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Gas Chromatography-Mass Spectrometry Profiles of Ten *Pilea* Species (Urticaceae)

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Abstract

The genus *Pilea* Lindley is the largest among the Urticaceae family. The large size and little commercial interest of this genus make difficult its taxonomy, with few revisions since Weddell. Very few revisions have been reported about the Colombian species since 1939, when Killip made a contribution to the genus for the northern Andes (Ecuador, Peru, Colombia, and Venezuela). To contribute to the knowledge of the *Pilea* genus in Colombia, field trips were made in the department of Valle del Cauca and ten herbarium samples were analyzed using gas chromatography-mass spectral profiles analysis; which allowed us to identify 33 compounds type monoterpene, sesquiterpene, diterpene, free fatty acids or their methyl, ethyl esters or amides, and triterpenes. It was concluded that Triterpenes were the best chemotaxonomical discriminants for the ten herbarium samples as ten species.

Keywords: Urticaceae, *Pilea*, terpenoids, steroids, fatty acids, gas chromatography, mass spectrometry, DPPH

1 Introduction

The genus *Pilea* Lindley is the largest among the 54-79 genera belonging to Urticaceae family, which contribute with about 500-715 species distributed around the world through the tropics and subtropics, except in Australia, New Zealand, and Europe [1-6]. This genus large size and little commercial interest make difficult its taxonomy, with few revisions since Weddell [3-6]; with some localized treatments increasing 562 names and 17 subgeneric groups. Very few revisions have been made to the Colombian species since 1939, when Killip [7] made a contribution to the genus for the northern Andes (Ecuador, Peru, Colombia and Venezuela). In order to contribute to the knowledge of the genus *Pilea* in Colombia, field trips were made in the department of Valle del Cauca. *Pilea* has approximately 90 species in Colombia, these are the most abundant in the three mountain ranges, at an altitude between 1000-3000 m. Five unpublished species and five new species were found to Colombia. Most species are confined to the shady understory and grow at the edge of water bodies (creeks, streams and waterfalls).

To support the process of taxonomic revision of the genus in Valle del Cauca, preliminary gas chromatography-mass spectrometry profiles of 10 species, collected by the herbarium of the Universidad del Valle, were performed in gas chromatography-mass spectrometry, using samples in amounts ranging between 0.0616 and 1.1992 g, to establish similarities and differences.

The ten GC-MS profiles of ethanolic extracts allowed us to identify 33 compounds type monoterpene, sesquiterpene, diterpene. Free fatty acids or their methyl, ethyl esters or amides, and triterpenes (Figures 1 and 2) were identified and the differences can be used as supplementary chemotaxonomic criteria for the identification of studied species.

Analysis by HPLC/MS-ESI of morphotype identified as Pilea microphylla (M-01) allowed the identification of two glycosylated flavonoids, apigenin-7-O-rutinoside[8, 9] and diosmetin-7-*O*-rutinoside [10].

2 Materials and Methods

2.1 Plant materials

The leaves of all species studied were taken from specimens at the Luis Sigifredo Espinal Tascón Herbarium CUVC, Universidad del Valle, which were collected and provisionally identified by Ana Isabel Vasquez Velez. Collection, voucher numbers, and sample masses are summarized in table 1.

Table 1. *Collection, voucher numbers, and sample masses of plant species used for extraction.*

Colection	Name	Voucher	Mass (g)
M-01	*Pilea microphylla*	CUVC-176	0.1030
M-05	*Pilea involucrata*	CUVC-145	0.0616
M-10	*Pilea* sp	CUVC-140	0.1926
M-12	*Pilea* sp	CUVC-151	0.1338
M-19	*Pilea scandens*	CUVC-171	0.2242
M-24	*Pilea* sp	CUVC-172	0.1210
M-25	*Pilea* sp	CUVC-151	0.2907
M-27	*Pilea* sp	CUVC-144	1.1992
M-29	*Pilea* sp	CUVC-157	0.8524
M-35	*Pilea* sp	CUVC-170	0.1430

2.2 Extraction

The leaves of each species were extracted by ultrasound in 95% ethanol (15 min x 4 mL x 3), evaporated at reduced pressure, and concentrated to 1 mL for free radical scavenging activity.

Thin layer chromatography (TLC), combined with the colorimetric estimation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) test [11] and gas chromatography mass spectrometry (GC-MS) analysis was used.

2.3 Gas Chromatograhpy-Mass spectrometry (GC-MS) Analysis

Each sample (1 uL) was injected to a RTX-5MS column (30 m x 0.25 mm I.D, 0.25 film) instaled in a Shimadzu GC-MS-QP2010 system equipped with an autosampler/autoinjector AOC-2010. Heliun was used as carrier gas at 1 mL/min controled by lineal speed, injector Split/splitless in Split mode at Split ratio: 4:1, injection temperature: 300 °C, temperature programming: 40 °C por 4 min, 15 °C/min up to 320 °C, 320 °C hold 10 min, interphase temperature: 280 °C, ionization source temperature: 240 °C, detector in Scan mode 70 eV, mass range: 35-700 Da.

3 Results and discussion

The thin layer chromatographic profiles on Sigel 60G F254 did not permit any compelling similarities and differences. For this reason, efforts were focused on the GC-MS profiles (Figure 5); which lead to the identification of 33 secondary metabolites, based on their electron ionization mass spectra, compared with the databases Wiley 8.0 database Standard Reference NIST Number 69, massbank, Spectral database for Organic Compounds SDBS (AIST Japan), and corroborated by the arithmetic and mechanistic analysis with charge location.

Table 1 summarizes the retention times, base and molecular ion peaks for the 33 identified compounds and their distribution through the 10 species analyzed. The structures of the compounds are summarized in Figures 1 and 2. Three compounds in common, hexadecanoic acid **(8)** [12], **(9E)**-octadecenamide **(15)** [13] and sitosterol **(20)** [14] were detected. Oleic acid **(10)** [15] was identified in 8 of the 10 species, followed by phytol **(9)** [16] found in 5 species. Other metabolites, especially triterpenoid type **(16-33)** show differences that separate the various species. These differences demonstrate that the ten samples may correspond to different species, consistent with the identification of some of them as species *Pilea microphyla* (M-01), *P. involucrata* (M-05), *P. scandens* (M-19), *P. gallowayana* (M-25) and *P. pteropodon* (M-29).

HPLC-DAD-ESI-MS analysis of *Pilea microphylla* extract reveals the presence of two glycosylated flavones, apigenin-7-*O*-rutinoside [8, 9] (Flavonoid 1, F1) and Diosmetin-7-*O*-rutinoside [10] (Flavonoid 2, F2). Aglycones were evidenced by their UV-Vis spectra and molecular mass by ESI-MS spectra in positive and negative mode (Figures 3 and 4).

Table 1. Distribution of metabolites in ten *Pilea* specie

					Species									
	t_R min	Compound names	Base	$[M]^{+\cdot}$	1	5	10	12	19	24	25	27	29	35
1	14,342	β-selinene [17]	93	204									3.19	
2	14,558	Teresantalol[18]	121								2.88			
3	16,1	Terpineol [19]	59								4.35			
4	16,8	Neophytadiene [20]	68	278			1.05		1.33		1.28	4.61		
5	16,867	6,10,14-trimethylpentadecan-2-one [21]	43	....					1.47		1.29			
6	17,108	Phytadiene [22]	57	278								3.61		
7	17,4	hexadecanoic acid methyl ester [23]	74	270					1.75					
8	17,658	hexadecanoic acid [24]	73	256	5.96	87.72	19.47	17.76	47.8	58.65	15.01	46.42	6.58	53.71
9	18,633	Phytol [25]	71	...			2.06	10.64	6.83	5.70		5.67		
10	18,8	9-(*Z*)-Octadecenoic Ácid (Oleic acid) [15]	55	264		2.63	3.43	6.98	15.1	7.70	7.78	8.04	1.82	
11	18,925	Octadecanoic Acid [26]	41	284					2.36					
12	18,942	Ethyl oleate [27]	55	310		2.91		1.08						8.18
13	20,142	(*9Z*)-Octadecenamide [13]	59	281			1.01		1.94	4.05		5.69		
14	20,258	bis(2-ethylhexyl) adipate [28]	129						3.02					
15	22,258	(*9E*)-Octadecenamide [13]	59	281	1.53	5.51	17.81	22.14	11.70	18.50	5.12	8.35	9.69	22.70
16	25,233	Ursa-9(11),12-dien-3-one [29]	422	422							13.75	1.35	16.13	
17	25,292	Lup-9(11),20(29)-dien-3,28-diol [30]	136	440	27.28									
18	25,467	Ursa-9(11),12-dien-3-ol [29]	424	424									9.66	
19	25,525	28-hydroxylup-20(29)-en-3-one	397	440	4.72									
20	25,625	Sitosterol	43	414	8.18	1.23	4.61	6.41	2.38	5.41	3.95	17.61	11.66	1.66
21	25,658	olean-9(11),12-dien-3-one	422	422									13.16	
22	25,758	Ursa-6,9(11)-dien-3-one [29]	422	422									10.46	
23	25,917	olean-9(11),12-dien-3-ol [30]	424	424									7.24	
24	26,017	3-β-lanosta-8,24-dien-3-ol Acetate [31]	453	468							35.46		10.61	
25	26,158	β-amirin [31]	218	426									14.38	
26	26,192	Arundoin [32]	273	440	9.82									
27	26,308	α-amyrenone [31]	218	424			11.87							
28	26,533	α-amirin [31]	218	426			20.45						15.00	
29	26,608	24-methylen-3-β-lanosta-8-en-3-ol [33]	425	440	4.63						5.54			
30	27,133	A'-neogamacer-22(29)-en-3-one [34]	189	440	36.44									
31	27,675	Ocotillone (20,24-epoxy-25-hydroxydammaren-3-one) [35, 36]	143				8.38							
32	28,242	3-β-lanosta-8,24-dien-3,20-diol [31]	69	442									1.54	
33	29,817	Uvaol [31]	203				8.42							

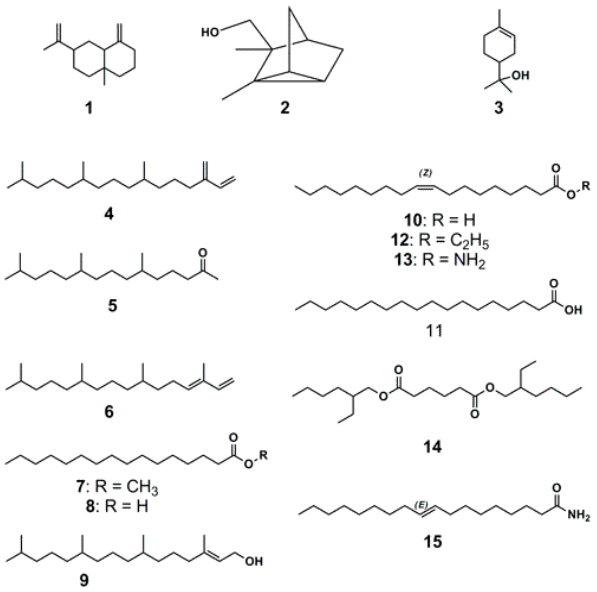

Figure 1. *Structures of metabolites type fatty acids, mono-, sesqui and diterpenoids of Pilea species.*

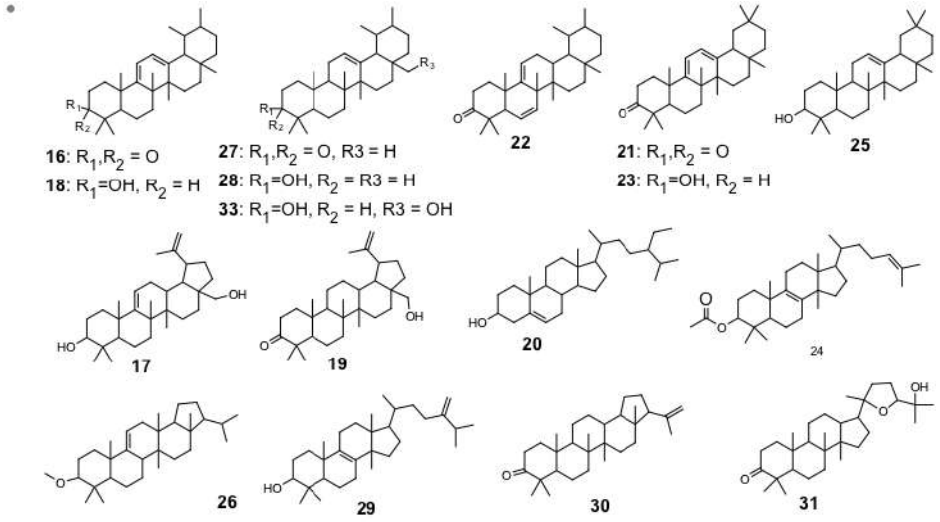

Figure 2. *Triterpenoid and steroid-type Secondary metabolites identified from Pilea species.*

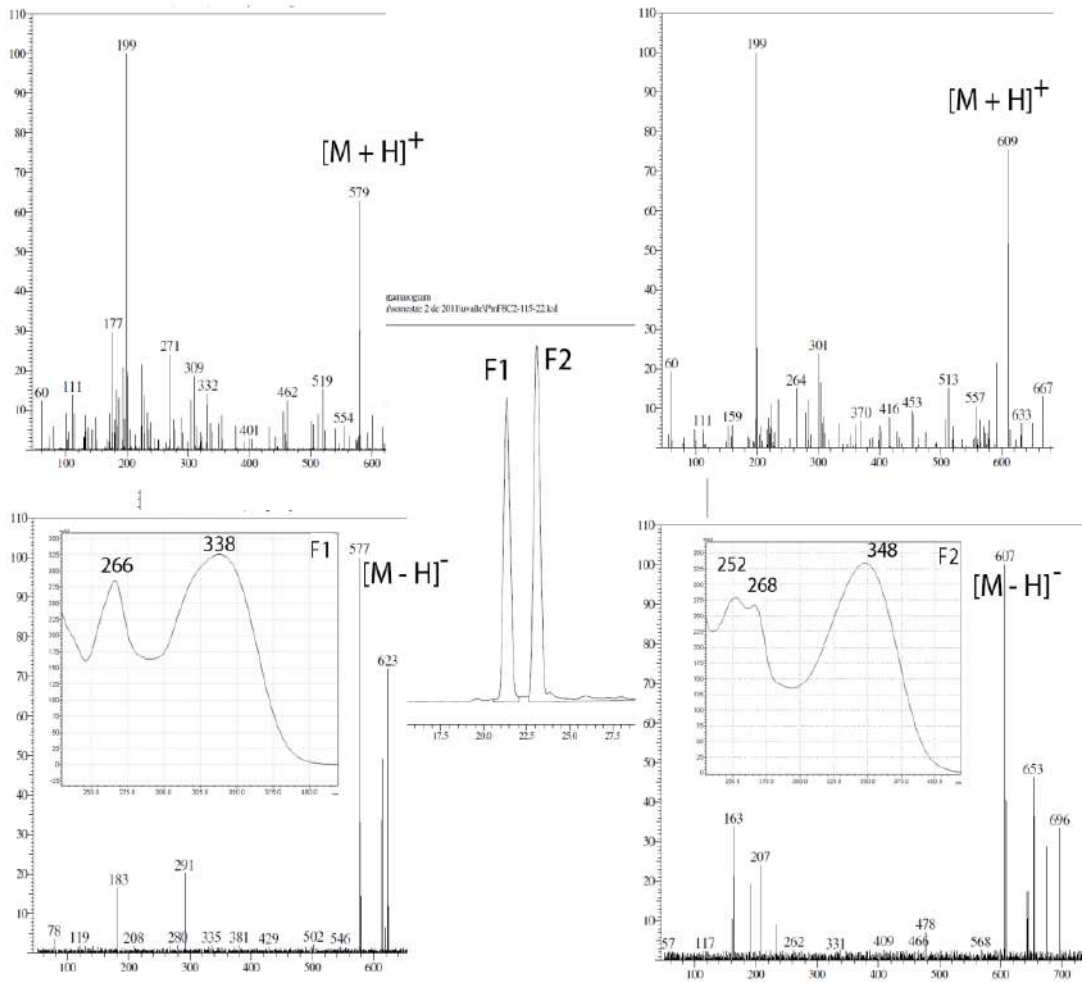

Figure 3. *HPLC-DAD-ESI-Mass Spectral Analysis of flavonoids from Pilea microphylla.*

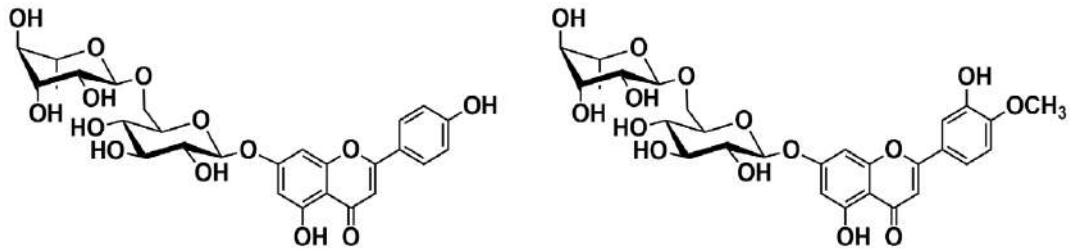

Figure 4. *Glycosyl Flavonoids from P. microphyla.*

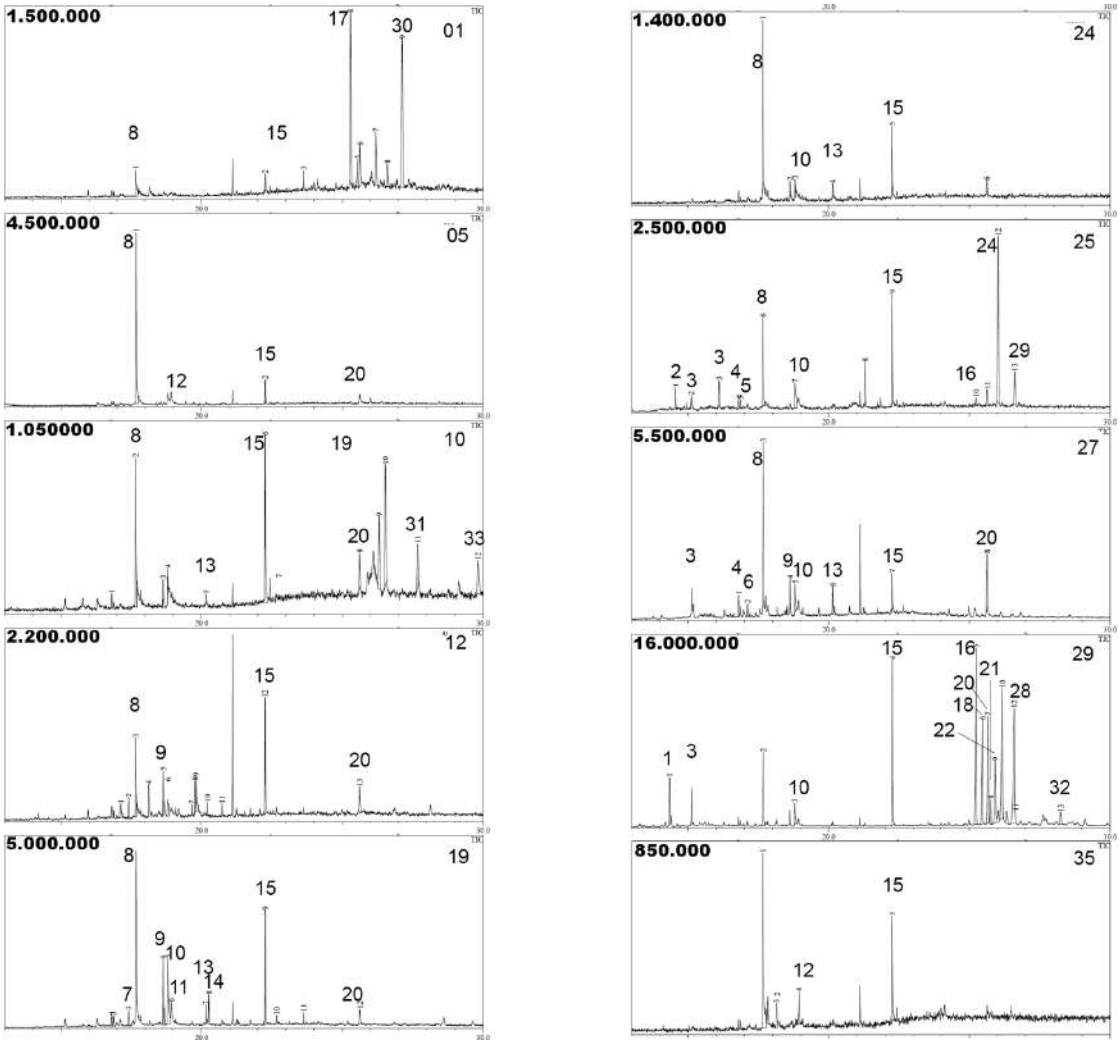

Figure 5. *Gas chromatograms with mass spectrometry detection in the scan mode of ten Pilea species*

4 Conclusions

According to the above results, the chromatographic profiles (Figure 5) and mass spectral compound identification (Figures 1, 2) allowed for the separation of the 10 plant samples as 10 species which have been morphologically characterized too. In the same way, GC-MS of triterpenoid and steroids from *Pilea* leaves constitute a chemotaxonomical tool to help on the identification of *Pilea* species.

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