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Chemical Composition and Antimicrobial Activity of Essential Oil of *Lepechinia radula* Benth Epling

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Abstract: The essential oil (EO) was obtained by hydrodistillation from the aerial parts of *Lepechinia radula* Benth Epling (Lamiaceae) from Ecuador. Thirty-four compounds accounting to 93.4% of the total oil were identified. The main constituents of the essential oil were δ-3-carene (19.9%), β-pinene (17.0%), (*E*)-β-caryophyllene (9.7%) and (*E*-*E*)-α-farnesene (9.4%). The essential oil of *L. radula* possessed strong antifungal activity against *Trichophyton rubrum* (ATCC® 28188) and *Trichophyton mentagrophytes* (ATCC® 28185).

Keywords: *Lepechinia radula*; Lamiaceae; δ-3-carene; *Trichophyton rubrum*; *Trichophyton mentagrophytes*.

1. Plant Source

Aerial parts of *L. radula* were collected at flowering stage in Guachanamá, on august 2011, in Loja Province (Southern Ecuador, latitude 4°4'29''S; longitude 79°56'24''W; altitude 2351 m). The plant was taxonomically identified at *"Herbarium of Universidad Nacional de Loja"* by Bolívar Merino. A voucher specimen number: PPN-la-034 has been deposited in the Herbarium of *"Universidad Técnica Particular de Loja"*.

2. Previous Studies

The genus *Lepechinia* belongs to the family Lamiaceae and has about 40 species distributed from south-western USA to Chile [1]. Previous pharmacological studies about *Lepechinia* spp. have reported hypoglycemic and vasorelaxant effects, antioxidant and antibacterial activity [2-4]. Regarding the volatile-essential oil components, some species of the genus *Lepechinia* have been studied so far; for instance: *L. salviaefolia* [5], *L. urbanii* [6], L. *paniculata* [7], *L. schiedeana* [8] and *L. mutica* [9].

In Ecuador the genus *Lepechinia* comprises 9 species [10], some of which are used in ethnomedicine. In Loja Province *L. radula* and *L. mutica*, are used to treat *"espanto" (a disease that is produced by unpleasant experiences, accidents, violent episodes, or moments of distress that produce*

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an emotional impact on the patient) [11,12]. *L. paniculata* is used to treat *"mal aire" (a disease caused by strong winds experienced while the person walks down a hill, by contact with cold air when the person leaves a sheltered place, or when a person walks through cemeteries or places where there are hidden treasures)* [12], besides of the treatment of headache and nervous system affection [13]. As well as *L. betonicifolia* and *L. bullata* have been used for the treatment of wound infections, punches and inflammations [14]. *Lepechinia radula* is a native shrub found in the Andean region of Ecuador. It is located in growing wild in both Azuay and Loja Provinces at 2000 - 2500 m a.s.l. [10,15]. Previous reports don't have been found about *L. radula*. Consequently the purpose of this study is to contribute to knowledge of chemical composition, physical properties and biological activity of essential oil of *L. radula.*

3. Present Study

Fresh leaves of *L. radula* (1000 g) were hydrodistilled for three hours using a Clevenger-type apparatus. Subsequently the essential oil samples were tagged and stored at 4°C until being used for analysis.

3.1. Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-FID (GC-FID):

GC-MS analysis was performed by using an Agilent gas chromatograph (model 6890N series). This chromatograph was coupled to a mass spectrometer-detector (model Agilent series 5973 inert); the spectrometer operated at 70 eV, scan rate: 2 scan/s and mass range was 40-350 m/. This was controlled by the data system MSD-Chemstation D.01.00 SP1; a polar HP-INNOWAX polyethylene glycol (Agilent 19091N-133); and a non-polar DB-5MS 5%-phenyl-methylpolyxilosane (Agilent 122-5532) both 30m x 0.25mm, thickness 0.25 μm film were used. Essential oil samples were diluted (1:100) in diclorometh ane. An automatic injector (series 7673) in split mode 1:50 was used. Helium was used as a carrier gas at 0.9 mL/min in constant flow mode. Injector and detector temperatures were set at 210 ºC and 250 ºC; respectively. The initial oven temperature was kept at 50 ºC for 3 min. Then it was gradually raised to 210 ºC at 2.5 ºC/min, and finally held for 3 min. Retention index of the compounds was determined based on the homologous of the standard aliphatic hydrocarbons TPH-6RPM of CHEM SERVICE C10-C25, which were injected after the oils at the same conditions. The identification of the essential oil components was based on the comparison of both MS data and their retention indices [16]. GC-FID analyses were carried out on an Agilent chromatograph (model 6890N series) by using a flame ionization detector (FID). The same capillary columns and analytical parameters as those used in the GC-MS measurement were also used in the GC-FID analysis.

3.2. Physical properties:

Physical characterization of *L. radula* essential oil was performed at 20 °C. A pycnometer (5 mL and an analytical balance (model METTLER AC100, ± 0.0001 g) were used to determine the density according to standard ANFOR NF T75-111. Refractive index was measured on a refractometer (model ABBE) on the basis of standard ANFOR NF 75-112. Finally, the standard ISO 592-1998 was used for optical activity measurement by means of a polarimeter (model AUTOPOL 880 Automatic Saccharimeter, ± 0.03, 10°C–30°C).

3.3. Antimicrobial activity:

Antimicrobial activity was determined by Minimun Inhibitorium Concentration *(MIC)* measurement according to the method detailed by [17,18]. Five Gram-negative bacteria [*Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumoniae* (ATCC9997), *Proteus vulgaris* (ATCC 8427), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (LT2)], two Gram-positive bacteria [*Enterococcus faecalis* (ATCC 29212), *Staphylococcus aureus* (ATCC 25923)] and two dermathophytes [*Trichophyton rubrum* (ATCC 28188) and *Trichophyton mentagrophytes* (ATCC 28185)] were used Solutions of the essential oils were prepared in dimethyl sulfoxide (DMSO) at a

concentration of 20 µL/mL. Gentamicine was used as a positive control for five bacteria with a MIC value of 0.39 µg/mL, while as ampicillin was used as a positive control for *E. faecalis* and *S. typhimurium*, with a MIC value of 3.12 µg/mL. Itraconazole was used a positive control with a MIC value of 0.48 µg/mL for fungi.

Thirty-four components were identified accounting for 93.4% of the oil. The chemical composition of the essential oil of *L. radula* is shown in Table 1 according to elution order from the DB5-MS column. The oil from the aerial part was dominated by monoterpenes δ-3-carene (19.9%), β-pinene (17.0%), β-phellandrene (8.6%). Also the sesquiterpenes (*E*)-β-caryophyllene (9.7%) and (*E*-*E*)-α-farnesene (9.4%) were identified as main compounds.

Previous investigations have reported the chemical composition of other species of *Lepechinia* from Loja Province. For example, the essential oil of *L. mutica* [9] was characterized by an essential oil dominated by β-phellandrene (30.0%), camphene (13.0%) and limonene (8.0%). Besides the representative compounds identified in the essential oil of *L. paniculata* were aromadendrene (24.0%), viridiflorene (12.4%) and β-phellandrene (7.7%) [7]. The chemical composition and biological activity of the essential oil of *Lepechinia radula* Benth Epling is reported for the first time.

Due to the heterogeneity of the compounds identified in the *Lepechinia* species, it is not possible to establish a characteristic pattern of compounds for the genus. The essential oil of *L. conferta* [1] and *L. shiedeana* [8] from Venezuela exhibited Ledol with 24.2% to 28.9% and (29.1%), respectively as the main compounds. In the case of, *L. floribunda* from Argentina [19] borneol (21.4%), β-caryophyellene (15.1%) and ledyl acetate (16.8%) were the major compounds; however, to the same specie collected in Bolivia, bornyl acetate (12.3-3.8%), β-caryophyllene (9.0%) and camphene (5.7-7.0%) were reported as the major compounds [20]. For essential oil from the leaves of *L. bullata*, the sesquiterpenes hydrocarbons: spirolepechinene and spirovetivane were isolated as the main compounds [21]. For *L. graveolens*, sesquiterpenes accounts by 61% of the total of compounds identified. In the essential oil of *L. meyeni* the monoterpenes constituted the most important fraction ca. 40%, followed by oxygenated sesquiterpenes ca 31%. [20]. The major constituents in the essential oil of *L. salviaefolia* were (-)-palustrol (19.1%), β-phellandrene (13.8%), borneol (11.8%) and camphene (7.2%) [5]. The essential oil of *Lepechinia calycina* collected in USA, was found to contain 1,8-cineole (19.7 %), camphor (17.5 %), δ-3-carene (17.4 %), camphene (7.8 %), as the main compounds etc. [22]. Table 1 also shows the material plant oil humidity, essential oil yield and its physical properties. The values of refraction index are comparable to those reported for other species [20]. On one hand, the essential oil of *L. radula* was considered inactive for both Gram-positive and Gram-negative bacteria; due to MIC values over to1000 µg/mL. On the other hand, *L. radula* essential oil exhibited a good antifungal activity against *T. rubrum* and *T. mentagrophytes* with a MIC value of 31.25 µg/mL and 62.50 µg/mL, respectively. According to Holetz et al. [37] if the extract shows a MIC less than 100 µg/mL, the antimicrobial activity is considered good, from 100 to 500 µg/mL the antimicrobial activity is considered moderate, and from 500 to 1000 µg/mL the antimicrobial activity is weak and over to 1000 µg/mL the extract is considered inactive.

Previous reports on *Lepechinia* genus [38,39] have shown good antimicrobial activity, as the case of the essential oil of the specie *L. caulescens* from Mexico, which exhibited in vitro activity against *Vibrio cholerae* (gram negative) with a MIC value of 4 µl/mL and a minimum bactericidal concentration (MBC) of 6 µl/mL [40]. Aditionally, the ethanol extract of *L. hastata* demonstrated a MIC of 87.50 µg/ml against *Staphylococcus aureus* (ATTC 25923) while as, the ethanol extract of *L. meyenii* displayed activity against *T. mentagrophytes* with an inhibition zone of 15 mm measured by the agar diffusion assay [41].

Table 1. Composition of the essential oils from the aerial parts of *Lepechinia radula*.

	Compounds	*L. radula*[a]	RI A[b]	RI[ref] A	RI P	RI[ref] P
1	α-Thujene	0.3±0.03	931	924[d]	1020	1029[g]
2	α-Pinene	1.2±0.13	937	932[d]	1016	1028[h]
3	Sabinene	2.6 ±0.71	972	969[d]	1112	1123[h]
4	β-Pinene	17.0±1.28	976	974[d]	1100	1113[h]
5	p-Mentha-1(7), 8-diene	0.2±0.02	1001	1003[d]	1186	1183[i]
6	δ-3-Carene	19.9±0.88	1006	1008[d]	1137	1148[j]
7	α-Terpinene	0.2±0.02	1014	1014[d]	1166	1197[i]
8	p-Cymene	0.2±0.02	1017	1020[d]	1258	1277[k]
9	Limonene	0.4±0.03	1021	1024[d]	1183	1194[l]
10	β-Phellandrene	8.6±0.21	1027	1025[d]	1196	1216[l]
11	γ-Terpinene	0.2±0.03	1055	1054[d]	1233	1238[l]
12	α-Terpinolene	1.4±0.08	1082	1086[d]	1271	1297[m]
13	Linalool	1.4±0.51	1101	1095[d]	1581	1570[n]
14	2-Methyl butyl 2-methyl butanoate	0.9 ±0.05	1103	1090[e]	-	-
15	α-Copaene	0.4±0.35	1369	1374[d]	1491	1471[m]
16	α-Gurjunene	0.1±0.05	1391	1409[d]	1527	1514[o]
17	(*E*)-β-Caryophyllene	9.7±0.74	1411	1417[d]	1610	1612[k]
18	β-Gurjunene	0.2±0.12	1421	1431[d]	1604	1610[p]
19	Trans-α-bergamotene	0.1±0.03	1428	1432[d]	-	-
20	α-Humelene	1.9±0.14	1447	1452[d]	1694	1687[k]
21	Allo-aromadendrene	0.2±0.07	1451	1458[d]	1665	1661[k]
22	(+)Epi-Bicyclosesquiphellandrene	0.1±0.03	1454	1470[f]	-	-
23	Germacrene-D	7.2±0.66	1473	1475[d]	1743	1726[k]
24	β-Selinene	0.7±0.10	1480	1484[d]	1752	1743[q]
25	Bicyclogermacrene	4.0±0.77	1487	1500[d]	1775	1755[r]
26	(*E*,*E*)-α-Farnesene	9.4±1.88	1489	1505[d]	1785	1760[f]
27	Germacrene A	0.4±0.03	1498	1509[d]	1731	1744[m]
28	δ-Cadinene	0.7±0.52	1512	1522[d]	1759	1758[i]
29	α-Cadinene	0.1±0.00	1529	1537[d]	-	-
30	Germacrene-B	1.9±0.25	1549	1559[d]	1884	1856[f]
31	Germacrene D-4-ol	1.4±0.12	1569	1574[d]	-	-
32	Caryophyllene oxide	0.7±0.07	1572	1582[d]	2069	2008[s]
33	Guaiol	0.8± 0.15	1590	1600[d]	-	-
34	α-Cadinol	1.5 ±0.36	1649	1652[d]	-	-
Monoterpene hydrocarbons			**49.6%**			
Oxygenated monoterpenes			**2.3%**			
Sesquiterpene hydrocarbons			**37.1%**			
Oxygenated sesquiterpenes			**4.4%**			
Total identified			**93.4%**			
Relative humidity[t] (%)			60.00±0.08			
Oil yield[u] (%)			0.37±0.01			
Relative density[v]			0.8713±0.004			
Refraction index[v]			1.473±0.0006			

Notes: [a] Percentage values are means of three determinations ± SD; [r] plant material; [s] Essential oil yields are given on fresh weight basis (w/w); [t] Essential oil at 20°C.
RI A, **RI P**, retention indices in the apolar column (DB-5MS) and in the polar column (HP-INNOWAX), respectively.
[b] compounds ordered according to the elution order in the column DB-5MS.
RI[ref.], references: [d]ref [16], [e-h]ref [23-26], [i]ref [11], [j-s]ref [27-36].

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