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Research Article

Comprehensive pharmacognostic and antioxidant profiling of *Curcuma macrochlamys* leaves using sequential extraction and GC-MS

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Abstract

Curcuma macrochlamys, a lesser-known species of the *Zingiberaceae* family, possesses promising pharmacological potential. This study aimed to investigate the pharmacognostic characteristics, total phenolic content, antioxidant activity, and chemical constituents of its leaf extracts. The leaves were successively extracted using *n*-hexane, ethyl acetate, and 96% ethanol. Macroscopic and microscopic analyses revealed key anatomical features, including spiral vessels, oleoresin cells, trichomes, and calcium oxalate crystals. The ethanolic extract yielded the highest phenolic content (22.688 ± 0.450 mg GAE/g) and exhibited the strongest antioxidant activity as determined by DPPH and FRAP assays. GC-MS analysis identified several bioactive compounds such as dodecanoic acid-methyl ester, methyl linolenate, and phytol, known for their antioxidant and antimicrobial properties. These findings highlight the potential of *C. macrochlamys* leaf extracts, particularly the ethanolic fraction, as a natural antioxidant source for pharmaceutical applications.

Keywords: *Curcuma macrochlamys*; antioxidant; phenolic content; GC-MS; pharmacognosy

Introduction

Curcuma is one of the most important genera within the Zingiberaceae family, widely recognized for its rich secondary metabolites, particularly phenolic compounds and polyphenol derivatives, which exhibit diverse biological activities. Several species of this genus have been extensively investigated for their pharmacological properties, especially antioxidant [1,2], antimicrobial, antiviral, anti-inflammatory, and anticancer activities [3]. However, *C. macrochlamys* remains scientifically underexplored, despite its potential to possess similar phytochemical constituents, given its close taxonomic relationship with other well-known species.

An initial approach to evaluating the therapeutic potential of medicinal plants involves the macroscopic and microscopic examination of plant organs, particularly leaves, to verify the identity and quality of crude drugs [4]. Microscopic studies of *Curcuma* leaves under light microscopy have frequently been conducted to elucidate anatomical and morphological features that are critical for species identification and pharmacognostic assessment. Investigations on *Curcuma sumatrana* revealed that its leaves are typically unifacial and display distinctive anatomical features, including prism-shaped calcium oxalate crystals, non-glandular trichomes, and prominent vascular bundles [5]. Comparative analyses among various *Curcuma* species have further highlighted consistent traits such as stomatal patterns, the occurrence of glandular and non-glandular trichomes, and the presence of distinct air canals in the petioles, all of which serve as reliable taxonomic indicators [6]. Altogether, both qualitative and quantitative anatomical observations obtained through light microscopy provide dependable criteria for distinguishing *Curcuma* species during vegetative stages, thereby supporting botanical taxonomy and the standardization of medicinal plants. Moreover, sequential extraction using solvents of different polarities facilitates the separation of bioactive compounds based on their solubility, enabling a more targeted identification of constituents [7]. Determination of total phenolic content is commonly carried out using the Folin–Ciocalteu method, which

detects phenolic compounds through a redox reaction that produces a blue coloration proportional to the phenol concentration in the sample [8].

While most previous studies have focused on rhizomes as the primary medicinal parts of *Curcuma* species, recent research has begun to investigate the pharmacological potential of leaves, owing to their diverse secondary metabolite content and more sustainable harvestability. Examining the leaves of various *Curcuma* species contributes to the development of alternative herbal products while promoting environmental conservation through the utilization of underexplored plant parts. Accordingly, this study aims to evaluate the macroscopic and microscopic characteristics, total phenolic content, antioxidant activities (DPPH and FRAP), and bioactive compound composition of *C. macrochlamys* leaf extracts as an initial effort to document its pharmacognostic potential.

Materials and Methods

Macroscopic and microscopic evaluation of fresh and dried leaf (Simplisia) of *C. macrochlamys*

The macroscopic evaluation involved observing the leaf shape, size, and color, as well as the characteristics of powdered simplicia prepared by oven-drying the leaves at 40 °C and sieving them through a 40-mesh screen. Microscopic examination was performed on both fresh leaves and powdered samples using a light microscope (The CST 10 Series Trinocular Camera Microscope). Transverse sections were prepared to observe tissue layers in cross-section, whereas longitudinal sections were used to examine tissue structures along their length, including the identification of specific diagnostic fragments in the samples.

Sample extraction preparation

A total of 15 g of *C. macrochlamys* leaves were subjected to successive extraction using solvents of increasing polarity: *n*-hexane, ethyl acetate, and 96% ethanol. The extraction was performed with ultrasonic assistance (Branson 3800, USA) at 42 kHz for 60 minutes at 40 °C. The solvents were successively replaced until the extract became clear, involving repeated extractions with *n*-hexane (9 cycles), ethyl acetate (10 cycles), and 96% ethanol (6 cycles). Each extraction stage was followed by filtration, drying of the residue, and evaporation of the filtrate using a vacuum rotary evaporator (Eyela 05B-2100, Japan) and a water bath (Memmert, Germany) at 50 °C until a concentrated extract was obtained.

Organoleptic evaluation and extract yield

The organoleptic properties of the three extracts were sensorially assessed using human senses, based on aroma, appearance, color, and taste. The extract yield was calculated as percentage of the extract weight relative to the initial weight of the fresh leaves [9].

Total phenolic content determination using microplate reader

The total phenolic content was determined following the method described by Bobo-Garcia et al. [10] using a 96-well microplate. A volume of 20 µL of extract was mixed with 100 µL of diluted Folin–Ciocalteu reagent (1:4) and incubated in the dark for 4 minutes, followed by the addition of 75 µL of 7.5% Na_2CO_3 solution. The mixture was further incubated for 2 hours, after which the absorbance was measured at 750 nm using a microplate reader (iMark, USA). The phenolic content was calculated from a gallic acid calibration curve and expressed as mg GAE/g of extract.

Antioxidant activity assay

DPPH method

The antioxidant activity was evaluated using the DPPH method according to Bobo-Garcia et al. with slight modifications [10]. Extract samples (*n*-hexane, ethyl acetate, and 96% ethanol) were tested at a concentration of 1000 µg/mL, while gallic acid (Sigma Aldrich, USA) was used as the standard at 40 µg/mL. A volume of 20 µL of each sample was mixed with 180 µL of 0.15 mM DPPH solution in a 96-well microplate and incubated for 40 minutes in the dark (wrapped in aluminum foil). Absorbance was measured at 595 nm using a microplate reader (iMark, USA), with analytical grade methanol as the blank. The absorbance values were converted using a quercetin standard curve, and the results were expressed as mg QE/g sample.

FRAP method

The antioxidant activity based on the FRAP (Ferric Reducing Antioxidant Power) method was determined by adding 30 µL of the sample, gallic acid standard (Sigma Aldrich, USA), and blank into a 96-well microplate, followed by the addition of 270 µL of FRAP reagent. The mixture was homogenized and incubated for 30 minutes at 37 °C in a Memmert incubator (Memmert GmbH, Germany), and the absorbance was measured at 595 nm. The assay was performed in triplicate with five replications. Antioxidant activity was expressed as FeEAC equivalents [11].

Metabolite profiling by GC-MS

The metabolite analysis of *C. macrochlamys* leaf extracts was performed using a GC-MS system (Agilent 7890A, Agilent Technologies, USA) equipped with a capillary column Agilent 19091S-433 HP-5MS (30 m × 250 µm × 0.25 µm; 5% phenyl methyl siloxane). The GC oven temperature was programmed to start at 50 °C for 3 minutes, followed

by a ramp of 5 °C/min to 250 °C, and held for 58 minutes. The injection was carried out in split mode with an injection volume of 1 µL and a split ratio of 2:1 at an inlet temperature of 280 °C. Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The transfer line temperature was maintained at 285 °C. The mass spectrometer operated in SCAN mode with a mass range of 35–550 m/z, using an ion source temperature of 230 °C and a quadrupole temperature of 150 °C.

Data analysis

Quantitative analyses of total phenolic content and antioxidant activities (DPPH and FRAP methods) were performed using Microsoft Excel Version 16.98 (Microsoft Corp., USA). The analysis process included constructing calibration curves and calculating sample concentrations based on linear regression equations.

Results and Discussion

This study employed a chemotaxonomic approach to identify and evaluate the anatomical characteristics and secondary metabolite profiles of *C. macrochlamys* leaves, which belong to the *Curcuma* genus (Zingiberaceae family), well known for its abundance of bioactive compounds. The evaluation was conducted through macroscopic and microscopic analyses of fresh leaves and powdered simplicia to reveal distinct diagnostic structural features.

Microscopic and macroscopic observations presented in **Figure 1** illustrate the anatomical features of *C. macrochlamys* leaves and their powdered form, providing specific diagnostic traits valuable for pharmacognostic assessment. The longitudinal leaf section revealed the presence of parenchyma cells (1), oleoresin cells (2), and epidermis (3), which serve as protective tissues and reservoirs for secondary metabolites. Stomata (4), together with guard cells (5) and stomatal pores (6), were visible on the abaxial surface, exhibiting morphological characteristics consistent with other members of the *Curcuma* genus [10]. Calcium oxalate crystals (8), typically prismatic or raphide in form, were also observed; these are common defense structures in Zingiberaceae species. The powdered simplicia contained key elements such as unicellular trichomes (10), oil cells (9), and fiber bundles (11), along with spiral-type vascular bundles (7), indicating xylem structures with secondary thickening. The presence of these microscopic fragments supports the quality of the plant material as a herbal raw ingredient and provides essential diagnostic parameters for the identification and standardization of *C. macrochlamys* leaf simplicia. Macroscopically, the upper leaf surface appeared green with reddish midribs and relatively narrow lamina, while the lower surface was predominantly red.

The transverse section of the *C. macrochlamys* leaf, illustrated in **Figure 2**, was prepared horizontally through the midrib, revealing distinctive anatomical structures. A single vascular bundle exhibited xylem (4) and phloem (3) arranged in a collateral configuration, with the xylem oriented toward the adaxial side and the phloem toward the abaxial side. The xylem tissue functions in transporting water and minerals from the roots to the leaves, whereas the phloem distributes photosynthetic products throughout the plant. Sclerenchyma (1) was identified as a mechanical supporting tissue, and an air cavity (2) was observed to facilitate intercellular gas exchange. Additionally, brown-colored oleoresin cells (7), characteristic oil- and resin-storing structures of the Zingiberaceae family were detected. Tetracytic-type stomata (6) with associated guard cells (12) were also present, consistent with previous observations in other *Curcuma* species [12,13].

The epidermal layer (5) protects the internal tissues, while beneath it lies the parenchyma (9), which contains chloroplasts serving as the primary site of photosynthesis. Non-glandular unicellular trichomes (10) were also observed, likely functioning as protective structures against environmental stress, as reported in various Zingiberaceae species. Prismatic calcium oxalate crystals (11), commonly found as secondary metabolites in the *Curcuma* genus, serve as diagnostic markers in botanical identification. Furthermore, the presence of fibers (8) contributes to the mechanical strength of the tissue. Finally, the vascular bundle with spiral thickening (13) confirms the presence of primary xylem tracheary elements, indicating adaptations for flexibility and efficient water transport in young plant tissues.

The results presented in **Table 1** show that 96% ethanol yielded the highest extract recovery (4.785%), followed by ethyl acetate (2.304%) and *n*-hexane (1.471%). This variation reflects the influence of solvent polarity on the efficiency of extracting bioactive compounds from *C. macrochlamys* leaves. Polar solvents such as ethanol are known to be more effective in extracting secondary metabolites particularly phenolics, flavonoids, and polysaccharides due to their greater solubility in polar media [8,14]. Moreover, ethanol exhibits superior cell wall penetration ability, which facilitates the release of bioactive compounds from plant tissues [15].

All three extracts displayed viscous consistency, characteristic aroma, and bitter taste, although distinct color differences were observed. The *n*-hexane extract appeared yellowish green, the ethyl acetate extract

was deep yellow, and the ethanol extract showed a brownish-yellow hue. These variations primarily result from the types of compounds selectively solubilized by each solvent. Non-polar solvents such as n-hexane predominantly extract lipid-soluble metabolites, including chlorophylls and carotenoids, whereas polar solvents like ethanol are more efficient in extracting phenolic and flavonoid compounds, which are susceptible to oxidative transformations during extraction.

Macroscopic evaluation of plant extracts encompassing visible characteristics such as color, texture, and overall appearance has been reported to correlate with antioxidant potential, as these traits often reflect the abundance of bioactive constituents, particularly phenolics and flavonoids. Extracts with deeper and more intense coloration generally contain higher levels of phenolic compounds, thereby exhibiting greater antioxidant activity. Supporting this, a recent study demonstrated that macroscopic properties, including color and viscosity, were consistent with antioxidant levels determined through chemical assays, underscoring the relevance of macroscopic assessment as an indicator in antioxidant studies.

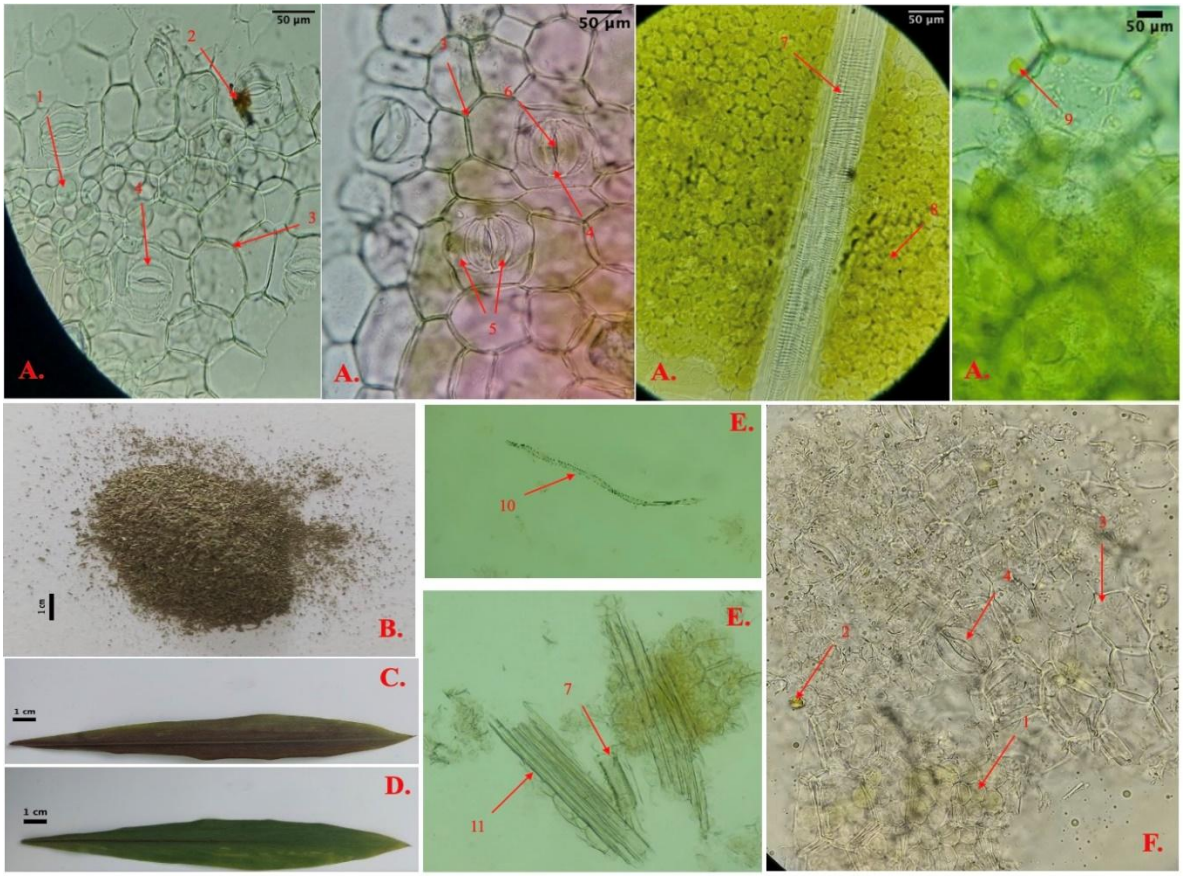

Figure 1. Microscopic and macroscopic characteristics of *C. macrochlamys* leaf and powdered simplicia. (A) Longitudinal section of the leaf under 400× magnification (40×10); (B) Powdered simplicia; (C) Abaxial (lower) leaf surface; (D) Adaxial (upper) leaf surface; (E) Microscopic view of powdered simplicia at 100× magnification (10×10); (F) Microscopic view at 400× magnification (40×10). Diagnostic structures identified: (1) parenchyma; (2) oleoresin cells; (3) epidermis; (4) stomata; (5) guard cells; (6) stomatal pore; (7) spiral-type vascular bundle; (8) calcium oxalate crystals; (9) oil cells; (10) trichomes; and (11) fiber bundles

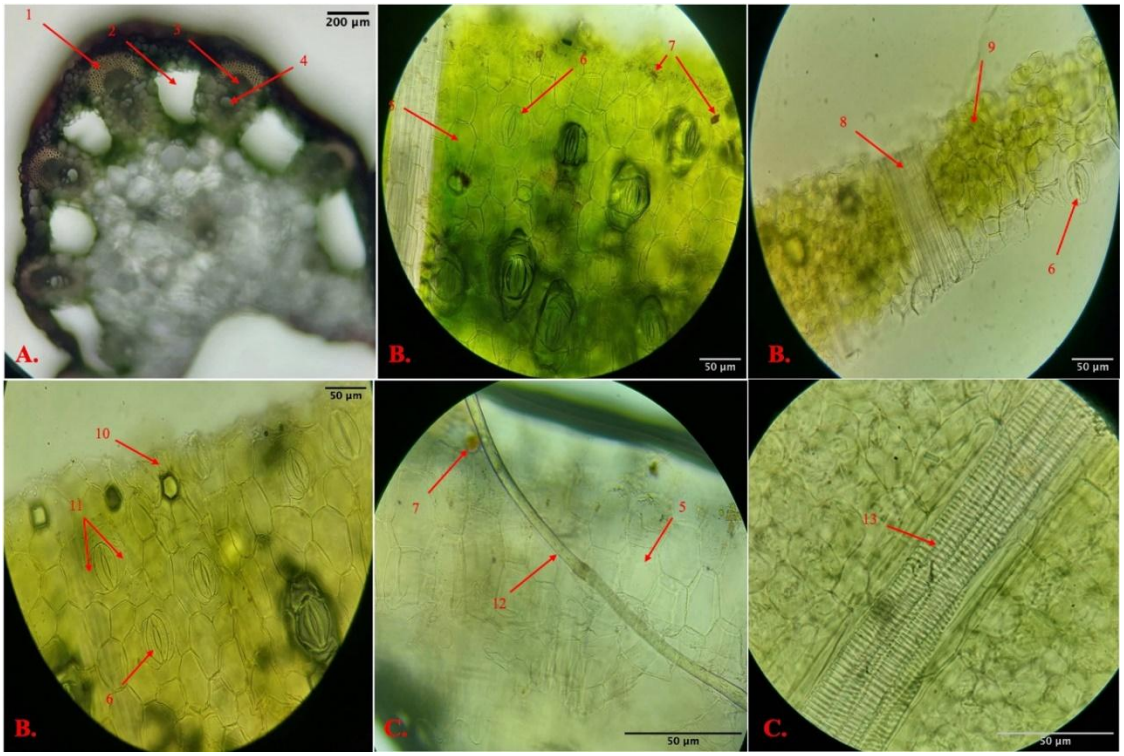

Figure 2. Transverse section of *C. macrochlamys* leaf showing internal anatomical features under light microscopy. (A) 10× magnification; (B) 40× magnification; (C) 100× magnification. 1. Sclerenchyma; 2. Air cavity (intercellular space); 3. Phloem; 4. Xylem; 5. Epidermis; 6. Stomata; 7. Oleoresin cell; 8. Fiber; 9. Parenchyma; 10. Calcium oxalate crystal; 11. Guard cell; 12. Trichome; 13. Vascular bundle with spiral xylem thickening.

Tabel 1. Comparative organoleptic and phenolic characteristics of leaf extracts of *C. macrochlamys*

Extract	**Yield (%)**	**Organoleptic Properties**	**Total Phenolic Content (mg GAE/g extract ± SD)**
n-hexane	1.471	Greenish-yellow, viscous, characteristic odor, bitter taste	20.448 ± 1.311
Ethyl Acetate	2.304	Dark yellow, viscous, characteristic odor, bitter taste	18.313 ± 0.340
Ethanol 96%	4.785	Brownish-yellow, viscous, characteristic odor, bitter taste	22.688 ± 0.450

Phenolic compounds are strategically localized in leaf vacuoles, cell walls, epidermal tissues, trichomes, vascular tissues, and chloroplasts, where they perform diverse roles including antioxidant defense, structural support, and protection against environmental stressors. This compartmentalization underlies their efficiency in scavenging reactive oxygen species and contributing to the overall antioxidant capacity of leaves [16–18].

The total phenolic content of the extracts was quantified using the Folin–Ciocalteu colorimetric method, with absorbance measured via a microplate reader. This reaction proceeds under alkaline conditions, wherein the Folin–Ciocalteu reagent oxidizes phenolic compounds to form a blue molybdenum–tungsten complex. The addition of Na_2CO_3 establishes a basic environment and accelerates the reaction, which occurs more rapidly at

higher temperatures [19]. The elevated phenolic content in the 96% ethanol extract can be attributed to the polar nature of phenolic compounds, which are highly soluble in polar solvents such as ethanol, methanol, acetone, and butanol. This is consistent with the color intensity produced in the reaction with Folin–Ciocalteu and Na_2CO_3 reagents, where the 96% ethanol extract exhibited the deepest blue coloration, followed by a lighter blue in the ethyl acetate extract and a bluish green hue in the *n*-hexane extract. The intensity of this blue coloration is directly proportional to the concentration of phenolic compounds in the extract [20].

The ethanolic extract exhibited the highest total phenolic content at 22.688 ± 0.450 mg GAE/g extract, followed by the *n*-hexane (20.448 ± 1.311 mg GAE/g) and ethyl acetate (18.313 ± 0.340 mg GAE/g) extracts. Phenolic compounds are key bioactive constituents that function as natural antioxidants through their hydroxyl (-OH) groups, which donate electrons to neutralize free radicals [21]. A similar trend has also been observed in other *Curcuma* species, particularly in leaf extracts. For instance, extraction of *Curcuma zedoaria* leaves using an ethanol–water mixture (90:10) yielded a total phenolic content of 125.75 ± 0.17 mg GAE/g extract and a flavonoid content of 6.12 ± 0.23 mg QE/g, as reported by Azahar et. al. [22].

These findings highlight the suitability of polar solvents for extracting *Curcuma* leaves and reinforce the relevance of the results obtained for *C. macrochlamys*. Thus, it is strongly supported that the chemical characteristics of *Curcuma* leaves particularly their phenolic and flavonoid content are significantly influenced by solvent polarity, with ethanol (often in combination with water) serving as an optimal solvent for bioactive compound extraction in *C. macrochlamys* and related species.

Interestingly, the phenolic content in the *n*-hexane extract was also relatively high (20.448 mg GAE/g), despite the general understanding that phenolic compounds are poorly soluble in non-polar solvents. This may be attributed to the presence of semi-polar phenolic compounds or interference from other substances capable of reacting with the Folin–Ciocalteu reagent, such as terpenoids or aromatic fatty acids [23]. The ethyl acetate extract exhibited the lowest phenolic content. Although ethyl acetate is a semi-polar solvent commonly employed in the fractionation of bioactive compounds, its effectiveness in extracting pure phenolics from *C. macrochlamys* leaves appears to be lower than that of ethanol. This may be due to its limited capacity to penetrate the leaf tissue structure or an inadequate polarity match with specific phenolic compounds present in the sample.

Gallic acid was used as the reference compound because it is one of the simplest phenolic acids, noted for its purity and stability. It contains one or more phenolic rings with hydroxyl groups attached to the aromatic ring, which makes it readily oxidized by donating hydrogen atoms to free radicals. The ability of phenolic compounds to form stable phenoxyl radicals underlines their strong antioxidant properties. Gallic acid has been shown to protect cells from oxidative stress, inhibit lipid peroxidation, and activate endogenous antioxidant mechanisms [24].

The results of the antioxidant activity test presented in **Table 2** indicate that the quercetin equivalent (QE) values of the ethyl acetate, 96% ethanol, and *n*-hexane extracts were significantly lower than that of the reference compound. Compared to gallic acid, which yielded a value of 2500.000 ± 19.454 mg QE/g, the antioxidant activities of the three extracts were approximately 68-87 times lower.

Table 2. Antioxidant activity of *C. macrochlamys* leaf extracts based on DPPH (mgQE/g) and FRAP (mol Fe^{2+}/g) using *n*-hexane, ethyl acetate, and 96% ethanol as solvents

Antioxidant assay	Samples			
	***n*-hexane**	**Ethyl Acetate**	**Ethanol 96%**	**Gallic acid**
DPPH (mgQE/g)	28,725 ± 1,620	34,902 ± 2,717	36,667± 4,727	2500,000 ± 19,454
FRAP FeEAC (mol/g)	87,722± 2,546	101,889± 10,046	106,611± 5,422	21818,055 ± 275,073

Based on the FeEAC values, the *C. macrochlamys* leaf extract obtained using 96% ethanol exhibited higher reducing activity than those extracted with ethyl acetate and *n*-hexane. A higher FeEAC value indicates greater antioxidant capacity in reducing Fe^{3+} to Fe^{2+}. Among the solvents tested, 96% ethanol proved to be the most effective for extracting antioxidant compounds from *C. macrochlamy* leaves. Shi et al. also reported strong antioxidant activity across 12 *Curcuma* species [25]. The DPPH and FRAP results in this study further confirmed that the 96% ethanol extract exhibited the highest antioxidant activity, consistent with its total phenolic content.

The gas chromatography–mass spectrometry (GC–MS) analysis results (**Table 3**) revealed that the n-hexane extract was dominated by lipophilic compounds, as expected for a non-polar solvent. In contrast, the ethanol extract, due to its amphiphilic character, was able to solubilize not only various phenolic compounds but also fatty acid esters. This observation aligns with previous GC–MS studies that reported the presence of methyl and ethyl esters in ethanol extracts of pumpkin seeds [26] and a predominance of aliphatic fatty acids and their methyl esters in ethanol extracts of *Taxithelium nepalense* [27]. Collectively, these findings confirm that ethanol, owing to its polar protic nature and strong solvating capacity, effectively extracts both phenolic metabolites and lipid-derived esters.

Hexadecanoic acid methyl ester and methyl oleate were identified as the major constituents representing saturated and unsaturated fatty acid esters, respectively, both recognized for their antioxidant and antifungal properties [28,29]. Phenol, 2,5-bis(1,1-dimethylethyl), a phenolic compound, has been computationally predicted to exhibit synthetic antioxidant potential as well as antiseptic, anti-inflammatory, antioxidant, and growth-stimulating activities [30]. Dodecanoic acid methyl ester (methyl laurate), detected in *C. macrochlamys* leaf extract, is a medium-chain fatty acid reported to possess promising anti-breast cancer activity [31]. Methyl linolenate is believed to contribute to both the antimelanogenic and antioxidant effects observed in B16F10 melanoma cells [32]. Phytol, a diterpene alcohol, was also identified, consistent with previous findings in *Curcuma caesia* [33]. Several of these compounds have also been reported in other species of the *Curcuma* genus, such as *Curcuma longa* [34].

The apparent discrepancy between total phenolic content (TPC) and GC–MS profiles observed in *C. macrochlamys* is not unique to this species but reflects a broader chemotaxonomic pattern. Within the *Curcuma* genus, ethanol extracts consistently exhibit higher phenolic content and antioxidant activity, whereas GC–MS analyses predominantly highlight fatty acid esters and terpenoids-compounds that do not directly contribute to TPC. A similar phenomenon was reported in ethanolic extracts of ginger, where strong antioxidant potential and high phenolic content were confirmed by HPLC, while GC–MS primarily revealed fatty acids and their derivatives [35].

Table 3. GC-MS-identified compounds in *C. macrochlamys* leaf extracts obtained using *n*-hexane, ethyl acetate, and 96% ethanol. Data includes retention time (RT), molecular formula, and compound class

No.	Compound	Retention Time (min)	Molecular Formula	Peak Area %		
				***n*-hexane**	**Ethyl Acetate**	**Ethanol 96%**
1.	Hexadecanoic acid, methyl ester	31.498	$C_{17}H_{34}O_2$	0.94	2.73	2.04
2.	Phenol, 2,5-bis(1,1-dimethylethyl)-	33.152	$C_{14}H_{22}O$	4.27	0.91	
3.	Phenol, 2,5-bis(1,1-dimethylethyl)-	33.155				0.56
4.	Dodecanoic acid, methyl ester	23.262	$C_{13}H_{26}O_2$		1.78	
5.	Dodecanoic acid, methyl ester	23.265				5.57
6.	Methyl Tetradecanoate	27.566	$C_{15}H_{30}O_2$		0.63	2.06
7.	Methyl Linolenate	37.376	$C_{19}H_{32}O_2$		1.03	
8.	Phytol	38.165	$C_{20}H_{40}O$		2.54	
9.	Methyl Octanoate	13.393	$C_9H_{18}O_2$			0.48
10.	Methyl Decanoate	18.550	$C_{11}H_{22}O_2$			0.74
11.	Methyl Stearate	35.128	$C_{19}H_{38}O_2$			0.37
12.	Methyl Oleate	35.480	$C_{19}H_{36}O_2$			1.30
13.	Lauric Acid	36.051	$C_{12}H_{24}O_2$			2.27
14.	Myristic Acid	39.399	$C_{14}H_{28}O_2$			0.76

Collectively, these findings underscore that GC–MS provides only a partial representation of antioxidant constituents and should ideally be complemented with techniques such as LC–MS or HPLC for a more comprehensive phytochemical profile. Furthermore, the coexistence of phenolic compounds and fatty acid esters indicates potential synergistic effects on antioxidant and antimicrobial activities, thereby reinforcing the pharmacological significance of *C. macrochlamys* leaf extract.

Conclusion

This study confirms that the leaf extract of *C. macrochlamys*, particularly that obtained using 96% ethanol, contains high levels of phenolic compounds and exhibits remarkable antioxidant activity. Microscopic analysis identified distinct pharmacognostic markers, whereas GC-MS profiling revealed several key bioactive metabolites. Collectively, these findings highlight the potential of *C. macrochlamys* leaves as a promising natural antioxidant source for future pharmaceutical applications.

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