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Influence of different preparation techniques on the composition and antioxidant action of curcumin and curcuminoids

[Influencia de diferentes técnicas de preparación en la composición y acción antioxidante de la curcumina y los curcuminoides]

Juliana Pelissari Marchi[1], Francislaine Aparecida dos Reis Lívero[1,2], Andreia Assunção Soare[2], Glacy Jaqueline da Silva[4], Ana Karina Vargas Soares[1], Camila Moreno Giarolo[1], Ezilda Jacomassi[1], Lauro Mera de Souza[3], Wanessa de Campos Bortolucci[4], Zilda Cristiani Gazim[4], Caio Franco de Araújo Almeida Campos[5], José Eduardo Gonçalves[5,6], Cintia de Souza Alferes Araújo[1], Samantha Wietzikoski[1] & Evellyn Claudia Wietzikoski Lovato[1]

[1]Postgraduate Program in Medicinal Plants and Phytotherapeutics in Basic Attention, Universidade Paranaense, Umuarama, Brazil
[2]Postgraduate Program in Animal Science with Emphasis on Bioactive Products, Universidade Paranaense, Umuarama, Brazil
[3]Institute of Research Pelé Pequeno Príncipe, Pequeno Príncipe Faculty, Curitiba, Brazil
[4]Postgraduate Program in Biotechnology, Paranaense University, Umuarama, Brazil
[5]Program of Master in Clean Technologies, Program of Master in Science, Technology and Food Safety and Cesumar Institute of Science, Technology and Innovation, University Center of Maringa, Maringa, Brazil
[6]Program of Master in Health Promotion and Cesumar Institute of Science, Technology and Innovation, University Center of Maringa, Brazil

Reviewed by:
Foluso Osunsanmi
University of Zululand
Republic of South Africa

Ricardo Dias de Castro
Federal University of Paraiba
Brazil

Correspondence:
Evellyn Claudia Wietzikoski **LOVATO**
evellyn@prof.unipar.br

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Abstract: Epidemiological evidence indicates that plant antioxidants activity can treat or help to prevent the development of various diseases. One species with great potential as an antioxidant is *Curcuma longa*. However, different extraction techniques can influence isolated chemical compounds. This study investigated chemical composition and antioxidant activity of two rhizome extracts of *C. longa*: hydroethanolic, obtained by exhaustion (HECLex); and dried by a spray dryer (HECLsd). The phytochemical composition was evaluated by GC/MS. Antioxidant activity was evaluated using DPPH and FRAP assays. Total phenolic compounds and soil analyses were performed. The main components of HECLex were ar-turmerone, γ-curcumene, α-turmerone, and β-sesquiphellandrene. The main components of HECLsd were 9,12,15-octadecatrienoic acid, 2,3-bis([trimethylsilyl]oxy)propyl ester, verrucarol, and 1-monolinoleoylglycerol trimethylsilyl ether. HECLsd had significantly higher levels of phenolic compounds and higher antioxidant capacity compared with HECLex. In conclusion, processes of the preparation of *C. longa* rhizomes alter the chemical components and consequently their biological activity.

Keywords: *Curcuma longa*; Free radicals; Nutraceutical; Phenolic compounds; Phytotherapy.

Resumen: La evidencia epidemiológica indica que la actividad de los antioxidantes de las plantas pueden tratar o ayudar a prevenir el desarrollo de diversas enfermedades. Una especie con gran potencial como antioxidante es *Curcuma longa*. Sin embargo, diferentes técnicas de extracción pueden influir en los compuestos químicos aislados. Este estudio investigó la composición química y la actividad antioxidante de dos extractos de rizoma de *C. longa*: hidroetanólico, obtenido por agotamiento (HECLex); y se seca con un secador por pulverización (HECLsd). La composición fitoquímica se evaluó mediante GC/MS. La actividad antioxidante se evaluó mediante ensayos DPPH y FRAP. Se realizaron análisis de suelos y compuestos fenólicos totales. Los componentes principales de HECLex fueron ar-turmerona, γ-curcumene, α-turmerone y β-sesquiphellandrene. Los componentes principales de HECLsd fueron ácido 9,12,15-octadecatrienoico, éster 2,3-bis ([trimetilsilil] oxi) propílico, verrucarol y éter 1-monolinoleoilglicerol trimetilsilil. HECLsd tenía niveles significativamente más altos de compuestos fenólicos y mayor capacidad antioxidante en comparación con HECLex. En conclusión, los procesos de preparación de los rizomas de *C. longa* alteran los componentes químicos y consecuentemente su actividad biológica.

Palabras clave: *Curcuma longa*; Radicales libres; Nutracéutico; Compuestos fenólicos; Fitoterapia

ABBREVIATIONS:
BHT: butyl hydroxy toluene; CG/MS: gas chromatography coupled to a mass spectrometer; HPLC: high performance liquid chromatography; DPPH: 2,2-diphenyl-1-picrylhydrazyl; EC_{50}: concentration of the substance that gives half-maximal response; FRAP: iron reducing capacity; HECLex: hydroethanolic extract of *Curcuma longa* obtained by exhaustion; HECLsd: hydroethanolic extract of *C. longa* dried using a spray dryer.

INTRODUCTION

Accumulating epidemiological evidence indicates that diets that are rich in fruits and vegetables can prevent or help reduce the incidence of various chronic noncommunicable diseases, such as brain and cardiovascular diseases, cancer, and diabetes. Some of these biological effects are attributable to secondary metabolites of the plants, especially flavonoids and phenolic compounds, which exert antioxidant effects *in vitro* and *in viv*o (Crozier *et al*., 2009). The antioxidant activity of molecules is extremely important for maintaining vital processes in humans because they can prevent oxidative damage that is caused by free radicals, since oxidative stress is often associated with the etiology and progression of various diseases, such as cancer, cardiovascular disease, and metabolic disease. Thus, the consumption of natural antioxidants can have health benefits, improving the quality of life of the population (Koehnlein *et al*., 2014).

One medicinal plant family that is rich in phenolic compounds with medicinal potential is Zingiberaceae. Several species of Zingiberaceae produce many bioactive compounds that are useful in foods (e.g., herbs and spices), flavorings, seasonings, and the pharmaceutical industry as antioxidant agents (Chan & Wong, 2015). One species of the Zingiberaceae family is *Curcuma longa*, popularly known as saffron and turmeric, among others (Lorenzi & Matos, 2008). It is an herbaceous perennial, deciduous, aromatic plant with large elliptical leaves, whitish or yellowish flowers, ovoid rhizomes, with sessile cylindrical tubers with an orange color inside (Lorenzi & Matos, 2008).

C. longa is a plant that is native to India and Southwest Asia, with a broad description of its ethnobotanical aspects and ethnopharmacological uses that are directly related to its chemical constituents (Marchi *et al*., 2016). In addition to its alimentary properties, this species exerts pharmacological actions, including hepatoprotective, gastroprotective, anticancer, anti-inflammatory, antimicrobial, anti-human immunodeficiency virus, hypolipidemic, hypoglycemic and antiaggregant effects (Cohly *et al*., 2003; Lorenzi & Matos 2008). *C. longa* also presents isolated antioxidant activity and potentiate the antioxidant action of vitamins C and E, reducing lipid peroxidation (Akram *et al*., 2010; Mallmann *et al*., 2012).

The use of *C. longa* extracts and its bioactive components is widespread, with beneficial effects on osteoarthritis, rheumatoid arthritis, gastrointestinal disorders (e.g., flatulence and dyspepsia), and skin disorders (e.g., wound healing) and antiinflammatory, antioxidant, and analgesic actions (Marczylo *et al*., 2007; Bastos *et al*., 2009; Mallmann *et al*., 2012; Kumar *et al*., 2016; Hewlings & Kalman, 2017; Xu *et al*., 2018). However, these effects of *C. longa* are influenced by changes in its chemical composition that are caused by variations in temperature, humidity, wind, air pollution, luminosity, ultraviolet radiation, location (altitude and latitude), nutrients (soil type), water availability, pathogen attack, planting, and harvesting period (Gobbo-Neto & Lopes, 2007). This is an extremely important factor when cultivating the species because the antioxidant activity of this plant depends on the chemical composition of the extract and mainly the extraction technique (Salgueiro & Castro, 2016).

The extraction of active components of *C. longa* can be accomplished using several techniques, such as extraction maceration from exhaustion using constant solvent renewal, lyophilization, spray drying, microwave, ultra-sonic and supercritical carbon dioxide assisted (Pereira & Stringheta, 1998; Wakte *et al*., 2011; Martins *et al*., 2013). To obtain a dry extract, the drying process must be performed such that it does not interfere with the characteristics and properties of the metabolites, does not result in undesirable changes, and maintains the quality of the dried product (Antal, 2015).

Lyophilization is generally used to dry small-scale food, medicines, and plant extracts. The main advantage of this technique is the drying of thermosensitive materials. The main disadvantage of this technique is its relatively high cost and long processing time (Antal, 2015). Spray drying has a lower final cost when the equipment is already available, is faster (i.e., solvent evaporation occurs in fractions of seconds), has the ability to control the uniformity of the size of solid particles, and has high solubility in water, thus broadening its possible applications (Oliveira & Petrovick, 2010). Spray drying can also be used for thermosensitive materials because contact with the hot air jet lasts only seconds

(Oliveira & Petrovick, 2010; Tacon & Freitas, 2012).

One of the great difficulties in increment the transition from bench to bedside involving natural products is the way of preparing plant extracts. Generally, on a small scale, at the laboratory level, plant extracts are prepared by exhaustion and on an industrial scale by spray dryer. Thus, studies that compare the phytochemical profiles of prepared and dried extracts by different techniques are essential to direct researchers to prepare the extract and evaluate its biological activities, in a way that can be produced on a large scale by the pharmaceutical industry in case of promising preclinical studies (Chaul *et al*., 2017). However, despite known influences of extraction and drying techniques on the metabolic constituents of plant species, different techniques for extracting and drying the crude extract that is obtained from rhizomes and consequently the antioxidant activity of *C. longa* have not been investigated. Thus, the present study evaluated the chemical composition and antioxidant activity of two extracts that were obtained from *C. longa* rhizomes: hydroethanolic extract that was obtained by exhaustion (HECLex) and hydroethanolic extract that was dried using a spray dryer (HECLsd).

MATERIALS AND METHODS

Soil analysis and botanical material

The soil analysis was carried out with the purpose of informing the soil conditions in which *C. longa* culture is implanted in the herb garden. Soil samples where *C. longa* was cultivated were collected at coordinates S23º07'44" and W52º19'08" and altitude of 635 m. The soil was collected at 0-20 cm depth in different points to obtain a composed sample. For the soil analysis calcium (Ca^{++}), magnesium (Mg) and aluminum (Al) were extracted with 1M KCl; potassium (K) were extracted according to Mehlich method, H+Al was determined by Shoemaker, McLean and Pratt method; carbon by Walkley and Black method; and sum of bases and cation exchange capacity. All soil analysis were performed by Solanálise, Central de Análises Ltda, Francisco Beltrão, Paraná, Brazil, according to previously methods described by Embrapa (Arruda *et al*., 2014).

Rhizomes of *C. longa* were collected after yellowing and leaf fall during the winter period, between June and July 2016. *C. longa* culture is part of the collection of medicinal plant species kept in the Medicinal Garden of the Paranaense University and a voucher was deposited under the registration number 2000. No specific permissions were required for these activities and the field studies did not involve endangered or protected species. This species is registered (No. A291401) in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen). During the period of rhizome collection, the temperature ranged from 12°C to 24°C, with 55-86% humidity and 10-18 km/h winds. The climate of the region is subtropical, with temperatures below 18°C in the winter (with low frost possibility) and above 22°C in summer (with greater rainfall possibility) (Kottek *et al*., 2006).

Extract preparation

The rhizomes were collected, washed, and dried in air-circulating greenhouses (Tecnal) at 50°C for 6 days (Vilela & Artur, 2008).

For preparation of the hydroethanolic extract that was obtained by exhaustion (HECLex), the material was pulverized in a knife mill (Willye TE-650) to 850 μm grain. The powder was subjected to a dynamic maceration process with solvent renewal using ethanol:water (70:30, v/v) until the material was depleted (Miranda *et al*., 2009; Brasil, 2010). The filtrate was then concentrated under reduced pressure on a rotary evaporator (Tecnal TE-211) at 40ºC until the crude extract HECLex was obtained. For preparation of the hydroethanolic extract (ethanol:water, 70:30, v/v) that was dried using a spray dryer (HECLsd), the rhizomes were ground in a blender until a grain size of 600 μm was obtained. After drying the extract with the addition of maltodextrin (10%) and aerosil (1%) adjuvants, the material was processed using a B-91 Mini Spray Dryer. The number of rhizomes that were used for the two extraction processes was 1:3 (plant:solvent, m/v), and the yields of HECLex and HECLsd were 18.41% and 8.46%, respectively.

Phytochemical analysis of the extracts

The analysis of extract samples (one sample of crude extract [grease] and one sample of crude extract [dried by spray drying]) of *C. longa* was performed using a gas chromatograph (Agilent 7890 B) coupled to a mass spectrometer (Agilent 5977A) that was equipped with an Agilent HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) under the following conditions: 280°C injector temperature, 2 μL injection volume at a ratio of 1:20, and helium as the carrier gas (flow adjusted to 1 ml/min). The initial column temperature was 60°C for 1 min, followed by gradual heating to 185°C at a rate of 1.5°C/min for 1 min, heating to 275°C at 9°C/min for 2 min, and heating to 300°C at 25°C/min for 1 min. The

temperatures of the transfer line, ion source, and quadrupole were 280°C, 230°C, and 150°C, respectively. Mass spectra were obtained in the range of 40-550 (m/z) that was provided by the solvent run time with a 3 min solvent delay time. Compounds were identified by comparing their mass spectra with mass spectra in the NIST 11.0 library and Adams (Adams, 2017).

Quantification of curcumin and curcuminoids

The quantification of curcumin and curcuminoids in HECLex and HECLsd was performed by high-performance liquid chromatography (HPLC; Varian 920 LC) in reversed-phase mode using a C18 column (150 × 4.6 mm × 2.7 µm particle size). The solvent was composed of 5 mM phosphoric acid-acetonitrile (55:45, v/v), and chromatography was performed isocratically at a flow rate of 600 µl/min at 40°C adapted from Koop *et al*. (2013). The calibration curve was generated with an authentic standard of curcumin (Sigma-Aldrich) that was prepared at 1, 5, 10, 15, 20, and 30 µg/ml in methanol. The samples were prepared at 500 µg/ml with injections of 10 µl. Detection was performed with a photodiode array detector (200-500 nm), and curcuminoids were monitored at 425 nm.

Determination of total phenolic compounds

The concentrations of phenolic compounds in the crude extract (grease) that was obtained by the dynamic maceration process with solvent depletion and the spray-dried crude extract were determined using the Folin-Ciocalteu method described by Soares *et al*. (2020) with some modifications. An aliquot of the sample (extract and water-white) will be diluted to a final volume of 1 ml of distilled water. In the different dilutions of the samples the following reagents were added: 0,150 mL of 1.9 M sodium carbonate (Na_2CO_3) and 0,05 mL of 1N Folin Ciocalteu phenol reagent (previously diluted with water 1:1 (v/v). After 1 h in the dark (without the presence of ambient light), absorbance was read at 725 nm in a Micronal spectrophotometer of each sample dilution (different concentrations), and white was used to subtract the purple-blue color from the reaction. The standard/calibration curve with gallic acid pertaining to the method was performed and obtained through Linear Regression. The results of the samples were compared with the standard curve of galic acid and the results were expressed as mg/ml gallic acid equivalents.

In vitro antioxidant evaluation: DPPH assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method is widely used to evaluate antioxidant capacity. This method is based on sequestering activity of the free radical DPPH (Oliveira, 2015). The sequestering activity of DPPH was determined according to the methodologies of Thaipong *et al*. (2006) and Choi *et al*. (2006). DPPH (0.024 g) was diluted in 100 ml of methanol. A 10 ml volume of this solution was added to 45 ml of methanol. Absorbance was read at 515 nm, with correction to near 1.1. To the 2.85 ml of the solution was added 0.15 mL of the sample. After 1 h in the dark at room temperature, absorbance was read at 515 nm. Sequestering activity is expressed as a percentage of free radical sequestration efficiency according to the following equation: % = $(1 - A_{Sample}/A_{Control}) \times 100$. The synthetic antioxidant butyl hydroxy toluene (BHT; 0.2 mg/ml) was used as a positive control. Sequestering activity is expressed as the EC_{50} (concentration of the substance that gives half-maximal response), determined by linear regression.

Total antioxidant capacity: FRAP assay

The ferric antioxidant powder (FRAP) assay measures iron reducing capacity. This method was originally developed as an alternative to determine iron reduction in biological fluids and aqueous solutions of isolated compounds (Thaipong *et al*., 2006). The FRAP assay in the present study was performed according to Benzie and Strain (1996) and Pulido *et al*. (2000). The reaction solution was prepared with 2.5 mL of a 10 mmol/L 2,4,6-tripyridyl-*S*-triazine (TPTZ) solution in 40 mmol/L HCl plus 2.5 ml of ferric chloride (FeCl 3. $6H_2O$; 20 mmol/L) in water and 25 ml of 300 mmol/L sodium acetate buffer, pH 3.6. For the reaction, 900 µL of FRAP reagent, 90 µL of distilled water, and 30 µL of the sample or standard (Trolox, Sigma) or blank (120 µL of distilled water + 900 µL FRAP reagent) were used. This preparation remained undisturbed for 30 min at 37°C and was then centrifuged at 3500 rotations per minute for 5 min and absorbance was read at 595 nm.

Statistical analysis

Differences in total phenolic compounds and antioxidant activity (DPPH and FRAP) between groups were analyzed using Student's *t*-test in duplicate. The data are expressed as mean ± standard error of the mean (SEM). Values of $p \leq 0.05$ were considered statistically significant. The data were analyzed using Statistic 13.3 software. Linear

correlations of antioxidant activity based on the DPPH capture method (EC_{50} determination) were analyzed using GraphPad Prism 5.0 software.

RESULTS

Soil characteristics

Table Nº 1 shows the chemical and granulometric characteristics of the soil where *C. longa* was cultivated.

TABLE No. 1
Chemical properties (pH and macro and micronutrients) of the soil that was used to cultivate *C. longa*

pH and Macronutrients									
	cmol/dm^3							mg/dm^3	g/dm^3
pH	Al^{3+}	$H^+ + Al^{3+}$	Ca^{2+}	K^+	Mg^{2+}	SB	CTC	P	C
3.7	0.46	4.96	0.59	0.16	0.18	0.93	5.89	17.41	7.88
Micronutrients									
(%)	cmol/dm^3				Ratio cmol/dm^3				
V	Fe^{2+}	Mg^{2+}	Cu^{2+}	Zn^{2+}	Ca/Mg	Ca/K	Mg/K		
15.79	39.32	51.95	1.65	1.63	3.28	3.69	1.12		

pH, pH in $CaCl_2$; P, phosphorus; C, carbon; Al^{3+}, aluminum; $H^+ + Al^{3+}$, potential acidity; Ca^{2+}, calcium; K^+, potassium; Mg^{2+}, magnesium; Cu^{2+}, copper; Zn^{2+}, zinc; BS, base saturation; CTC, cation exchange capacity; V, base saturation. The soil was of the Caiuá sandstone formation, classified as Dystrophic Red Latosol (LVd19) with a granulometric composition of 91.25% sand, 3.75% silt, and 5.0% clay. The pH was acidic (pH 3.70), with reddish color, medium fertility due to the organic matter content (13.55 g/dm^3), and saturation for bases (V: 15.79%)

Compounds found in HECLex and HECLsd extracts

The chemical composition of HECLex was analyzed by gas chromatography coupled with mass spectrometry (GC/MS). A total of 117 compounds were identified, representing 99.43% of the volatile components of the sample (Table No. 2). Oxygenated sesquiterpenes were the dominant compounds, comprising 67.05%, followed by hydrocarbon sesquiterpenes (23.22%). The major constituents were ar-turmerone (9.70%), γ-curcumene (8.55%), α-turmerone (7.30%), β-turmerone (15.32%), and β-sesquiphellandrene (6.78%).

TABLE No. 2
Chemical composition of the hydroethanolic extract of *C. longa* that was obtained by the dynamic maceration process with solvent depletion (HECLex)

Peak	*Compound*	*Relative Area (%)*	*RI calculated*	*Method of identification*
1	*O*-guaiacol	0.13	1095	a, b, c
2	α-copaene	0.42	1354	a, b, c
3	β-elemene	1.92	1363	a, b, c
4	*trans*-caryophyllene	0.08	1400	a, b, c
5	α-gurjunene	-	1401	a, b, c
6	α-cedrene	-	1407	a, b, c
7	*cis*-caryophyllene	0.11	1416	a, b, c
8	*trans*-α-bergamotene	-	1430	a, b, c
9	γ-elemene	0.07	1432	a, b, c
10	aromadendrene	0.12	1440	a, b, c
11	*trans*-β-farnesene	0.06	1442	a, b, c

12	α-humulene	0.15	1450	a, b, c
13	deydroaromadendrene	0.11	1460	a, b, c
14	ar-curcumene	2.97	1467	a, b, c
15	γ-curcumene	8.55	1481	a, b, c
16	β-curcumene	1.25	1516	a, b, c
17	β-sesquiphellandrene	6.78	1520	a, b, c
18	zingiberene	0.12	1535	a, b, c
19	selina-3,7(11)-diene	0.31	1545	a, b, c
20	germacrene B	0.09	1551	a, b, c
21	ledene	0.13	1554	a, b, c
22	longipinanol	0.31	1563	a, b, c
23	caryophylene oxide	0.99	1569	a, b, c
24	n.i.	0.14	1574	a, b, c
25	ar-turmerol	1.66	1583	a, b, c
26	*cis*-β-elemenone	0.60	1589	a, b, c
27	globulol	0.13	1592	a, b, c
28	viridiflorol	2.01	1597	a, b, c
29	cedrol	0.21	1600	a, b, c
30	ledol	0.84	1609	a, b, c
31	oplopenone	0.40	1616	a, b, c
32	1,7-diepi-α-cedrenal	1.45	1623	a, b, c
33	caryophylla-4(12),8(13)-dien-5β-ol	0.46	1630	a, b, c
34	patchouli alcohol	0.36	1650	a, b, c
35	(+)gymnomitrol	0.15	1654	a, b, c
36	β-turmerone	15.32	1662	a, b, c
37	ar-turmerone	9.70	1670	a, b, c
38	α-(Z)-santalol	1.80	1675	a, b, c
39	8-cedren-13-ol	0.94	1681	a, b, c
40	germacrone	0.69	1687	a, b, c
41	α-turmerone	7.30	1695	a, b, c
42	longifolol	1.32	1725	a, b, c
43	β-(Z)-santalol	0.21	1727	a, b, c
44	*iso*-longifolol	0.12	1729	a, b, c
45	curcumenol	3.35	1730	a, b, c
46	zerumbone	0.21	1731	a, b, c
47	2E,6E-farnesol	0.20	1745	a, b, c
48	shyobunone	0.29	1747	a, b, c
49	germacrol	2.89	1748	a, b, c
50	*p*-nonylphenol	0.25	1750	a, b, c
51	valerenal	0.25	1750	a, b, c
52	α-methyl-(3,4,or 6 methyl)-α-ionone	0.10	1753	a, b,c
53	(+)-1,10-dihydronootkatone	0.40	1755	a, b, c
54	caryophylla-3,8(13)-dien-5β-ol	1.57	1756	a, b, c

55	caryophyllenol	1.29	1757	a, b, c
56	*trans*-nuciferol	0.30	1765	a, b, c
57	α-(E)-atlantone	0.68	1770	a, b, c
58	zingiberenol	1.57	1798	a, b, c
59	nootkatone	0.68	1809	a, b, c
60	1,4-*cis*-1,7-*trans*-acorenone	0.07	1835	a, b, c
61	aristolenepoxide	0.11	1845	a, b, c
62	(+)-8(15)-cedren-9-ol	0.11	1851	a, b, c
63	1-bisabolone	0.28	1874	a, b, c
64	(+)-1,10-dihydronootkatone	0.59	1883	a, b, c
65	5β,7βH,10α-eudesm-11-en-1α-ol	0.17	1897	a, b, c
66	levomenol	0.28	1916	a, b, c
67	α-*trans*-sesquicyclogeraniol	0.23	1923	a, b, c
68	(±)-(E)-dihydrofarnesal	0.17	1950	a, b, c
69	α-*trans*-sesquicyclogeraniol	0.25	1954	a, b, c
70	γ-*cis*-sesquicyclogeraniol	0.23	1987	a, b, c
71	n.i.	0.19	1995	a, b, c
72	custonolide	1.13	2025	a, b, c
73	nootkaton-11,12-epoxide	0.17	2031	a, b, c
74	1,2-longidione	0.65	2045	a, b, c
75	valarenic acid	0.16	2057	a, b, c
76	1,2-longidione	0.31	2065	a, b, c
77	Polygodial	0.78	2070	a, b, c
78	malonic acid, phenyl-, diethyl ester	0.37	2078	a, b, c
79	α-santonin	0.06	2098	a, b, c
80	hexadecanoic acid, ethyl ester	0.10	2109	a, b, c
81	(+)-copaiferic acid	0.06	2118	a, b, c
82	λ-8(20),13-dien-15-oic acid, (E)-(+)	0.11	2151	a, b, c
83	9,12,15-octadecatrienoic acid, ethyl ester (Z,Z,Z)	0.14	2208	a, b, c
84	Sclareol	0.25	2224	a, b, c
85	λ-14-ene-8,13-diol (13R)	0.11	2226	a, b, c
86	oleic acid ethyl ester	0.15	2240	a, b, c
87	Mandenol	0.37	2261	a, b, c
88	linoleic acid ethyl ester	0.23	2357	a, b, c
89	octadecanoic acid, ethyl ester	0.30	2370	a, b, c
90	octadecanoic acid, 17-methyl, methyl ester	0.46	2378	a, b, c
91	methyl abietate	0.21	2391	a, b, c
92	λ-8(20),13(16),14-trien-18-oic acid, 15,16-epoxy	0.26	2407	a, b, c
93	2,6,10,14-hexadecatetraenoic acid, 3,7,11,15-tetramethyl, methyl ester, (E,E,E)	0.49	2411	a, b, c
94	copalic acid methyl ester	0.22	2467	a, b, c
95	16-hydroxy-(-)-kauranoic acid	0.91	2490	a, b, c
96	7,10,13-eicosatrienoic acid, methyl ester	0.94	2503	a, b, c

97	λ-8(20)-13-diene-15,19-dioic acid (E)	0.34	2551	a, b, c
98	gibberellic acid	0.49	2626	a, b, c
99	cholest-5-en-3-one	0.28	2778	a, b, c
100	cholestan-3-ol, 2-methylene, (3β,5α)	0.32	2842	a, b, c
101	Squalene	0.32	2952	a, b, c
102	Stigmasterol	0.38	2956	a, b, c
103	β-sitosterol	0.19	2967	a, b, c
104	5α-pregn-16-en-20-one, 3β,12α-dihydroxy, diacetate	0.27	2973	a, b, c
105	ergosta-5,22-dien-3-ol, acetate, (3β,22E)	0.07	2992	a, b, c
106	n.i.	0.06	2995	a, b, c
107	n.i.	0.12	3000	a, b, c
108	Botulin	0.15	3007	a, b, c
109	Betulinol	0.12	3009	a, b, c
110	n.i.	0.00	3012	a, b, c
111	Betulol	0.08	3015	a, b, c
112	betulinic acid	0.03	3020	a, b, c
113	betulic acid	0.40	3023	a, b, c
114	β-carotene	0.18	3029	a, b, c
115	Lycopene	0.04	3032	a, b, c
116	n.i.	0.05	3033	a, b, c
117	n.i.	0.01	3038	a, b, c
Sesquiterpene hydrocarbons		23,24		
Oxygenated sesquiterpenes		66,65		
Diterpenes		2,75		
Triterpenes		1,1		
Tetraterpenes		0,22		
Phytoesterols		0,57		
Other compounds		3,67		
Not identified		1,77		

[a]Compounds listed according to order of HP-5MS UI column elution. [b]Retention Index (IR), calculated using a homologous series of *n*-alkanes on a capillary column (HP-5MS) (Adams, 2017). [c]Identification based on comparison of mass spectra in NIST 11.0 libraries. Relative Area (%), percentage of the area occupied by the compound within the chromatogram; n.i., not identified. t=traces

The analysis of the chemical composition of HECLsd revealed twenty-one compounds, representing 97.08% of the volatile components of the sample (Table No. 3). The major constituents were α-curcumene (7.54%), α-zingiberene (13,07%), β-sesquiphellandrene (11.23%), α-tumerone (6.71%), β-tumerone (20.06%), ar-turmerone (12.90%) and germacrone (6.65%).

TABLE No. 3
Chemical composition of the hydroethanolic extract of *C. longa* that was obtained by the spray dryer process (HECLsd)

Peak	*Compound*	*Area (%)*	*RI Calculated*	*Method of Identification*
1	α-cedrene	0.86	1375	a, b, c
2	*trans*-β-farnense	-	1479	a, b, c
3	α-curcumene	7.54	1483	a, b, c
4	α-zingiberene	13,07	1490	a, b, c
5	β-bisabolene	1.91	1502	a, b, c
6	*cis*-α-bisabolene	-	1510	a, b, c
7	β-sesquiphellandrene	11.23	1518	a, b, c
8	longiborneol	0.79	1584	a, b, c
9	β-elemenone	4.42	1596	a, b, c
10	α-tumerone	6.71	1597	a, b, c
11	n.i.	0,93	1624	a, b, c
12	β-tumerone	20.06	1660	a, b, c
13	ar-turmerone	12.90	1663	a, b, c
14	bergaptene	1.19	1688	a, b, c
15	germacrone	6.65	1693	a, b, c
16	1-bisabolone	1.95	1739	a, b, c
17	(±)-t*rans*-dihydrofarnesal	1.05	1767	a, b, c
18	n.i.	0.58	1798	a, b, c
19	allyl ionone	1.57	1832	a, b, c
20	valerenic acid	2.17	2088	a, b, c
21	hexadecanoic acid, methyl ester	3.22	2132	a, b, c
	Sesquiterpene hydrocarbons	34.61		
	Oxygenated sesquiterpenes	55.72		
	Other compounds	6.96		
	Not identified	1.51		

[a]Compounds listed according to order of HP-5MS UI column elution. [b]Retention Index (IR) calculated using a homologous series of *n*-alkanes on a capillary column (HP-5MS) (Adams, 2017). [c]Identification based on comparison of mass spectra in NIST 11.0 libraries. Area (%), percentage of area occupied by the compound within the chromatogram; n.i., not identified.

Bisdemethoxycurcumin, demethoxycurcumin, and curcurmin are present in extracts

The HPLC analysis indicated the presence of three peaks that were consistent with curcuminoids, observed at 7.08, 8.44, and 10.05 min. These peaks presented similar absorbance spectra with λ_{max} at 418-426 nm. The elution profiles of curcuminoids were similar to previous reports,[6] suggesting the presence of bisdemethoxycurcumin (BDM), desmethoxycurcumin (DMC), and curcumin (main peak). The standard that was used for quantification presented a single peak at 10.05 min. Thus, the quantification of curcuminoids was based on the calibration curve that was obtained from curcumin, which gave a correlation coefficient of 0.995. Total curcuminoids were obtained from the sum of the areas of the three peaks (3.1% for HECLsd and 5.5% for HECLex). Based on the individual areas, the ratio of curcuminoids (BDM:DMC:curcumin) was 1:2.1:3.5 for HECLsd and 1:2.4:4.5 for HECLex (Figure No. 1).

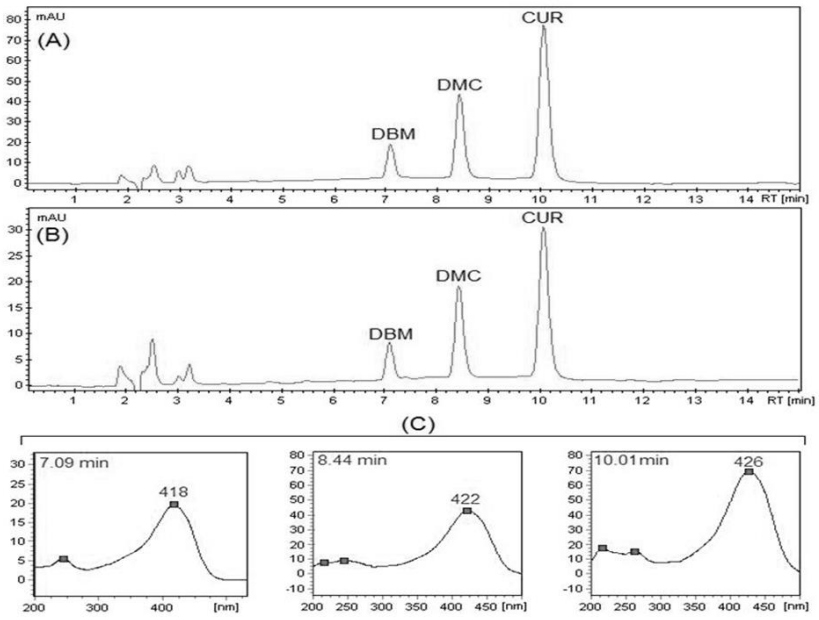

Figure No. 1
Analysis of curcuminoids from *Curcuma longa* by high-performance liquid chromatography, showing chromatographic profile of HECLex (A) compared with HECLsd (B). Curcuminoids were identified as bisdemethoxycurcumin (BDM), demethoxycurcumin (DMC), and curcurmin (CUR). (C) Absorbance spectra of each peak from the chromatograms, showing their similarities

HECLsd has more total phenolic compounds than HECLex

Table No. 4 shows the concentrations of total phenolic compounds in HECLex and HECLsd. Student's *t*-test revealed a significant increase in total phenolic compounds in HECLsd (72.28 µg gallic acid equivalents/mg of extract) compared with HECLex (19.00 µg gallic acid equivalents/mg of extract; t = -18.24, p=0.002).

TABLE No. 4
Concentrations of total phenolic compounds in hydroethanolic extract of *C. longa* that was obtained by a dynamic maceration process with solvent depletion (HECLex) or a spray dryer process (HECLsd)

Parameter	*HECLex*	*HECLsd*
Total phenolic compounds (µg gallic acid equivalents/mg of extract)	19.00 ± 0.28	72.28 ± 2.90*

The data are expressed as mean ± SEM. *p<0.01, compared with HECLex (Student's *t*-test)

Free-radical scavenging activity of HECLsd and HECLex

Student's *t*-test revealed higher antioxidant capacity, determined by the DPPH assay, for HECLsd (EC_{50} = 0.2305 mg extract/ml, r^2 = 0.9915) compared with HECLex (EC_{50} = 1.1065 mg/mL, r^2 = 0.6584; t = 1238.85, p=0.000001). The FRAP assay determination of iron reducing capacity showed that HECLsd (18.752 nmols Trolox equivalents/mg of extract) compared with HECLex (4.148 nmols Trolox equivalents/mg of extract; t = 18.75, p=0.007, Student's *t*-test; Table No. 5).

TABLE No.5
Free-radical scavenging activity of the hydroethanolic extract of *C. longa* that was obtained by a dynamic maceration process with solvent depletion (HECLex) or a spray dryer process (HECLsd)

Parameter	*HECLex*	*HECLsd*
DPPH (values of EC50 mg extract.mL^{-1})	1.1065 ± 0.0005	0.2305 ± 0.0005*
FRAP (nmols Trolox equivalents/mg of extract)	4.148 ± 0.016	18.752 ± 1.261*

The data are expressed as mean ± SEM. *p<0.01, compared with HECLex (Student's t-test). EC_{50} values were obtained by interpolation of linear regression analysis

DISCUSSION

To understand the various human health benefits of bioactive compounds of *C. longa*, the phytochemistry of these compounds needs to be analyzed, and extract preparation needs to be standardized, including cultivation, extraction, and drying. Planting, extraction, and drying can directly influence the chemical composition of bioactive compounds of medicinal plants and consequently their biological actions (Koffi *et al*., 2010; Pavarini *et al*., 2012; Altemimi *et al*., 2017; Gallego *et al*., 2019).

In the present study, the soil analysis indicated low levels of calcium, magnesium, aluminum, carbon, organic matter, the sum of bases, and the saturation of bases. Average levels of potassium and iron were observed, with high levels of hydrogen + aluminum and the saturation of aluminum, manganese, copper, and zinc. The average level of potassium is presumed to not interfere with the number of rhizomes, only the number of leaves (May *et al*., 2005). Potassium can increase the growth (i.e., height) of the leaves in the mother plant. However, the interaction between nitrogen and potassium can increase the number of leaves of the pit plant (mother plant and tiller) and tiller. However, these compounds do not interfere with the production of rhizomes (May *et al*., 2005). The combined levels of. nitrogen, phosphorus, and potassium may be related to pigment color in *C. longa* rhizomes and plant development (Cecilio-Filho *et al*., 2000).

Another factor that directly interferes with the composition of plant metabolites is the pH of the soil (Compant *et al*., 2019; Mykhailenko *et al*., 2020). In the present study, the pH of the *C. longa* soil bed that was used was 3.7. The HPLC analysis indicated lower total curcuminoids and curcumin for HECLsd compared with HECLex. However, evaluation of the chemical compositions of the two extracts by GC/MS indicated the low expression of curcuminoids in HECLex (8.55% γ-curcumene) and the absence of curcuminoids in HECLsd. One explanation for this finding may be related to the pH (3.7) of the soil. Greater stability of the curcumin molecule is found in a pH range of 4.0 to 7.0 (Rusig & Martins, 1992). A pH > 7.0 makes curcumin unstable, increasing the rate of degradation of the molecule (Tonnesen & Karlsen, 1985; Rusig & Martins, 1992). The absence of curcumin in HECLsd may be related to temperature. The process of preparing the extract by spray drying raises the temperature considerably (175ºC), which results in the loss of volatile compounds (detected by GC/MS) and concentrating the active ingredients in the sample. A greater number of components (21 components) was detected in HECLsd sample, in contrast to HECLex (117 components). Temperature and luminosity also interfere with the stability of curcumin and curcuminoids. Up to 100°C, there is no significant loss of curcumin. Above 125°C, occurs 15.25% of degradation of the compound compared with initial levels (Rusig & Martins, 1992).

Phenolic compounds and flavonoids are secondary metabolites of plants that have important antioxidant actions. Antioxidant compounds can slow or inhibit the oxidation of lipids or other molecules, preventing the onset or propagation of oxidation chain reactions (Duarte-Almeida *et al*., 2006; Swalla *et al*., 2020). Total phenolic compounds contribute to antioxidant activity by inhibiting lipid peroxidation, neutralizing or sequestering free radicals, and promoting metal chelation. Therefore, they exert actions on the initiation and propagation stages of lipid peroxidation (Sousa *et al*., 2007; Pisoschi *et al*., 2021). An important relationship also exists between the position of hydroxyl groups and their proximity to -CO_2H groups relative to phenyl groups. A closer proximity of these groups is associated with greater antioxidant capacity of the hydroxyl group in the meta position (Hrazdina *et al*., 1970; Tanvir *et al*., 2017). We found that HECLsd had a higher concentration of total phenolic compounds in the analyzed extracts compared with HECLex.

The evaluation of antioxidant activity by the DPPH and FRAP assays also indicated that HECLsd had a higher antioxidant capacity compared with

HECLex. This result may be attributable to the presence of the major components 9,12,15-octadecatrienoic acid, 2,3-bis([trimethylsilyl]oxy)-propyl ester (Z,Z,Z), verrucarol, and 1-monolinoleoylglycerol trimethylsilyl ether. These results corroborate a previous study that investigated the total antioxidant capacity of *C. longa* and *C. xanthorrhiza* using the DPPH and FRAP assays. The results showed that *C. longa* had a strong antioxidant capacity and strong free-radical scavenging activity relative to the DPPH and FRAP reference compounds (Asourl *et al*., 2013; Akinola *et al*., 2014).

The analysis of the chemical composition of HECLsd indicated that 9,12,15-octadecatrienoic acid, 2,3-bis([trimethylsilyl]oxy)propyl ester (Z,Z,Z), verrucarol, and 1-monolinoleoylglycerol trimethylsilyl ether were major components. Several studies have isolated these compounds from other plant species, such as *Vitex neguno* (Kumar *et al*., 2010), *Portulaca oleracea* (Osman & Hussein, 2015), *Archidium ohioense* (Godwi *et al*., 2015), *Begonia malabarica* (Aswathy *et al*., 2015) and *Jatropha gossypifolia* (Bharathy *et al*., 2012). These compounds were shown to exert several biological activities, including antioxidant, antiinflammatory, antimicrobial, antipyretic, anticancer, antiulcerogenic, hepatoprotective, cardioprotective, hypocholesterolemic, insecticidal, nematicidal, antihistaminic, antiarthritic, antiecemic, antiacne, and antiandrogenic effects (Sermakkani & Thangapandian, 2012; Sheela & Uthayakumari, 2013; Godwi *et al*., 2015; Aswathy *et al*., 2015; Tyagi & Agarwal, 2017). 1-Monolinolethylglycerol, which was also present in HECLsd in the present study, exerts antiarthritic, hepatoprotective, antimicrobial, antiasthma, diuretic, anti-inflammatory, antioxidant, and antidiabetic effects (Bharathy *et al*., 2012; Sheela & Uthayakumari, 2013; Senthil *et al*., 2016; Tyagi & Agarwal, 2017). Thus, HECLsd presented higher antioxidant capacity compared with HECLex, despite the absence of curcumin. This antioxidant capacity may be attributable to the presence of 9,12,15-octadecatrienoic acid and 1-monolinolethylglycerol.

The major substances that were found in HECLex were ar-turmerone, γ-curcumene, α-turmerone, β-turmerone, and β-sesquiphellandrene. Previous studies have investigated the antibacterial and antifungal activity of these compounds (Raina *et al*., 2005; Essien *et al*., 2015) and also found low antioxidant activity of α-turmerone (Sun *et al*., 2017). These previous findings corroborate the present results, in which HECLex presented low antioxidant capacity. Similar results were reported previously (Johnson *et al*., 2008) in which phenolic components of different plants and a crude extract of *C. longa* were evaluated, with values that were similar to the present study.

In conclusion, the results of our study show that different processes of extracting and preparing *C. longa* rhizomes can alter the expression of chemical components and consequently their biological activity. The HECLsd presented less curcumin and curcuminoids than HECLex, however, HECLsd presented higher antioxidant activity and total phenolic compounds than HECLex. Further studies are necessary to isolate the compounds that were identified with both extraction processes and evaluate their antioxidant activity.

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