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Gas Chromatography-Mass Spectrometry Study from the Leaves Fractions Obtained of *Vernonanthura patens* (Kunth) H. Rob

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ABSTRACT

Preliminary phytochemical study of methanol extracts from *Vernonanthura patens*'*s* leaves located in Marcabelí (El Oro province), Ecuador. The methodology consisted of chromatographic column separations with increasing polarity solvents and the analysis of fractions by Gas Chromatography-Mass Spectrometry coupled system (GC-MS). The structure of 53 compounds was proposed. Analyzed *V. patens* species showed the presence of terpene and aliphatic hydrocarbons, free fatty acids and their methyl and ethyl esters, oxygen sesqui, triterpenoids and sugars. For ecuadorian species, there are no records of chemical studies.

Keywords: *Vernonanthura patens*; Phytochemical Study; Gas Chromatographic-Mass Spectrometry Analysis

1. Introduction

Vernonanthura patens (Kunth) H. Rob [*Asteraceae*]—a wild shrub from South America, is distributed from southern Mexico to Rio de la Plata-Argentina [1,2]. The common name of this species in Ecuador is "laritaco". Traditionally, leaves' decoctions are used to fight malaria, postpartum, stomach problems, skin rashes, diarrhea and as an anthelmintic [3,4]. In Ecuador, *V. patens* is used to wash wounds, relieve headaches, it is also used as the anti-inflammatory compound and cough suppressant to treat certain types of cancer. It is also reported its use for leishmaniasis [5] and athlete's foot treatment [6]. Despite the complexity of components reported for the genus, there is little information on the species *V. patens* in particular. The earliest information listed on the chemical composition of the species dates from 1975, which only highlights the absence of sesquiterpene lactones in the species which constituted a chemical marker of género [7]. However, in 1986, 10 compounds were identified among those, including sesquiterpene [8], lactones. These were the only previous reports on the chemical composition of the species, none of which grows in Ecuador. The present study was aimed to develop a preliminary phytochemical analysis of methanolic extracts of *Vernonanthura patens*'s leaves, followed by a further fractionation by column chromatography and analysis of the fractions by Gas Chomatograpy-Mass Spectrometry coupled system (GC-MS).

2. Methods

Vernonanthura patens adult leaves were collected from plants in vegetative state, during the months of December to February (2009 and 2010), in El Oro province of Ecuador.

Drying of leaves was performed in an oven by recirculating air at 45˚C for eight hours. Dried leaves were ground in a knife mill to a powder (2 mm of particle diameter), particles.

Sixty-seven grams of the dry drug were extracted by maceration with pure methanol in a closed container and darkness for eight days. The extract was evaporated until dryness in a rotary evaporator and the residue (7 g) was fractionated by chromatography column packed with activated silica gel (60 to 200 mesh). Hexane, hexane/ethyl acetate 90:10; hexane/ethyl acetate 80:20 and ethyl acetate, were used as solvent systems.

The obtained fractions were analyzed by GC-MS, in an Agilent 7890A gas chromatograph, with Agilent 5975 detector (Avondale, PA, USA) equipped with a HP column of 5 m long (0.25 mm diameter and 0.25 cm internal diameter). The carrier gas was helium and the analytical conditions were: initial temperature: 100˚C (increasing 8˚C per minute till 250˚C final temperature); inlet temperature and mass detector: 250˚C and 300˚C respectively. The mass detector was used in scan mode ("scan") with a range of 100 to 400 mass units.

The structural assignments were made by the library database selecting only those structures that reached 90% or more probability.

Six fractions were analyzed by GC-MS: two hexane fractions ("A" and "B"); two from hexane/ethyl acetate mixture (90:10 and 80:20 respectively); one ethyl acetate fraction and the insoluble residue from the initial methanol extract.

3. Results

From the A hexane extract, the structures of 29 compounds were assigned (**Figure 1**, **Table 1**) and from B hexane extract there were 8 compounds (**Figure 2**, **Table 2**).

From the ethyl acetate 90:10 and 80:20 mixes the structures of 12, 10 and 2 compounds were proposed respectively (**Figures 3** and **4**, **Table 3**). In the ethyl acetate fraction 4 compounds were identified (**Figure 5**), while in the alcoholic residue we identified 9 compounds (**Figure 6**, **Table 4**).

Twenty-nine and eight compounds were proposed by CG-MS in hexane fractions "A" (**Figure 1**, **Table 1**) and "B" (**Figure 2**, **Table 2**) respectively. Analysis of fractions eluted with hexane/ethyl acetate mixtures (90:10 and 80:20) and ethyl acetate showed 10, two and seven compounds respectively (**Figures 3-5**; **Table 3**). Seven structures were tentatively assigned when methanol residue of initial extract was studied (**Figure 5** and **Table 4**).

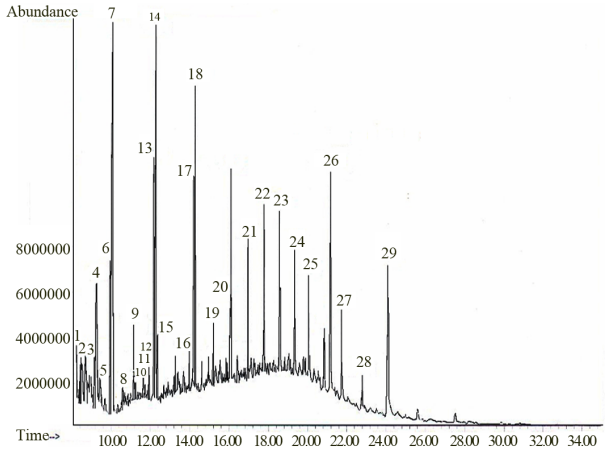

Figure 1. Analytical gas chromatogram of the A hexane fraction.

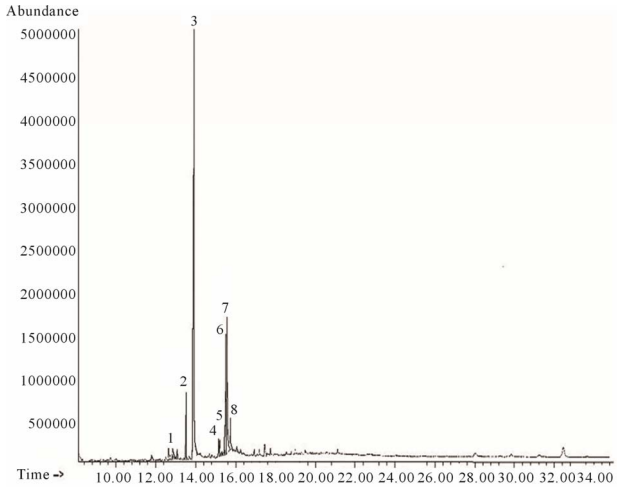

Figure 2. Analytical gas chromatogram of the B hexane fraction.

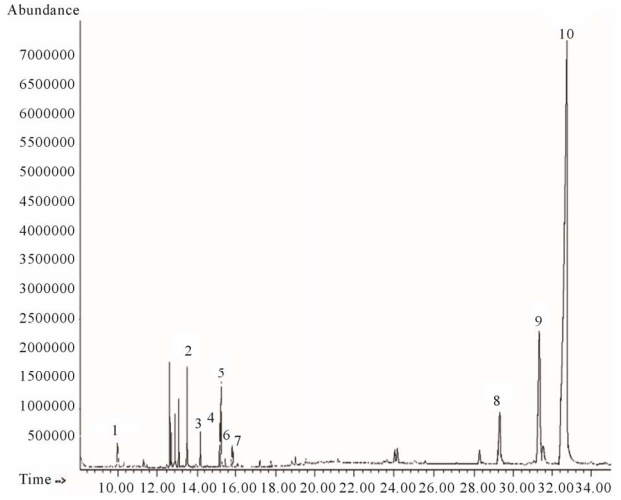

Figure 3. Analytical gas chromatogram of hexane-ethyl acetate 90:10 fractions.

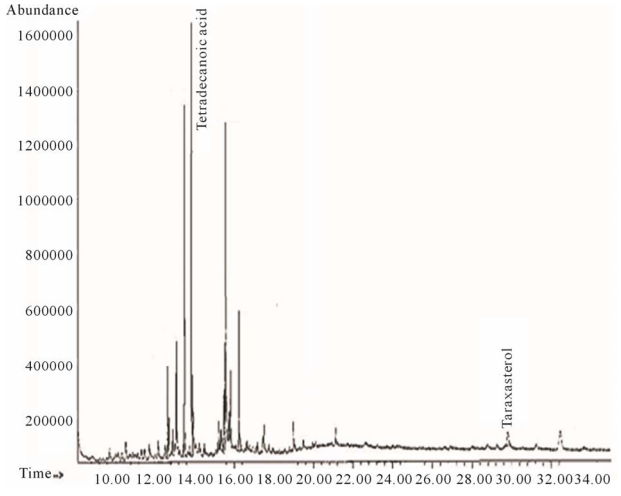

Figure 4. Analytical gas chromatogram of hexane-ethyl acetate 80:20 fraction.

Table 1. Compounds identified in the A hexane fraction.

Peak	Tr Min.	Compound	Abundance %
1	8.435	α-Caryophyllene (sesquiterpene)	1.67
2	8.678	Napthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-(1α,4a.α,8a.α) (bicyclic sesquiterpene, type selinene)	2.10
3	9.156	Naphthalene, 1,2,4a,5,6,8-hexahydro-4,7-dimethyl-1-(1-methylethyl) (bicyclic sesquiterpene, type selinene isomer)	1.69
4	9.234	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl) (bicyclic sesquiterpene, type selinene isomer)	5.73
5	9.426	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-[1S-(1.α,4a.β,8a.α)] (bicyclic sesquiterpene, type selinene isomer)	1.49
6	9.950	2-Tetradecene, (E)-	4.81
7	10.090	Tetradecane	16.82
8	10.583	2,6,10-Trimethyl-pentadecane	0.79
9	11.153	2,6,11-Trimethyl-pentadecane	1.40
10	11.226	2,6,10-Trimethyl-dodecane	0.63
11	11.646	Tritetracontane	0.57
12	11.937	Hexadecane	0.60
13	12.191	1-Octadecene	6.41
14	12.295	Heptadecane	10.82
15	12.357	4-Methyl-heptadecane	0.92
16	13.976	Octadecane	0.56
17	14.199	3-Eicosene	3.68
18	14.272	Eicosane	5.68
19	15.180	Heneicosane	0.97
20	16.031	1-Docosene	1.19
21	16.944	Nonadecane	2.05
22	17.769	Tetracosane	2.68
23	18.563	11-Decyl-tetracosane	2.86
24	19.325	9-Octyl-heptadecane	2.50
25	20.057	5,14-Dibutyl-octadecane	1.55
26	21.157	Squalene	4.51
27	21.738	Nonacosane	1.98
28	22.791	11-Pentyl-heneicosane	1.03
29	24.104	Hentriacontane	6.03

4. Discussion

The hexane fraction "A" contained 29 hydrocarbons compounds (**Figure 1**, **Table 1**). Five of the identified compounds were sesquiterpenes, one was a triterpene and the rest were aliphatic of high molecular mass, which were classified as fatty compounds [9]. The most abundant compounds were tetradecane (peak 7) and heptadecane (peak 14); hentriacontano, 1-octadecene, eicosane, an isomer of selinene (peak 4), 2-tetradecene and squalene, showed a relative abundance.

The hexane fraction "B" consisted of mainly fatty acids (**Figure 2**, **Table 2**). The most preponderant compound was tetradecanoic acid (peak 3), in correspondence with

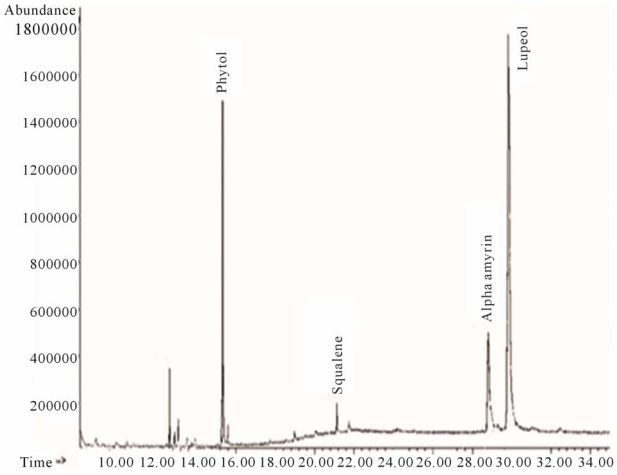

Figure 5. Chromatogram of the ethyl acetate fraction.

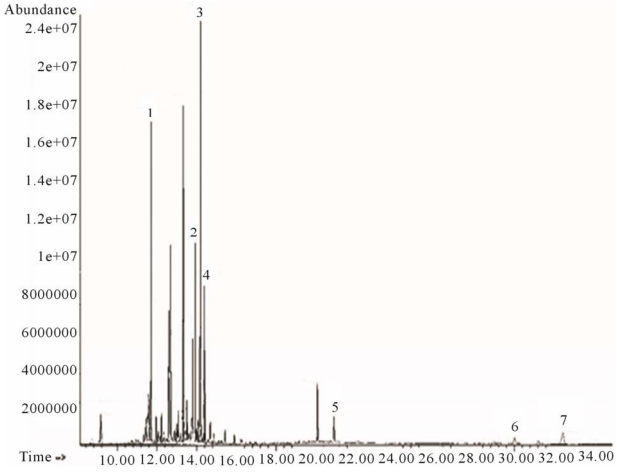

Figure 6. Chromatogram of the ethyl acetate fraction.

Table 2. Compounds identified in the B hexane fraction.

Peak	Tr Min	Compound	Abundance %
1	12.835	Pentadecanoic acid	1.69
2	13.514	Hexanoic acid methyl ester	5.42
3	13.898	Tetradecanoic acid	56.78
4	15.143	9,12-Octadecadienoic acid-methylester	1.63
5	15.206	9,12,15-Octadecatrienoic acid-1-ol	1.17
6	15.496	9.12-Octadecadienoic acid	10.81
7	15.559	9,12,15-Octadecatrienoic acid-methyl ester	13.78
8	15.725	Octadecanoic acid	2.59

tetradecane presence as majority hydrocarbon in hexane fraction "A". There was also a relative abundance of di- and tri-unsaturated fatty acids as methyl esters forms. Presence of methyl esters is characteristic of some *Asteraceae species.*

Table 3. Compounds identified in hexane-ethyl acetate 90:10 fractions.

Peak	Tr Min.	Compound	Abundance %
1	10.001	Caryophyllene oxide	1.25
2	13.514	Hexadecanoic acid methyl ester	2.63
3	14.178	Hexadecanoic acid ethyl ester	0.81
4	15.143	9,12-Octadecadienoic acid methyl ester	0.93
5	15.211	9,12,15-Octadecatrienoic acid methylester	2.11
6	15.750	9,12-Octadecadienoic acid ethyl ester	0.48
7	15.813	9,12,15-Octadecatrienoic acid ethylester	0.59
8	29.277	3-Friedelanon-8-en	4.54
9	31.270	3-Acetate-12-oleaneno	12.31
10	64.05	Lupeol acetate	64.05

Table 4. Compounds identified in methanol extract residue.

Peak	Tr min	Compound	Abundance %
1	11.689	Xylitol	13.08
2	13.919	D-mannitol	8.31
3	14.168	Inositol	19.69
4	14.370	D-glucose	5.35
5	20.918	Stigmasterol	t
6	30.014	Lupenone	t
7	32.479	Lupeol acetate	t

Methyl and ethyl esters of fatty acids were also detected in the hexane/ethyl acetate 90:10 fraction (**Figure 3**, **Table 3**), Three of them were already assigned in the hexane fractions. High abundance of a sesquiterpenoid (caryophyllene oxide) and two triterpenoid acetates was observed, although the most abundant compound was Lupeol acetate. It should be noticed the presumable presence of friedelanon, which has been reported in other species of *Vernonanthura* genus [8].

Two compounds structures were assigned by analysis of the hexane/ethyl acetate 80:20 fraction (**Figure 4**). The most abundant was tetradecanoic acid, which was also detected in the hexane fraction "B". The other proposed compound was taraxasterol, a triterpene.

Four terpene compounds were proposed from the ethyl acetate fraction (**Figure 5**): A diterpene (phytol), and three triterpenes (squalene, α-amyrin and lupeol). Squalene was already assigned in the hexane fraction "A", and lupeol was the most abundant compound of the ethyl acetate fraction.

The residue of the initial methanol extract was silan-

ized for its chromatographic analysis. GC-MS analysis (**Figure 6**, **Table 4**) showed the presence of carbohydrate-like compounds at the first 15 minutes retention time. Xylitol, D-mannitol, D-glucose and inositol, the most abundant compound (peak 3), were the only structures which could be assigned. The compounds exceeded 15 minutes retention time were triterpenoids; both of them (lupenone and stigmasterol) were assigned for the first time in this study.

Fifty-three compounds were proposed in this study of methanol extract of *Vernonanthura patens*' leaves, which constitute the first report of its chemical composition.

5. Conclusions

The *V. patens* species, was shown in its leaves' composition, terpene and aliphatic hydrocarbons, free fatty acids and their methyl and ethyl esters, oxygen sesqui and triterpenoids and sugars. Sesquiterpene lactones reported for the plant species were not detected.

Further researches are in need to determine the compounds which are responsible for the biological activity previously reported for this plant species.

6. Acknowledgements

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