. patagonicum (Phil.) Cabrera	Kaempferol-3-O-glucoside	(Mendiondo & Juárez, 2001)
	Kaempferol-3-O-rutinoside	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
Fulcaldea laurifólia (Bonpl.)	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
Poir.	Kaempferol-3-O-rutinoside	
	Kaempferol and/or quercetin-3-O-	
	glucuronide	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
Schlechtendalia luzulaefolia	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
Less.	Kaempferol-3-O-rutinoside	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
Mutisioideae		
Mutisieae		
Gerbera anandria (L.) Sch.	Quercetin	(He *et al.*, 2014)
Bip.	Apigenin-7-O-6-D-glucopyranoside	
G. x hybrida	Apigenin	(Bashandy *et al.*, 2015)
	Kaempferol	
	Pelargonidin	
G. jamersonii Adlam	Apigenin-7-glucoside	(Asen, 1984)
Cultivars	Apigenin-4'-glucoside	
	Apigenin-7-malonylglucoside	
	Kaempferol-3-glucoside	
	Kaempferol-4'-glucoside	
	Kaempferol-3-malonylglucoside	
	Luteolin-7-glucoside	

	Luteolin-4'-glucoside	
	Quercetin-3-glucoside	
	Quercetin-4'-glucoside	
	Quercetin-3-malonylglucoside	
G. piloselloides Cass.	Apigenin-7-O-glucoside	(Wang *et al.*, 2014)
	Apigenin-7-O-rutinoside	
	Kaempferol-3,7-bismanoside	
	Luteolin-7-O-glucoside	
	Luteolin-7-O-rutinoside	
Mutisia acuminata Ruiz &	Isorhamnetin-3-glucoronide	(Daily *et al.*, 1988; Catalano
Pav.	Pelargonidin diglycoside	*et al.*, 1995; Juárez *et al.*,
	Quercetin-3-glucoronide	2003)
	Quercetin	
M. friesiana Cabrera	Isorhamnetin-3-O-rutinoside	(Viturro, Molina, &
	Quercetin-3-O-rutinoside	Schmeda-Hirschmann, 1999)
Nassauvieae		
Dolichlasium glanduliferum	Jaceosidin	(Guerreiro, 1989)
Lag. Ex Hook. & Arn.		
D. lagascae Gillies ex. D. Don	Isosakuranetin	(Zdero *et al.*, 1986)
	Pinocembrim	
Leucheria runcinata D. Don	3, 6,7,3'-Tetra-O-methyl-5,4'-	(Bittner *et al.*, 1994)
	dihydroxyflavone	
	6,4'-dimethyl-5,7-dihydroxyflavone	
Perezia multiflora Less.	Kaempferol-3-O-glucoside	(De Israilev & Gonzalez,
	Kaempferol-3-O-rhamnoside	1994)
Proustia pungens Poepp. ex	Acacetin	(Bittner *et al.*, 1989)
Less.	Acacetin-7-methyl-ether	(Valant-Vetschera &
	Genkwanin	Wollenweber, 2007)
	Isosakuranetin	

	Sakuranetin	
	Rhamnazin	
	Rhamnocitrin	
P. pyrifolia DC.	Dihydroquercetin	(Delporte *et al.*, 2005)
	Quercetin	
Trixis vauthieri DC.	7-O-methyldihydrokaempferol	(Bohlmann *et al.*, 1981)
	5,4'-Dihydroxy-7-methoxyflavone	
	Sakuranetin	
	3,5,4'-Trihydroxy-7-3'-dimethoxyflavanone	
	Padmatin	
	3,4',5,7-tetrahydroxyflavanone	
	3,4',5-Trihydroxy-7-methoxyflavone	
Onoserideae		
Aphylloclados denticulatus (J. Rémy ex Gay.) Cabrera	Apigenin	(Maldonado, Hoeneisen, & Silva, 1988b)
Gypothamnium pinifolium (Phil.)	Kaempferol-3-O-glucoside	(Bohm & Stuessy, 1995)
	Kaempferol-3-O-rutinoside	
	Quercetin-3-O-glucoside	
	Quercetin-3-O-rutinoside	
Plazia daphnoides Wedd.	Acacetin	(Zdero, Bohlmann, & Niemeyer, 1988)
	Genkwanin	
	Isosakuranetin	
	Naringenin	
	Sakuranetin	
Wunderlichioideae		
Wunderlichia crulsiana Taub.	5,3', 4'-trihydroxy-7-methoxy flavone	(André, Dias, & Vichnewski, 2002)
	5,7,3',4'-tetrahydroxy flavone	
	Apigenin-7-O-$-D-(4"trans-O-p-coumaroyl) glucoside	

	Apigenin-7-O-$-D-(4",6" trans-di-O-p-coumaroyl) glucoside	
W. mirabilis Riedel ex Baker	7-O-Methylacacetin	(Bohlmann *et al.*, 1984a)
Gochnatioideae		
Gochnatia foliolosa D. Don ex Hook. & Arn.	3,7-dimetilkaempferol Quercetin-3-Me 3'-metilquercetin Quercetin-3,7-diMe Quercetin-3,3'-diMe Quercetin Ayanin Eriodictyol Rhamnetin Apigenin Genkwanin Rhamnocitrin Ramnazin Kumatakenin	(Faini, Torres, & Castillo, 1984; Valant-Vetschera & Wollenweber, 2007)
G. glutinosa D. Don ex Hook. & Arn.	Sakuranetin Genkwanin 5,7,4'-trihydroxy-3,3'-dimethoxyflavone 5,3',4'-trihydroxy-7-methoxyflavanone 3,7-dimethoxy-5,3',4'-trihydroxyflavone Isokaempferide Kumatakenin Ermanin Kaempferol-7,4'-diMe Kaempferol-3,7,4'- triMe	(Valant-Vetschera & Wollenweber, 2007)

	Quercetin	
	Quercetin-3-Me	
	Rhamnetin	
	Isorhamnetin	
	Quercetin-3,7-diMe	
	Quercetin-3,3'-diMe	
	Eriodictyol	
	Eriodictyol-7-Me	
G. vernonioides Kunth	Sakuranetin	(Bohlmann *et al.*, 1984b)
Moquiniastrum argentinum	Hispidulin	(Garcia & Guerreiro, 1988)
(Cabrera) G. Sancho	Luteolin 7-methyleter	
M. barrosoae (Cabrera) G.	kaempferol 3-O- -D-(6''-O-E-p-	(Santos Júnior *et al.*, 2010)
Sancho	cumaroil)-glicopiranoside	
M. blanchetianum (DC.) G.	Kaempferol-3-O--D-glucopyranoside	(Lima *et al*., 2003)
Sancho	Kaempferol 3-O- -D-(6''-O-E-p-	
	cumaroil)-glicopiranoside	
M. polymorphum (Less.) G.	3-O-Metilquercetin	(Sacilotto, Vichnewski, &
Sancho	Rutin	Herz, 1997; Moreira *et al.*,
	Hyperoside	2000)
	Genkwanin	
	Desmethoxycentaureidin	
M. pulchrum (Cabrera) G.	Genkwanin	(Lucarini *et al.*, 2015)
Sancho	Scutellarin	
	Apigenin	
Nahuatlea obtusata (S. F. Blake) V. A. Funk	Luteolin	(Maldonado, Flores, & Ortega, 1988a)

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