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Principal Component Analysis Of *Heliantheae* (Asteraceae) *Sensu* Stuessy And *Karis and Ryding* Based On Chemical Data

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ABSTRACT

This paper shows some congruencies and incongruencies between the chemical data and morphological analysis of the *Heliantheae tribe sensu Stuessy* and *Karis and Ryding*. The chemical data were obtained from a database containing information about monoterpenes, sesquiterpenes, sesquiterpene lactones, diterpenes, triterpenes, flavonoids, polyacetylenes, coumarins, benzofuranes and benzopyranes isolated from the species of the tribe. The number of occurrences of these secondary metabolites were standardized and analysed using the principal component analysis. The importance of the chemical data to the understanding of the position of the subtribes is discussed on the basis of two main botanical classifications.

KEYWORDS

Heliantheae;
Asteraceae;
Principal componet analysis(PCA).

INTRODUCTION

The family *Asteraceae* has been intensely studied by researchers from several areas, such as chemistry and pharmacy due its large chemical and morphological diversity. Several classifications systems intend to cluster the genera into tribes according to morphological[6,9,11,23,36], macromolecular[21,22,25] and micromolecular[1,12,13] criteria. In the last decades two important publications summarized the knowledge of the most important specialists in this subject. In the first one, the Proceedings of the Reading Symposium[19], the morphology, chemistry and the phenetics of the family were emphasized. In the second one[7], rearranged all genera described since then and described the possible phylogenetic relationships

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among them. In another paper[8], proposed slight modifications of the earlier system. For instance, the subtribe *Cardueae* was elevated to the subfamily status.

Since the symposium, several authors attribute different interpretations to groups for which the classification is still not clear, for example, the tribes *Mutiseae* and *Helenieae*. In the tribe *Heliantheae* for instance, the division described in[8] is the same as that suggested by[23] who performed a large morphological analysis of the *Heliantheae* and *Helenieae* tribes using about 60 genera that represented several of the subtribes belonging to Heliantheae. On the basis of macromolecular data and using cladistical analyses, an evolutionary tree was produced.

The purpose of this paper is to use chemical data related to isolated secondary metabolites of the tribe *Heliantheae* (*Asteraceae*) and to compare them with the two classifications described in the literature. In order to perform our analyses a database containing chemical information about the family was used.

The chemical database

The chemistry of the *Heliantheae* is extremely complex and several classes of secondary metabolites were isolated from representatives of the tribe (Figure 1). Polyacetylenes (P) have been exhaustingly studied[3], a complementary review has been published[10] and this was updated using references cited in the Chemical Abstract. Monoterpenes (M) were revised by Ferreira (1999). Sesquiterpene lactones (L) and diterpenes (D) were revised by Seaman (1982) and Seaman *et al.* (1990), respectively. Triterpenes (T) have also been reviewed by Macari (1994). The chemistry and the evolution of the flavonoids (F) in the whole family are very well discussed in a recent book[4] and in two papers by Emerenciano *et al* and Ferreira *et al*[14,16]. Benzofuranes and benzopyranes (B) were reviewed by Proksch and Rodriguez (1983). Sesquiterpenes (S) are part of our database denominated SISTEMAT[29] and coumarins (C) were reviewed by Murray (1991) and updated by Brant (2003).

Stuessy's classification system

Stuessy (1977) attempted to establish phylogenetic relationships among the subtribes of the *Heliantheae* on the basis of their number of chromosomes and morphology. Among 15 subtribes recognized by the author (Figure 2), three evolutionary lines were emphasized: the first one having the subtribe *Verbesininae* in the center with chromosome numbers based mainly on x = 15, x = 16 and 17 and their aneuploid derivatives, the second one centred around the tribe *Coreopsidinae* tribe with chromosomic bases of x=12, and the third one having the tribe

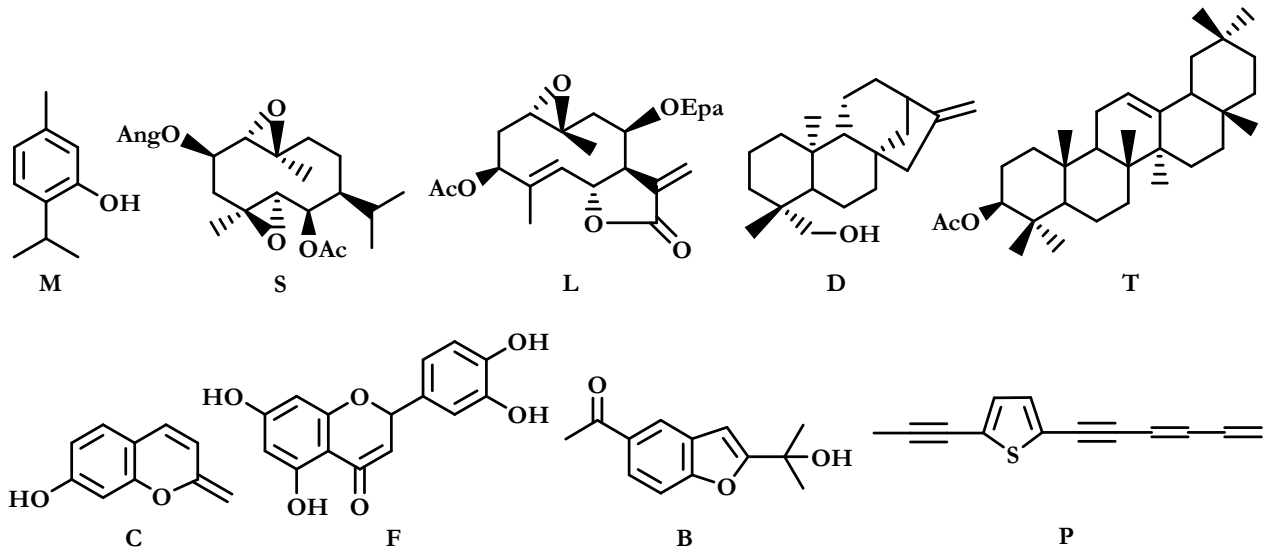

Figure 1: Examples of secondary metabolites isolated from species of the tribe *Heliantheae*

Monoterpenos(M); Sesquiterpenes (S), Diterpenes (D), Polyacetylenes (P) and Triterpenes(T); Sesquiterpenes Lactones (L); Coumarins (C); Flavonoids (FLAV); Benzofuranes (B).

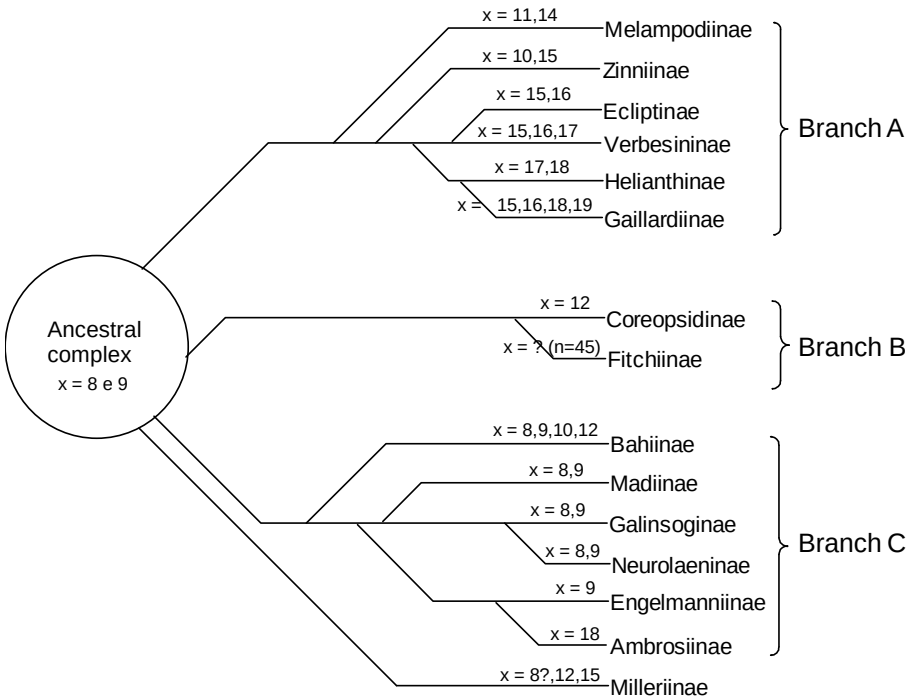

Figure 2: Hypothetic relationships among the Heliantheae subtribes according to Stuessy (1977). The number in parenthesis indicates the number of chromosome in each subtribe.

Galisonginae in the center with chromosome numbers varying from x=8 until x=18. The subtribes *Gaillardinae* and *Bahiinae*, classified by Hoffmann (1890) as belonging to the tribe *Helenieae* were transferred to the *Heliantheae* by *Stuessy*. As a reason for this procedure, the author claimed morphological similarities between these two tribes.

Considering n=8 and n=9 the smallest chromosomie numbers found in the *Heliantheae* and the fact that the herbaceous habit is always present in the most of their representatives, Stuessy suggested that the ancestral complex possessed attributes of the three evolutionary lines, specially of the two largest. Morphological characteristics belonging to the evolutionary line *Galinsoginae* necessarily reflect the ancestral condition.

Others systems of classification envolving *Heliantheae* must be cited. Among them it is important to emphasize Robinson (1981) and Baldwin (2002). However, a comparison of the four systems will subject of a next work.

Methods

The data relative to *Heliantheae sensu Stuessy* and *sensu Karis* and *Ryding* were extracted from the chemistry and taxonomic database of the *Asteraceae* which resulted in a smaller database containing about 6000 occurrences of compounds in species grouped by genera in subtribes (TABLE 1 and 3, generated by PCA analysis[37] respectively). From the data shown in TABLE 1, it is observed, for example, that for the subtribe *Helianthinae* (HEL–third row) 245 species (second column) were chemically studied from the 292 extant species (third column). From this subtribe 57 monoterpenes (M), 30 sesquiterpenes (S), 364 lactones (L), 271 diterpenes (D), 18 triterpenes (T), 24 coumarins (C), 398 flavonoids (F), 232 polyacetylenes (P) and 168 benzofuranes (B) were isolated.

The occurrences are obtained by counting how many times a compound of a given group appears in a determined species. A compound X can appear n times within a delimited taxon, genus, subtribe, family, etc. We defined NCOR (number of occurrences) as the number of times that a unique substance appears in the studied taxon (TAX), counting the number of times that it appears in each species (ESP). NCOR can be mathematically defined as:

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TABLE 1: Total occurrence numbers of some classes of secondary metabolites in *Heliantheae* tribe (*sensu Stuessy*)

Subtribe	Stu	Exis	M	S	L	D	T	C	F	P	B
ECL	55	183	46	1	72	137	7	8	2	117	0
GAI	96	114	80	6	358	0	0	0	50	42	0
HEL	245	292	57	30	364	271	18	24	398	232	168
MEL	60	156	46	6	179	154	0	2	36	48	3
VER	84	315	315	48	141	352	11	0	6	68	4
ZIN	22	42	0	10	72	6	0	0	0	40	0
COR	168	480	14	39	3	1	1	4	190	836	0
FIT	1	6	0	0	7	0	0	0	0	3	2
AMB	96	96	147	32	418	3	6	0	34	59	0
BAH	51	161	53	17	146	0	6	2	8	36	12
ENG	11	31	0	0	4	31	5	0	0	6	0
GAL	24	111	29	15	2	1	5	0	0	87	0
MAD	48	87	0	0	0	6	0	0	0	0	0
NEU	22	34	11	1	56	13	0	0	13	12	0
MIL	43	79	61	1	27	71	1	0	166	34	4

Stu and Exis: refer to the numbers of species studied chemically and existing respectively; M, S, L, D, T, C, F, P and B: refer to the number of occurrences pertaining to the Monoterpene, Sesquiterpene, Sesquiterpene lactone, Diterpene, Triterpene, Coumarin, Flavonoid, Polyacetylene and Benzofurane classes, respectively, isolated from the respective subtribe. The subtribes are indicated as Ecliptinae (ECL), Gaillardiinae (GAI), Helianthinae (HEL), Melampodiinae (MEL), Verbesininae (VER), Zinninae (ZIN), Coreopsidinae (COR), Fitchiinae (FIT), Ambrosiinae (AMB), Bahiinae (BAH), Engelmanniinae (ENG), Galinsoginae (GAL), Madiinae (MAD), Neurolaniinae (NEU) and Milleriinae (MIL).

TABLE 2: Corrected occurrence numbers of secondary metabolites in *Heliantheae* tribe (*sensu Stuessy*)

Subtribe	Stu	Exis	MC	SC	LC	DC	TC	CC	FC	PC	BC
ECL	55	183	153.1	3.33	239.6	455.8	23.3	26.62	6.65	389.3	0
GAI	96	114	95	7.13	425.1	0	0	0	59.4	49.88	0
HEL	245	292	67.93	35.8	433.8	323	21.5	28.6	474	276.5	200
MEL	60	156	119.6	15.6	465.4	400.4	0	5.2	93.6	124.8	7.8
VER	84	315	1181	180	528.8	1320	41.3	0	22.5	255	15
ZIN	22	42	0	19.1	137.5	11.45	0	0	0	76.36	0
COR	168	480	40	111	8.57	2.85	2.86	11.43	543	2389	0
FIT	1	6	0	0	42	0	0	0	0	18	12
AMB	96	96	147	32	418	3	6	0	34	59	0
BAH	51	161	167.3	53.7	460.9	0	18.9	6.31	25.3	113.6	38
ENG	11	31	0	0	11.27	87.36	14.1	0	0	16.91	0
GAL	24	111	134.1	69.4	9.25	4.62	23.1	0	0	402.4	0
MAD	48	87	0	0	0	10.88	0	0	0	0	0
NEU	22	34	17	1.55	86.55	20.09	0	0	20.1	18.55	0
MIL	43	79	112.1	1.84	49.6	130.4	1.84	0	305	62.47	7.3

Stu and Exis: refer to the numbers of species studied chemically and existing respectively, respectively; MC, SC, LC, DC, TC, CC, FC, PC and BC: refer to the number of corrected occurrence number pertaining to the Monoterpene, Sesquiterpene, Sesquiterpene lactone, Diterpene, Triterpene, Coumarin, Flavonoid, Polyacetylene and Benzofurane classes, respectively, isolated from the respective subtribe. The subtribes are indicated The subtribes are indicated as Ecliptinae (ECL), Gaillardiinae (GAI), Helianthinae (HEL), Melampodiinae (MEL), Verbesininae (VER), Zinninae (ZIN), Coreopsidinae (COR), Fitchiinae (FIT), Ambrosiinae (AMB), Bahiinae (BAH), Engelmanniinae (ENG), Galinsoginae (GAL), Madiinae (MAD), Neurolaniinae (NEU) and Milleriinae (MIL).

TABLE 3: Total occurrence numbers of secondary metabolites in Heliantheae tribe *sensu* (Karis & Ryding)

Subtribe	Stu	Exis	M	S	L	D	T	C	F	P	B
RUD	31	31	10	18	31	2	0	1	10	139	0
ZIN	35	109	24	10	72	6	1	0	0	95	1
VER	189	541	75	61	583	472	173	70	122	123	151
HEL	153	292	37	11	251	268	1	20	284	71	25
GAL	109	189	0	0	0	1	4	0	0	47	0
MEL	111	200	99	40	444	108	7	4	65	107	15
COR	163	443	13	19	10	0	1	4	190	827	2
ENG	11	31	0	0	4	31	5	0	0	6	0
AMB	80	83	147	32	418	3	6	0	34	59	0

Stu and Exis: refer to the numbers of species studied chemically and existing respectively, M, S, L, D, T, C, F, P and B: refer to the number of occurrences pertaining to the Monoterpene, Sesquiterpene, Sesquiterpene lactone, Diterpene, Triterpene, Coumarin, Flavonoid, Polyacetylene and Benzofurane classes, respectively, isolated from the respective subtribe. The subtribes are indicated as Rudbeckiinae (RUD), Zinninae (ZIN), Verbesininae (VER), Helianthinae (HEL), Galinsoginae (GAL), Melampodiinae (MEL), Coreopsidinae (COR), Engelmanniinae (ENG), Ambrosiinae (AMB).

$$NCOR = \sum_{\mathbf{c}\text{lass}(n)} COMP[TAX],$$

where COMP is counted by species and class represents a 'delimited' chemical metabolite. NCOR indicates the supremacy of a chemical class over the others.

For instance, whereas the number of extant species in *Ambrosiinae* (AMB) and *Gailardiinae* (GAI) subtribes (according to Bremer) corresponds to 96 and 114 respectively, the NCOR for monoterpenes, in *Ambrosiinae*, is equivalent to 147 and in *Gailardiinae* (GAI) is equal to 80, which result in numbers that are not really comparable.

Another reason that inhibits the direct use of the number of occurrences in chemotaxonomic studies is the fact that some species have not been chemically studied yet, and as a result it is necessary to normalize these numbers. In others chemotaxonomic articles, these facts have not been considered[18].

In this paper, suppose two hypothetic taxa TA and TB. Consider, for instance, that TA has the number of occurrences equivalent to 50 and number of studied species equivalent to 100 and TB has the same number of occurrences of TA but a different number of studied species, 200. Consequently, we will have as a result N occurrences of some compounds in TA having the double value of the number of occurrences in TB. In order to consider this fact, the variable NCORc was introduced through the equation: NCORc = NCOR*(Nesp/Exist). The original data *sensu Stuessy* and *Karis* and *Ryding* are shown in TABLE 1 and 3, respectively. In TABLE 2 and 4 the corrected data for the chemical classes were named MC (Monoterpenes Corrected), SC (Sesquiterpenes Corrected), LC (Sesquiterpene lactones Corrected), DC (Diterpenes Corrected), TC (Triterpenes Corrected), CC (Coumarins Corrected), FC (Flavonoids Corrected), PC (Polyacetylenes Corrected) and BC (Benzofuranes Corrected).

RESULTS

Figure 2 indicates that the subtribe *Milleriinae* is completely separated from the others. This behavior is associated with the large diversity of flavonoids and high modified coumarins available in this kind of tribe, which is extremely consistent to the Stussey diagram.

In Figure 3 which presents the separation of the subtribes according to the PCA analysis it is verified that a cluster is formed by the subtribes *Helianthinae* (HEL), *Verbesininae* (VER) and *Ecliptinae* (ECL) which are positioned according to Stuessy in his first lineage. However, the subtribe *Zinninae* (ZIN) that also belongs to this linage appears in a different position compared to the clusters mentioned above. The remained tribes, *Melampodiinae* (MEL) and *Gaillardiinae* (GAI) are not separated through the PCA's analyses.

Stuessy's third lineage appears in the PCA analy-

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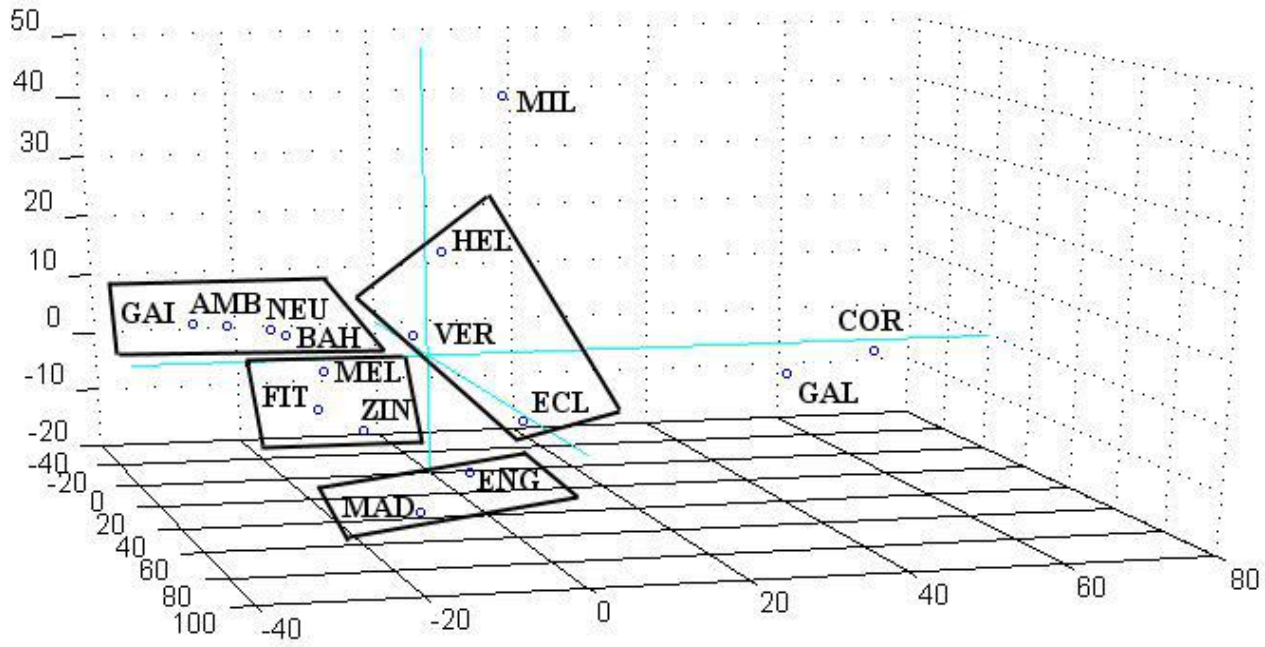

Figure 3: Score plot of the samples of TABLE 2 (*sensu* Stuessy) in planes defined by the principal components PC1–PC2–PC3 obtained from the data of TABLE 1

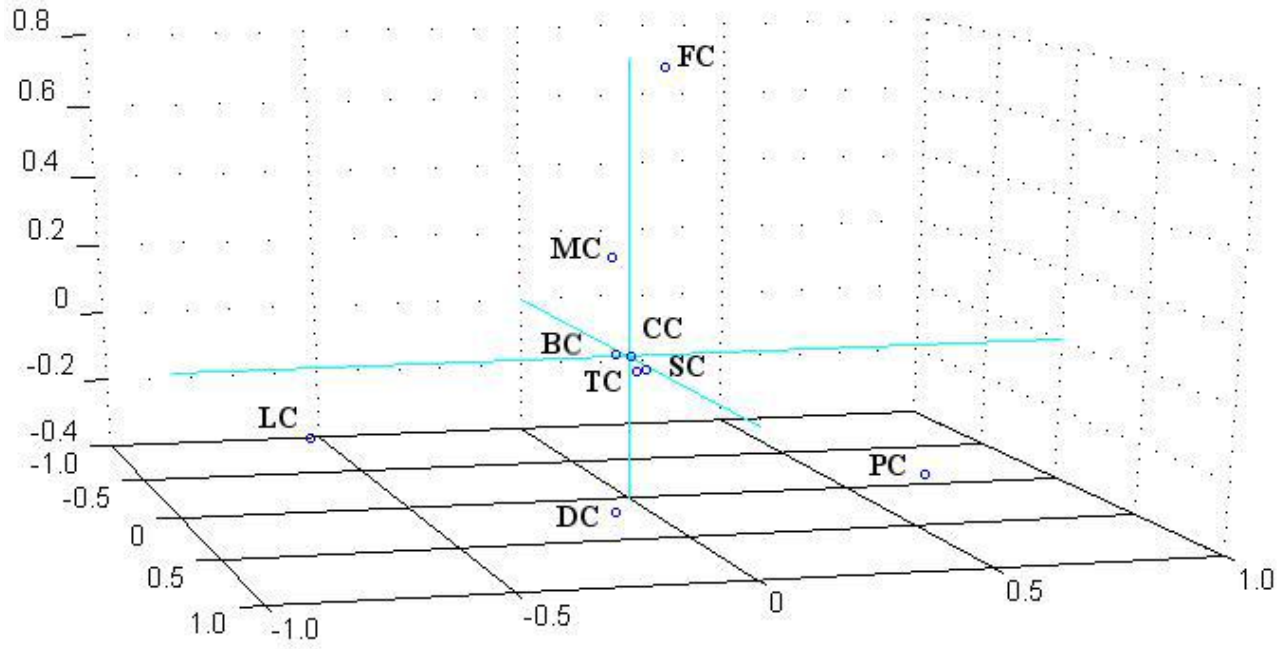

Figure 4: 3D Plot of the X-loadings of 9 variables in PC1–PC2–PC3 coordinate system, obtained from the data of TABLE 2 (Stuessy)

sis separated from the others. Considering the chemical similarities and the presence of diterpenes that characterize them strongly, the subgroup composed by the subtribes *Madiinae* (MAD) and *Engelmanniinae* (ENG) constitute a very well defined cluster. Another subgroup, located separated from the group described before is formed by *Neurolaeminae* (NEU), *Ambrosiinae* (AMB) and *Bahiinae* (BAH). However,

these tribes are linked to the *Gaillardiinae* (GAI) that belongs to the first lineage according *Stuessy*. TABLE 2 shows the small expression of diterpenes (DC), triterpenes (TC) and coumarins (CC) and the high number of sesquiterperne lactones (LC). In view of this fact *Gaillardiinae* (GAI) also appears as a subtribe of the third lineage which is completely inconsistent with the Stuessy´s classification.

It is very interesting to observe that in Stuessy´s classification, the third lineage is centralized, from the botanical point of view, in *Gaillardiinae* (GAI). From the chemical point of view, this subtribe is completely separated from the others since the great production of polyacetylenes and as a result it appears separated from the others in the diagram. It is also important to observe the influence of the variable flavonoid (Figure 4) to the separation of the *Milleriinae* subtribe that appears apart from the others (Figure 3) and in agreement with Bremer´s classification (Figure 2).

The subtribes *Engelmanniinae* (ENG) and mainly *Madiinae* (MAD) both with x=8 and x=9, reported by Stuessy as belonging to the evolutionary line *Galinsoginae* (GAL) emerge, in the PCA analyses (Figure 3), in isolated positions.

Figure 5 shows that the subtribes are separated *sensu Karis* according to PCA's analyses. In addition to the fact that the genera considered in each subtribe by *Karis* and *Ryding* are different, the number of occurrences in each taxon modifies according to this classification and obviously the number of corrected occurrences also changes (TABLE 4). Comparing our results to the cladistic analysis of *Karis* and *Ryding* we found several similarities between the chemistry and the botanical's view. The first clade of the diagram *sensu Karis* shows that the tribes *Rudbeckiinae* (RUD), *Zinniinae* (ZIN) and *Verbesininae* (VER) are together in a cluster and they are under strong influence of monoterpenes, sesquiterpenes, benzofuranes, triterpenes and coumarins (Figure 6). *Helianthinae* (HLT) and *Melampodiinae* (MEL) produce another cluster in the space defined by PC1-PC2-PC3 which is consistent to Karis' diagram. Although *Coreopsidinae* (COR) and *Engelmanniinae* (ENG) show great disagreement related to the diversity of flavonoids and polyacetylenes (TABLE 4) they are positioned in the right side of the diagram. Figure 5 shows that the subtribes *Ambrosiinae* (AMB) and *Galinsoginae* (GAL) occupy different positions in the diagram which is consistent with the chemical data. In the former the

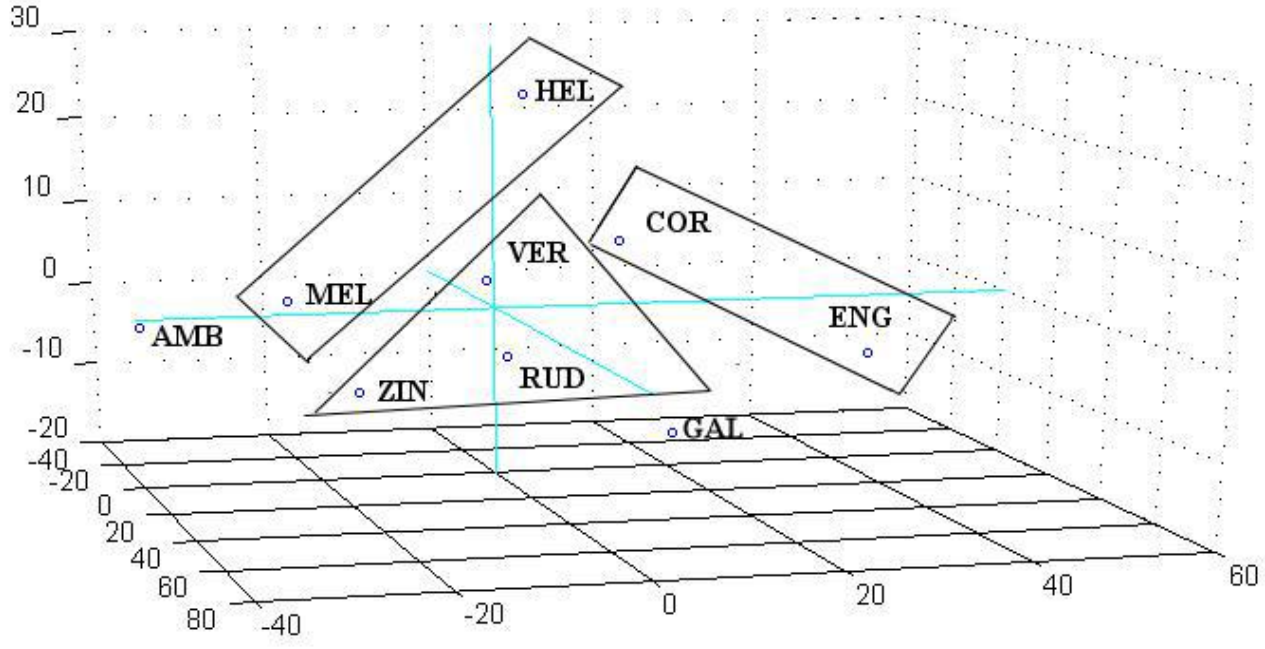

Figure 5: Score plot of the samples of TABLE 4 (sensu *Karis* and *Ryding*) in planes defined by the principal components PC1–PC2–PC3, obtained from the data of TABLE 3

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TABLE 4: Corrected occurrence numbers of secondary metabolites in *Heliantheae* tribe (*sensu Karis* and *Ryding*)

Subt	Stu	Exis	MC	SC	LC	DC	CT	CC	FC	PC	BC
RUD	31	31	10	18	31	2	0	1	10	139	0
ZIN	35	109	7.71	3.211	23.12	1.93	0.32	0	0	30.5	0.32
VER	189	541	26.2	21.31	203.67	165	60.44	24.5	42.62	43	52.75
HEL	153	292	19.4	5.764	131.52	140	0.52	10.5	148.8	37.2	13.1
GAL	108	167	0	0	0	0.65	2.59	0	0	30.4	0
MEL	98	200	48.5	19.6	217.56	52.9	3.43	1.96	31.85	52.4	7.35
COR	163	443	4.78	6.991	3.68	0	0.37	1.47	69.91	304	0.74
ENG	6	23	0	0	1.04	8.09	1.30	0	0	1.57	0
AMB	96	83	170	37.01	483.47	3.47	6.94	0	39.33	68.2	0

Stu and Exis: refer to the numbers of species studied chemically and existing respectively; MC, SC, LC, DC, TC, CC, FC, PC and BC: refer to the number of corrected occurrence number pertaining to the Monoterpene, Sesquiterpene, Sesquiterpene lactone, Diterpene, Triterpene, Coumarin, Flavonoid, Polyacetylene and Benzofurane classes, respectively, isolated from the respective subtribe. The subtribes are indicated as Rudbeckiinae (RUD), Zinninae (ZIN), Verbesininae (VER), Helianthinae (HEL), Galinsoginae (GAL), Melampodiinae (MEL), Coreopsidinae (COR), Engelmanniinae (ENG), Ambrosiinae (AMB).

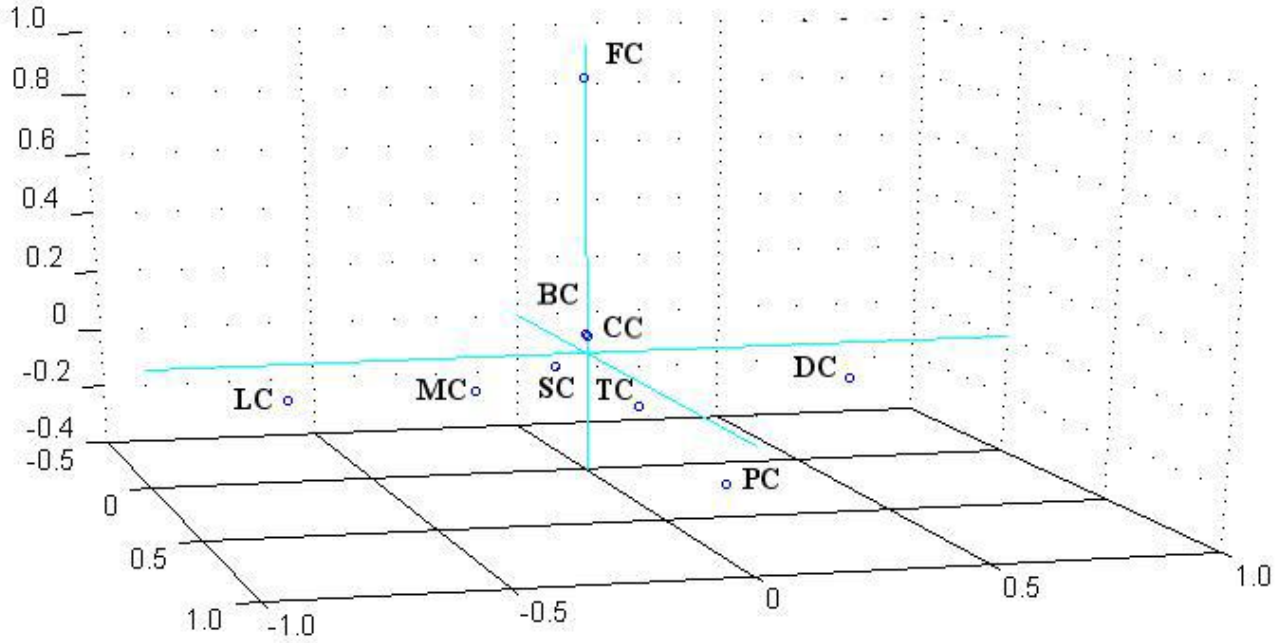

Figure 6: 3DPlot of the X-loadings of 9 variables in PC1–PC2–PC3 coordinate system, obtained from the data of TABLE 4, (*sensu Karis* and *Ryding*)

chemistry is based on sesquiterpene lactones and the second the chemistry is centralized in polyacetylenes.

CONCLUSIONS

This work shows a comparison of chemical data related to isolated secondary metabolites in *Asteraceae* at the level of the subtribes considering two botanical classifications. Polyacetylenes, flavonoids and sesquiterpene lactones revealed to be strong chemotaxonomic markers at the tribe level.

Stuessy's classification[32] includes the most part of the genera described and although does not possess phyletic character it could be compared, in the context of this work, with the chemical focus obtained through the analysis of the main component.

The subtribe *Milleriinae* (MIL), in his classification, is not included in these lineages, fact that is consistent with the chemical data. The second Stuessy's lineage is not in agreement with the chemical data. The subtribes *Fitchiinae* (FIT) and *Coreopsidinae* (COR) are totally apart from it due the great number of polyacetylenes in *Coreopsidinae* (COR).

The third Stuessy's lineage is considered as a group of taxa not defined. Although the subtribe *Milleriinae* (MIL) shows some morphological similarities to third lineage, in Stuessy's classification it is placed in a subtribe separated from the others three ones. In another work that concurs chemically with this point of view, it appears in the PCA diagram in a very distant position compared to the others subtribes group in the third lineage. The reason for such behavior is that *Milleriinae* (MIL) is under strong influence of flavonoids, diterpenes and monoterpenes.

The cladistic analysis of *Karis* and *Ryding* is very incomplete because it has been performed using species from 64 genera. Considering that the tribe possesses the numbers of genera varying from 190 until 200, this sample used by the authors is considered small. The author dispose of some subtribes belonging to Stuessy's classification and created other ones such as *Rudbeckiinae*. The chemical data are fundamental to arrange five subtribes which *Karis* placed together in his cladistic classification: *Melampodiinae* (MEL), *Zinniinae* (ZIN), *Verbesininae* (VER), *Rudbeckiinae* (RUD) and *Galinsoginae* (GAL). *Ambrosiinae* (AMB) and *Engelmanniinae* (ENG) constitute another branch in *Karis*' diagram and are also very far from the others one from the chemical point of view.

The use of PCA in the taxonomic studies can be compared with the two types of classification obtained to *Heliantheae* and some correlations between the chemical and botanical data are seen. As well as the others subgroups there are still some doubts about the evolution of this tribe and we considered that the introduction of the one or more chemical descriptors could be useful to improve the discussion about the evolution of the groups within the *Asteraceae* family.

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