academicJournals

Vol. 10(33), pp. 553-558, 3 September, 2016
DOI: 10.5897/JMPR2016. 6217
Article Number: 05376B460431
ISSN 1996-0875

http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Chemical Composition antimicrobial and free radical scavenging activity of essential oil from leaves of *Renealmia thyrsoidea* (Ruiz &Pav.) Poepp. & Endl.

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Received 2 August, 2016; Accepted 25 August, 2016

***Renealmia thryrsoidea* (Ruiz & Pav.) Poepp. & Endl is a plant used by the Amazonian indigenous people of Ecuador for its various medicinal properties. Its leaves exhibit a remarkable aroma with a hint of spiciness. The essential oil extracted from its leaves was analyzed by GM/MS, using two systems with columns of different polarity, in both was confirmed the presence of terpinolene (26.32%), α-felandrene (17.16%), γ-terpinene (6.55%), β-pinene (5.97%) and p-cymol (4.70%). Free radical scavenging activity was analyzed through 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) spectrophotometric methods; by applying ABTS method, the activity was comparable to that of the essential oil of *Thymus vulgaris*. The trials of antimicrobial activity show a strong inhibition against Gram negative bacteria as *Escherichia coli* and *Pseudomonas aeruginosa*.**

Key words: *Renealmia thryrsoidea*, essential oil, DPPH, ABTS, minimum inhibitory concentration (MIC).

INTRODUCTION

Essential oils are a group of secondary metabolites that currently have a productive significance (Schmidt, 2015). Many of them are valued for their qualities as potential medicinal products (Noriega, 2009) and cosmetics (Muñoz-Acevedo et al., 2015). Ecuador is a country with a very high plant diversity (Bendix et al., 2013) and many of these species are rich in essential oils that have various medicinal uses (Malagon et al., 2003; Sacchetti et al., 2005; Guerrini et al., 2009).

The *Renealmia* genus (Zingiberaceae family) is distributed throughout South America with 75 species (Maas, 1997). In Ecuador, the number of species reaches 25, 4 of which are endemic (Jorgensen and León-Yanez, 1999).

Renealmia thyrsoidea (Ruiz & Pav.) is a widely distributed species in tropical America, in countries such as Bolivia, Colombia, Ecuador, Guyana, Nicaragua, Panama, Peru, Suriname, Trinidad and Tobago and Venezuela. In Ecuador this spices is distributed in the three continental regions: the coast, the highlands, and Amazon; with a noted presence in the provinces of Cotopaxi, Imbabura, Morona Santiago, Napo, Pastaza,

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Pichincha, Sucumbios, Tungurahua and Zamora Chinchipe (Jorgensen and León-Yanez, 1999). An important reported use is that of acting as an antidote for snake bites (Davis and Yost, 1983). The antimalarial and antipyretic properties of *R. thyrsoidea* were reported by Schultes et al. (1990) and Céline et al. (2009), this plant possesses analgesic and anti-flu properties (de La Torre et al., 2008), and anti-leishmaniasis uses are reported in various regions (Yannick, 2009). The fruits are edible (de la Torre et al., 2008) and a dye is extracted from them to be used in ritual practices (Maas, 1997).

Several of the ethnic groups present in the Amazon region of Ecuador employ the species. The importance of this research lies in the assessment of the plant's essential oils for pharmacological and medicinal traits.

MATERIALS AND METHODS

Plant

The leaves of *R. thyrsoidea* were collected in the community of San Luis de Inimkis, Canton Macas, province of Morona Santiago, with the following geographic coordinates: 02° 26' South latitude and 78° 11' West longitude; at an altitude of 1070 m.o.s.l. The botanical identification took place in the herbarium Alfredo Paredes of the Universidad Central del Ecuador by the botanist Carlos Cerón. The fresh leaves were collected from mature plants and distilled in vapor stream, with an equipment of 250 liters capacity belonging to the Fundacion Chankuap recursos para el futuro, in the city of Macas.

Chemical composition

The essential oil composition was determined using gas chromatography and mass spectrometry equipped with two columns of different polarity systems. The sample was prepared by dissolving 25 µl of essential oil in 1 ml of acetone. For coupling 1, a gas chromatograph GC Agilent 7890 A was coupled to a mass spectrometer MS 5975. The column used was a DB1-MS, with a length of 60 m, 0.32 mm internal diameter and 0.25 um thickness. The analysis started at a temperature of 60°C with a rate of 4°C per min up to 280°C. The carrier gas was helium at a flow of 3.5 ml min^{-1}. The injection volume was 1 µl. For coupling 2, a gas chromatograph GC Agilent 6890 A was coupled to a mass spectrometer MS 5973. The column used was a DB-Wax, with a length of 30 m, 0.25 mm internal diameter and 0.25 um thick. The analysis started at a temperature of 60°C with a velocity gradient of 3°C per min up to 240°C. The carrier gas was helium at a flow of 3.3 ml min^{-1}. The injection volume was 1 µl. In both cases, electron ionization was used with energy of 70 eV and a range of m/z 35 to 400 Da. The component identification was performed by comparing the mass spectra to those present in the MASSLIB database and by determining the retention index for each component using as reference the standard series C8-C30 of hydrocarbons.

Free radical scavenging activity

Spectrophotometric methods DPPH (diphenyl-2-picrylhydrazyl) and ABTS [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt] have been a permanent technique to evaluate the free radical scavenging activity in essential oils (Bardaweel et al., 2015; Ornano et al., 2015). Fort the DPPH assay, varying amounts of *R. thyrsoidea* essential oil were taken and dissolved in dimethyl sulfoxide (DMSO) to a volume of 100 µl. To each solution, 2.9 ml of DPPH (1×10^{-4} molar in ethanol) was added. The solution was stirred vigorously for 30 min in the dark at room temperature. The absorbance was measured at 517 nm in a Shimadzu UV 1240 mini. Similarly, the analysis proceeded with the ABTS test, to each solution dissolved in DMSO 0.9 ml of ABTS 40 mM (previously radicalized with a $K_2S_2O_8$ solution) was added. The absorbance was measured at 734 nm in a Shimadzu UV mini 1240. Antiradical activity for each mixture was calculated according to the following formula:

$$\text{DPPH or ABTS } \% = \frac{Ab - Aa}{Ab} \text{x } 100$$

Where Aa and Ab are the absorbance of blanks and samples, respectively after 30 min (DPPH) and 1 min ABTS. The activity of the essential oil was evaluated by calculating the IC_{50}, which is 50% inhibition of oxidation of DPPH and ABTS; this was calculated from the data of the calibration curves obtained from the data of the concentration versus percentage inhibition. As referents of activity, *Tymus vulgaris* essential oil and butylated hidroxianisole (BHA) were used.

Antimicrobial activity (MIC evaluation)

The antimicrobial activity was evaluated using the disc diffusion method often described as an applicable methodology to evaluate the antimicrobial activity of essential oils (Rivera et al., 2015; Pesavento et al., 2015). The strains tested were Grampositive bacteria: *Staphylococcus aureus* subsp. *aureus* ATCC 6538 and *Streptococcus mutans* ATCC 25175; Gramnegative bacteria: *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 9027. The yeasts *Candida tropicalis* ATCC 13803 and *Candida albicans* ATCC 10231 were also tested. The antimicrobial activity is described as the minimum inhibitory concentration (MIC) in mg ml^{-1}. The essential oil of *T. vulgaris* was used as a reference for any appreciable activity (Rossi et al., 2011).

RESULTS AND DISCUSSION

Production of the essential oil

The essential oil yield was 0.047% (w/w). Essential oil density was 0.873 mg ml^{-1}.

Chemical composition

In both studies with both DB1 and DB-Wax column the most abundant compounds were: terpinolene (26.32%), α-felandrene (17.16%), γ-terpinene (6.55%), β-pinene (5.97%) and p- cymol (4.70%). The analysis with DB1 column reveals the presence of components 116; DB-Wax column revealed the presence of 107 compounds with the same equivalent percentages, as shown in Table 1.

Free radical scavenging activity

IC_{50} values indicate the ability to inhibit by 50%, the

Table 1. Components of essential oil of leaves of *R. thyrsoidea*.

CG/MS with column DB1-MS				CG/MS with a column DB-Wax			
No	**Compound**	**Retentionindex**	**Area (%)**	**No**	**Compound**	**Retentionindex**	**Area (%)**
1	α-thujene	923	0.20	1	6-p-menthen-2-on	765	0.02
2	α-pinene	930	2.38	2	α-thujene	1038	0.20
3	α-fenchene	941	0.04	3	α-pinene	1045	2.38
4	Camphene	943	0.05	4	1,4-cadinadien	1046	0.15
5	Verbenene	946	0.23	5	α-fenchene	1082	0.04
6	3-cyclohexeno, 4-methyl-	956	0.02	6	camphene	1091	0.05
7	Sabinene	965	0.02	7	β-pinene	1125	5.97
8	β-pinene	971	5.97	8	sabinene	1134	0.02
9	dehydrocineol/1,8-epoxy-2-p-menthen	978	0.02	9	verbenene	1139	0.23
10	β-myrcene	981	1.78	10	δ-2-carene	1149	0.03
11	δ-2-carene	991	0.03	11	β-myrcene	1164	1.78
12	α-phellandrene	998	17.16	12	δ-3-carene	1167	2.16
13	δ-3-carene	1005	2,16	13	α-phellandrene	1178	17,16
14	α-terpinene	1009	1.24	14	α-terpinene	1196	1.24
15	p-cymol	1012	4.70	15	dehydrocineol/1,8-epoxy-2-p-menthen	1206	0.02
16	β-phellandrene	1019	1.23	16	limonene	1213	3.40
17	Limonene	1021	3.40	17	β-phellandrene	1225	1.23
18	1,8 cineol	1025	Tr	18	1,8 cineol	1225	Tr
19	β-cisocimene	1025	0.08	19	β-cisocimene	1234	0.08
20	β-trans-ocimene	1036	1.89	20	γ-terpinene	1249	6.55
21	γ-terpinene	1048	6.55	21	β-trans-ocimene	1252	1.89
22	Fenchone	1066	0.01	22	p-cymol	1281	4.70
23	p- dimethylstyrol	1072	0.41	23	terpinolen	1287	26.32
24	Terpinolen	1079	26.32	24	menthatrien, 1,3,8-p-	1397	0.07
25	Linalool	1083	0.06	25	fenchone	1411	0.01
26	Fenchol	1097	0.07	26	p- dimethylstyrol	1443	0.41
27	menthatrien, 1,3,8-p-	1098	0.07	27	α-cubebe	1466	0.06
28	menthen-1-ol, trans-2-p-	1105	0.07	28	menthen, 4,8-epoxy-1-p-	1475	0.47
29	Kampfer	1119	0.02	29	α-copaene	1498	2.02
30	trans-pinocarveol	1122	1.05	30	pinocamphone	1519	0.05
31	menthen, 4,8-epoxy-1-p-	1126	0.47	31	α-ylangene	1521	0.03
32	methylcamphenilol	1131	0.03	32	kampfer	1529	0.02
33	pinocamphon	1135	0.05	33	iso-pinocamphon	1547	0.48
34	Pinocarvon	1136	0.02	34	linalool	1548	0.06
35	menthadien-8-ol, 1,5-p-	1137	0.02	35	menthen-1-ol, trans-2-p-	1563	0.07
36	iso-pinocamphon	1148	0.48	36	pinocarvon	1570	0.02
37	α-8hidroxy terpinen	1153	0.13	37	α-santalene	1577	1.12
38	menthadien-4-ol, 1,8-p-	1156	Tr	38	fenchol	1585	0.07
39	p- 8- cymenol	1157	0.34	39	α-bergamotene	1586	0.28
40	4-terpinenol	1160	0.29	40	bornylacetate	1588	0.03
41	Myrtenal	1167	0.03	41	methylcamphenilol	1596	0.03
42	α-terpineol	1170	0.70	42	γ-maaliene	1604	0.02
43	Myrtenol	1176	0.06	43	4-terpinenol	1607	0.29
44	Verbenon	1178	0.06	44	aromadendrene	1614	0.56
45	trans-piperitol	1187	0.04	45	myrtenal	1627	0.03
46	benzylaceton	1205	0.05	46	β-epi-santalene	1638	0.18
47	Citronellol	1208	0.03	47	trans-pinocarvylacetate	1647	0.06
48	menthen-2-on, 6-p-	1218	0.02	48	alloaromadendrene	1647	0.98
49	trans-myrtanol	1220	0.03	49	β-santalene	1652	Tr

Table 1. Contd.

50	perillaaldehyd	1243	0.01	50	trans-pinocarveol	1655	1.05
51	Phellandral	1246	0.03	51	trans-β- farnesene	1666	0.10
52	bornylacetate	1273	0.03	52	α-humulene	1670	0.07
53	Carvacrol	1275	0.05	53	menthadien-4-ol, 1,8-p-	1680	Tr
54	trans-pinocarvylacetate	1278	0.06	54	γ-muurolene	1688	0.38
55	benzalaceton	1316	0.02	55	ledene	1694	0.77
56	α-cubebene	1346	0.06	56	δ-selinene	1695	0.20
57	α –ylangene	1368	0.03	57	α-amorphene	1700	0.10
58	α-copaene	1373	2.02	58	α-terpineol	1701	0.70
59	dimethylanthranilat	1376	Tr	59	verbenone	1713	0.06
60	γ-guajene	1406	0.31	60	β-selinene	1720	0.06
61	b-ylangene	1412	Tr	61	menthadien-8-ol, 1,5-p-	1723	0.02
62	α-santalene	1415	1.12	62	α-muurolene	1724	0.19
63	γ-maaliene	1420	0.02	63	phellandral	1725	0.03
64	α-bergamotene	1429	0.28	64	8-hydroxy-α-terpinene	1728	0.13
65	aromadendrene	1433	0.56	65	β-bisabolene	1729	0.33
66	β-epi-santalene	1439	0.18	66	trans-piperitol	1742	0.04
67	trans-β-farnesene	1445	0.10	67	δ-cadinene	1756	1.30
68	α-humulene	1446	0.07	68	γ-cadinene	1757	0.24
69	β-santalene	1451	Tr	69	citronellol	1764	0.03
70	alloaromadendrene	1453	0.98	70	perillaaldehyde	1767	0.01
71	trans-isoeugenolmethylether	1458	0.02	71	myrtenol	1784	0.06
72	cadina-1(6),4-dien ii	1464	0.19	72	α-cadinene	1788	0.06
73	γ-muurolene	1467	0.38	73	trans-calamenene	1827	0.12
74	γ-amorphene	1470	0.10	74	8-p-cymenol	1834	0.34
75	trans-β-bergamotene	1475	0.07	75	benzylacetone	1852	0.05
76	β-selinene	1477	0.06	76	trans-myrtanol	1853	0.03
77	δ-selinene	1481	0.20	77	palustrol	1926	0.10
78	Ledene	1487	0.77	78	maaliol	1981	Tr
79	α-muurolene	1489	0.19	79	ledol	2027	0.36
80	β-bisabolene	1497	0.33	80	γ-guajene	2030	0.31
81	γ-cadinene	1502	0.24	81	trans-nerolidol	2034	0.49
82	trans-calamenene	1505	0.12	82	cubeban-11-ol	2046	0.23
83	δ-cadinene	1511	1.30	83	cubenol	2050	0.24
84	1,4-cadinadiene	1520	0.15	84	cubenol	2050	0.06
85	α-calacorene	1524	0.03	85	α-calacorene	2053	0.03
86	α-cadinene	1526	0.06	86	cubenol, 1,10-diepi-	2055	0.05
87	Euasaron	1533	0.02	87	globulol	2067	0.54
88	trans-nerolidol	1544	0.49	88	dimethylanthranilat	2068	Tr
89	Maaliol	1545	Tr	89	viridiflorol	2080	0.21
90	Palustrol	1555	0.10	90	guajol	2080	1.27
91	spathulenol p.1 (hm)	1558	0.10	91	propanol, 2-(4a,8-dimethyl-2,3,4,4a,5,6,7,8-octahydro-	2091	0.13
92	Globulol	1569	0.54	92	guaien-11-ol, 5	2104	0.55
93	Viridiflorol	1575	0.21	93	benzalacetone	2105	0.02
94	cubeban-11-ol	1578	0.23	94	spathulenol p.1 (hm)	2131	0.10
95	Guajol	1580	1.27	95	γ-eudesmol	2161	0.22
96	propanol, 2-(4a,8-dimethyl-2,3,4,4a,5,6,7,8-octahydro-	1585	0.13	96	a-santalal	2164	0.10
97	Ledol	1587	0.36	97	τ-cadinol	2167	0.27
98	cubenol, 1,10-diepi-	1597	0.05	98	trans-isoeugenolmethylether	2170	0.02
99	guaien-11-ol, 5	1605	0.55	99	carvacrol	2195	0.05
100	Cubenol	1610	0.24	100	δ-cadinol	2196	0.09

Table 1. Contd.

101	γ-eudesmol	1612	0.22	101	bulnesol	2203	0.77
102	τ-cadinol	1620	0.27	102	bisabolol, α- p.1	2213	0.03
103	δ-cadinol	1624	0.09	103	α-eudesmol	2217	0.25
104	Cubenol	1626	0.06	104	β-eudesmol	2229	0.29
105	β-eudesmol	1629	0.29	105	α-cadinol	2229	0.19
106	α-cadinol	1632	0.19	106	campherenol	2291	0.30
107	α-eudesmol	1634	0.25	107	γ-bicyclohomofarnesal	2366	0.03
112	tras-a-santalol	1676	0.09	Total 96.81%			
113	farnesylsaeuremethylester, e,e-	1758	0.04				
114	γ-bicyclohomofarnesal	1770	0.03				
115	prop-2-en-1-on, 1-(2,6-dihydroxy -4-methoxyphenyl)-3	2324	0.49				
116	2,4(8)-menthadienol-(1)		0.25				
Total		97.98%					

Table 2. Free radical scavenging activity of *R. thryrsoidea* leaf essential oil, by applying DPPH and ABTS methods.

Essential oils	DPPH IC_{50} mg ml^{-1}	ABTS IC_{50} mg ml^{-1}
R. thyrsoidea	20.86 ± 0.4	1.44 ± 0.1
T. vulgarys	2.42 ± 0.1	2.92 ± 0.2
BHA	0.059 ± 0.001	0.025 ± 0.001

Table 3. Antimicrobial activity of the essential oil of leaves of *R. thryrsoidea.*

Microorganismos	*R. thryrsoidea* MIC (mg ml^{-1})	*T. vulgaris* MIC (mg ml^{-1})
Gram negative bacteria		
Escherichiacoli ATCC 8739	0.35	0.1
Pseudomonas aeruginosa ATCC 9027	0.35	0.1
Gram positive bacteria		
Staphylococcus aureus subsp. *aureus* ATCC 6538	1.39	0.39
Streptococcus mutans ATCC 25175	5.56	0.1
Yeast		
Candida tropicalis ATCC 13803	1.39	0.1
Candida albicans ATCC 10231	0.35	0.1

concentration of free radicals present. For this test, the reference essential oil was that of *T. vulgaris* whose thymol and carvacrol components are widely known for this property (Mastelić et al., 2008; Lee et al., 2005). The results are shown in Table 2.

Antimicrobial activity

There are different results depending on the microbial strains evaluated. A good activity was observed in the Gram negative bacteria tested, close to the natural pattern registered with the essential oil of *T. vulgaris*, represented by the values in Table 3.

Conclusion

The study provides an insight into the chemical components present in the essential oil from the leaves of *R.*

thyrsoidea. Most notable was the presence of monoterpenes. As for the antioxidant activity appreciable activity highlighted in the evaluation of electron scavenging capacity ABTS methodology, whereas by the method DPPH oil is less active. Interestingly, anti-microbial activity evaluated in both Gram negative strains, whose values of minimum inhibitory concentration reaches 0.35 mg ml^{-1}.

Conflict of interests

The authors have none to declare.

ACKNOWLEDGEMENTS

We would like to thank Symrise for funding and supporting this research.

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