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Chemical composition, antioxidant and antimicrobial activities of *Lepechinia rufocampii* Epling & Mathias essential oil from the highlands of Ecuador

[Composición química, actividades antioxidantes y antimicrobianas del aceite esencial de *Lepechinia rufocampii* Epling & Mathias de las tierras altas del Ecuador]

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Abstract: The chemical composition, antioxidant and antimicrobial activities of the essential oil from leaves and flowers of *Lepechinia rufocampii* Epling & Mathias were studied. GC-FID and GC-MS analyses allowed the identification and quantification of 122 constituents, representing 98.7% of the essential oil. Aliphatic compounds, mainly methyl ketones (62.4%) and sesquiterpene hydrocarbons (19.5%) were found to be the most abundant compounds, while oxygenated monoterpenes were the minor. The most abundant compounds were undecan-2-one (34.6%), nonan-2-one (21.1%), and (E)-caryophyllene (8.3%). Antioxidant activity was examined using DPPH, ABTS, and FRAP assays. The essential oil had a low scavenging effect and it showed ferric reducing activity. Antimicrobial activity of the essential oil was observed against pathogenic bacteria and a pathogenic yeast. The essential oil showed very good activity against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella enterica* serovar, but low activities against *Pseudomonas aeruginosa* and *Candida albicans*. The MIC value of the essential oil varied from 1.04-33.05 µL/mL, with the lowest for *Salmonella enterica* serovar.

Keywords: *Lepechinia rufocampii*; Essential oil; Chemical composition; Antioxidant activity; Antimicrobial activity

Resumen: Se estudió la composición química, actividades antioxidantes y antimicrobianas del aceite esencial de hojas y flores de *Lepechinia rufocampii* Epling & Mathias. Los análisis por GC-FID y GC-MS permitieron la identificación y cuantificación de 122 constituyentes, que representan el 98.7% del aceite esencial. Los compuestos alifáticos, principalmente metilcetonas (62.4%) y los hidrocarburos sesquiterpénicos (19.5%) resultaron ser los compuestos más abundantes, mientras que los monoterpenos oxigenados fueron los minoritarios. Los compuestos más abundantes fueron undecan-2-ona (34.6%), nonan-2-ona (21.1%) y (E)-cariofileno (8.3%). La actividad antioxidante se examinó mediante ensayos DPPH, ABTS y FRAP. El aceite esencial tuvo un bajo efecto eliminador y mostró actividad reductora de hierro. Se observó actividad antimicrobiana del aceite esencial contra bacterias patógenas y una levadura patógena. El aceite esencial mostró muy buena actividad contra *Staphylococcus aureus*, *Escherichia coli* y *Salmonella enterica* serovar, pero baja actividad contra *Pseudomonas aeruginosa* y *Candida albicans*. El valor de CIM del aceite esencial varió de 1.04 a 33.05 µL/mL, siendo el más bajo para *Salmonella enterica* serovar.

Palabras clave: *Lepechinia rufocampii*; Aceite esencial; Composición química; Actividad antioxidante; Actividad antimicrobiana

INTRODUCTION

Lepechinia Willd. is a genus of plants belonging to the Lamiaceae family and comprises almost 43 species disseminated from the Southwest USA to Chile (Camina *et al*., 2018; Ramírez *et al*., 2018). Most of the *Lepechinia* spp in South America are found between 1500-4000 m, usually in dry open habitats of the Andean highlands (Drew & Sytsma, 2013). In Ecuador, only 9 native and 4 endemic species are reported (Jørgensen & León-Yánez, 1999), some of which are used in ethnomedicine (Andrade *et al*., 2009; Armijos *et al*., 2014; Malagón *et al*., 2016; Armijos *et al*., 2021). *Lepechinia rufocampii* Epling & Mathias is a flowering herbaceous shrub known by the common name *salvia gateada*. It is an endemic species of the inter-Andean zone of Ecuador that has been reported mainly in the province of Azuay (Jørgensen & León-Yánez, 1999). The use of *L. rufocampii* infusions for treating cough and tuberculosis has been described in traditional medicine, as well its use as antispasmodic, diuretic, stimulant and to control the menstrual cycle. It has been also used for facial treatments, molar pain, angina and as an antifungal (Tinoco-Valencia, 2020).

Regarding the chemical composition of the essential oils, some species of the genus *Lepechinia* have been studied so far, including *L. betonicifolia* (Caballero-Gallardo *et al*., 2011; Ebadollahi *et al*., 2020; Calva *et al*., 2022), *L. bullata* (Eggers *et al*., 1999), *L. calycina* (Lawrence & Morton, 1979), *L. caulescens* (Avila-Acevedo *et al*., 2005), *L. conferta* (Borges *et al*., 2006), *L. chamaedryoides*, *L. floribunda* (Velasco-Negueruela *et al*., 1994; Lopez-Arze *et al*., 2009; Pellegrini *et al*., 2014; Camina *et al*., 2018), *L. graveolens* (Lopez-Arze *et al*., 2009), *L. heteromorpha* (Gilardoni *et al*., 2019), *L. meyeni* (Lopez-Arze *et al*., 2009), *L. mutica* (Malagón *et al*., 2003; Ramírez *et al*., 2017; Ramírez *et al*., 2018), *L. paniculata* (Valarezo *et al*., 2012; Gilardoni *et al*., 2019; Panamito *et al*., 2021), *L. radula* (Morocho *et al*., 2017; Gilardoni *et al*., 2019), *L. salviae* (Eggers *et al*., 2001; Díaz *et al*., 2019), *L. schiedeana* (Cicció et al., 1999; Stashenko *et al*., 1999; Puertas-Mejía *et al*., 2002; Rojas *et al*., 2004), *L. urbanii* (Zanoni & Adams, 1991), and *L. vulcanicola* (Brand *et al*., 2015). Up to date, only one Up to date, only a primary study of the essential oil from leaves of *L. rufocampii* has been reported with the identification by GC-FID of five major compounds. The antibacterial activity of the essential oil against *Escherichia coli* and *Salmonella typhimurium* was also evaluated (Calderón-Cevallos & Guerrero-Ricaurte, 2013).

This paper reports the chemical composition, antioxidant and antimicrobial activities of the essential oil from the leaves and flowers of *Lepechinia rufocampii* Epling & Mathias grown wild in the highland region of Ecuador.

MATERIALS AND METHODS

Plant material and essential oil isolation

Aerial parts were collected at 3000 m above sea level in Cañar canton (2°29′1.32″ S, 78°58′42.24″ W), Ecuador during January 2019. Sampling was performed in triplicate from different plants. A voucher specimen was kept at the Herbarium of the University of Azuay (accession number 13283). After harvested, the manual weeding of plants was performed to obtain only the good parts of leaves and flowers eliminating everything that could affect essential oil purity. The essential oil was isolated from 200 g of fresh material by hydrodistillation for 3 h in a modified Clevenger-type apparatus. Distillations were performed separately for each sampling. After extraction, the oil was dried with anhydrous sodium sulfate and stored at 4ºC until being analyzed.

Chemical characterization of the essential oil

The essential oil was characterized by GC-MS and GC-FID. GC-FID was performed for quantification of the constituents, using a Hewlett-Packard 6890N series II (Agilent, Santa Clara, CA, USA) equipped with DB-5ms column (30 m x 0.25 mm i.d. x 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA) fused silica capillary column and flame ionization detector. Hydrogen was used as carrier gas at 1 mL/min. Temperature program was at follows: 70ºC (2 min), 70ºC to 250ºC at 4ºC/min, 250ºC (10 min). The injector and detector temperatures were 220°C and 240°C, respectively. One µL of a diluted sample of the essential oil (10% in diethyl ether, v/v) was injected in split mode (1:20). Quantification was carried out using relative percentage abundance and normalization method with correction response factors based on grouping the components by their functional groups (Costa *et al*., 2008). Percentage data were the mean values of two injections per sample.

GC-MS analyses were performed using a QP-

2010 Ultra (Shimadzu, Japan) equipped with a DB-5ms (30 m × 0.25 mm i.d. × 0.25 µm film thickness, J & W Scientific, Folsom, CA, USA) and DB-Wax (30 m × 0.25 mm × 0.25 µm; J & W Scientific, Folsom, CA, USA) capillary columns. The temperature program, carrier flow rate and injection mode for both columns were the same followed for GC-FID. Identification was achieved by comparison of mass spectra of compounds with those of authentic standards, as well as based on the comparison of their fragmentation patterns in the mass spectra with those from NIST 05, Wiley 6, Adams 2007, and NBS 75 k and with in-house Flavorlib library. Also, Linear retention indexes (LRI) determined with reference to homologous series of *n*-alkanes (C_8-C_{24}), were compared against those found in literature and NIST Standard Reference Database (https://webbook.nist.gov/chemistry).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay

A free radical test was performed according to the according to the DPPH assay described previously (Brand-Williams *et al*., 1995). Briefly, 750 µL of five concentrations of the essential oil to evaluate in a range of concentrations between 0.2-10 mg/mL were added to 1.5 mL of DPPH* ethanolic solution (0.075 mg/mL). The absorbance of the reaction mixture was determined at 515 nm after 20 min at room temperature in the dark.

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay

Experiments were performed according to the method reported by Kuskoski *et al*. (2004). An aliquot of the essential oil (100 µL) was added to 1 mL of $ABTS^{*+}$ solution. The absorbance was measured spectrophotometrically at 750 nm after 10 min. The reference synthetic antioxidant Trolox at a concentration of 50 a 700 µmol/L in methanol was tested under the same conditions and the results were expressed in IC_{50} values**.**

Ferric-reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant activity was determined in FRAP assay (Pellegrini *et al*., 2003). The methodology was based on the reduction of the Fe^{3+} – TPTZ complex in ferrous form at low pH. Briefly, the sample (20 µL) was mixed with 900 µL of freshly prepared FRAP reagent. The absorbance at 593 nm was measured after an incubation of 30 min at 37ºC. The FRAP values were derived from the comparison between the absorbance variations in the test mixture with those obtained from the calibration curve prepared. The FRAP values were expressed in units of ascorbic acid equivalent. All determinations were performed in triplicate.

Antimicrobial assays

The *in vitro* antimicrobial activity of essential oils was tested by the agar disc-diffusion assay and the determination of minimum inhibitory concentrations (MIC), for the sensible microbial strains (Wikler *et al.*, 2006). The antibacterial activity of the essential oil was determined against five pathogenic strains, namely *Staphylococcus aureus* (ATCC25923), *Escherichia coli* (ATCC 25922), *Salmonella enterica serovar* (ATCC14028) and *Pseudomonas aeruginosa* (ATCC 27853), while the antifungal activity was assayed against the pathogenic yeast *Candida albicans* (ATCC 14053). Strains were kept on Nutrient Agar and Sabouraud slants at 4ºC, for bacteria and yeasts respectively. Bacterial strains were activated in Nutrient Agar at 37°C for 24 hours, while the yeast was activated in Sabouraud at 28ºC for 48 hours before testing. Aliquots of 10 µL of the essential oil solutions in 5% DMSO at 1, 3, 6, 12 and 24 mg/mL were tested. Ciprofloxacin (30 µg) was used as bacterial positive control and Ketoconazole (33 µg) discs were used for yeast. Activity was measured in terms of zone of inhibition (ZOI). The net zone of inhibition was determined by subtracting the disc diameter (6 mm) from the total zone of inhibition revealed by the test disc in terms of clear zone around the disc. The MIC of the essential oil was determined by micro dilution broth assay using 96 'U' bottom micro-titer plates. Experiments were performed in triplicate. The scale of measurement was the following (disk diameter not included): low ZOI < 5 mm, moderate ZOI = 5-9.9 mm), good (ZOI = 10-15 mm) or very good (ZOI > 15 mm (Chaturvedi *et al*., 2018).

RESULTS AND DISCUSSION

The essential oil yield from leaves and flowers of *L. rufocampii* was 0.31% (v/w), which was similar to 0.29% (v/w) for the essential oil from leaves acquired in a market in Cuenca, Ecuador (Calderón-Cevallos & Guerrero-Ricaurte, 2013).

The essential oil obtained from aerial parts of *L. rufocampii* was analyzed by GC-FID and GC-MS.

One hundred and twenty-two components, which represents 98.7% of the total sample, were identified using GC retention index and mass spectral data (Table No. 1). Aliphatic compounds, mainly methyl ketones (62.4%) and sesquiterpene hydrocarbons (19.5%) were found to be the principal groups of constituents. The most abundant compounds were undecan-2-one (34.6%), nonan-2-one (21.1%), and (*E*)-caryophyllene (8.3%).

The data presented here differ from the results reported by Calderón-Cevallos & Guerrero-Ricaurte (2013), who only identified α-pinene, β-pinene, limonene, linalool and menthone (13.7% of the total composition) as major compounds in the leaf essential oil of this species.

Table No. 1
Composition of the essential oil of *Lepechinia rufocampii* Epling & Mathias

Compound	RI_A[a]	RI_A[ob]	RI_P	RI_P[o]	%[c]
2-Methylbutyl acetate	880	884	1111	1115	tr[d]
Heptanal	905	902	1187	1184	tr
α-Thujene	933	930	1013	1019	0.1 ± 0.0
α-Pinene	941	939	1029	1026	0.1 ± 0.0
Camphene	953	954	1060	1063	0.1 ± 0.0
Thuja-2,4(10)-diene*	961	960	1109	1113	tr
Sabinene	972	975	1118	1115	0.2 ± 0.0
β-Pinene	978	979	1121	1125	1.2 ± 0.1
Octan-3-one	980	984	1266	1270	tr
Myrcene	986	989	1165	1160	1.2 ± 0.1
Octan-2-one	990	991	1294	1297	tr
Octan-3-ol	992	991	1395	1394	tr
Octan-2-ol	993	995	1410	1412	tr
Octanal	1000	999	1299	1295	tr
α-Phellandrene	1002	1003	1179	1177	tr
α-Terpinene	1014	1017	1190	1189	0.6 ± 0.1
p-Cymene	1022	1025	1262	1265	1.2 ± 0.1
Limonene	1028	1029	1198	1195	0.8 ± 0.1
β-Phellandrene	1031	1030	1201	1200	0.2 ± 0.0
1,8-Cineole	1033	1031	1212	1212	0.1 ± 0.0
(*Z*)-β-Ocimene	1035	1037	1230	1227	0.1 ± 0.0
(*E*)-β-Ocimene	1046	1050	1248	1250	0.1 ± 0.0
γ-Terpinene	1058	1060	1258	1254	0.6 ± 0.1
Octan-1-ol	1065	1068	1561	1564	tr
cis-Sabinene hydrate	1069	1070	1469	1467	tr
Terpinolene	1087	1089	1270	1271	tr
Nonan-2-one	1091	1090	1392	1389	21.1 ± 2.0
Linalool	1096	1097	1555	1557	0.1 ± 0.0
Nonan-2-ol	1098	1098	1531	1534	0.2 ± 0.0
Nonanal	1102	1101	1396	1400	0.1 ± 0.0
1-Octen-3-yl acetate	1114	1113	1400	1402	0.2 ± 0.0
3-Octyl acetate	1122	1123	1348	1344	0.1 ± 0.0
α-Campholenal	1127	1126	1499	1496	tr
Geijerene	1140	1143	1334	1338	0.4 ± 0.1
Camphor	1146	1146	1501	1497	0.2 ± 0.0
2-Octyl acetate	1147	1147	1504	1493	0.1 ± 0.0

Pinocarvone	1166	1165	1578	1575	tr
Nonan-1-ol	1167	1169	1667	1663	tr
Borneol	1170	1169	1703	1700	tr
cis-Pinocamphone*	1172	1175	1534	1530	1.2 ± 0.2
Terpinen-4-ol	1175	1177	1602	1606	tr
Naphthalene	1180	1181	1763	1764	tr
Decan-2-one	1190	1192	1497	1495	1.8 ± 0.1
Methyl salicylate	1193	1192	1752	1755	tr
Myrtenal	1199	1196	1629	1632	0.1 ± 0.0
Decanal	1206	1202	1518	1515	tr
Octyl acetate	1211	1214	1484	1481	0.1 ± 0.0
trans-Carveol	1215	1217	1838	1836	tr
β-Citronellol	1222	1226	1752	1750	tr
(*Z*)-3-Hexenyl 2-methylbutanoate	1230	1232	1469	1472	tr
2-Nonyl acetate	1236	1236	1466	1466	1.5
Carvone	1246	1243	1727	1725	tr
iso-Geijerene C*	1249	1250	1492	1490	0.1 ± 0.0
Linalyl acetate	1254	1254	1563	1560	0.3 ± 0.0
Geranial	1268	1267	1730	1733	tr
Decan-1-ol	1271	1270	1764	1763	tr
Dihydroedulan II*	1281	1278	-	-	tr
Bornyl acetate	1284	1287	1576	1579	0.2 ± 0.0
Safrole	1285	1287	1876	1874	tr
Undecan-2-one	1292	1294	1598	1595	34.6 ± 3.1
Carvacrol	1296	1299	2219	2222	0.4 ± 0.1
Undecan-2-ol	1305	1307	1713	1712	0.1 ± 0.0
Nonyl acetate	1311	1312	1583	1581	0.2 ± 0.0
Myrtenyl acetate	1329	1327	1706	1701	tr
δ-Elemene	1334	1338	1466	1460	tr
α-Terpinyl acetate	1348	1349	1704	1700	0.1 ± 0.0
Citronellyl acetate	1355	1353	1666	1663	0.1 ± 0.0
Neryl acetate	1366	1362	1723	1725	tr
Carvacryl acetate	1370	1373	1886	1880	1.8 ± 0.1
α-Ylangene	1373	1375	1466	1470	tr
α-Copaene	1376	1377	1486	1492	0.1 ± 0.0
β-Bourbonene	1384	1388	1501	1504	0.1 ± 0.0
β-Elemene	1391	1391	1593	1590	0.2 ± 0.0
Dodecan-2-one	1399	1396	1699	1704	0.8 ± 0.1
Methyl eugenol	1401	1406	2033	2031	0.1 ± 0.0
α-Gurjunene	1408	1410	1525	1527	2.1 ± 0.2
α-Cedrene	1411	1412	1581	1579	tr
(*E*)-Caryophyllene	1421	1419	1596	1598	8.3 ± 0.7
β-Cedrene	1423	1421	1603	1606	0.2 ± 0.0
2-Undecyl acetate	1427	1430	1799	1802	0.2 ± 0.0
β-Copaene	1434	1432	1630	1631	tr
Neryl acetone	1439	1436	1835	1838	0.1 ± 0.0
(*E*)-β-Farnesene	1454	1457	1664	1660	0.2 ± 0.0

α-Humulene	1457	1455	1675	1670	0.3 ± 0.0
allo-Aromadendrene	1461	1460	1644	1646	0.1 ± 0.0
cis-Muurola-4(14),5-diene*	1465	1467	1674	1678	0.1 ± 0.0
γ-Gurjunene	1479	1477	1713	1714	0.1 ± 0.0
γ-Curcumene	1481	1483	1693	1690	2.7 ± 0.2
Germacrene D	1485	1485	1697	1690	1.9 ± 0.2
(*Z*,*E*)-α-Farnesene	1493	1491	1745	1747	1.1 ± 0.1
Tridecan-2-one	1495	1496	1817	1814	1.3 ± 0.1
α-Muurolene	1501	1500	1731	1729	tr
(*E*,*E*)-α-Farnesene	1506	1505	1756	1755	0.1 ± 0.0
β-Bisabolene	1508	1506	1719	1715	1.1 ± 0.1
Shyobunone*	1511	1510	1859	1859	0.5 ± 0.0
γ-Cadinene	1513	1514	1760	1760	0.1 ± 0.0
Cubebol	1517	1515	1928	1930	tr
δ-Cadinene	1520	1523	1764	1762	0.4 ± 0.0
trans-Calamenene	1531	1529	1841	1844	tr
10-*epi*-Cubebol*	1534	1535	1888	1890	tr
Dihydro-eugenyl acetate	1537	1538	-	-	tr
α-Cadinene	1540	1539	1813	1815	tr
α-Calacorene	1548	1546	1914	1916	tr
Elemicin	1555	1557	2267	2264	0.1 ± 0.0
Germacrene B	1559	1561	1801	1805	0.1 ± 0.0
(*E*)-Nerolidol	1561	1563	2038	2040	0.1 ± 0.0
Tetradecan-2-one	1567	1569	1877	1874	tr
Caryophyllene alcohol	1573	1572	2031	2033	0.2 ± 0.0
Germacrene D-4-ol*	1577	1576	2041	2044	1.1 ± 0.1
Caryophyllene oxide	1585	1583	1986	1983	1.1 ± 0.1
Piperonyl acetone*	1588	1590	-	-	tr
Humulene epoxide I	1591	1593	2046	2043	0.1 ± 0.0
Viridiflorol	1594	1593	2096	2099	0.9 ± 0.1
Humulene epoxide II	1610	1608	2077	2070	0.1 ± 0.0
1,10-di-*epi*-Cubenol*	1615	1619	2052	2054	tr
α-Muurolol	1642	1646	2185	2182	tr
α-Cadinol	1650	1654	2223	2221	0.2 ± 0.0
(*E*)-amyl cinnamic alcohol	1661	1661	-	-	0.3 ± 0.0
α-Bisabolol	1682	1686	2224	2228	0.1 ± 0.0
Shyobunol*	1689	1688	1957	1953	0.8 ± 0.0
(*E*)-Phytol	2124	2121	2636	2633	tr
n-Tricosane	2300	2300	2300	2300	tr
Monoterpene hydrocarbons					5.3 ± 0.4
Oxygenated monoterpenes					2.5 ± 0.1
Sesquiterpene hydrocarbons					19.5 ± 1.7
Oxygenated sesquiterpenes					4.6 ± 0.2
Aliphatic compounds					62.4 ± 5.7
Aromatic compounds					3.8 ± 0.4
Others					0.6 ± 0.1

Due to the heterogeneity of the volatile constituents identified in *Lepechinia* spp, it is very difficult to establish a characteristic pattern of compounds for the genus. The compositions of essential oils of several species of *Lepechinia* from the southern region of Ecuador is shown in Table No. 2. It is interesting to note that none of these studies reported methyl ketones as major constituents, but they show that chemical composition of the essential oils from related species can vary greatly.

Table No. 2
Composition of the essential oil of Lepechinia spp. from the southern region of Ecuador

Species	Major compounds	Reference
L. mutica	β-phellandrene (30%)	Malagón *et al*., 2003
L. mutica	shyobunol (10.8%), δ-3-carene (8.7%), δ-cadinene (6.9%), globulol (5.9%), (*E*)-caryophyllene (4.6%)	Ramírez *et al*., 2017
L. paniculata	aromadendrene (24.6%), β-phellandrene (7.7%)	Valarezo *et al*., 2012
L. paniculata	α-pinene (18.4%), (*E*)-caryophyllene (15.4%), β-pinene (10.9%), δ-3-carene (10.6%), (*E*)-caryophyllene (9.9%).	Panamito *et al*., 2021
L. radula	δ-3-carene (19.9%), β-pinene (17.0%), β-phellandrene (8.6%), (*E*)-caryophyllene (9.7%), (*E*,*E*)-α-farnesene (9.4%)	Morocho *et al*., 2017
L. heteromorpha	viridiflorene (27.3%), ledol (21.2%)	Gilardoni *et al*., 2019
L. betonicifolia	β-pinene (30.4%), sabinene (27.9%)	Calva *et al*., 2022

The results from the antioxidant activities determined, for the first time for the specie, by three *in vitro* methods: DPPH, ABTS, and FRAP assays are summarized in Table No. 3. In general, the essential oil presented a weak antioxidant activity through all the assays. A possible explanation for the low antioxidant activity of *L. rufocampii* essential oil could be attributed to the low level of phenols and terpenoids. The poor activity of the oils may be attributed to the weak ability of their main components to scavenge $DPPH^{*}$ and $ABTS^{*+}$ free radicals. An earlier study that screened the antioxidant activity of pure oil components found that carbonyl compounds exhibited a low scavenging activity (Ruberto & Baratta, 2000). Fe^{3+} reduction can be used as an indicator of electron-donating activity and therefore reflects an important mechanism of phenolic antioxidant action. The essential oil at 0.5 mg/mL had the best significant reductive potential, similar to those found for Trolox. This better activity with this method may be explained by the possible different mechanisms involved in the assays.

The antimicrobial activity of the *L. rufocampii* essential oil was assessed against four pathogenic bacterial strains and a pathogenic fungus strain. The antimicrobial activity of the essential oil was estimated in terms of net zone of inhibition and minimum inhibitory concentration (Table No. 4). The essential oil showed low (ZOI < 5 mm) and very good (ZOI > 15 mm) activities against the microbial strains. The essential oil showed very good activity against *S. aureus*, *E. coli* and *S. enterica* serovar, but low activities against *P. aeruginosa* and *C. albicans*. The MIC value of the essential oil varied from 1.04-33.05 µL/mL, with the lowest for *S. enterica* serovar. The antimicrobial activity of the essential oils may be due to the occurrence of high proportion of methyl ketones. The methyl ketone nonan-2-one had been reported as an active antifungal compound (Vaughn *et al*., 1993). By contrast, the antimicrobial activity of undecan-2-one, reported as major compound of *L. rufocampii* essential oil, is known to be low against bacterial strains but elevated against yeasts (Gibka *et al*., 2009), but only *E. coli* was common in both studies.

Table No. 3
Antioxidant effectiveness of *Lepechinia rufocampii* essential oil

Sample	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	Concentration (mg/mL)	FRAP (µM of ascorbic acid equivalents)
Essential oil	12.98 ± 0.24	39.56 ± 2.75	4	166.9 ± 0.1
			2	145.0 ± 4.2
			1	135.2 ± 4.0
			0.5	127.0 ± 2.1
Trolox	0.01 ± 0.01	0.025 ± 0.01	0.05	120.0 ± 4.1

Values are the mean of three determinations with standard deviation

Table No. 4
Antimicrobial activity against some microorganisms of Lepechinia rufocampii essential oil

	ZOI (mm)	MIC (µL/mL)
Staphylococcus aureus	18.00 ± 0.12	1.07 ± 0.01
Ciprofloxacin	23.70 ± 0.21	0.19 ± 0.30
Escherichia coli	21.01 ± 0.03	1.65 ± 0.01
Ciprofloxacin	31.30 ± 0.32	0.12 ± 0.30
Salmonella enterica serovar	18.01 ± 0.01	1.04 ± 0.01
Ciprofloxacin	32.00 ± 0.56	0.39 ± 0.17
Pseudomonas aeruginosa	4.17 ± 0.01	33.05 ± 0.11
Ciprofloxacin	31.70 ± 0.36	0.27 ± 0.37
Candida albicans	4.01 ± 0.12	16.50 ± 0.01
Ketoconazole	31.30 ± 0.11	0.29 ± 0.01

CONCLUSIONS

A complete characterization of the chemical profiling and antioxidant and antimicrobial properties was made for the essential oil from the leaves and flowers of *Lepechinia rufocampii*. One hundred and twenty-two compounds were identified in the essential oil. Aliphatic compounds, mainly methyl ketones (62.4%) and sesquiterpene hydrocarbons (19.5%) were found to be the most abundant compounds, while oxygenated monoterpenes were the minor. The most abundant compounds were undecan-2-one (34.6%), nonan-2-one (21.1%), and (*E*)-caryophyllene (8.3%). The essential oil had a low scavenging effect and it showed ferric reducing activity. The essential oil showed very good activity against *S. aureus*, *E. coli* and *S. enterica* serovar, but low activities against *P. aeruginosa* and *C. albicans*.

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