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ARTÍCULO DE INVESTIGACIÓN

Composición química y actividades biológicas del aceite esencial de rizomas de curcuma (*Curcuma longa* L.) cultivado en el Ecuador amazónico

Chemical composition and biological activities of essential oil from turmeric (Curcuma longa L.) rhizomes grown in Amazonian Ecuador

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ABSTRACT

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant widely cultivated in the tropical and subtropical regions of the world. The essential oil from rhizomes has many pharmacological activities reported. The present paper reports the chemical composition and the antioxidant and antimicrobial activities of the essential oil from rhizomes grown in the Amazonian Ecuador. The essential oil was obtained by steam distillation. Analyses of the essential oil were performed by gas chromatography with flame ionization and mass selective detectors. Eighty-eight compounds were identified in the essential oil. Oxygenated compounds were the most represented class of volatiles (63.6%), including *ar*-turmerone (45.5%) and α-turmerone (13.4%) as major compounds. Sesquiterpene hydrocarbons were the second class (18.0%) with α-zingiberene (5.3%) as predominant. Another major constituent was the monoterpene hydrocarbon α-phellandrene (6.3%). Antioxidant properties of the essential oil were determined by radical-scavenging capacity of the oil (DPPH) and ferric-reducing antioxidant power (FRAP) methods. The essential oil had a low antioxidant activity by both methods, while it has antimicrobial activity against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and *Pennicilium citrinum*. No antimicrobial activity was found against *Escherichia coli*, *Salmonella Enteritidis* and *Aspergillus niger*.

Keywords: *Curcuma longa*; rhizomes; essential oil; composition; antioxidant activity; antimicrobial activity

RESUMEN

La cúrcuma (*Curcuma longa* L.) es una planta perenne herbácea y con rizomas ampliamente cultivada en las regiones tropicales y subtropicales del mundo. Se han reportado múltiples actividades farmacológicas del aceite esencial de los rizomas. En este trabajo se estudió la composición química, así como las actividades antioxidante y antimicrobiana del aceite

esencial de los rizomas cultivados en el Ecuador amazónico. El aceite esencial se obtuvo por destilación por arrastre con vapor. Los análisis se hicieron por cromatografía de gases con detectores de llama de hidrógeno y selectivo de masas. Se identificaron 88 compuestos en el aceite esencial. Los compuestos oxigenados fueron la clase química más representativa con 63,6 %. Entre ellos, la *ar*-turmerona (45,5 %) y α-turmerona (13,4 %) fueron los más abundantes. Los hidrocarburos sesquiterpénicos fueron la segunda mayor clase (18,0 %) con el α-zingibereno (5,3 %) como predominante. Otro constituyente mayoritario fue el hidrocarburo monoterpénico α-felandreno (6,3 %). La capacidad antioxidante fue evaluada en el aceite esencial mediante los métodos del 1,1-difenil-2-picril-hidrazilo (DPPH) y el poder antioxidante reductor del férrico (FRAP). El aceite esencial mostró una baja actividad antioxidante por ambos métodos, mientras que tuvo actividad antibacteriana, contra bacterias gram positiva (*Bacillus subtilis* and *Staphylococcus aureus*) y *Pennicilium citrinum*. No se encontró actividad antimicrobiana frente a *Escherichia coli*, *Salmonella Enteritidis* y *Aspergillus niger*.

Palabras clave: *Curcuma longa*; rizomas; aceite esencial; composición; actividad antioxidante; actividad antimicrobiana

INTRODUCTION

Turmeric (*Curcuma longa* L.) is a rhizomatous herbaceous perennial plant of the Zingiberaceae family, native to tropical South Asia, but is now widely cultivated in the tropical and subtropical regions of the world (Remadevi *et al.*, 2007). The deep orange-yellow powder known as turmeric is prepared from boiled and dried rhizomes of the plant. It has been commonly used as spice and medicine, particularly in Asia. The curcuminoid pigments and volatile oil, which are the major secondary metabolites of the rhizome, have been shown to be largely responsible for the pharmacological activities of turmeric powder, extracts and oleoresins (Remadevi *et al.*, 2007; Sasikumar, 2001).

There are extensive *in vitro* and *in vivo* investigations on essential oil and extracts of turmeric extracts which showed hepato- and cardioprotective, hypoglycemic, anti-amyloidogenic, antifungal, parasiticidal, antioxidant, insect repelling, chemo-resistance and radio-resistance activities (Remadevi *et al.*, 2007; Balakrishnan, 2007; Li *et al.*, 2018). Although the fungicidal and bactericidal properties are traditionally known (Remadevi *et al.*, 2007; Sasikumar, 2001; Balakrishnan, 2007; Li *et al.*, 2018), reports about antifungal properties of the essential oil are scant and show a wide discrepancy about its inhibitory concentration in the substrate for the same fungal species (Saju *et al.*, 1998; Behura *et al.*, 2000; Jayaprakasha *et al.*, 2001). This wide variation may be related to the chemical composition of the essential oil, which varies considerably among the cultivars, maturity stage, and cultivation prcedures (Singh *et al.*, 2002; Ming *et al.*, 2002; Raina *et al.*, 2002; Pino *et al.*, 2003; Singh *et al.*, 2010; Liju *et al.*, 2011; Gounder y Lingamallu, 2012; Avanço *et al.*, 2017). In Ecuador, turmeric is commercially grown in the Amazonian region, where it is used as a food seasoning. However, only one study has been done about the chemical composition of its essential oil and biological activities (Sacchetti *et al.*, 2005). Therefore, the present study was done to analyze the chemical composition and biological activities of the essential oil from turmeric (*Curcuma longa* L.) rhizomes grown in Amazonian Ecuador.

MATERIALS AND METHODS

Materials and isolation of essential oil

Rhizomes of *C. longa* were collected by *Fundación Chankuap*' (Macas, Ecuador) in May 2017 from wild trees on the outskirts of the Wasak'entsa reserve in eastern Ecuador and positively identified by the National Herbary of Pontificia Universidad Católica del Ecuador (voucher Nr. HERUTEQ1057). Fresh rhizomes (800 g) were steam distilled for 6 h in a pilot-scale distiller. The oil yield was 0.8% v/w.

Gas chromatography

Analyses of the essential oil was performed by gas chromatography with a flame ionization detector (GC-FID) on a Konik 4000A (Konik, Barcelona) equipped with a 30 m x 0.25 mm i.d. x 0.25 mm DB-5ms (J & W Scientific, Folsom, CA, USA) column. The analysis parameters were: oven temperature program, 60 ºC (2 min), 60–220 ºC (4 ºC/min) and 220 ºC (5 min); hydrogen carrier gas flow rate 1 mL/min; injector and detector temperatures 250 ºC. Samples (1 µL) were injected using split ratio 1:50, and previously diluted in *n*-pentane (1:6 v/v). The quantification of compounds was performed using relative percentage abundance and normalization method.

The essential oil was also examined by gas chromatography-mass spectrometry (GC-MS) using a QP-2010 Ultra (Shimadzu, Japan) with the same capillary column, temperature program and helium carrier gas flow rate as in GC-FID. EIMS, electron energy, 70 eV; ion source and connecting parts temperature, 250 ºC. Acquisition was performed in scanning mode (mass range m/z 35–400 u). Compounds were identified using their linear retention indices and mass spectra. Linear retention indices, calculated using linear interpolation relative to retention times of C_8–C_{24} of *n*-alkanes, were compared with those standards and data from the literature (Adams, 2001). Mass spectra were compared with corresponding reference standard data reported in the literature (Adams, 2001) and mass spectra from NIST 05, Wiley 6, NBS 75 k, and in-house Flavorlib libraries. In many cases, the essential oil was subject to co-chromatography with authentic compounds.

Assay of 2,2-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity

The antioxidant activity of the essential oil was measured 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 was mixed with 750 µL of five concentrations of the essential oil to evaluate in a range of concentrations between 25-500 µg/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 the absorbance was determined at 515 nm, until the reaction plateau step was reached. Ascorbic acid was used as antioxidant standard. Three independent tests were performed for each sample. Then, the IC_{50} values (total antioxidant compound necessary to decrease the initial DPPH radical concentration by 50%) were determined.

Ferric-reducing antioxidant power (FRAP) assay

The FRAP of the essential oil was measured by the method reported earlier (Benzie, 1996). Briefly, acetate buffer (300 mmol/L, pH=3.6), TPTZ (2,4,6-tripyridyl-s-triazine; Sigma) 10 mmol/L in 40 mmol/L HCl and $FeCl_3 \cdot 6H_2O$ (20 mmol/L) were mixed in the ratio of 10:1:1 to obtain the working FRAP reagent. The essential oil (90 µL) was mixed with 900 µL of FRAP reagent. A solution of ascorbic acid was used as standard and Trolox, a stable antioxidant was used as positive control. The mixtures were incubated at room temperature for 4 min and the absorbance was measured at 593 nm. The FRAP was expressed in units of ascorbic acid equivalent.

Antimicrobial screening

For determination of minimal inhibitory concentration (MIC) 5, 0.5, 0.05 and 0.005 µL/mL of the essential oil were placed in different test tubes and 1 mL of dimethyl sulfoxide added to each of them. One milliliter of peptone water (Mueller Hinton broth) was added followed by addition of 1 mL of 24h–culture broth of the microorganism. The test tubes were all sealed with sterile corks and subsequently incubated at 32 °C for bacteria and 25 °C for fungus during 48 h. After incubation the tubes were observed for clearance or turbidity. The tube with highest degree of clearance was taken as the MIC. Three independent tests were performed for each sample. This procedure was separately carried out for the six test microorganisms: *Bacillus subtilis* ATCC 6633 (G+), *Staphylococcus aureus* ATCC 25923 (G+), *Escherichia coli* ATCC 25922 (G-), *Salmonella Enteritidis* ATCC 13036 (G-), *Aspergillus niger* ATCC 16404 and *Pennicilium citrinum* ATCC 9849.

RESULTS AND DISCUSSION

A total of 88 volatile compounds were identified in the essential oil from *C. longa* rhizomes (98.8% of the total composition) (Table 1). As can be seen, oxygenated sesquiterpenes were the most represented class of volatiles with 63.6 %. Among them, *ar*-turmerone and α-turmerone were the most abundant. Sesquiterpene hydrocarbons were found as the second major chemical class (18.0%) with α-zingiberene being the main component. Another individual major constituent was the monoterpene hydrocarbon α-phellandrene.

Table 1. Chemical composition (%) of the essential oil from *C. longa* rhizomes.

#	Compound	LRI	%	Identity	#	Compound	LRI	%	Identity
1	(*Z*)-3-Hexen-1-ol	859	tr	A	44	Thymol methyl ether	1233	tr	A
2	Heptan-2-one	892	tr	A	45	Neral	1238	0.2	A
3	Heptan-2-ol	904	tr	A	46	Carvone	1244	tr	A
3	Tricyclene	927	tr	B	47	Carvotanacetone	1247	tr	B
4	α-Thujene	930	tr	A	48	Geraniol	1250	tr	A
5	α-Pinene	939	0.4	A	49	Piperitone	1253	tr	A
6	Camphene	954	0.4	A	50	Geranial	1267	0.3	A
7	Benzaldehyde	960	tr	A	51	Bornyl acetate	1287	tr	A
8	Sabinene	975	tr	A	52	Thymol	1290	0.2	A
9	β-Pinene	979	tr	A	53	Undecan-2-one	1294	tr	A
10	6-Methyl-5-hepten-2-one	984	tr	A	54	Carvacrol	1299	tr	A
11	Myrcene	991	0.3	A	55	δ-Elemene	1337	tr	A

12	δ-2-Carene	1000	tr	B	56	Citronellyl acetate	1353	tr	A
13	α-Phellandrene	1003	6.3	A	57	Eugenol	1359	tr	A
14	δ-3-Carene	1011	0.2	A	58	α-Ylangene	1375	tr	A
15	α-Terpinene	1017	0.2	A	59	α-Copaene	1377	0.1	A
16	*p*-Cymene	1023	1.5	A	60	Methyl (*E*)-cinnamate	1381	tr	A
17	Limonene	1028	1.0	A	61	β-Elemene	1391	tr	A
18	1,8-Cineole	1031	2.6	A	62	7-*epi*-Sesquithujene	1394	0.1	B
19	(*Z*)-β-Ocimene	1037	tr	A	63	α-Cedrene	1412	0.1	A
20	(*E*)-β-Ocimene	1050	tr	A	64	(*E*)-Caryophyllene	1419	0.8	A
21	γ–Terpinene	1060	0.4	A	65	β-Gurjunene	1432	tr	B
22	*cis*-Sabinene hydrate	1071	tr	B	66	*trans*-α-Bergamotene	1435	0.1	A
23	Terpinolene	1089	2.1	A	67	(*E*)-Cinnamyl acetate	1446	tr	A
24	Nonan-2-one	1090	tr	A	68	(*E*)-β-Farnesene	1457	0.4	A
25	Rosefuran	1095	tr	B	69	α-Acoradiene	1466	0.1	B
26	Linalool	1097	0.1	A	70	β-Acoradiene	1471	tr	B
27	(*E*)-6-Methyl-3,5-heptadien-2-one	1107	tr	B	71	γ–Curcumene	1478	0.2	A
28	1,3,8-*p*-Menthatriene	1112	tr	B	72	*ar*-Curcumene	1482	3.6	A
29	*cis*-2-*p*-Menthen-1-ol	1122	tr	B	73	α-Zingiberene	1491	5.3	A
30	*trans*-*p*-Menth-2-en-1-ol	1141	tr	B	74	*trans*-Muurola-4(14),5-diene	1494	0.2	B
31	Ipsdienol	1145	tr	B	75	(*E,E*)-α-Farnesene	1502	0.4	A
32	Myrcenone	1150	tr	B	76	β-Bisabolene	1506	1.6	A
33	Citronellal	1153	tr	A	77	β-Curcumene	1516	0.3	A
34	4-Acetyl-1-methylcyclohexene	1158	tr	B	78	β-Sesquiphellandrene	1523	4.0	B
35	*trans*-β-Terpineol	1164	tr	A	79	(*E*)-γ-Bisabolene	1531	0.3	B
36	Borneol	1169	0.1	A	80	(*Z*)-Nerolidol	1533	0.4	A
37	Terpinen-4-ol	1177	0.1	A	81	α-Dehydro-*ar*-himachalene	1537	0.1	B
38	*p*-Cymen-8-ol	1183	0.1	A	82	*ar*-Turmerol	1583	1.5	A
39	α-Terpineol	1189	0.2	A	83	*ar*-Dihydro-turmerone	1596	1.7	A
40	Myrtenal	1192	tr	B	84	*ar*-Turmerone	1664	45.5	A
41	α-Phellandrene epoxide	1197	0.1	B	85	α-Turmerone	1697	13.4	A
42	*trans*-Piperitol	1208	tr	B	86	(*E*)-γ-Atlantone	1709	1.0	B
43	β-Citronellol	1226	tr	A	87	(*E*)-α-Atlantone	1779	0.6	B

Note: *Identity A*: identification based on the linear retention times (LRI) and mass spectra of pure compounds; B: identification based on LRI and mass spectra comparison with databases or literature data. *tr*: traces (< 0.1 %).

The chemical composition of the turmeric rhizomes essential oil varies considerably according to several factors (Balakrishnan, 2007). These results disagree with those reported in the literature in both quality and quantity. The essential oil previously analyzed from the same region was dominated by α-phellandrene (20.4%), α-turmerone (19.8%), 1,8-cineole (10.3%), γ-terpinene (6.1%) and β-turmerone (7.4%) (Sacchetti *et al.*, 2005). The volatile oil obtained by hydrodistillation of turmeric rhizomes grown in the North Indian Plains contained 1,8-cineole (11.2%), α-turmerone (11.1%), β-caryophyllene (9.8%), *ar*-turmerone (7.3%) and β-sesquiphellandrene (7.1%) as major compounds (Raina *et al.*, 2002), while the essential oil from Gorakhpur (India) was dominated by *ar*-turmerone (24.4%) and α-turmerone (20.5%) (Singh *et al.*, 2010). The essential oil isolated from rhizomes grown in Kerala (India) was rich in *ar*-turmerone (61.8%), curlone (12.5%) and *ar*-curcumene (6.1%)

(Liju *et al*., 2011); while that from Mysore (India) was dominated by *ar*-turmerone (21.0-30.3%), α-turmerone (26.5-33.5%) and β-turmerone (18.9 - 21.1 %) (Gounder y Lingamallu, 2012). The main components of the essential oil from Brazil were α-turmerone (42.6%), β-turmerone (16.0 %) and *ar*-turmerone (12.9%) (Avanço *et al*., 2017); while the composition of the essential oil from Cuban rhizomes was close to those found in the present work: *ar*-turmerone (47.7%) and α-turmerone (16.1%) (Pino *et al*., 2003).

Antioxidant properties of the essential oil were determined by two methods: radical-scavenging capacity of the oil (or DPPH bleaching) and ferric-reducing antioxidant power (FRAP) assay (Table 2). The IC_{50} value was14.5 mg/mL for the essential oil with a maximal effect of 30 % at 60 mg/mL. The radical scavenging potential of turmeric essential oil was much lower when compared to those of standard ascorbic acid and Trolox. This performance was lower than those reported previously for a sample obtained from the same region (Sacchetti *et al*., 2005) and a sample from Mysore (India) (Gounder y Lingamallu, 2012).

Table 2. Antioxidant effectiveness of the essential oil.[1]

Sample	DPPH	FRAP	
	IC_{50} (mg/mL)	Concentration (mg/mL)	(µM of ascorbic acid equivalents)
Essential oil	14.5 ± 2.9	4	389.0 ± 12.0
		2	311.2 ± 19.5
		1	304.6 ± 10.2
Trolox	0.012 ± 0.004	0.1	402.3 ± 20.1

[1]Antioxidant effectiveness expressed as IC_{50} (DPPH scavenger activity) and FRAP (in units of ascorbic acid equivalent). Values represent an average of three determinations with standard deviation.

In the second assay, at low pH ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to ferrous [Fe^{2+}] form, an intense blue colored complex with absorption maximum at 593 nm by the electron donating action of antioxidant (Benzie, 1996). The FRAP was 389.0 ± 12.0 µM of ascorbic acid equivalents and the essential oil showed less activity than Trolox. Probably, the sesquiterpene ketones *ar*-turmerone and α-turmerone exert either the synergistic or additive actions towards the total antioxidant activity. It may also be possible that these compounds alone or in synergy with other compounds present in the essential oil are responsible for the observed antioxidant activity of rhizomes of *C. longa* (Singh *et al*., 2010).

The MIC of the essential oil was ranged between 0.05-5 µL/mL (Table 3). The essential oil showed better activity against *St. aureus* and *B. subtilis* followed by *P. citrinum*. As it is commonly found, Gram+ bacteria were more susceptible to the essential oil than Gram- ones (Kalemba y Kunicka, 2003). In general, these results are in accordance with those reported for this essential oil (Singh *et al*., 2002; Sacchetti *et al*., 2005; Singh *et al*., 2011) and they are attributed to the presence of tumerones as responsible for showing antimicrobial activity against specific pathogens (Dhingra *et al*., 2007; Singh *et al*., 2011).

Table 3. Minimal inhibitory concentrations of the essential oil against tested microorganisms.

Microorganism	MIC (µL/mL)
Bacillus subtilis ATCC 6633	0.5
Staphylococcus aureus ATCC 25923	0.05
Escherichia coli ATCC 25922	-
Salmonella Enteritidis ATCC 13036	-
Aspergillus niger ATCC 16404	-
Pennicilium citrinum ATCC 9849	5.0

Note: -: No inhibition.

CONCLUSIONS

Essential oil composition of *Curcuma longa* rhizomes show the presence of 88 volatile constituents, of which the most prominent were *ar*-turmerone (45.5%) and α-turmerone (13.4%). The essential oil had a low antioxidant activity by using the DPPH assay and it showed ferric reducing antioxidant power, while it has antimicrobial activity against gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and *Pennicilium citrinum*. No antimicrobial activity was found against *Escherichia coli*, *Salmonella Enteritidis* and *Aspergillus niger*.

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