Rev. CENIC Cienc. Quím. Vol. 54. (publicación continua): 275-282. Año. 2023. e-ISSN: 2221-2442.

ARTICULO DE INVESTIGACION

CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF ESSENTIAL OIL FROM *MARGYRICARPUS PINNATUS (LAM.) KUNTZE*

COMPOSICIÓN QUÍMICA Y ACTIVIDADES BIOLÓGICA DEL ACEITE ESENCIAL DE *MARGYRICARPUS PINNATUS (LAM.) KUNTZE*

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Recibido: 08 de noviembre de 2023; Aceptado: 07 de diciembre de 2023;

ABSTRACT

Margyricarpus pinnatus (Lam.) Kuntze, commonly name nigua or piquiyuyo in Ecuador, is a subshrub and grows primarily in the montane tropical biome. The chemical composition, antioxidant and antimicrobial activities of its essential oil from the leaves and flowers of plants grown in the Ecuadorian Andes were studied. The essential oil was obtained by hydrodistillation with a yield of 0.36 % v/m. With the aid of gas chromatography with mass and flame ionization detectors (GC-MS and GC-FID), 134 volatile constituents making up 97.5 % of the total oil constituents were identified. The major groups of compounds were aromatic compounds (51.9 %), oxygenated monoterpenes (16.3 %) and monoterpene hydrocarbons (12.5 %). The most abundant compounds were carvacrol (23.1%) and carvacryl acetate (19.2 %). Antioxidant activity was examined using DPPH and ABTS assays. The essential oil had a high scavenging effect and reduction of ferric ion capacity through FRAP assay. The results show that the essential oil scavenged DPPH· radical and ABTS·$^{+}$ radical capacity as reflected by the IC_{50} values 1.18 ± 0.27 mg/mL and 4.47 ± 0.31mg/mL, respectively. These results agreed with the literature for essential oils that phenols possess high antioxidant activity. The antimicrobial activity was determined against four pathogenic bacterial strains and a pathogenic fungus strain using a modified agar-diffusion method. The results of the antimicrobial tests showed that Escherichia coli and Candida albicans were very sensitive to the effects of the essential oil.

Keywords: *Margyricarpus pinnatus*, essential oil, chemical composition, antioxidant activity, antimicrobial activity.

RESUMEN

Margyricarpus pinnatus (Lam.) Kuntze, comúnmente llamado nigua o piquiyuyo en Ecuador, es un subarbusto y crece principalmente en el bioma tropical montano. Se estudió la composición química, actividades antioxidantes y antimicrobianas de su aceite esencial procedente de las hojas y flores de plantas cultivadas en los Andes ecuatorianos. El aceite esencial fue obtenido por hidrodestilación con un rendimiento de 0,36 % v/m. Con la ayuda de cromatografía de gases con detectores de ionización de masa y de llama (GC-MS y GC-FID), se identificaron 134 componentes volátiles que representan el 97,5 % del total de componentes del aceite esencial. Los principales grupos de compuestos fueron compuestos aromáticos (51,9 %), monoterpenos oxigenados (16,3 %) e hidrocarburos monoterpénicos (12,5 %). Los compuestos más abundantes fueron carvacrol (23,1%) y acetato de carvacrilo (19,2 %). La actividad antioxidante se examinó mediante los ensayos DPPH y ABTS. El aceite esencial tuvo un alto efecto eliminador y una reducción de la capacidad de iones férricos mediante el ensayo FRAP. Los resultados muestran que el aceite esencial elimina la capacidad de radicales DPPH· y ABTS·$^{+}$, como lo reflejan los valores de IC_{50} de 1,18 ± 0,27 mg/mL y 4,47 ± 0,31 mg/mL, respectivamente. Estos resultados coincidieron con la literatura sobre aceites esenciales de que los fenoles poseen una alta actividad antioxidante. La actividad antimicrobiana se determinó contra cuatro cepas de bacterias patógenas y una cepa de hongo patógeno utilizando un método de difusión en agar modificado. Los resultados de las pruebas antimicrobianas mostraron que *Escherichia coli* y *Candida albicans* fueron muy sensibles a los efectos del aceite esencial.

Palabras claves: *Margyricarpus pinnatus*, aceite esencial, composición química, actividad antioxidante, actividad antimicrobiana.

INTRODUCTION

Medicinal plants constitute the main component of popular medicine, knowledge about its usefulness has been accumulated through several centuries based on different medicinal systems, are supported by numerous written documents, coming from the most diverse cultures and are consumed throughout the world for the treatment of numerous diseases. In recent decades their use has increased to such an extent that The World Health Organization (WHO) has estimated that 80 % of people living in developing countries exclusively use traditional medicine (OMS, 2014).

Margyricarpus pinnatus (Lam.) Kuntze (Rosaceae), commonly name *nigua* or *piquiyuyo* in Ecuador, is a subshrub and grows primarily in the montane tropical biome. The native range of this species is Colombia to S. South America. It is a quietly beautiful, rich, green, heather-like

plant with pure white pearl-like fruit. It is traditionally used to treat unspecified medicinal disorders, as a medicine, and it has environmental uses and for food (Plants of the World Online, 2023).

A review of the literature reveals that the chemical composition of the essential oil of this species has been the subject of a previous study from plants grown in Colombia (Alfonso-Rojas et al., 2009). Herein, results from studies of the chemical composition and antioxidant and antimicrobial activities of essential oil obtained from the aerial part of *Margyricarpus pinnatus* (Lam.) Kuntze grown wild in the highlands of Ecuador are reported.

MATERIALS AND METHODS

The aerial part (leaves and flowers) of *M. pinatus* were collected during flowering in March 2019 at 3000 m above sea level in Cañar canton (2°29′1.32″ S, 78°58′42.24″ W), Ecuador. A voucher specimen was kept at the Herbarium of the University of Azuay (accession number 7547).

Essential oil was isolated from 200 g of fresh material by hydrodistillation for 3 h in a modified Clevenger-type apparatus (Pino et al., 2009). Distillations were performed in triplicate. The yield of essential oil was 0.36% v/m of the raw-material mass.

Identification of the compounds was performed using GC-MS on a QP-2010 Ultra (Shimadzu, Japan) equipped with a 30 m × 0.25 mm i.d. × 0.25 µm DB-5ms (J & W Scientific, Folsom, CA, USA) or DB-Wax (J&W Scientific, Folsom, CA, USA) fused-silica capillary columns. The following conditions were used for both columns: oven temperature was programmed from 70 °C (after 2 min) to 250 °C at 4 °C/min and held for 10 min, helium as carrier gas at 1 mL/min, injector and FID temperatures were set at 250 °C. Compounds were identified by comparing linear retention indices determined using a homologous series of *n*-alkanes (C_8–C_{24}) and their mass spectral libraries (NIST 05, Wiley 6, Adams 2007, and NBS 75 k and in-house Flavorlib created with pure standards).

Quantification was made using a Hewlett-Packard 6890N series II (Agilent, Santa Clara, CA, USA) equipped with a similar DB-5ms column. Quantification of compounds was carried out

using relative percentage abundance and normalization method with correction response factors based on grouping the essential oil components by their functional groups (Costa et al., 2008). Percentage data are the mean values of two injections per sample.

The antioxidant activity was measured for two *in vitro* assays: 2,2-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS). The antioxidant capacity was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (Tabart, 2008). In the test tubes, 1.5 mL of DPPH* (0.075 mg/mL) in ethanol were mixed with 750 µL of five concentrations of the essential oil to evaluate in a range of concentrations between 0.2-10 mg/mL A control sample (absolute ethanol) and a reference sample (750 µL absolute ethanol and 1.5 mg/mL of DPPH solution) were also used. The decrease in absorbance was determined at 515 nm, and Trolox was used as antioxidant standard. The IC_{50} values (total antioxidant compound necessary to decrease the initial DPPH radical concentration by 50%) were determined. Analyses were performed in triplicate.

The antioxidant capacity was also determined in terms of hydrogen donating or radical scavenging ability, using the stable $ABTS^{*+}$ radical (Re et al., 1995). It was carried out according to the procedure described by Kuskoski et al. (2004). The $ABTS^{*+}$ radical was formed after the reaction of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration) incubated at room temperature and in the dark for 16 h. Once the $ABTS^{*+}$ radical was formed, it was diluted with methanol until obtaining an absorbance value of 0.700 at 750 nm. A 100 µL aliquot of the essential oil was added to 1 mL of $ABTS^{*+}$ solution. The absorbance, after 10 min, was measured spectrophotometrically at 750 nm. 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. Analyses were performed in triplicate.

The antibacterial activity was assayed against some human pathogenic strains from to the American Type Culture Collection, Maryland, USA: *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 fungus *Candida albicans* (ATCC 14053). Strains were kept on Nutrient Agar and Sabouraud slants at 4 ºC, for bacteria and fungus respectively. Bacterial strains were activated in Nutrient Agar at 37 °C for 24 hours, while the fungus was activated in Sabouraud at 28 ºC for 48 hours before testing. The *in vitro* antimicrobial activity of the essential oil was tested by the agar disc-diffusion assay and the determination of minimum inhibitory concentrations (MIC) as per CLSI guidelines, for the sensible microbial strains (Wikler et al., 2006). 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 fungus. 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. All experiments were performed in triplicate.

RESULTS AND DISCUSSION

A total of 134 volatile constituents making up 97.5 % of the total oil constituents from *M. pinnatus* grown in the highlands of Ecuador were identified (Table 1). The essential oil composition included mainly aromatic compounds (51.9 %). The major groups of compounds were aromatic compounds (51.9 %), oxygenated monoterpenes (16.3 %) and monoterpene hydrocarbons (12.5 %). Its main constituents were carvacrol (23.1%) and carvacryl acetate (19.2 %).

Table 1. Composition of the essential oil from *Margyricarpus pinnatus*

Compound	RI_A[a]	RI_P	%	Compound	RI_A[a]	RI_P	%
n-Octane	800	800	tr[b]	3-Methylbutyl hexanoate	1256	1457	tr
(*Z*)-3-Hexen-1-ol	860	-	tr	2-Phenylethyl acetate	1259	1816	0.3
Hexan-1-ol	871	-	tr	Geranial	1268	1730	tr
1-Methyl-3-isopropylcyclopentane	873	-	0.1	Citronellyl formate	1275	1631	tr
2-Methylbutyl acetate	880	1111	0.2	Bornyl acetate	1284	1576	0.3
2,6-Dimethyl-1,5-heptadiene	886	-	tr	Thymol	1290	2150	2.2
(*E*)-Salvene	892	-	tr	Carvacrol	1296	2220	23.1
n-Nonane	900	-	tr	Myrtenyl acetate	1329	1705	tr
Heptanal	903	1181	tr	*trans*-Carvyl acetate	1341	1760	tr
Santolina triene	908	1013	tr	Citronellic acid	1347	2251	0.1
α-Thujene	933	1012	1.6	α-Cubebene	1349	1467	0.1
α-Pinene	941	1029	0.8	Thymyl acetate	1351	1810	tr
α-Fenchene	949	1056	tr	Citronellyl acetate	1355	1665	5.2
Camphene	953	1060	0.2	Carvacryl acetate	1370	1886	19.2
Thuja-2,4(10)-diene	961	1109	tr	Geranyl acetate	1382	1758	0.2
Benzaldehyde	963	1511	tr	β-Bourbonene	1384	1501	tr
Sabinene	972	1118	0.5	β-Cubebene	1387	1540	0.2
1-Octen-3-ol	976	1455	0.3	(*E*)-Jasmone	1393	-	tr
β-Pinene	978	1121	0.8	Methyl eugenol	1401	2033	0.4
Octan-3-one	981	1266	tr	α-Gurjunene	1408	1525	0.1
Myrcene	986	1165	1.1	(*E*)-Caryophyllene	1421	1596	0.4
Octan-2-one	990	1294	tr	Cuminyl acetate	1432	1954	tr
Octan-3-ol	992	1395	0.2	β-Gurjunene	1434	1602	1.9
6-Methyl-5-hepten-2-ol	994	-	tr	Coumarin	1437	-	tr
2,3-Dehydro-1,8-cineole	996	1193	tr	Aromadendrene	1442	1609	tr
m-Mentha-1(7),8-diene	999	-	tr	Citronellyl propanoate	1448	-	tr
p-Mentha-1(7),8-diene	1001	-	tr	Geranyl acetone	1455	1864	tr
α-Phellandrene	1003	1179	0.2	α-Humulene	1457	1674	0.2
δ-3-Carene	1012	1161	0.2	Valerena-4,7(11)-diene	1459	-	0.1
α-Terpinene	1014	1190	1.4	γ-Gurjunene	1479	1713	0.1
p-Cymene	1023	1263	6.5	Germacrene D	1485	1696	0.5
Limonene	1028	1198	2.6	(*E*)-Methyl isoeugenol	1492	2179	0.2
β-Phellandrene	1031	1201	0.4	Viridiflorene	1494	1698	0.1
1,8-Cineole	1033	1212	0.4	Bicyclogermacrene	1500	1700	0.2
(*E*)-β-Ocimene	1047	1249	0.1	Epizonarene	1502	1694	tr
2,6-Dimethyl-5-heptenal	1055	1355	tr	(*E,E*)-α-Farnesene	1507	1756	0.2
γ-Terpinene	1058	1258	2.6	Germacrene A	1511	1743	tr
cis-Sabinene hydrate	1068	1469	0.6	γ-Cadinene	1513	1760	tr
1-Nonen-3-ol	1077	1557	tr	δ-Cadinene	1520	1764	2.7
Terpinolene	1087	1270	0.1	*trans*-Calamenene	1531	1841	0.1
Nonan-2-one	1091	1390	0.5	*trans*-Cadina-1(2),4-diene	1536	1775	tr
p-Cymenene	1093	1435	tr	α-Cadinene	1541	1813	tr
Linalool	1096	1555	0.2	α-Calacorene	1548	1914	tr
trans-Sabinene hydrate	1099	1402	2.1	Elemicin	1555	2265	tr
1-Octen-3-yl acetate	1115	1404	5.5	β-Calacorene	1569	1955	tr
3-Octyl acetate	1121	1348	1.3	Maaliol	1575	-	0.1
trans-p-Mentha-2,8-dien-1-ol	1123	-	tr	Spathulenol	1578	2132	1.5
cis-Limonene oxide	1136	-	tr	Caryophyllene oxide	1585	1986	tr

Table 1. (cont.)

Compound	RI_A[a]	RI_P	%	Compound	RI_A[a]	RI_P	%
trans-p-2-Menthen-1-ol	1138	1551	tr	Globulol	1587	2084	0.2
Geijerene	1141	1335	tr	Viridiflorol	1594	2096	tr
Camphor	1144	1500	0.3	Humulene epoxide II	1604	2076	tr
Citronellal	1153	1484	0.5	*iso*-Spathulenol	1639	2225	0.2
Umbellone	1165	-	tr	(*Z*)-Asarone	1641	2255	0.2
Nonan-1-ol	1167	1667	tr	α-Cadinol	1654	2223	0.1
Borneol	1170	1703	0.1	*neo*-Intermedeol	1659	-	tr
cis-Pinocamphone	1172	1534	tr	*cis*-Calamenen-10-ol	1662	-	tr
Terpinen-4-ol	1175	1602	0.8	Shyobunol	1689	1957	tr
p-Cymen-8-ol	1182	1854	tr	*n*-Heptadecane	1700	-	tr
α-Terpineol	1187	1693	0.2	(*Z,Z*)-Farnesol	1714	-	tr
3-Nonenyl acetate	1200	-	tr	Oplopanone	1741	-	tr
Decanal	1206	1517	tr	Benzyl benzoate	1763	-	tr
Octyl acetate	1211	1484	tr	*n*-Octadecane	1800	-	tr
trans-Carveol	1215	1839	2.8	(*Z,E*)-Farnesyl acetate	1820	-	tr
cis-Sabinene hydrate acetate	1220	-	tr	Monoterpene hydrocarbons			12.5
β-Citronellol	1222	1752	1.8	Oxygenated monoterpenes			16.3
cis-Carveol	1227	-	tr	Sesquiterpene hydrocarbons			6.6
Thymyl methyl ether	1236	1579	0.2	Oxygenated sesquiterpenes			2.1
Pulegone	1236	1641	0.2	Aliphatic compounds			8.1
Cuminaldehyde	1241	1790	tr	Aromatic compounds			51.9
Carvone	1246	1727	0.3	Others			tr
Linalyl acetate	1254	1563	0.3				

[a]RI_A and RI_P, experimental linear retention indices on DB-5ms and DB-Wax column, respectively. [b]tr (<0.1%).

According to our knowledge, there are only one report on the composition of the essential oil from *M. pinnatus* grown in Colombia. Major compounds in the leaf oil were limonene (57.8 %) and α-pinene (9.7 %), whereas sabinene (24.2 %), limonene (9.1 %) and pinocarvone (9.7 %) were higher in the fruit oil (Alfonso-Rojas et al., 2009). The chemical composition differences of these essential oils seem to show chemotype existence and indicate the high influence of environmental factors such as geographic location and light intensity (Figueiredo, et al., 2008).

Antioxidant properties of the essential oil were determined, for the first time, by two *in vitro* methods: DPPH and ABTS assays (Table 2). The essential oil had a high scavenging effect and reduction of ferric ion capacity through FRAP assay. The results show that the essential oil scavenged DPPH· radical and $ABTS^{\cdot+}$ radical capacity as reflected by the IC_{50} values 1.18 ± 0.27 mg/mL and 4.47 ± 0.31mg/mL, respectively. These results agreed with the literature for essential oils that phenols possess high antioxidant activity (Amorati et al., 2013). Although the scavenging activity against ABTS radicals and FRAP capacity was lower in *M. pinnatus* essential oil compared to the reference compound, it is worth mentioning that Trolox is considered as a potent antioxidant.

Table 2. *Antioxidant effectiveness (IC_{50}) of M. pinnatus essential oil*

Sample	DPPH (mg/mL)	ABTS (mg/mL)
Essential oil	1.18 ± 0.27	4.47 ± 0.31
Trolox	0.01 ± 0.01	0.025 ± 0.01

Mean of three determinations with standard deviation.

The antimicrobial activity was determined against four pathogenic bacterial strains and a pathogenic fungus strain using a modified agar-diffusion method (Solís-Quispe et al., 2019). The essential oil exhibited low (ZOI < 5 mm), moderate (ZOI = 5-9.9 mm), good (ZOI = 10-15 mm) or very good (ZOI > 15 mm) activities against studied microbial strains. The results of the antimicrobial tests showed that *E. coli* and *C. albicans* were very sensitive to the effects of *M. pinnatus* essential oil. The results agreed with the literature for essential oils with high content of phenols have high antimicrobial activity (Nourbakhsh et al., 2021).

Table 3. Antimicrobial activity of *M. pinnatus* essential oil

	ZOI (mm)	MIC µL/mL)
Staphylococcus aureus (ATCC25923)	4.07 ± 0.13	16.18 ± 0.21
Ciprofloxacin	23.70 ± 0.21	0.19 ± 0.30
*Escherichia coli (*ATCC 25922)	16.67 ± 0.14	1.17 ± 0.01
Ciprofloxacin	31.3 ± 0.32	0.12 ± 0.30
Salmonella enterica serovar (ATCC14028)	6.37 ± 0.33	2.33 ± 0.12
Ciprofloxacin	32.0 ± 0.56	0.39 ± 0.17
Pseudomonas aeruginosa (ATCC 27853)	2.08 ± 0.12	12.01 ± 0.51
Ciprofloxacin	31.70 ± 0.36	0.27 ± 0.37
Candida albicans (ATCC 14053)	11.57 ± 0.01	3.07 ± 0.01
Ketoconazole	31.30 ± 0.11	0.29 ± 0.01

ZOI: net zone of inhibition. MIC: minimum inhibitory concentration. Values are means ± standard deviation of three separate experiments.

CONCLUSIONS

One hundred thirty-four volatile compounds were identified in the essential oil. aromatic compounds (51.9 %), oxygenated monoterpenes (16.3 %) and monoterpene hydrocarbons (12.5 %). The most abundant compounds were carvacrol (23.1%) and carvacryl acetate (19.2 %). To the best of our knowledge, as far as the literature is concerned, the results presented in this work can be considered as the first report about the biological properties of the essential oil of *Margyricarpus pinnatus* (Lam.) Kuntze. The essential oil had a high scavenging effect and reduction of ferric ion capacity through FRAP assay. The essential oil exhibited activity against all microorganism strains under test, particularly against *Escherichia coli* and *Candida albicans*.

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Este articulo no presenta conflicto de interes

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