

VOLATILE ORGANIC COMPOUNDS AND ANTIOXIDANT CAPACITY OF *Curcuma longa* L. AND *Curcuma caesia* Roxb. LEAVES: EXPLORING BIOACTIVITY IN NATURAL PRODUCTS

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ABSTRACT

Natural products contain diverse bioactive compounds with therapeutic and pharmacological potential, including antimicrobial, antioxidant, anticancer, antifungal properties, and respiratory benefits for conditions. Bioactive volatile organic compounds were identified using headspace-gas chromatography in *Curcuma longa* L (CL). and *Curcuma caesia* Roxb. (CC) leaves, with CL leaves containing 90.48% terpenes and CC leaves containing 77.5% terpenes and 22.5% non-terpenes. The total phenolic content and antioxidant capacity were evaluated, employing a 2x7 factorial design analyzed through one-way and factorial ANOVA. Methanol:water and ethanol extracts from CL exhibited superior radical sequestering capacity (IC₅₀ of 0.35 mg mL⁻¹) compared to CC extracts. The highest Ferric Reducing Antioxidant Power value of 95.28 µMol FeSO g⁻¹ was achieved analyzing methanol:water. Ethanol:water was considered most appropriate, as it is the most suitable solvents for industrial food processing due to safety and compatibility aspects.

Keywords: *Zingiberaceae*, by-product use, extraction, natural antioxidant.

INTRODUCTION

The extraction method plays a crucial role in obtaining phenolic compounds from plant leaves. When selecting a plant leaf extract for use as a natural antioxidant, certain parameters must be considered to ensure the best possible extraction of phenolic compounds. The choice of solvent is especially important, and it should be non-toxic, have an adequate volume and polarity, and be suitable for the specific plant being used (PEDRO et al., 2018).

The solvent is a critical factor that directly influences the extraction process of bioactive compounds from plant leaves, as its chemical nature can cause varying amounts of extraction depending on its concentration and interactions with other food constituents. Recovery of phenolic compounds also differs between different plants, and extraction conditions must be optimized to ensure maximum extraction (ZŁOTEK et al., 2016).

Phenolic compounds are secondary metabolites that are strongly associated with macromolecules such as polysaccharides, cell wall components, dietary fiber, proteins, and lipids (TAIZ et al., 2016). However, quantifying phenolic compounds that are complexed with these macromolecules can be challenging, as a significant fraction of polyphenols may be ignored due to their retention in the residue after extraction (DOMÍNGUEZ-RODRÍGUEZ et al., 2017).

Despite the challenge of extracting phenolic compounds that are complexed with macromolecules in plant tissues, it is possible to disrupt the chemical bonds between these molecules. The hydrophilic/hydrophobic character of the substances plays a crucial role in determining the most suitable solvent for their extraction, as does enzymatic action. Generally, polar organic solvents such as acetone, ethanol, methanol, and ethyl acetate are effective at releasing these compounds from cellular structures (CHENG et al., 2014).

The objective of this study was twofold: first, to identify the bioactive compounds present in CL and CC leaves using headspace-gas chromatography-mass spectrometry (HS-GC-MS), and second, to evaluate the natural extracts of CL and CC using seven different types of solvents, including mixed and non-mixed solvents, in terms of their total phenolic compound (TPC) content and antioxidant capacity using DPPH and FRAP (Ferric Reducing Antioxidant Power) methods.

MATERIAL AND METHODS

Collection and preparation of the samples

The CL leaves were collected from the Turmeric Growers Cooperative (Cooperaçãofrão) in Mara Rosa city, Goiás State, Brazil (14°00'10.9"S and 49°07'11.8"W), whereas the CC leaves were collected from an organic farm in Nossa Senhora Aparecida, Hidrolândia city, Goiás State, Brazil.

The plant leaves were harvested at dawn and stored in nylon bags to prevent exposure to light and air. They were then transported to the Universidade Federal de Goiás - UFG in Goiânia city, Goiás State, Brazil, under controlled temperature (20°C) to prevent any potential degradation of the samples. The samples were stored at -20°C until further analysis.

The leaves were carefully selected based on their visual appearance, including their typical green color and integrity. They were then washed with distilled water and sanitized in a sodium hypochlorite solution (0.1 mL L⁻¹) for 15 minutes, followed by rinsing with distilled water and drying with a paper towel to remove any surface moisture. The stalks were removed from the leaves, and the remaining portions were cut into 2 cm pieces and placed on perforated stainless-steel trays for convective drying in an air circulation oven (Tecnal TE-394/3, Piracicaba city, São Paulo State, Brazil) at a temperature of 40°C ± 2°C for approximately 24 hours. After drying, the leaf fragments were vacuum-packed (Selovac Microvac, São Paulo city, São Paulo State, Brazil) in low-density polyethylene bags to maintain their original characteristics and prevent contamination. The samples were stored in a cool, dark place until further analysis. Prior to extraction, the leaves of each species were crushed (Figure 1) in an electric grain (Bistro-Blade, Bodum, Denmark) and passed through 60 mesh sieves to prepare the extracts.



Figure 1. Flowchart of the stages of preparation and analysis of Curcuma leaves.

Headspace – Gas Chromatography coupled with mass spectrometry analysis (HS-GC-MS)

To identify and trace the chemical compounds present in CL and CC leaves, the samples were subjected to analysis using HS-GC-MS. This involved the use of a gas chromatograph (Shimadzu Nexis GC2030) coupled to a mass spectrometer, equipped with a capillary column (SH-Stabilwax-ms, 30m, 250 μ m id, 0.25 μ m). Prior to analysis, the samples were heated via headspace (AOC-6000 autosampler) at 80°C for 30 minutes, and a volume of 2.5 mL was injected into the chromatograph.

The analysis was conducted using a split mode with a ratio of 10:1 and an equilibrium time of 3 min. The oven temperature was programmed to start at 40°C and maintained for 1 minute, followed by a heating ramp from 5°C/min to 160°C, increased to 10 °C/min to 250°C, and then maintained for 15 min. The total analysis time was 49 min. Helium 5.0 was used as the carrier gas at a pressure of 4.7 psi, a flow rate of 0.79 mL/min, and a linear velocity of 32.4 cm/s. The injector, interface, and ion source temperatures were maintained at 250°C, and the mass spectrometer operated in scan mode, recording ions in the range of 25 to 500 m/z with a scan time of 150 ms.

Preparation of the extracts with different solvents

The plant extracts were prepared from the dehydrated and crushed leaves using a modified version of the method described by MICHIELS *et al.* (2012). A completely randomized design was employed, with a 2x7 factorial arrangement comprising two types of leaves (CL and CC) and seven types of solvents, including both mixed and non-mixed solvents (see Table 1). The experiment was replicated three times, with center points included in each replication.

Extraction was carried out with a ratio of 1:20 (w/v), where 1 g of crushed leaves was mixed with 20 mL of solvent in test tubes wrapped with aluminum foil to avoid exposure to light. The tubes were then stirred for 1 hour on a rotary shaker (AP22, Phoenix Lufenco, São Paulo). Seven types of solvents were used, including mixed and non-mixed solvents (Table 1).

Table 1. Mixed and non-mixed solvents used in the preparations of the leaf extracts of CL and CC.

Solvent	Acronym	Proportion (%)
Water	W	100
Ethanol	E	100
Ethanol + water	EW	70:30
Methanol	M	100
Methanol + water	MW	70:30
Methanol + acetic acid	MAA	99,5:0,5
Acetone + water + acetic acid	AWA	10:28:2

After the pooling of supernatants, the resulting mixture was filtered through 125 mm Whatman filter paper, and the filtrate was transferred into a 50 mL volumetric flask. The flask was then made up to volume with the respective mixed or non-mixed solvents, resulting in extracts with a concentration of 2% or 0.02 g mL⁻¹. The extracts were thoroughly mixed, transferred to amber flasks, and stored at 25°C until further analysis.

Extracts characteristics

Total phenolic compounds (TPC)

The TPC content was determined using the spectrophotometric method described by (CHAN; LIM; LIM, 2007), with some modifications. Briefly, 300 µL of leaf extract was mixed with 1.5 mL of 10% (v/v) Folin-Ciocalteu reagent (GORINSTEIN *et al.*, 2003) and 1.2 mL of 7.5% (w/v) sodium bicarbonate solution in an aluminum foil-wrapped test tube. The mixture was homogenized and incubated in the dark at 25°C. After 30 minutes, the absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The TPC content was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW) using a calibration curve of gallic acid (Sigma-Aldrich, St. Louis, MO, USA). All analyses were performed in triplicate, and the results were expressed as mean ± standard deviation.

After 30 minutes of incubation in the dark, the absorbance of the mixture was measured at a wavelength of 765 nm (infrared) using a spectrophotometer (SP-2000 UV, Bel Engineering,

Monza, Italy), which had been previously adjusted with a blank test containing 300 μL of extracting solvent. A standard curve of gallic acid ($y = 0.0282x - 0.0078$, $R^2 = 0.9991$) was constructed to quantify the total phenolic content (TPC) in the samples. The TPC content was expressed as mg of gallic acid equivalent (GAE) per 100 g of material.

Radical scavenging activity (DPPH)

The antioxidant activity of the leaf extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay to measure radical scavenging activity, following the method described by Brand-Williams et al. (1995) with slight modifications. Briefly, 1 mL of each leaf extract was mixed with 2 mL of 0.02 mg/mL DPPH solution in methanol and allowed to react in the dark for 30 minutes. The absorbance was then measured at 517 nm (green) using a spectrophotometer (SP-2000 UV, Bel Engineering, Monza, Italy) that was previously adjusted with methanol as a blank. The DPPH radical scavenging activity was expressed as the IC₅₀ value, which represents the concentration of extract required to scavenge 50% of DPPH (MENDES *et al.*, 2018). Trolox was used as a positive control for comparison.

Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay measures the ferric reduction capacity of a sample, where the antioxidant potential promotes the reduction of ferric ion (Fe^{+3}) to ferrous ion (Fe^{+2}), and is typically used to evaluate the antioxidant power of a sample. This reaction is evidenced by the formation of a blue-colored complex (Fe^{2+}). The method suggested by PULIDO *et al* (2000) was used to determine the ferric reducing antioxidant power. The FRAP reagent was prepared by mixing sodium acetate buffer (300 mM, pH 3.6), a 10 mM solution of 2,4,6-tripiridyl-s-triazine (TPTZ) in 40 mM hydrochloric acid (HCl), and 20 mM ferric chloride ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$) in a 10:1:1 (v/v/v) ratio.

To perform the assay, 2,700 μL of the FRAP reagent was homogenized with 90 μL of the leaf extract sample and 270 μL of distilled water. The test tubes were incubated in a water bath at 37°C for 30 min, and the analyses were taken in a spectrophotometer (SP-2000 UV, Bel Engineering, Monza, Italy) at 595 nm, with the FRAP reagent used to calibrate the spectrophotometer. The quantification of the compounds was determined from the calibration standard curve, which was prepared with different concentrations of a 2000 μM ferrous sulfate

solution (ranging from 500 to 2000 μM). The results were expressed as μM ferrous sulfate per gram (dry weight) of the sample. The standard curve equation was $y=0.001x+0.0275$, with an R^2 value of 0.9905.

RESULTS AND DISCUSSION

Identification of compounds present in CL and CC leaf extract by HS-GC-MS.

To understand the chemical composition of the CL and CC leaf extracts, a HS-GCMS analysis was performed to identify the compounds present. The identification of these compounds is important for determining the potential health benefits and applications of these plant extracts. The CL leaf extract contained 42 compounds, including: 19) Hydrocarbons; (14) Alcohols; (1) Aldehyde; (1) Ester; (2) Ethers and (5) Ketones. The CC leaf extract contained 40 compounds, including: (14) Hydrocarbons; (15) Alcohols; (2) Aldehydes; (1) Ester; (2) Ethers and (6) Ketones.

The terpene group was the most abundant chemical class in CL, accounting for 90.48% of the total compounds, with Monoterpene hydrocarbons, Oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, and diterpenes contributing 31.58%, 23.68%, 23.68%, 18.42%, and 2.63%, respectively. The non-terpene group accounted for 9.52%. Similarly, in CC, the terpene group was the dominant class, representing 77.5% of the total compounds, while non-terpene groups accounted for 22.5%. Among the terpenes, monoterpene hydrocarbons (29.04%), oxygenated monoterpenes (35.48%), sesquiterpenes (12.9%), and oxygenated sesquiterpenes (22.58%) were identified, while diterpenes were not detected.

The findings of this study are consistent with a prior investigation (OLAYEMI et al., 2018) that detected compounds in *Cymbopogon Citratus* leaves, accounting for 99.0% of the total oil. Monoterpene compounds dominated the detected compounds, representing 88.9% of the total oil, including monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and other non-terpene groups at 20.3%, 68.6%, 1.7%, and 8.4%, respectively. The study also identified non-terpene compounds, representing 10.8% of the total oil (OLAYEMI et al., 2018).

Terpenes are a diverse group of compounds that possess important biological and pharmacological activities, such as antimicrobial, antifungal, antioxidant, anti-inflammatory, insecticidal, analgesic, anticancer, gastroprotective, antibacterial, antidepressant, antiproliferative, anxiolytic, and neuroprotective effects, among others (KIYAMA, 2020; SOMMANO et al., 2020; ZHANG et al., 2019). In particular, monoterpenes have been shown to possess antioxidant, anti-inflammatory, antihyperlipidemic, and antinociceptive properties, as well as estrogenic effects that are relevant for Alzheimer's disease therapy. Moreover, many other monoterpenes have demonstrated promising neuroprotective activity through various mechanisms, while several monoterpenoid alcohols have exhibited good antiparkinsonian activity (HANUŠ; HOD, 2020; KIYAMA, 2020). Additionally, some terpenes have been found to have larvicidal activity (ANDRADE-OCHOA et al., 2018).

SESSOU et al. (2012) reported that the fresh leaves of *Cymbopogon citratus* contained 9.9% hydrogenated monoterpenes, 85.3% oxygenated monoterpenes, 2.2% hydrogenated sesquiterpenes, and 0.1% oxygenated sesquiterpenes, representing a total of 97.5% of the oil composition. This high proportion of terpenes is significant as they are a source of bioactive compounds with diverse molecular structures and potential therapeutic effects (ACAR et al., 2020).

The major compounds identified in the leaves of CL and CC were Eucalyptol, (+)-2-Bornanone, Methyl Alcohol and Camphene. The molecular formula (MF) and retention time of these major compounds in CL leaves are as follows: Eucalyptol (MF C₁₀H₁₈O, RT 8.514-8.544), (+)-2-Bornanone (MF C₁₀H₁₆O, RT 16.552), Methyl Alcohol (MF CH₄O, RT 3.027) and Camphene (MF C₁₀H₁₆, RT 5.353). These are shown in Figure 2 and Table 2. Similarly, for CC, the retention time of major compounds were Eucalyptol (RT 8.544), (+)-2-Bornanone (RT 16.554), Methyl Alcohol (RT 3.022) and Camphene (RT 5.353), as shown in Figure 3 and Table 3.

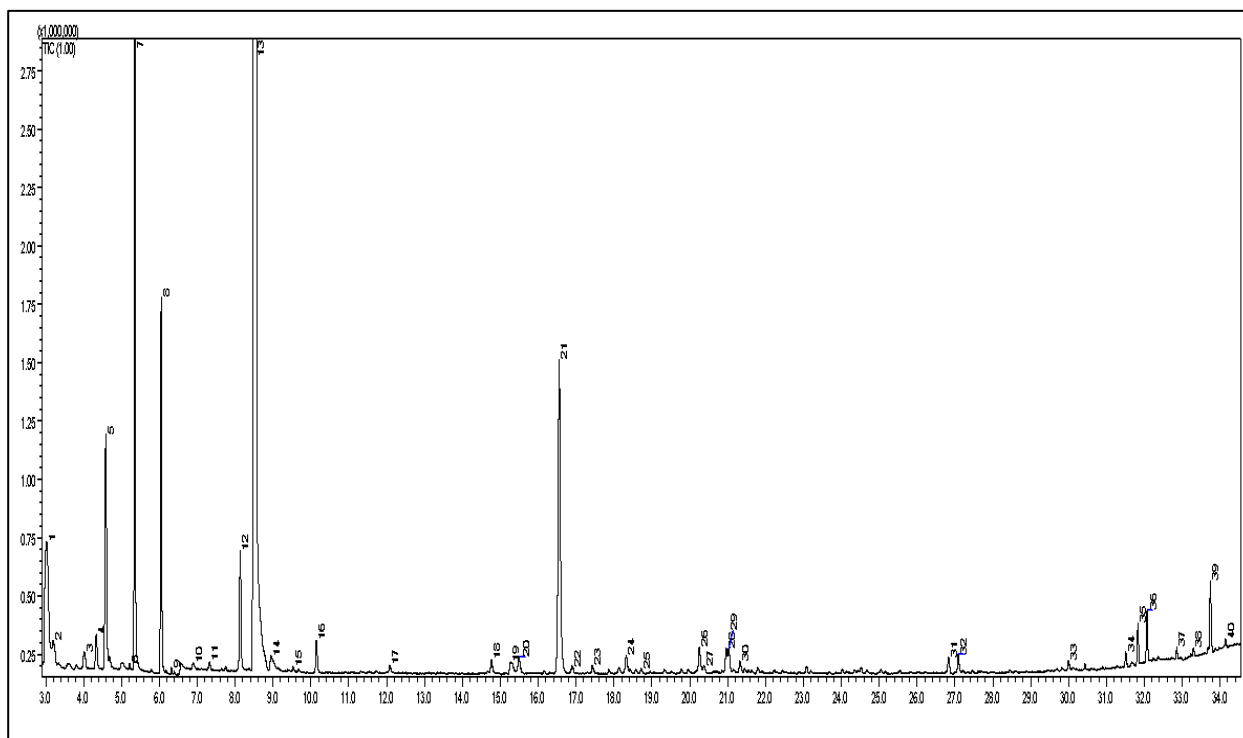


Figure 2. Chromatogram of chemical compounds from the fresh leaves of CL.

Table 2. Identification of chemical compounds from the fresh leaves of CL.

Name of compounds	Molecular Formula	Retention Time (RT)	Relative content (%)	MS Similarity (%)
Methyl Alcohol	CH ₄ O	3.022	4.64	96
Butanal, 3-methyl-	C ₅ H ₁₀ O	3.199	0.65	94
2,3-Butanedione	C ₄ H ₆ O ₂	4.024	0.35	95
Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	C ₁₀ H ₁₆	4.338	0.62	95
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	C ₁₀ H ₁₆	4.588	4.15	95
Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene-, (1R)-	C ₁₀ H ₁₆	5.216	0.07	92
Camphene	C ₁₀ H ₁₆	5.353	7.86	95
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	C ₁₀ H ₁₆	6.048	4.40	95
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	C ₁₀ H ₁₆	6.323	0.06	94
Dicyclopropyl carbine	C ₇ H ₁₂ O	6.898	0.11	82
.beta.-Myrcene	C ₁₀ H ₁₆	7.326	0.09	94
D-Limonene	C ₁₀ H ₁₆	8.136	1.80	95
Eucalyptol	C ₁₀ H ₁₈ O	8.544	60.28	95

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Table 3. (Continue...) Identification of chemical compounds from the fresh leaves of CL.

Name of compounds	Molecular Formula	Retention Time (RT)	Relative content (%)	MS Similarity (%)
Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-p-Mentha-1,5,8-triene	C ₁₀ H ₁₈ O	8.955	0.40	85
	C ₁₀ H ₁₄	9.541	0.07	89
Benzene, 1-methyl-3-(1-methylethyl)-	C ₁₀ H ₁₄	10.147	0.51	96
5-Hepten-2-one, 6-methyl-	C ₈ H ₁₄ O	12.084	0.10	94
trans-Linalool oxide (furanoid)	C ₁₀ H ₁₈ O ₂	14.765	0.24	95
Acetic acid	C ₂ H ₄ O ₂	15.277	0.38	96
2-Furanmethanol, 5-ethenyltetrahydro-.alpha.,.alpha.,5-trimethyl-, cis-(+)-2-Bornanone	C ₁₀ H ₁₈ O ₂	15.493	0.36	88
	C ₁₀ H ₁₆ O	16.554	7.06	98
Benzaldehyde	C ₇ H ₆ O	16.899	0.12	97
Linalool	C ₁₀ H ₁₈ O	17.429	0.12	94
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	C ₁₅ H ₂₄	18.320	0.31	95
	C ₁₀ H ₁₈ O	18.730	0.09	92
Isoborneol	C ₁₀ H ₁₈ O	20.256	0.50	96
Cyclohexanemethanol, .alpha.,.alpha.-dimethyl-4-methylene-.alpha.-Terpineol	C ₁₀ H ₁₈ O	20.376	0.17	91
	C ₁₀ H ₁₈ O	20.965	0.44	92
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	C ₁₀ H ₁₈ O	21.027	0.46	88
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	C ₁₅ H ₂₄	21.331	0.21	96
Caryophyllene oxide	C ₁₅ H ₂₄ O	26.836	0.23	92
Isospathulenol	C ₁₅ H ₂₄ O	27.089	0.26	81
Epicurzerenone	C ₁₅ H ₁₈ O ₂	30.000	0.13	93
1-Hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one	C ₁₄ H ₂₂ O ₂	31.509	0.21	73
Caparratriene	C ₁₅ H ₂₆	31.828	0.53	72
(-)-Neoclovene-(I), dihydro-	C ₁₅ H ₂₆	32.071	0.69	72
(1aR,3aS,7S,7aS,7bR)-1,1,3a,7-Tetramethyldecahydro-1H-cyclopropa[a]naphthalen-7-ol	C ₁₅ H ₂₆ O	32.855	0.14	72
1,3a-Ethano(1H)inden-4-ol, octahydro-2,2,4,7a-tetramethyl-	C ₁₅ H ₂₆ O	33.297	0.10	71
Curcumenol	C ₁₅ H ₂₂ O ₂	33.745	0.97	94
Curcumenone	C ₁₅ H ₂₂ O ₂	34.143	0.09	94

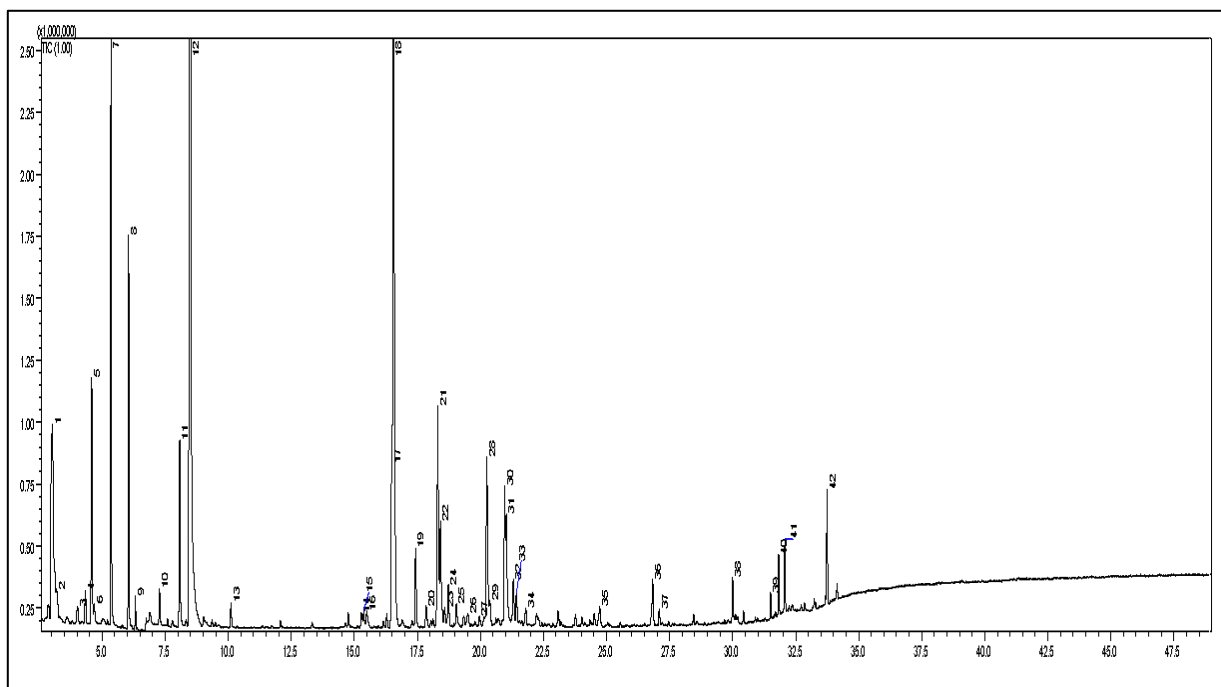


Figure 3. Chromatogram of chemical compounds from the fresh leaves of CC.

Table 4. Identification of chemical compounds from the fresh leaves of CC

Name of compounds	Molecular Formula	Retention Time (RT)	Relative content (%)	MS Similarity (%)
Methyl Alcohol	CH ₄ O	3.027	5.33	97
Butanal, 3-methyl-	C ₅ H ₁₀ O	3.192	0.47	86
2,3-Butanedione	C ₄ H ₆ O ₂	4.017	0.26	86
Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-	C ₁₀ H ₁₆	4.336	0.42	94
(1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene	C ₁₀ H ₁₆	4.587	2.99	96
Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	C ₁₀ H ₁₆	4.689	0.27	80
Camphene	C ₁₀ H ₁₆	5.353	5.32	95
Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-, (1S)-	C ₁₀ H ₁₆	6.048	3.29	95
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	C ₁₀ H ₁₆	6.324	0.25	96
.beta.-Myrcene	C ₁₀ H ₁₆	7.276	0.33	96
D-Limonene	C ₁₀ H ₁₆	8.079	2.11	94
Eucalyptol	C ₁₀ H ₁₈ O	8.514	42.82	95
o-Cymene	C ₁₀ H ₁₄	10.107	0.26	96
Acetic acid	C ₂ H ₄ O ₂	15.286	0.20	78

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Table 5. (Continue...) Identification of chemical compounds from the fresh leaves of CC.

Name of compounds	Molecular Formula	Retention Time (RT)	Relative content (%)	MS Similarity (%)
Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-trans-Linalool oxide (furanoid)	C ₁₅ H ₂₄	15.371	0.19	90
(-)-.beta.-Bourbonene	C ₁₅ H ₂₄	16.480	1.66	90
(+)-2-Bornanone	C ₁₀ H ₁₆ O	16.552	14.09	96
Linalool	C ₁₀ H ₁₈ O	17.430	1.04	97
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	C ₁₅ H ₂₄	17.848	0.32	89
Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4.beta.)]-Caryophyllene	C ₁₅ H ₂₄	18.308	3.13	96
Bicyclo[2.2.1]heptan-2-ol, 2,3,3-trimethyl-	C ₁₀ H ₁₈ O	18.418	1.60	95
3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-	C ₁₀ H ₁₈ O	18.583	0.28	92
Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1R-(1.alpha.,7.beta.,8a.alpha.)]-	C ₁₅ H ₂₄	18.724	0.57	95
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	C ₁₅ H ₂₄	19.045	0.27	91
Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-	C ₁₅ H ₂₄	19.500	0.27	93
Isoborneol	C ₁₀ H ₁₆ O	19.961	0.16	95
Cyclohexanemethanol, .alpha.,.alpha.-dimethyl-4-methylene-	C ₁₀ H ₁₈ O	20.254	2.76	97
L-.alpha.-Terpineol	C ₁₀ H ₁₈ O	20.374	0.36	92
Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	C ₁₀ H ₁₈ O	20.960	1.71	93
Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	C ₁₀ H ₁₈ O	21.024	1.53	87
Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]-	C ₁₅ H ₂₄	21.306	0.54	97
(-)-Carvone	C ₁₅ H ₂₄	21.430	0.38	93
Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dimethyl-5-isopropenyl-, trans-	C ₁₀ H ₁₄ O	21.796	0.23	92
Caryophyllene oxide	C ₁₅ H ₂₀ O	24.730	0.39	95
Isospathulenol	C ₁₅ H ₂₄ O	26.829	0.65	91
Epicurzerenone	C ₁₅ H ₂₄ O	27.087	0.19	81
Curcumenol	C ₁₅ H ₁₈ O ₂	30.002	0.52	94
Cyclodeca[b]furan-2(3H)-one, 3a,4,5,8,9,11a-hexahydro-3,6,10-trimethyl-, [3S-(3R*,3aR*,6E,10E,11aR*)]-	C ₁₅ H ₂₂ O ₂	31.510	0.24	82
Cyclohexene, 4-pentyl-1-(4-propylcyclohexyl)-	C ₁₅ H ₂₂ O ₂	31.828	0.54	73
Curcumenol	C ₂₀ H ₃₆	32.070	0.68	72
Curcumenol	C ₁₅ H ₂₂ O ₂	33.744	1.09	94

Eucalyptol compound

Eucalyptol, also known as 1,8-cineole, is a saturated monoterpene commonly found in many essential oils. It is a major component of *Eucalyptus globulus* oil, representing up to 80% of its composition (JUERGENS, U., 2014). The present study found that eucalyptol comprised 82.26% and 42.82% of the total oil in CL and CC leaves, respectively.

Eucalyptol is a widely distributed compound and can be found as a major component in various plants. For instance, (SOBREIRA DANTAS NÓBREGA DE FIGUÊIREDO *et al.*, 2019) found that the essential oil of *Hyptis martiusii* Benth contained 25.93% of eucalyptol, while (BOUKHATEM *et al.*, 2020) reported that eucalyptol constituted 61.36% of the essential oil extracted from lavender leaves (*Lavandula stoechas* L.).

Previous studies have demonstrated that eucalyptol exhibits a diverse range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant, antifungal, anticancer, and bronchodilatory effects (FAZELAN *et al.*, 2020; JUERGENS, U., 2014). Eucalyptol is widely used for the treatment of various diseases, such as asthma and respiratory problems, including chronic obstructive pulmonary disease (COPD) (GONDIM *et al.*, 2019; JUERGENS, Lisa Joy *et al.*, 2017, 2018). Moreover, eucalyptol may also offer complementary and alternative therapeutic options (ACAR *et al.*, 2020).

Camphene compound

Camphene is a bicyclic monoterpene hydrocarbon that is a major constituent of many essential oils. It is also known as 2,2-dimethyl-3-methylidenebicyclo[2.2.1]heptane. Camphene is found in various plants such as *Laurus nobilis*, *Achillea millefolium*, *Valeriana officinalis* L., *Valeriana officinalis* L. var. *Latifolia* Miq., *Valeriana jatamansi* Jones and *Nardostachys chinensis* Bat.), and Curcuma, among many others (ACAR *et al.*, 2020; FENG *et al.*, 2019; SEVINDIK *et al.*, 2019).

In this study, the camphene compounds extracted from CL and CC leaves were found to be 7.86% and 5.32%, respectively. In comparison, SEVINDIK *et al.* (2019) reported much higher camphene percentages of 28.46% and 12.05% in the leaves and flowers of *Laurus nobilis*, respectively. Camphene has been found to exhibit a range of biological activities, including antidiabetic, antibacterial, anticancer, antioxidant, antifungal, anti-inflammatory, and neuropathic

pain properties (CHÁVEZ-SILVA et al., 2018; MOULAZADEH et al., 2021; SEVINDIK et al., 2019). Additionally, camphene may be particularly relevant to diseases mediated by diabetes and obesity (ACAR et al., 2020).

The (+)-2-Bornanone compound

(+)-Borneol is an oxygenated bicyclic monoterpene compound found in the essential oils of various plants, including *Lauraceae*, *Halimium halimifolium*, *Curcuma*, and *Lavandula stoechas* (CANLI et al., 2019; EL GAMOUZ et al., 2022). This compound has demonstrated antihyperalgesic, antinociceptive, and anti-inflammatory properties (JIANG et al., 2015), as well as antimicrobial activity against over twenty different microorganisms, including several MDR strains (CANLI et al., 2019).

Methyl Alcohol compound

Methyl alcohol is a volatile substance emitted by plants in large amounts. While plants normally generate methanol in the process of demethylation of macromolecules such as DNA and proteins, the main source of methanol derivatives are cell wall pectin, which are demethylesterified by pectin methylesterases (PMEs) (DOROKHOV; SHESHUKOVA; KOMAROVA, 2018). However, it has been shown that exposure to MeOH can cause toxic brain damage that affects the central nervous system (HLUSICKA et al., 2019). It is important to note that this toxic effect is not related to the normal production of methanol in plants but rather to the inhalation of high concentrations of methanol.

Methanol, an abundant and volatile substance emitted by plants, has been found to have diverse biological effects. While methanol is toxic to some insects, it also mediates defense reactions against certain herbivores. Studies have also demonstrated the potential of methanol as a therapeutic agent. For example, BAEK et al. (2020) found that methanol extracted from *I. obliquus* has cytotoxic activity against human lung cancer cells and may have potential for use in lung cancer treatment. Similarly, (DIXIT et al., 2013) reported that methanol has insecticidal properties and could be used in insect control. The main source of methanol in plants is the demethylation reaction of macromolecules, including DNA and proteins, but it is primarily derived from cell wall pectin that is demethylesterified by pectin methylesterases (PMEs) (DOROKHOV; SHESHUKOVA; KOMAROVA, 2018). However, it is important to note that

methanol can also cause toxic brain damage when ingested and affect the central nervous system (HLUSICKA et al., 2019).

Phenolic compounds (PC) extraction with different solvents and antioxidant capacity

The choice of solvents used for extraction can greatly influence the phenolic content and antioxidant capacity of plant extracts obtained from CL and CC leaves, as demonstrated in Table 4. This is because solvent type and polarity can affect the solubility of phenolic compounds by modulating single electron transfer (SET) and hydrogen atom transfer (HAT), which are important factors in measuring antioxidant capacity (PÉREZ-JIMÉNEZ; SAURA-CALIXTO, 2006).

Table 6. The PC, antioxidant capacity: IC₅₀ (DPPH) and FRAP of CL and CC leaf extracts prepared with mixed and non-mixed solvents.

Species	Solvent	PC ³	DPPH ⁴	FRAP ⁵
CL	Methanol	730,56±0,28 ^{eA}	0,56±0,01 ^{bA}	75,79±1,71 ^{cdA}
	Methanol + water (70:30)	836,04±0,99 ^{bA}	0,35±0,01 ^{aA}	95,28±0,01 ^{aA}
	Ethanol	428,44±0,33 ^{fA}	0,35±0,01 ^{aA}	44,14±0,76 ^{eA}
	Ethanol + water (70:30)	762,53±0,57 ^{cB}	0,56±0,01 ^{bB}	79,43±0,72 ^{bcA}
	Water	271,180,74 ^{gB}	0,56±0,01 ^{bA}	24,47 ±0,75 ^{fB}
	Acetone + water + acetic acid (70:28:2)	852,70±0,93 ^{aA}	0,55±0,02 ^{bB}	81,76±0,36 ^{bA}
	Methanol + acetic acid (99,5:0,5)	735,86±1,13 ^{dA}	0,59±0,03 ^{bA}	74,45±0,91 ^{dA}
CC	Methanol	361,31±0,79 ^{fB}	0,90±0,03 ^{dB}	46,34±1,23 ^{cB}
	Methanol + water (70:30)	709,94±0,48 ^{cB}	0,58±0,01 ^{cB}	77,89±0,36 ^{abB}
	Ethanol	155,96±0,82 ^{gB}	1,33±0,07 ^{eB}	22,36±0,04 ^{eB}
	Ethanol + water (70:30)	860,19±0,95 ^{aA}	0,43±0,00 ^{aA}	49,22±0,30 ^{cB}
	Water	492,63±0,25 ^{dA}	0,55±0,00 ^{bA}	74,61±1,55 ^{bA}
	Acetone + water + acetic acid (70:28:2)	736,25±0,33 ^{bB}	0,45±0,01 ^{aA}	81,75±0,07 ^{aA}
	Methanol + acetic acid (99,5:0,5)	370,34±0,44 ^{eB}	0,95±0,01 ^{dB}	41,09±1,11 ^{dB}

¹ Different lower-case letters in each column for each leaf species indicate that the means differed significantly by Tukey's test ($p < 0.05$); while different uppercase letters in each column for each solvent also differed significantly by Tukey's test ($p < 0.05$); ² Proportion of each mixed solvents in parentheses; ³ mg GAE 100 g⁻¹; ⁴ IC₅₀: mg mL⁻¹; ⁵ μMol FeSO g⁻¹.

In this study, the extraction of phenolic compounds (PCs) from leaves was found to be significantly affected by the plant species, the type of solvent used (mixed or non-mixed), and the interaction between species and solvent, as revealed by the Factorial Anova analysis (Supplementary Table 1). Specifically, for CL leaves, the most effective solvent for extracting PCs was found to be AWA, followed by MW, EW, MAA, M, E, and W, respectively. In contrast, for CC leaves, EW was found to be the most efficient solvent for PC extraction, followed by AWA, MW, W, MAA, M, and E (Table 4). It should be noted that the polarity and type of solvent can greatly influence the solubility of phenolic compounds by affecting single electron transfer (SET) and hydrogen atom transfer (HAT), which are key factors in measuring antioxidant capacity (PÉREZ-JIMÉNEZ; SAURA-CALIXTO, 2006).

The combined use of EW, MW, and AWA can be considered a good option for extracting PCs from both CL and CC leaves. However, the choice of the most efficient solvent for PC extraction depends on the specific characteristics of the food matrices being analyzed (MICHIELS et al., 2012; REZAIE et al., 2015).

The results of Table 4 indicate that water and ethanol were the least effective solvents to extract PCs from CL leaves, whereas ethanol, methanol, and MAA were the least effective solvents for CC leaves. On the other hand, methanol was found to be the most effective solvent among the non-mixed solvents for extracting PCs from both CL and CC leaves, with a higher extraction efficiency of approximately 169% compared to water, which had lower extraction power for CL leaves. Therefore, methanol could be a promising solvent for extracting PCs from these plant species.

In the case of CC leaves, water was found to be the most effective solvent for extracting PCs, resulting in a 215% higher yield than ethanol, which was the least effective solvent (Table 4). However, it is worth noting that highly polar solvents like water can also extract impurities such as reducing sugars, organic acids, and soluble proteins, which may affect the determination of PC levels in the extracts (VIZZOTTO; PEREIRA, 2011). Therefore, careful consideration should be given to selecting the appropriate solvent for PC extraction depending on the specific requirements of the analysis.

It is worth noting the significantly higher amount of PC (397%) extracted from CL leaves with methanol in the present study (Table 4) compared to the previous study on the same species,

which reported 183.76 mg of GAE per 100 g of sample (BRAGA et al., 2018). However, it should be noted that the choice of the most efficient solvent for phenolic extraction may vary depending on the food matrix studied. For example, in a study by MICHIELS et al. (2012), acetone-based solvents were found to be more effective than methanol-based solvents for phenolic extraction yields from fruits and vegetables.

Phenolic compounds are characterized by a hydroxyl group attached to an aromatic hydrocarbon group. The use of mixed solvents and water is more efficient than non-mixed solvents, as glycosylated PCs are more soluble in water. This was observed in the present study (Table 4), as treatments composed of mixed solvents and water produced the best results. Additionally, methanol and ethanol can extract non-glycosylated compounds due to the high availability of the free electron pair. Therefore, the high content of PCs extracted by combining methanol with water or ethanol with water can be explained by the high selectivity of alcoholic solvents. Unlike other solvents, alcohols have a high affinity for these chemical compounds because they participate in carbon-oxygen and oxygen-hydrogen chemical interactions (ROSAN, 2001)

In a study conducted by PEDRO et al. (2018) on goji berry, organic solvents mixed with water were found to be the most effective in extracting PCs, which is similar to the present study. The optimal solvents were MW (70:30 v/v), EW (70:30 v/v), EW (50/50 v/v), AW (70:30 v/v), and AWA (70:28:2 v/v/v), which is consistent with the best performing solvents identified in the current study (Table 4), except for AWA (70:28:2 v/v/v), which did not produce the best extraction results of study of PEDRO et al. (2018).

Therefore, it can be inferred that there were more intermolecular bonds between the PC molecules present in the leaves of CL with the polar part of the AWA (70:28:2) mixed solvent and in the leaves of CC with the polar part of the EW (70:30) solvent. Consequently, these solvents exhibited greater efficiency in extracting PCs from the raw materials, resulting in extracts with higher PC concentrations, as observed in the present study (Table 4).

The TPC values obtained from the leaves of CL and CC with hydro-ethanolic extract in this study (Table 4) were higher than those reported for other species, such as Jambolão (*Syzygium cumini* L.) leaves and fruits with values of 237.52 and 109.17 mg GAE 100 g⁻¹, respectively (OLAYÉ *et al.*, 2020). Similarly, the TPC values reported for leaves of *Achyranthes*

aspera L., *Eclipta alba* L., and *Vitex negundo* L. using methanol and hydrochloric acid (68.84 ± 0.61 , 55.32 ± 0.47 , and 72.11 ± 0.73 mg GAE 100 g^{-1} , respectively) in the study (RANA et al., 2019) were lower than those obtained in this study (Table 4). The TPC values obtained with AWA (70:28:2 v/v/v) extract in the present study were higher than those found in the variety *Broccoli Italica* and leek cultivar Blue Liege extracted with 120 mg GAE 100 g^{-1} and 80 mg GAE 100 g^{-1} , respectively (MICHIELS et al., 2012), but lower than that reported for freeze-dried basil leaf *Ocimum basilicum* L. with TPC value of 15000 mg GAE 100 g^{-1} (ZŁOTEK et al., 2016).

The free radical inhibition capacity is an important parameter to highlight, and the results are expressed as IC₅₀, which refers to the concentration of extract capable of reducing the initial concentration of the DPPH radical by 50%. Therefore, a lower IC₅₀ value indicates higher antioxidant activity (OLIVEIRA, 2015). In the case of CL leaves, the extracts with MW and ethanol (E) demonstrated the best response in the DPPH radical scavenging assays and did not differ significantly ($p < 0.05$) between them (Table 4). The IC₅₀ values for these extracts were, on average, 68% higher than those obtained with the MAA solvent, which resulted in the lowest response. The extracts obtained with other solvents did not differ significantly ($p > 0.05$) from each other.

Regarding CC leaves, the mixture of ethanol and water (EW) and AWA presented the best response and did not differ significantly from each other (Table 4), followed by water, MW, methanol, MAA, and ethanol. Only methanol and MAA did not differ significantly ($p > 0.05$).

Comparing the DPPH radical scavenging capacity between the two leaves studied, there was a difference ($p < 0.05$) between the same solvents or mixed solvents, except for the aqueous extract, which did not vary between the extracts of the different turmeric species ($p > 0.05$). The CL leaf showed higher values with MW, ethanol, methanol, and MAA when compared to CC (Table 4).

It is worth mentioning that ethanol and methanol are the most commonly used solvents in DPPH assays because these alcoholic media facilitate the donation of a hydrogen atom from the alcohol itself (ethanol or methanol), thereby increasing the solubility and constant rate of hydrogen transfer to the DPPH radical. However, in aqueous solutions, the solubility is much lower and

may interfere with the evaluation of antioxidant capacity, making the DPPH radical less accessible for the reaction with the samples being evaluated (OLIVEIRA, 2015).

The DPPH assay involves both a fast electron transfer reaction (SET) and a slow hydrogen atom transfer reaction (HAT), mainly in hydrogen-accepting solvents such as methanol and ethanol (HUANG et al., 2005). To ensure optimal extraction of antioxidant compounds, multi-step extractions are recommended. In the present study, two extractions were performed for 1 hour each, using stirring at room temperature.

A study by TANVIR et al., (2017) on the DPPH free radical scavenging activity of aqueous and ethanolic extracts of CC rhizomes revealed that the ethanolic extracts exhibited higher sequestering activities than the corresponding aqueous extracts. This finding indicates the influence of solvent on the measurement of antioxidant properties. Similar observations can be made for the CL leaves in the current study, as shown in Table 4. It is worth noting that the antioxidant activity is dependent on the concentration of the extract, as demonstrated in a study by MOULAZADEH et al. (2021) on *Lawsonia inermis* leaves, where the antioxidant activity increased with increasing concentration of the ethanolic extract. This high activity was attributed to the high content of phenolic compounds and flavonoids, which were also found in the ethanolic extracts in our study (Table 4), along with vitamin C, carotenoids, lycopene, and chlorophyll.

MANAS (2014) reported that the IC₅₀ value for aqueous extract of long turmeric from West Bengal, India was 5.99 mg mL⁻¹, while kiwifruit leaves (*Actinidia arguta*) had an IC₅₀ value of 53.95 µg mL⁻¹ on alcoholic extract (ALMEIDA et al., 2018). These values show lower and higher antioxidant capacity, respectively, when compared to the IC₅₀ values of the aqueous extract (CL 0.56 and CC 0.55 mg mL⁻¹) and ethanolic extract (CL 0.35 and CC 1.33mg mL⁻¹) in the present study.

However, LIU; NAIR (2012) reported good antioxidant activity for both CL and C. mango leaves from Michigan - USA, as measured by MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The aqueous extract was significantly better than the methanolic extract, and this difference may be due to the synergistic or additive effects of the compounds present in the extract. These authors also found that both leaves contained few curcuminoids, the main bioactive compounds present in their rhizomes. Therefore, it is possible that the antioxidant

capacity of the leaves in the present study also comes from other polyphenolic compounds besides curcuminoids.

MAU et al. (2020) reported a better IC₅₀ value (0.19 mg mL⁻¹) for the aerial parts of sweet potato than the leaves in the present study (Table 4). Meanwhile, FAKHFAKH et al. (2017) found a similar value (0.35 mg mL⁻¹) to that of CL leaf for the ethanolic extract of *Malva aegyptiaca* L. leaf. Takwa et al. (2018), studying basil leaf, found less expressive values (595 µg mL⁻¹) when compared to CL. In a study on asparagus leaves, a by-product, IC₅₀ values of 13.8-7.6 mg mL⁻¹ were found at different grinding times (0-6 hours) (CHITRAKAR et al., 2020).

The existence of antioxidants or reducing substances in the plant extracts facilitates the conversion of the ferric complex (Fe³⁺) to the ferrous form (Fe²⁺), which is the principle of the FRAP assay (PREMRAM et al., 2018). The decrease of Fe³⁺ in the solution leads to a decrease in color, which implies the potent reducing power of plant extracts (OLAYÉ et al., 2020). For the ferric reducing antioxidant power of CC leaves, the extract with AWA and MW showed the highest antioxidant capacity (p < 0.05), with no significant difference between the two, followed by the extracts with water, EW, methanol, MAA, and ethanol (Table 4).

Regarding CL leaves, the extract with methanol:water exhibited the highest antioxidant capacity (FRAP), followed by AWA, EW, methanol, MAA, ethanol, and water. The mixed solvents with water obtained better results than the same non-mixed solvents for both leaves (Table 4).

The present study compared the FRAP values of different solvents used for extracting phytochemicals from the leaves of CL and CC. The results showed that AWA and MW solvents had higher FRAP values for CL (95.285 µMol FeSO g⁻¹) and CC (81.75 µMol FeSO g⁻¹), respectively, compared to aqueous and ethanolic extracts of rhizomes of two varieties of CL reported (TANVIR et al., 2017). Water and ethanol were also used for extracting phytochemicals from the leaves of CL and CC, and they showed better yields compared to the study cited above. Water is a commonly used polar inorganic solvent for extracting bioactive phytochemicals, while ethanol can provide higher yields of antioxidant compounds in varieties of the Zingiberaceae family.

Other studies have reported higher FRAP values for aqueous and ethanol extracts of various plants, such as *Phyllanthus emblica*, *Tectona grandis*, and *Ficus bengalensis*, which showed higher stability of antioxidant properties. The presence of curcuminoids and other polyphenols found in the *Curcuma* genus can improve lipoprotein oxidation, prevent lipid peroxidation, and stabilize the cell membrane, suggesting their important role in decreasing the risk of atherosclerosis. Methanol and hydrochloric acid extracts of leaves of *Achyranthes aspera* L., *Eclipta alba* L., and *Vitex negundo* L. also showed higher FRAP values compared to the results obtained in roots, reinforcing the antioxidant potential of CL and CC leaf extracts.

The present study showed that methanol solvent had higher antioxidant activity in the leaves of CL ($75.79 \mu\text{M FeSO g}^{-1}$) and CC ($46.34 \mu\text{M FeSO g}^{-1}$) compared to the FRAP value shown in rhizomes of ginger (*Zingiber officinale*) using methanol. Regarding the results with AWA, EW, AA solvents obtained with CL and CC, similar results were verified in (XU; CHANG, 2007), where different solvents were evaluated for extracting phytochemicals from different beans.

Although extracts containing acetone and methanol showed good results for TPC and antioxidant capacity, from a toxicological point of view, ethanol and water are safer solvents than acetone, methanol, and other organic solvents and, therefore, more acceptable by the food industry. EW extract was considered the most suitable for food application due to its best response for both PC content and antioxidant.

CONCLUSIONS

In conclusion, this study has demonstrated the effectiveness of using the HS-GC-MS technique for analyzing the terpene compounds in CL and CC leaves. These compounds are important for plants as they contribute to their aroma and flavor. The study also highlights the significant influence of solvent type on the extraction of phenolic compounds and the determination of antioxidant capacity in the leaf extracts. Mixed solvents were found to be more effective than non-mixed solvents, with the EW solution (70:30, v:v) showing the best response. This solvent is also desirable for industrial food processing due to its safety and compatibility aspects. The results of this study suggest that the leaf extracts of CL and CC have the potential to be used as natural antioxidants in food applications. Overall, this research provides valuable

insights into the optimization of extraction methods for plant-based natural compounds and their potential applications in the food industry.

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