Artículo original

The volatile constituents of leaves from *Lepechinia bullata* (Kunth) Epling from Venezuelan Andes.

Constituyentes volátiles de las hojas de *Lepechinia bullata* (Kunth) Epling de los Andes venezolanos

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RESUMEN

El aceite esencial de las hojas de *Lepechinia bullata* (Lamiaceae) de los Andes venezolanos fue obtenido por hidrodestilación y su composición fue determinada por CG y CG-EM. Los constituyentes mayoritarios fueron *β*-felandreno (14,9 %), premnaspirodieno (14,8 %), borneol (13,3 %), farneseno (8,1 %) acetato de bornilo (5,5 %). El presente estudio es el primero en reportar la presencia de 33 constituyente y la composición porcentual (94,7 %) del aceite esencial de las hojas de *L. bullata*.

PALABRAS CLAVE

Lepechinia bullata, Lepechinia, Lamiaceae, aceites esenciales.

ABSTRACT

The essential oil from the leaves of *Lepechinia bullata* (Lamiaceae) from the Venezuelan Andes was obtained by hydrodistillation and its composition was determined by GC and CG-MS. The major constituents were *β*-phellandrene (14.9 %), premnaspirodiene (14.8 %), borneol (13.3 %), farnesene (8.1 %) and bornylacetate (5.5 %). The present study is the first that reports the presence of 33 constituents and their percentage composition (94.7 %) in the essential oil from leaves of *L. bullata*.

KEY WORDS

Lepechinia bullata, Lepechinia, Lamiaceae, essential oil.

INTRODUCTION

The genus *Lepechinia* (Lamiaceae), comprising about 40 species of herbs and shrubs, is widely distributed in the Americas [1]. Four species have been reported for Venezuela: *L. bullata*, *L. schiedeana*, *L. salviaefolia* and *L. conferta*, which are found in the Andes of Colombia and Venezuela, at altitudes of 1600–3800 m above sea level [2].

Some species from *Lepechinia* are used for their antitumoral and insulin-mimetic properties [3], to treat uterine infections or to calm stomach pains [1]. Sesquiterpenes [2], diterpenes [4-8], triterpenes and flavonoids have been isolated from different species of this genus [9]. The essential oils of *L.* floribunda [10], *L. calcina* [11], *L. salviaefolia* with three quimiotypes [type 1 (palustrol, borneol and *β*-phellandrene), type 2 (premnaspirodiene, *β*-phellandrene and borneol) and type 3 (δ-3-carene, τ-cadinol and borneol)] [12], *L. urbanii*, *L. salvia*, *L. graveolens*, *L. conferta*, *L. caulescens* and *L. schiedeana* have been studied [13-20].

Lepechinia bullata (Briq.) Epl. (Lamiaceae) is a common medicinal plant [21], Leaves and flowers of *L. bullata* are anatomically different from those of *L. salviaefolia,* it is easy to distinguish between them because *L. bullata* has eliptic leaves, almost as wide as long, its flowers form a dense panicle, the borders of the calyx is dentate, and the spikes are small. On the other hand *L. salviaefolia* has nearly lanceolate leaves, at least twice as long as wide, its flowers form a slack panicle, and the spikes of the calyx are sharp and larger [22]. Earlier investigations dealt with the occurrence of abietane diterpenes in the aerial parts of *L. bullata*, the 6,7-dehydroroyleanone, horminone, and 7-*O*-methylhorminone were isolated of the MeOH extract has been found to display cytotoxic activity in cultured nasopharyngeal carcinoma and murine leukemia cells [4]. Eggers *et al* (1999) [12] isolated spirolepechinene, a new sesquiterpene hydrocarbon, and the known compounds spirovetivane and premnaspirodiene from the essential oil of *L. bullata*. On the other hand Cegarra *et al* (2006) [23] reported the presence of ten monoterpenes (*α*-thujene, *α*-pinene, camphene, sabinene, *β*-pinene, myrcene, limonene, 1,8-cineol, linalool and borneol) and two sesquiterpenes (caryophyllene and *α*-humulene) but did not report the percentage composition of the oil of *L. bullata* leaves. The present study is the first that reports percentage composition of each of the 33 components found in the essential oil from the leaves of *L. bullata*

MATERIAL AND METHODS

Plant material. Aerial parts of *L. bullata* were collected at Paramo La Culata (Mérida State, Venezuela) at an altitude of 3100 m. A voucher specimen (LR 02) was deposited at the MERF Herbarium, Faculty of Pharmacy, University of Los Andes. The identity of the plant was confirmed by Prof. Alexander Cegarra from the Faculty of Forestry of University of Los Andes.

Extraction of essential oils. Leaves were steam distilled

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for four hours using a Clevenger type apparatus, the oil sample was dried over anhydrous sodium sulfate, and stored in a dark vial at 4 ºC.

GC analysis. A Perkin Elmer Autosystem gas chromatograph with a flame ionization detector was used for Kováts indices determination (Adams, 2007). The analyses were carried out using a 5 % diphenyl, 95 % dimethyl polysiloxane (AT-5, Alltech Associates, Inc., Deerfield, Illinois, USA) capillary column, 60 m x 0.25 mm id x 0.25 µm film. The oven for the AT-5 column was programmed from 60 ºC (1.0 min) to 260 ºC at 4 ºC/min. Injector and detector temperatures were both 250 ºC and a flow rate of 1.0 mL/min of heat constant volume was used. The Kováts retention indices (RI) were determined relative to the retention times of a series of *n*-paraffin hydrocarbons (C_7-C_{22}) with a logarithmic scale.

GC/MS analysis. The analysis was performed on a Hewlett Packard 6890 series II gas chromatograph linked to a Hewlett Packard 5973 mass detector equipped with a HP automatic injector and a 30 m long crosslinked 5 % phenylmethyl siloxane (HP-5MS, Hewlett Packard, USA; mm id; 0.25 µm film thickness) capillary column. The ionization energy was 70 eV. A sample of 1.0 µl of 2 % soln. of the oil in *n*-heptane was injected with split ratio of 100:1. The temperature of the injection block was 250 ºC. The GC oven temperature program was the same used for GC analysis. Identification of the oil components was established by comparison of their retention times with those of authentic samples, by Kováts indices on the columns already described under gas chromatography, and by computer comparison of mass spectra with a Wiley ms data library (6th ed), followed by comparisons of ms data with published literature [24].

RESULTS AND DISCUSSION

Hydrodistillation of the fresh aerial parts of *L. bullata* yielded 0.05 % of oil. Gas chromatography of the freshly isolated oil revealed the presence of 33 components. The retention indices and percentage composition are given in Table 1. The essential oils of other *Lepechinia* show a different composition from that of *L. bullata* oil, since these oils contain *δ*-3-carene, borneol, palustrol, ledol y *o*-cresol [10-20]. The oil from *L. bullata* was found to contain sesquiterpenes (39.9 %) and monoterpenes (32.1 %) as major components, but oxygenated monoterpenes were also abundant (22.8 %). The most abundant constituents were *β*-phellandrene (14.9 %), premnaspirodiene (14.8 %), borneol (13.3 %), farnesene (8.1 %) and bornylacetate (5.5 %). The *β*-phellandrene rich oil fractions are marketed as industrial perfumes for detergents and the like [25]. The composition of the essential oil from the leaves of *Lepechinia bullata* has been previously reported by Cegarra *et al* (2006) [23] who reported the presence of only eleven compounds, but did not mention their percentage composition. On the other hand Eggers *et al* (1999) [12], also studied the composition of the essential oil from *L. bullata* leaves, but only isolated the most abundant constituents of the essential oil of *L. bullata* collected at Santo Domingo (Venezuelan Andes): spirolepechinene and premnaspirodiene, in order to determine their molecular structures. Therefore, the present study is the first that reports the presence of 33 constituents and the percentage composition of each of the components in the essential oil from leaves of *L. bullata* collected at Paramo La Culata. The essential oil from leaves collected at Santo Domingo was reported to contain about 20 % of spirolepechinene and 45 % of premnaspirodiene, while the oil from leaves collected at La Culata contained only 14.8 % premnaspirodiene, 3.1 % spirolepechinene, and it most abundant component was *β*-phellandrene (14.9 %). This difference in composition suggests there are chemical varieties of *L. bullata*.

TABLE 1.

Constituents and percentage composition of the essential oil of *Lepechinia bullata* leaves.

Nº	Area (%)	Component	IK
1	0.2	2-hexenol	860
2	0.2	tricyclene	925
3	1.7	*α*-thujene	931
4	2.5	*α*-pinene	936
5	5.3	camphene	952
6	0.6	sabinene	974
7	1.4	*β*-pinene	978
8	0.8	myrcene	989
9	1.8	*α*-phellandrene	1003
10	0.5	*δ*-3-carene	1010
11	0.5	*α*-terpinene	1017
12	0.3	*p*-cymene	1025
13	14.9	*β*-phellandrene	1032
14	1.0	*γ*-terpinene	1060
15	0.5	*trans*-sabinene hydrate	1069
16	0.4	*α*-terpinolene	1090
17	1.7	linalool	1099
18	0.2	octenyl acetate	1112
19	0.3	ment-2-en-1-ol	1125
20	13.3	borneol	1173
21	1.1	4-terpineol	1183
22	5.5	bornyl acetate	1291
23	0.2	isoledene	1378
24	0.3	*α*-gurjunene	1416
25	4.8	*trans*-caryophyllene	1427
26	3.1	alloaromadendrene	1449
27	3.1	spirolepechinene	1461
28	0.3	*α*-humulene	1464
29	8.1	*α*-farnesene	1506
30	14.8	premnaspirodiene	1517
31	0.7	*γ*-2-cadinene	1525
32	0.8	globulol	1588
33	3.6	τ-cadinol	1622

CONCLUSIONS

In the present study is the first that reports the presence of 33 constituents and the percentage composition of each of the components in the essential oil from leaves of *L. bullata*. The most abundant constituents were *β*-phellandrene, premnaspirodiene, borneol, farnesene and bornylacetate. The sesquiterpenes were the main detected components (39.9 %) of the essential oils analyzed.

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