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RESEARCH ARTICLE

Biological activities and phytochemical constituents of mace extract derived from *Endocomia macrocoma*

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Abstract

This study explores the phytochemical composition and biological activity of petroleum ether extract (PEEM) derived from the mace of *Endocomia macrocoma*. Methods employed include qualitative phytochemical screening, HPTLC, GC-MS, LC-MS, as well as free radical scavenging and antimicrobial assays. Preliminary screening revealed the presence of terpenoids, glycosides, alkaloids and tannins. Antioxidant activity was confirmed via HPTLC-DPPH and ABTS assays showing yielding IC_{50} values of 143.68 ± 2.82 µg/mL and 105.28 ± 1.36 µg/mL, respectively. As the first comprehensive analysis of *E. macrocoma* mace, this study highlights its potential as a natural source of antioxidants and antimicrobial agents, supporting traditional medicinal use and offering prospects for pharmaceutical development.

Keywords: antioxidant activity; antimicrobial properties; *Endocomia macrocoma*; fatty acids; mace extract

Introduction

Plants have long served as essential sources of pharmaceutical agents due to their rich reservoir of bioactive compounds (1). India, recognized globally for its botanical wealth, plays a pivotal role in medicinal plant cultivation. The World Health Organization (WHO) has documented approximately 21000 medicinal plants used for therapeutic purposes globally, of which 2500 species are found in India, with around 150 species being commercially exploited on a large scale (2, 3).

Fruits are rich sources of diverse phytochemicals, including flavonoids, carotenoids, phenolics, tannins, saponins and alkaloids. The composition and concentration of these bioactive compounds vary significantly across fruit species, contributing to their unique therapeutic effects and potent antioxidant properties (4, 5). Given their biomedical potential, there is an increasing need for focused research to isolate and characterize these constituents, with an eye toward applications in the pharmaceutical and nutraceutical industries (6).

Nutmeg, derived from the fruit of *Myristica fragrans*, consists of the seed and its aril, the latter known as mace. Historical accounts suggest that nutmeg and mace reached the Malabar and Coromandel coasts of India during the first millennium AD, initially valued for culinary rather than medicinal purposes (7). Traditionally, mace has been employed to address a wide range of ailments, including urinary incontinence, cardiovascular diseases, neurological disorders, reproductive dysfunctions and gastrointestinal infections (8). Mace is also associated with various health benefits, such as aiding digestion, alleviating inflammation, managing fevers, asthma and treating dermatological and urinary tract conditions (9, 10). Besides *Myristica fragrans*, other genera within the Myristicaceae family-such as *Knema*, *Horsfieldia*, *Virola* and *Endocomia*-also produce mace-like arils with differing phytochemical profiles (11).

Endocomia macrocoma, a lesser-known member of this family, is native to coastal and island ecosystems. Unlike the brownish mace of *M. fragrans*, the mace of *E. macrocoma* is vivid red. The plant bears dehiscent fruits, in clusters of 4-5. The ovoid fruit, enclosed by a yellow fleshy pericarp, contains oblong seeds surrounded by a bright red aril measuring approximately 3-5 × 2-3 cm. These arils exhibit longitudinal sutures and a distinct fleshy texture (12).

Seeds of *Endocomia macrocoma* subsp. *prainii* has been identified as a novel source of crude kernel wax. Key compounds identified include myristic acid methyl ester, ethyl myristate (C14) and methyl palmitate (C16) (13).

To date, no study has investigated the phytochemical composition or biological activity of *E. macrocoma* mace. This

study addresses that gap by analyzing the petroleum ether extract of *E. macrocoma* mace and evaluating its antioxidant and antimicrobial properties, highlighting its potential as a natural therapeutic agent.

Materials and Methods

Chemicals and microbial strains

All reagents and solvents used, including methanol, petroleum ether, chloroform, toluene, ethyl acetate, formic acid, ethanol, sodium hydroxide, sulfuric acid, sodium carbonate, glacial acetic acid, ferric chloride, Whatman No.1 filter paper, Folin-Ciocalteu's reagent, Dragendorff's reagent, silica gel aluminum sheets, DPPH (2,2-diphenyl-1-picrylhydrazyl), ascorbic acid, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and potassium persulfate, were procured from Merck (Mumbai, Maharashtra, India). Anisaldehyde sulphuric acid reagent, nutrient agar media, nutrient broth and sabouraud dextrose agar were purchased from Sigma Aldrich Pvt Ltd, Bangalore, India.

Microbial strains including gram-positive *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* (ATCC 33400); gram-negative *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922); and fungal strains *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC 281) were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India.

Methodology

Collection of mace and preparation of extracts

Fruits of *Endocomia macrocoma* subsp. *prainii* were collected in May 2022 from the Botanical Survey of India, South Andaman and Nicobar Islands, with support from Dr. A Gangaprasad (Botanist, Centre for Biodiversity Conservation, University of Kerala). Mace (arils) were washed thoroughly, air-dried and oven-dried at 50 °C. The dried material (79.8 g) was powdered and extracted with petroleum ether using cold maceration method. The extracts were filtered through Whatman No.1 filter paper and concentrated using a rotary flash evaporator (Heidolph, Hei-VAP Value, Germany), yielding 1.09 g of crude extract which indicate (1.37 %), referred to as PEEM. The extract was stored in airtight containers at 4 °C for further analysis (Fig. 1).

Phytochemical screening

Preliminary phytochemical screening of PEEM was conducted following standard protocols (14) with minor modifications. A 1.5 % solution of PEEM in petroleum ether was used for tests.

Flavonoids were confirmed by mixing 3 mL of PEEM with 4 mL of 1N sodium hydroxide, resulting in a yellow coloration. Steroids were detected when 1 mL of PEEM was mixed with 2 mL of chloroform and 1 mL of sulfuric acid, forming a reddish-brown ring. Terpenoids were detected by mixing 1 mL of PEEM with 2 mL of chloroform and 1.5 mL of sulfuric acid and checking for a brown ring. Phenols were detected by mixing 1 mL of PEEM with few drops of 5 % ferric chloride and checking presence of blue or green colouration. Quinones were detected by mixing 1 mL of PEEM with 1 mL of concentrated sulfuric acid, producing a red coloration. Glycosides were detected by mixing 1 mL of PEEM with 2 mL of glacial acetic acid and a few drops of ferric chloride, followed by 1 mL of sulfuric acid, which resulted in a brown ring. Anthocyanins and betacyanins were checked by mixing 2 mL of PEEM with 1 mL of 2 N sodium hydroxide and heating; a bluish-green color can idicate anthocyanins, while a yellow color can suggest betacyanins. Alkaloids were detected using Dragendorff's test, where 2 mL of PEEM mixed with Dragendorff's reagent and checked for an orange-red precipitate. Tannins were detected by boiling 0.5 g of PEEM in 20 mL of water, filtering the solution and mixing 1 mL of the filtrate with ferric chloride, checking for a reddish-green coloration. Saponins were detected by mixing 0.5 g of PEEM with 2 mL of water followed by boiling, shaking and checking for foam. Lastly, coumarins were detected by heating 1 mL of PEEM with sodium hydroxide-moistened filter paper, which exhibited yellow fluorescence under UV light.

High-Performance Thin Layer Chromatography (HPTLC-DPPH)

100 mg of PEEM was dissolved in 10 mL of HPLC-grade methanol, sonicated for 10 min and centrifuged at 2500 rpm. Aliquots (1.0-6.0 µL) were applied to silica gel aluminum sheets using a Hamilton syringe and Linomat 5 applicator (Camag, Switzerland). Chromatogram was developed in toluene:ethyl acetate (9:1, v/v) in a twin trough glass chamber. The developed plate was air-dried, derivatized with anisaldehyde sulfuric acid and scanned under visible and 366 nm UV light using a TLC Scanner IV with VisionCATS software. DPPH-active bands were analyzed for antioxidant activity. DPPH solution of 0.2 mM was prepared in methanol then sprayed the developed TLC plate evenly with DPPH solution. The TLC plate was incubated in the dark for 30 min at room temperature. Observation of yellowish bands against a purple background under visible light. Quantify active bands using a TLC Scanner IV at 517 nm.

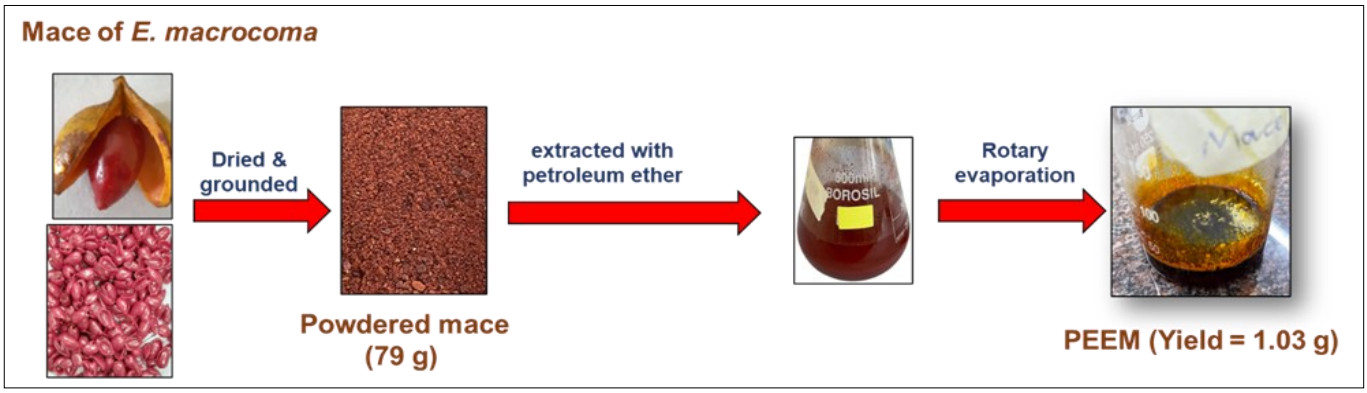

Fig. 1. Schematic representation of the preparation of mace extract of *Endocomia macrocoma*.

DPPH free radical scavenging assay

PEEM (50-200 µg/mL) was mixed with 1 mL of 0.2 mM DPPH (OD ~0.8) and incubated in the dark for 30 min. Absorbance was measured at 517 nm (LMSPUV1000B spectrophotometer). Ascorbic acid was used as the positive control. The percentage of inhibition was calculated (15).

The percentage inhibition is calculated by the difference between the control optical density (OD) and the sample OD is divided by the control OD and the result is multiplied by 100.

ABTS radical scavenging assay

$ABTS^{\bullet+}$ was generated by reacting 14 mM ABTS with 4.9 mM $K_2S_2O_8$ for 16 hrs in the dark. The solution was diluted to an absorbance of 0.700±0.020 at 734 nm. PEEM samples (50-200 µg/mL) were added to 1 mL of this solution and absorbance was measured at 734 nm (16).

The percentage inhibition is calculated as the difference between A_0 and A_1, divided by A_0, then multiplied by 100,

where A_0 = control absorbance and A_1 = sample absorbance.

Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS was performed using a Hypersil GOLD C18 column (100×2.1 mm, 3 µm) under gradient elution (30 min) with 0.1 % formic acid in water (A) and 100 % acetonitrile (B). The Q-TOF analyzer (Model: G6550A) was operated in negative ESI mode (m/z 126-1200). Source parameters: gas temperature 250 °C, flow rate 13 L/min, nebulizer pressure 35 psig.

Gas Chromatography-Mass Spectrometry (GC-MS)

Samples were derivatized using methanolic NaOH and HCl, extracted with hexane and analyzed using Shimadzu Nexis GC-2030 with an SH-l-5Sil MS capillary column (30 m × 0.25 mm × 0.25 µm). Injection temperature: 250 °C; carrier gas: helium (24 mL/min); split ratio: 20. Temperature program: initial 70 °C (2 min), ramped to 200 °C at 8 °C/min, then to 280 °C at 4 °C/min.

Antibacterial activity

The agar well diffusion method was employed to test PEEM (25, 50, 75 and 100 µg/mL) against *S. aureus*, *S. pneumoniae*, *P. aeruginosa* and *E. coli*. Wells were loaded with extract and incubated at 37 °C for 18-24 hrs. Inhibition zones were measured (in mm) in triplicate and the average recorded.

Antifungal activity

Antifungal activity was evaluated by the well diffusion method against *A. niger* and *C. albicans* on SDA plates. Plates were incubated at 37±2 °C for 48 hrs and inhibition zones (in mm) were measured.

Results

Phytochemical screening of PEEM

Preliminary phytochemical analysis of the petroleum ether extract of *Endocomia macrocoma* mace (PEEM) revealed the presence of terpenoids, glycosides, alkaloids and tannins (Table 1).

Table 1. Qualitative phytochemical analysis of mace extracts of *Endocomia macrocoma* (PEEM)

Test No.	Test	Results
1	Glycosides	+
2	Alkaloids (Drangendorff reagent)	+
3	Tannins	+
4	Terpenoids	+
5	Phenols	-
6	Quinones	-
7	Flavonoids	-
8	Anthocyanin & Betacyanin	-
9	Steroids	-
10	Saponins	-
11	Coumarins	-

(+) present, (-) absent

PEEM- Petroleum ether extract of mace of *E. macrocoma*

HPTLC-DPPH assay

The HPTLC profiling of PEEM under UV light (366 nm) showed three distinct bands at Rf values of 0.32, 0.37 and 0.45, indicating the presence of various phytoconstituents (Fig. 2A). Upon derivatization with DPPH, five prominent yellow zones were observed at Rf values of 0.19, 0.25, 0.33, 0.76 and 0.87, suggesting antioxidant-active compounds (Fig. 2B).

DPPH free radical scavenging activity

The DPPH radical scavenging activity of PEEM was concentration dependent. At 50 µg/mL, PEEM exhibited 16.63 % inhibition, which increased to 27.17 % at 75 µg/mL. Maximum inhibition of 65.80 % was observed at 200 µg/mL. The IC_{50} value of PEEM was calculated as 143.68±2.82 µg/mL (Table 2). Ascorbic acid, used as the standard, demonstrated higher potency with a lower IC_{50} value.

ABTS radical scavenging activity

Similarly, PEEM exhibited concentration-dependent ABTS radical scavenging activity. At 50 µg/mL, inhibition was 4.82 %, increasing to 10.9 % at 75 µg/mL and reaching 55.91 % at 200 µg/mL. The IC_{50} value was 105.25±1.36 µg/mL (Table 3), indicating significant antioxidant potential, though lower than that of the ascorbic acid standard.

LC-MS analysis of PEEM

LC-MS analysis revealed a dominance of carboxylic acids in PEEM. The major identified compounds were 4-oxocyclohexanecarboxylate (abundance: 13169 a u) and 2-methylglutaric acid (5434 a u) (Table 4). These findings confirm the abundance of carboxylic acid derivatives in the extract. Chromatograms and compound structures are shown in Fig. 3.

GC-MS analysis of PEEM

GC-MS profiling identified several fatty acids and related compounds. The major constituents included oleic acid (44.99 %), n-hexadecanoic acid (31.21 %), octadecanoic acid (4.57 %) and palmitoleic acid (4.35 %). Minor components included sesquiterpenoids, hydrocarbons (dotriacontane and tetracontane), aldehydes, esters and phthalates (Table 5). The chromatogram and corresponding bar chart illustrating compound percentages are presented in Fig. 4.

Antibacterial activity

The antibacterial activity of PEEM was evaluated against *S. aureus*, *E. coli*, *S. pneumoniae* and *P. aeruginosa* using the agar well diffusion method (Fig. 5).

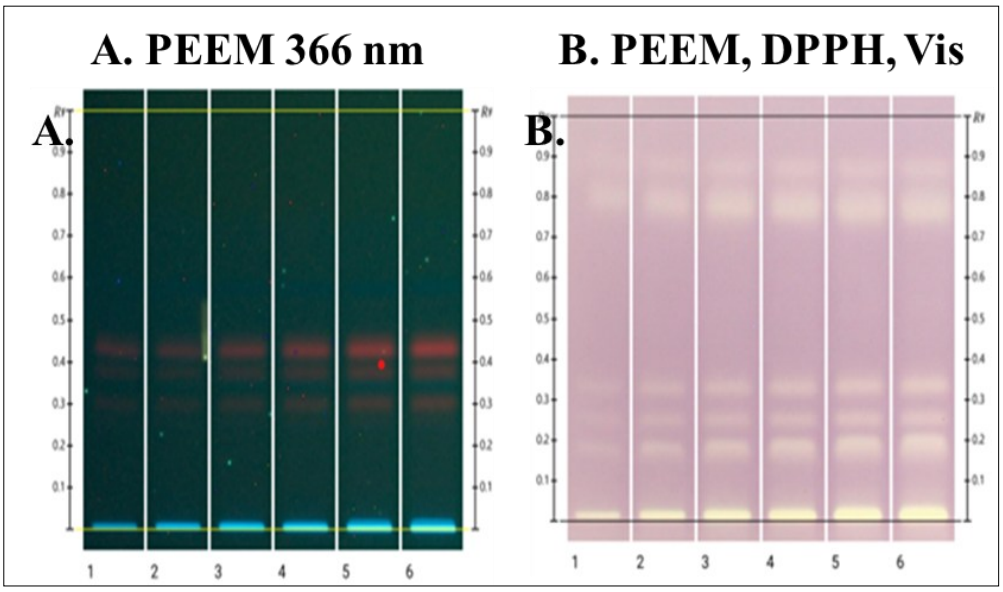

Fig. 2. HPTLC-DPPH of mace extracts of *Endocomia macrocoma* (PEEM). (A.) Developed at 366 nm of PEEM and (B.) Plate image after dipping in DPPH reagent under VIS.

Table 2. Free radical scavenging capacity (IC_{50} value) of mace extracts of *Endocomia macrocoma* (PEEM) conducted by DPPH method

Test compound	Concentrations (µg/mL)	Percentage inhibition	SD	IC_{50} (µg/mL)
	50	16.63	0.308	
	75	27.17	0.364	
PEEM	100	42.14	0.459	
	150	52.49	0.513	143.68±2.82
	200	65.80	0.824	
	10	37.52	0.361	
	25	49.53	0.429	
Ascorbic acid	50	71.16	0.474	25.08±0.39
	75	78.55	0.635	
	100	86.32	0.913	
	control	0	0	

Table 3. Free radical scavenging capacity (IC_{50} value) of mace extracts of *Endocomia macrocoma* (PEEM) conducted by ABTS method

Test Compound	Concentrations (µg/mL)	Percentage inhibition	SD	IC_{50} (µg/mL)
	50	4.82	0.213	
	75	10.9	0.267	
PEEM	100	21.49	0.311	105.28±1.36
	150	38.31	0.476	
	200	55.91	0.754	
	10	9.64	0.127	
	25	21.54	0.216	
Ascorbic acid	50	33.11	0.265	74.65±0.82
	75	45.01	0.438	
	100	69.93	0.543	
	Control	0	0	

Table 4. Non-volatile compounds of petroleum ether mace extract *of Endocomia macrocoma* (PEEM) using LC-MS and their chemical formula

SL.	Compound	Formula	RT	Mass	Quantity (a u)	Class of chemicals
1	4-Oxocyclohexanecarboxylate	$C_7H_{10}O_3$	15.032	142.0636	13169	carboxylic acid
2	2-methyl-glutaric acid	$C_6H_{10}O_4$	9.086	146.0578	5434	carboxylic acid

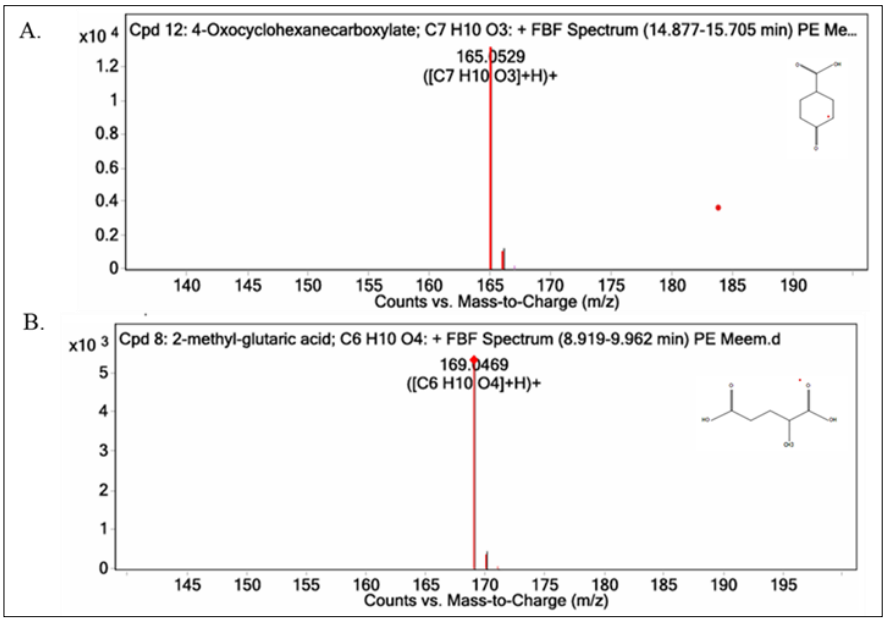

Fig. 3. LC-MS chromatogram of mace extract of *Endocomia macrocoma* (PEEM). (A-B) Chromatography and structure of compounds of PEEM.

Table 5. Volatile compounds of petroleum ether mace extract of *Endocomia macrocoma* (PEEM) and their percentage area using GC-MS

SL.	Compound name	Chemical class	R Time	Area percentage
1.	Oleic acid	Fatty acid	28.24	44.99
2.	n-Hexadecanoic acid	Fatty acid	25.84	31.21
3.	Octadecanoic acid	Fatty acid	28.47	4.57
4.	Palmitoleic acid	Fatty acid	25.41	4.35
5.	Tetradecanoic acid	Fatty acid	22.733	2.56
6.	(S)-2,6,6-Trimethyl-2-((2S,5S)-5-methyl-5-vinyltetrahydrofuran-2-yl)-2H-pyran-3(6H)-one	Sesquiterpenoids	32.27	1.19
7.	Dotriacontane	Saturated hydrocarbon (alkane)	35.75	1.13
8.	Tetracontane	Saturated hydrocarbon (alkane)	34.65	1.07
9.	Dotriacontanal	Aldehyde	32.84	1.01
10.	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	Lipid	30.60	0.93
11.	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Phthalate ester	35.10	0.91
12.	Hexanoic acid, 4-hexadecyl ester	Fatty acid	29.27	0.91
13.	cis-10-Heptadecenoic acid	Fatty acid	26.75	0.58
14.	Eicosanoic acid	Fatty acid	30.89	0.58
15.	Tetratriacontane	Wax	37.02	0.57
16.	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Fatty acid	27.49	0.46
17.	Hexadecanoic acid, methyl ester	Fatty acid	25.16	0.49
18.	Henicosanal	Aldehyde	30.48	0.31
19.	Nonacosanal	Aldehyde	31.68	0.30
20.	9-Octadecenoic acid, methyl ester, (E)-	Fatty acid	27.58	0.30
21.	Farnesol formate	Sesquiterpene	24.98	0.30
22.	3,5,9-Undecatrien-2-one, 6,10-dimethyl-, (E,E)-	Sesquiterpene	19.83	0.30
23.	Z-11-Pentadecenol	Fatty acid	22.45	0.27
24.	Octadecanoic acid, 9,10-dihydroxy-, methyl ester, bis(trifluoroacetate)	Fatty acid	24.55	0.21
25.	3-Methylcyclopentyl acetate	Alkyl ester	5.85	0.17
26.	5-Hepten-2-one, 6-methyl-	Ketone	7.73	0.16

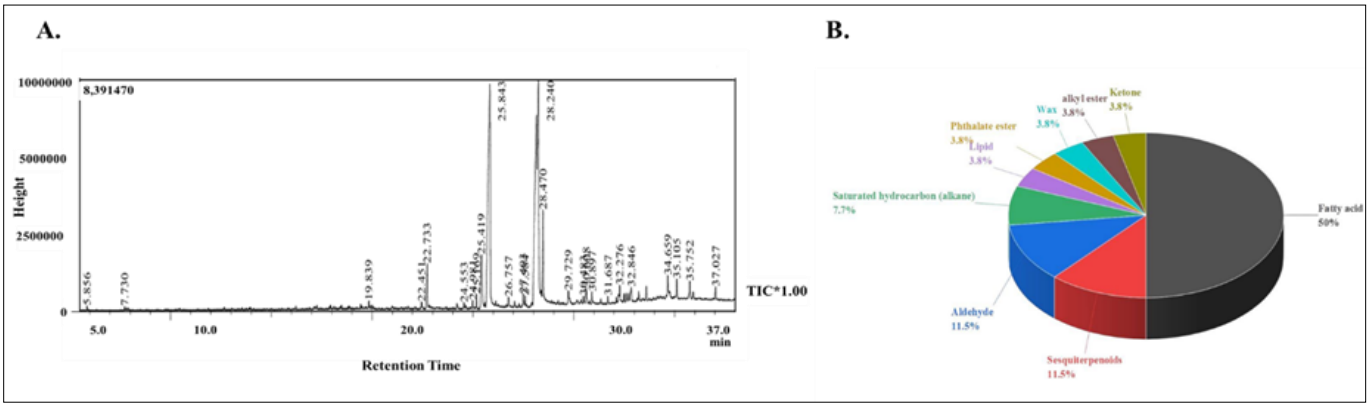

Fig. 4. GC-MS of mace extract of *Endocomia macrocoma* (PEEM): (A.) Chromatogram of PEEM, (B.) Bar chart of volatile compounds and its percentage of PEEM.

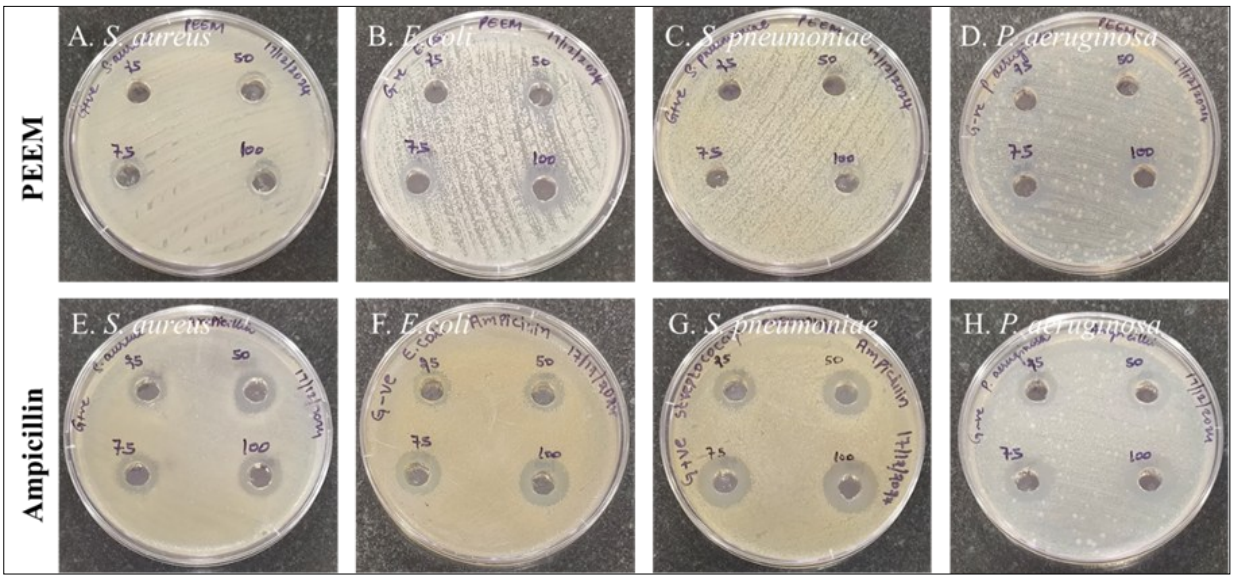

Fig. 5. Anti-bacterial activity of mace extract of *Endocomia macrocoma* (PEEM). Representative images of agar plates containing (A.) *Staphylococcus aureus* (B.) *Escherichia coli* (C.) *Streptococcus pneumonia* (D.) *Pseudomonas aeruginosa* and their growths in the presence of different concentrations of PEEM (25, 50, 75 and 100 µg/mL). Representative images of agar plates containing (E.) *Staphylococcus aureus* F.) *Escherichia coli* (G.) *Streptococcus pneumonia* (H.) *Pseudomonas aeruginosa* in the presence of different concentrations of ampicillin (25, 50, 75 and 100 µg/mL).

The antimicrobial activity revealed that *S. aureus* showed no inhibition at 25 µg/mL but exhibited moderate inhibition zones ranging from 7 to 8 mm at 50-100 µg/mL. For *E. coli*, inhibition zones increased with concentration, reaching 12 mm at 100 µg/mL. *S. pneumoniae* was inhibited only at 100 µg/mL, with a 7 mm inhibition zone. *P. aeruginosa* showed no activity at 25 and 50 µg/mL, but inhibition zones of 9 mm and 11 mm were observed at 75 and 100 µg/mL, respectively.

PEEM showed moderate antibacterial activity, especially against *E. coli* and *P. aeruginosa*, with efficacy increasing with concentration. Ampicillin served as a positive control, exhibiting significantly larger inhibition zones.

Antifungal activity

Antifungal testing revealed that PEEM exhibited no activity against *Candida albicans* at all tested concentrations (25-100 µg/mL). However, for *Aspergillus niger*, PEEM demonstrated moderate activity, with inhibition zones of 12 mm and 14 mm at 75 and 100 µg/mL, respectively (Fig. 6). These findings suggest a concentration-dependent antifungal effect limited to filamentous fungi.

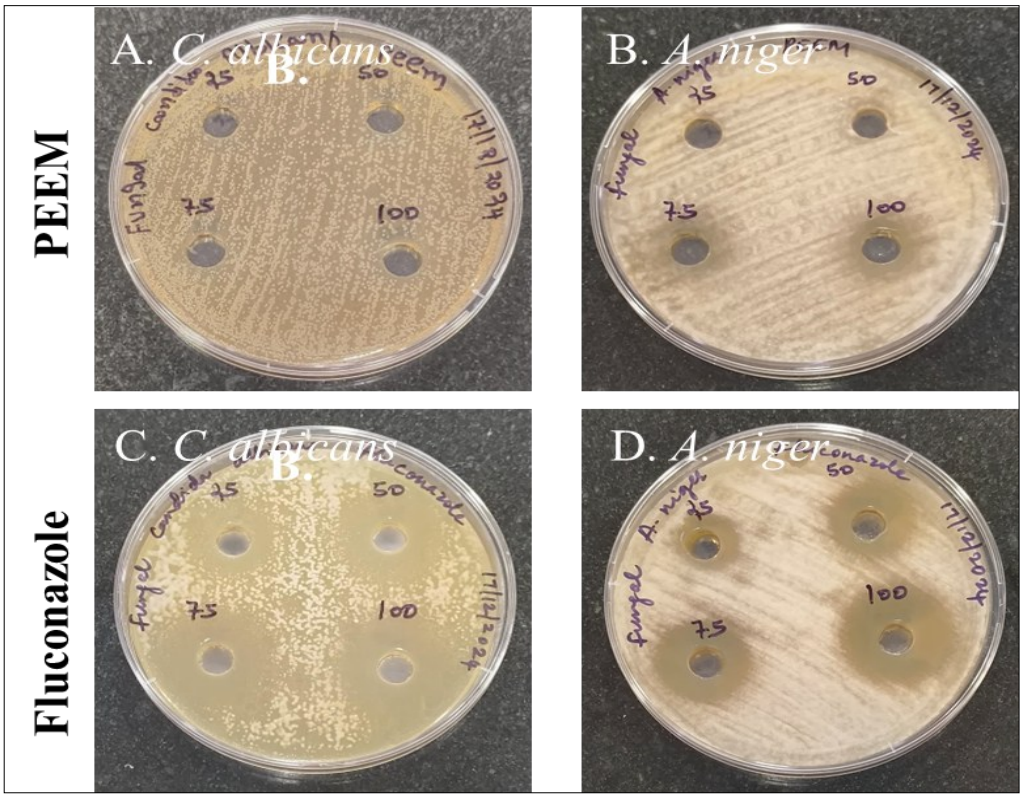

Fig. 6. Anti-fungal activity of mace extract of *Endocomia macrocoma* (PEEM). (A-B.) Representative images of agar plates showing growth of *Candida albicans* and *Aspergillus niger* in the presence of different concentrations of PEEM (25, 50, 75 and 100 µg/mL). (C-D.) Representative images of agar plates showing growth of *Candida albicans* and *Aspergillus niger* in the presence of different concentrations of different concentrations of Fluconazole (25, 50, 75 and 100 µg/mL).

Discussion

This study presents the first comprehensive analysis of the phytochemical composition and biological activities of *Endocomia macrocoma* mace using petroleum ether extraction (PEEM). The findings underscore the therapeutic potential of this underexplored species, particularly in antioxidant and antimicrobial applications.

Phytochemical screening revealed a rich profile of bioactive constituents, with PEEM extracts notably containing fatty acids, terpenoids, glycosides, alkaloids and tannins. These results align with previous findings in other Myristicaceae species, indicating that the mace aril is a reservoir of bioactive secondary metabolites.

HPTLC-DPPH profiling demonstrated the presence of antioxidant-active constituents, with several yellow zones observed post-DPPH derivatization, confirming radical scavenging capabilities. This was corroborated by DPPH and ABTS assays, where PEEM exhibited moderate antioxidant activity, with IC_{50} values of 143.68 ± 2.82 µg/mL and 105.25 ± 1.36 µg/mL, respectively. These results suggest that the non-polar components extracted by petroleum ether contribute significantly to antioxidant activity, consistent with early findings (17).

GC-MS analysis revealed oleic acid (44.99 %) and palmitic acid (n-hexadecanoic acid, 31.21 %) as the dominant constituents. Oleic acid has well-documented antibacterial and anti-inflammatory properties (18-20), supporting its potential role in PEEM's activity against *Staphylococcus aureus*. Palmitic acid, also abundant, is known for its antioxidant, antimicrobial and anticancer properties (21-24), further validating the diverse bioactivity of PEEM. The observed antioxidant effects may be attributed to these fatty acids and other compounds like terpenoids, known for their radical-scavenging potential (25). Previous studies reported that organic acids enhance antioxidant activity, which aligns with the abundance of such acids observed via LC-MS in PEEM (26).

The antibacterial results showed that PEEM exerted concentration-dependent inhibitory effects, particularly against *E. coli* and *P. aeruginosa*, as well as moderate inhibition of *S. aureus*. These effects are likely due to the high content of fatty acids. Oleic acid disrupts bacterial membranes (17), while palmitic acid affects membrane integrity and inhibits biofilm formation (22, 28-30). Such selectivity supports the potential use of PEEM in targeting gram-negative bacterial infections, which are often more resistant to antibiotics.

In contrast, antifungal activity was limited. PEEM was effective only *against Aspergillus niger* at higher concentrations and showed no inhibition against *Candida albicans*. This selective efficacy suggests that the extract may be more suited to combat filamentous fungi rather than yeasts, possibly due to differences in cell wall structure and lipid composition.

Comparison with the well-studied *Myristica fragrans* (nutmeg) reveals both similarities and distinctions. While both species are rich in fatty acids, *M. fragrans* exhibits higher levels of volatile terpenoids such as sabinene, α-pinene and myristicin (30). In contrast, *E. macrocoma* is distinguished by its abundance of fatty acid methyl esters and elevated oleic acid content, which may account for differences in bioactivity. Although the antioxidant and antimicrobial activities of *E. macrocoma* are comparable to those of *M. fragrans*, they appear slightly less potent-likely due to differences in phytochemical profiles and aril pigmentation, which can influence secondary metabolite biosynthesis.

Overall, the diverse bioactivities exhibited by *E. macrocoma* mace-particularly its antioxidant and antibacterial effects highlight its promise as a source of natural therapeutic agents. The presence of biologically relevant fatty acids, terpenoids and organic acids supports potential applications in managing oxidative stress-related conditions such as cardiovascular and neurodegenerative diseases. Additionally, its antimicrobial activity, especially against resistant gram-negative bacteria, suggests a role in developing novel antibacterial treatments.

These findings provide scientific validation for the traditional use of mace in folk medicine, particularly for gastrointestinal disorders, infections and inflammation, as previously reported (10, 8). The study lays a foundation for future pharmacological investigations and bioactivity-guided fractionation to isolate and characterize the most potent compounds in *E. macrocoma* mace.

Conclusion

This study provides the first comprehensive phytochemical and bioactivity evaluation of *Endocomia macrocoma* PEEM. The extract is rich in bioactive fatty acids, particularly oleic and palmitic acids, which likely contribute to its significant antioxidant and antimicrobial properties. PEEM demonstrated concentration-dependent free radical scavenging activity and antibacterial efficacy, especially against *E. coli* and *P. aeruginosa*. These findings validate the applicable medicinal use of mace and highlight the potential of *E. macrocoma* as a promising source of natural therapeutic agents for oxidative stress and infectious diseases.

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Authors' contributions

ASA carried out the free radical scavenging and anti-microbial studies. APS participated in extraction. NV participated in the LC-MS analysis. EJA participated in the GC-MS. ARN participated in the phytochemical screening. AG participated in providing fruit. VV performed the conceptualisation, writing, review, editing, supervision and project administration. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical issues: None

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