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ORIGINAL RESEARCH ARTICLE

GC-MS Profiling and Antidandruff Potential of *Eugenia aromatica* Extracts

Nawaf Abubakar[1], Kaumi Alkali[2] and Abidina Abba[1]
[1]Department of Applied Biology, Federal University of Technology, Babura, Jigawa State, Nigeria
[2]Department of Biological Sciences, Alansar University, Maiduguri, Borno State, Nigeria

ABSTRACT
Dandruff, characterized by scalp flaking and itching, is associated with *Malassezia, Staphylococcus,* and *Propionibacterium.* This study aimed to evaluate the susceptibility of *Eugenia aromatica* (Clove) extracts against fungi, *M. restricta* and *M globossa*, and bacteria, *S. epidermidis* and *P. acne.* Plant extraction and GCSM analysis were carried out using a standard method; varying concentrations of 500, 700, and 100 mg/mL were prepared for Aqueous, Methanolic, Ethanolic, n-Hexane, and Chloroform extracts. Antimicrobial activity was assessed using the agar well. The ethanolic extract of *E. aromatica* showed the highest zones of inhibition at 1000mg/ml, with *S. epidermidis* showing 53 mm and *P. acne* showing 55 mm. *M. restricta* and *M. globossa* were more sensitive to the ethanolic extract of *E. aromatica,* with the highest zone of inhibition at 1000mg/ml, with *M. restricta* showing a 60 mm diameter and M. *globossa* demonstrating a 59 mm diameter. Fifty-one (51) Active compounds were identified using GC-MS analysis, including Eugenol (45.90%), cis-13-Octadecenoic acid (16.69%), Octadecanoic acid (6.93%), and n-Hexadecanoic acid (2.55%). These findings validate the traditional use of clove in herbal remedies for treating fungal and bacterial skin conditions. Future research should isolate these active compounds, test their synergistic effects, and evaluate their safety for therapeutic applications.

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INTRODUCTION
Dandruff is one of the most prevalent scalp conditions affecting both adolescents and adults, characterized by the excessive shedding of dead skin cells and the accumulation of loosely adherent white or grey flakes, often without accompanying inflammation (Schwartz *et al.,* 2010). While dandruff is confined to the scalp, its more severe form, seborrhoeic dermatitis, presents with red patches and yellow-gray scales extending to other sebaceous areas (Plewig *et al.,* 2008). Globally, dandruff affects approximately 50% of the population, with peak incidence in early adulthood and a decline after age 50 (Baroni *et al.,* 2008). Its multifactorial etiology involves sebaceous secretions, individual susceptibility, and microbial imbalance, particularly involving *Malassezia* species, *Staphylococcus epidermidis*, and *Propionibacterium acnes* (Deangelis *et al.,* 2005; Zhijue, 2016). *Malassezia*, formerly *Pityrosporum*, thrives in humid environments, poor hygiene, and crowded conditions, increasing the risk of infection (Shuster, 1999; Rippon, 2010).

Efficacy of *Eugenia aromatica* specifically against dandruff-causing pathogens, particularly in regions such as northern Nigeria, where both the burden of dandruff and reliance on traditional remedies are high. Inadequate veterinary and healthcare systems, alongside widespread misuse of commercial antimicrobials, have compounded resistance issues (WHO, 2001; Iwu, 2012). Researchers such as Afolayan, (2003) and Adesiji *et al.,* (2012) have highlighted the promise of medicinal plants in addressing resistant infections. Additionally, traditional remedies such as *Allium sativum*, *Myristica fragrans*, and *E. aromatica* are widely used to treat bacterial and fungal infections, yet remain scientifically under-investigated (Nelson-Harrison *et al.,* 2002; Gilani and Rahman, 2005). Given the impact of genetic and environmental factors on phytochemical profiles, localized studies are crucial to validate the therapeutic efficacy of these plants (Appel *et al.,* 1997; Wonggiratthiti, 2000). This study aims to evaluate the antimicrobial activity of *Eugenia aromatica* extracts against *M. restricta*, *M. globosa, S. epidermidis,* and *P. acnes.*

Although several antifungal agents—such as ketoconazole, zinc pyrithione, and selenium sulfide—are commonly used for treatment, they present notable limitations. These include the development of resistant fungal strains, reduced efficacy with prolonged use, and undesirable side effects such as scalp irritation, dryness, and hair discoloration (Gupta & Nicol, 2016; Rallis *et al.,* 2019). Additionally, synthetic antifungal formulations are often costly and may disrupt the scalp's natural microbiota, leading to relapse after treatment discontinuation (Zhao *et al.*, 2021). Consequently, there is

Correspondence: Nawaf Abubakaa. Department of Applied Biology, Federal University of Technology, Babura, P.M.B 2022, Jigawa State, Nigeria. ✉ nawafpg@gmail.com.
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a growing need for alternative, plant-derived antifungal compounds that are both effective and safe for long-term use (Adefegha *et al.,* 2017).

The novelty of employing *E. aromatica* (clove) against dandruff-causing pathogens lies in its rich phytochemical composition and broad antimicrobial activity, which has been underexplored specifically in the context of dandruff management (Naveed *et al.,* 2021). Unlike most conventional antifungal studies that rely on crude screening, this approach integrates Gas Chromatography–Mass Spectrometry (GC–MS) profiling with Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) determinations (Egbung *et al.,* 2022). The GC–MS technique enables the identification of bioactive constituents such as eugenol, β-caryophyllene, and eugenyl acetate, providing a chemical fingerprint that correlates with antifungal potency (Chaieb *et al.,* 2007). Following this, MIC and MFC assays quantitatively assess the precise inhibitory and fungicidal thresholds of *E. aromatica* extracts against *Malassezia* and other dandruff-associated fungi (*Candida albicans, Aspergillus niger,* etc.), offering a rigorous, evidence-based evaluation of efficacy (Okoh *et al.,* 2020).

This integrated approach represents a significant advancement over previous phytochemical studies, which often lacked comprehensive bioactivity correlation (Mahmoud *et al.,* 2023). The combination of GC–MS chemical profiling with antifungal susceptibility testing not only establishes a mechanistic link between specific compounds and antifungal activity but also highlights *E. aromatica* as a potential source of natural, scalp-compatible antifungal agents. The novelty therefore**,** resides in demonstrating, for the first time, the targeted efficacy of *E. aromatica* against dandruff pathogens using a dual analytical-biological validation framework. Such a strategy paves the way for the development of standardized, plant-based antidandruff formulations that could overcome the limitations of existing synthetic treatments.

MATERIALS AND METHODS

Collection, Identification, and Processing of Plant Samples

The plant used in the current investigation was *Eugenia aromatica.* The main market in Sokoto is where the samples were bought. For identification and authentication, the sample was taken to the Department of Biological Sciences' herbarium at Usmanu Danfodiyo University in Sokoto, Sokoto State, Nigeria, in a sterile, clean plastic bag. As explained by Kumar *et al.* (2000), voucher specimens of the sample were made and placed in the same herbarium. After being cleaned with tap water, the plant samples were allowed to air dry in the shade. A high-capacity grinding machine was used to ground the dried material into a fine powder. Before use, the produced plant powders were stored in sterile polythene bags (Patil *et al.,* 2010).

3.7 Extraction Procedure

Two hundred (200) grammes of *E. aromatica* powdered samples were extracted individually using ethanol, methanol, water, hexane, and chloroform. One litre each of distilled water, hexane, chloroform, ethanol, and methanol was added to a 2000 ml beaker to create the extracts. After stirring and covering with aluminium foil, the suspensions were stored for twenty-four hours. The resulting mixture was filtered through muslin cloth, and crude extracts were obtained by independently evaporating each filtrate to dryness using a hot plate heated at 40°C. Each plant's crude extract was weighed and refrigerated until needed.

Preparation of Plant Extract Concentration

A stock solution was prepared by dissolving 20g of the solid plant extracts in 100ml of normal saline, yielding a stock of 200mg/ml. The concentrations were prepared from the stock solution using the dilution formula (equation 5) as follows.

$$C_1V_1 = C_2V_2$$

Where: C_1 = present concentration, V_1 = Volume to use, C_2 = required concentration, V_2 = required volume

Subsequently, 500 mg/ml, 750 mg/ml, and 1000 mg/ml concentrations were used to assess the antimicrobial activity of *E. aromatica* extracts. To prepare these concentrations, a given amount of 12.5 ml, 18.75 ml, and 25 ml of the stock solution of the extract *E. aromatica* was drawn using a syringe and each dissolved in a conical flask containing 5 ml of sterile distilled water, respectively.

Sub-culturing of Fungal Colonies

Using a sterilised loop and inoculation needle, the resulting fungal colonies were aseptically transferred to new Sabouraud Dextrose Agar (SDA) media after the advancing edges of the isolates were cut. These were continued until pure cultures were obtained. The isolates' pure cultures were maintained in McCartney bottles at 40°C in the dark on the Sabouraud Dextrose Agar (SDA) slope (Morsy et al., 2009).

Determination of Antimicrobial Sensitivity of Plant Extracts

Test microorganisms

The test microorganisms were the bacteria (*Staphylococcus epidermis* and *Propionibacterium acnes*) and fungi (*Malessezia restricta* and *Malessezia globossa*) isolated from dandruff samples in this study. The isolates were maintained on Sabouraud Dextrose Agar (SDA) (fungi) and Nutrient Broth Agar (NBA) (bacteria).

Standardization of Test Microorganisms (McFarland Standard)

A loopful of the bacterial culture and fungal spores was inoculated into separate 100 ml NA and SDA, and the mixture was then incubated on a shaker at 20 0C overnight to produce active cultures. To achieve 5 × 10 cfu/ml, the cells were harvested by centrifuging at 4000 rpm for 5 minutes, washed with normal saline, resuspended in normal saline, and diluted in normal saline.

Antimicrobial Sensitivity Testing of Plant Extracts

The agar well method, first described by Murray et al. (1995), modified by Olurinola (1996), and subsequently adopted by Valgas *et al.* (2007) and Magaldi et al. (2004), was used to test the antifungal and antibacterial activity of the various plant extracts. After dispensing 20 millilitres of Mueller Hinton Agar (MHA) into sterile universal bottles, 0.2 millilitres of each bacterial and fungal culture were added, gently mixed, and then transferred to sterile Petri dishes. Three cups or wells were created in each Petri dish using a number 3-cup borer (6mm) diameter that had been adequately sterilised by flame after setting. Each cup's base would be sealed with a drop of molten MHA. After that, 50 µL of the extracts at concentrations of 500 mg/mL, 750 mg/mL, and 1000 mg/mL are added to the cups or wells, and they are left to diffuse on the workbench for 45 minutes. For bacteria, the plates were incubated for 24 hours at 30°C; for fungi, they were incubated for 5 days at room temperature. The antibiotic zone scale was used to measure the zones of inhibition in millimeters. Tetracycline for all bacterial strains and fluconazole antifungal disc for fungus served as the positive controls. The diameter of the inhibition zone (IZ) surrounding the wells was used to assess the antibacterial and antifungal activity.

Minimum Inhibitory Concentration (MIC)

The tube dilution method, as outlined by Ajaiyeoba *et al.* (2003), was used to determine the MIC of the bacterial and fungal strains. Fungal starin's MIC was measured using a peptone medium, while the bacterial strain's MIC was measured using nutrient broth media. The various extracts were serially diluted in order to determine the MIC. Eight test tubes were used in the experiment. The concentration was serially diluted so that each test tube contained 1/10 the concentration of the previous tube, with the first tube containing 500 mg/ml. After adding 0.1 ml of the test organism, which had been previously standardised to 0.5 McFarland, to the mixture, the mixture was incubated for 24 hours (for bacteria) at 37 °C and for 5–7 days (for fungi) at room temperature. The MIC was determined as the lowest concentration of the turbidity-free extract.

Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

This was an offshoot of the previously determined MICs. The MBC and MFC of the plant extracts were determined by subculturing from all the tubes that showed no turbidity in the MIC tests onto a sterile nutrient agar plate. The lowest concentration at which no growth was observed after incubation was taken as the MBC.

Gas chromatography/Mass Spectroscopy (GC-MS) analysis

The GC-MS-QP2010 plus (Shimadzu, Japan) with a flame ionisation detector (FID) was used for the analysis. To reduce peak broadening, the injection was carried out in split-less mode for three minutes at 2500C using a 0.75mm ionisation detector inlet. Helium was used as the carrier gas for chromatographic separations on a DB-WAX analytical column (30m, 0.25mm, 0.25mm; J&W Folsom, C.A.) at a steady flow rate of 0.8 ml/min. Ion source temperature of 200 °C, ionisation voltage of 70 eV, and mass scan range of m/z 23-450 at 2.76 scans/s were the operating settings for MS (electron impact ionisation mode) (McNair et al., 2019)

Values were expressed as percentages, mean (± SEM). Comparisons between groups were conducted using analysis of variance (ANOVA); p-values < 0.05 were considered significant. Chi-square was used to analyze significant differences between observed and expected frequencies in prevalent studies, with *p*-values of <0.05 were considered significant

RESULTS

Evaluating the antimicrobial activity of different extracts of *E. aromatica* against fungi, *M. restricta* and *M globossa*, and bacteria, *S. epidermidis* and *P. acnes* was performed to determine the most effective solvent for the extraction of active ingredients. All the clove extracts exhibited antimicrobial activity against the tested isolates, with varying susceptibility patterns. The ethanolic extract of *E. aromatica* was the most effective, showing high antifungal and antibacterial activity against the tested microorganisms, as shown in Table 1 and Table 2. The bacterial strains *S. epidermidis* and *P. acne* were more resistant to *E. aromatica* extracts, with inhibition zones of 30 mm and 34 mm, respectively, at a 500mg/ml extract concentration. The highest zones of inhibition were recorded at 1000mg/mL, with *S. epidermidis* showing 53 mm and *P. acne* showing 55 mm (Tables 1 and 3). Efficacy increased with increasing extract concentration. The fungi, *M. restricta* and *M. globossa*, were more sensitive to the ethanolic extract of *E. aromatica*. The highest zone of inhibition was recorded at 1000mg/mL, with *M. restricta* showing 60 mm diameter and M. *globossa* demonstrating 59 mm diameter. The lowest zones of inhibition were recorded at 500mg/mL, with *M. restricta* showing inhibition zone diameters of 53 mm and *M globossa* showing 51 mm diameter (Table 2 and Table 4).

The methanol extract of *E. aromatica* demonstrated antimicrobial activity against all tested strains: *M. restricta*, *M globossa*, *S. epidermidis,* and *P. acne.* The highest zones of inhibition were recorded at 1000mg/ml with inhibition zone diameters of 57, 54, 50, and 52 mm, respectively.

Table 1: Inhibition Zone of *E. aromatica* Extracts Against *S. epidermidis*

Concentration (mg/ml)	Mean zone of inhibition (mm) ± Standard error					
	Control	Aqueous	Methanolic	Ethanolic	n-Hexane	Chloroform
500	30±0.02[a]	30±0.9[a]	35±0.07[b]	36±0.01[b]	21±0.02	25±0.05
750	30±0.02	32±0.05	47±0.9	48±0.06	27±0.03	30±0.1[a]
1000	30±0.02[ab]	34±0.5[c]	50±0.9	53±1.0	30±0.07[ad]	32±0.1[bcd]

Values are mean ±standard error (n=3), Mean values with the same superscript in a raw are not significantly different (P≤0.05)

Table 2: Inhibition Zone of *E. aromatica* Extracts Against *M. restricta*

Concentration (mg/ml)	Mean zone of inhibition (mm) ± Standard error					
	Control	Aqueous	Methanolic	Ethanolic	n-Hexane	Chloroform
500	40±0.1[a]	52±0.07	45±0.03	53±0.01	40±0.08[a]	41±0.04
750	40±0.1	56±0.03[a]	51±0.07	58±0.01 [a]	43±0.9[b]	43±1.1[b]
1000	40±0.1	64±0.2	57±1.0[a]	60±0.02[a]	45±0.07[b]	48±1.3 [b]

Values are mean ±standard error (n=3). Mean values with the same superscript in a row are not significantly different (P<0.05)

Table 3: Inhibition Zone of *E. aromatica* Extracts Against *P. acne*

Concentration (mg/ml)	Mean zone of inhibition (mm) ± Standard error					
	Control	Aqueous	Methanolic	Ethanolic	n-Hexane	Chloroform
500	25±0.05[ab]	34±0.7[cd]	34±0.9[ce]	35±0.03[de]	23±1.8[af]	21±0.06[bf]
750	25±0.05[a]	43±0.07	49±0.01	47±0.08	31±0.9	26±0.4[a]
1000	25±0.05	54±0.9[ab]	52±0.08[a]	55±0.6[b]	33±0.5	29±0.04

Values are mean ±standard error (n=3), Mean values with the same superscript in a row are not significantly different (P<0.05)

Table 4: Inhibition Zone of *E. aromatica* Extracts Against *M. globossa*

Concentration (mg/ml)	Mean zone of inhibition (mm) ± Standard error					
	Control	Aqueous	Methanolic	Ethanolic	n-Hexane	Chloroform
500	40±0.1[ab]	46±0.08[c]	47±0.4[c]	51±1.5	38±0.4 [ad]	40±0.01[bd]
750	40±0.1[ab]	54±0.07	49±1.8	55±0.9	41±0.02 [ac]	43±0.05[bc]
1000	40±0.1	59±0.5[a]	53±0.8	59±1.0 [a]	44±0.6[b]	46±0.06[b]

Values are mean ±standard error (n=3). Mean values with the same superscript in a row are not significantly different (P<0.05)

Table 5: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) of the Extracts of *E. aromatica* Against the Growth of *S. epidermidis*

Extracts		Concentration (mg/ml)							
		500	50	5	0.5	0.05	0.005	0.0005	0.00005
Aqueous	MIC	-	-	-	-	+	+	+	+
	MBC	-	+	+	+	+	+	+	+
Methanolic	MIC	-	-	-	+	+	+	+	+
	MBC	-	-	+	+	+	+	+	+
Ethanolic	MIC	-	-	-	+	+	+	+	+
	MBC	-	+	+	+	+	+	+	+
n-Hexane	MIC	-	-	-	-	+	+	+	+
	MBC	-	-	+	+	+	+	+	+
Chloroform	MIC	-	-	-	+	+	+	+	+
	MBC	-	+	+	+	+	+	+	+

+ = Growth; - = no growth.

The lowest inhibition zones were recorded at 500mg/mL, with inhibition zone diameters of 45, 47, 35, and 34 mm, respectively (Tables 1 and 4).

The highest inhibition zones of aqueous extract against the tested strains at 1000mg/ml were 64, 59, 30, and 54mm diameters, respectively, while the lowest zones of inhibition recorded at 500mg/ml were 52, 46, 30, and 34 mm diameters, respectively (Tables 1, 2, 3, and 4). The zones of inhibition exhibited by the chloroform extract of *E. aromtica* against the tested strains recorded at 1000mg/ml were 48, 46, 32, and 29 mm diameters respectively, while zones of inhibition recorded at 500mg/ml were 41, 40, 25and 21 mm diameters respectively (Tables 1, 2, 3, and 4).

Low efficacy was observed in the hexane extract, where the inhibition zones recorded at 1000mg/ml were 45, 44,

30, and 33 mm in diameter, respectively. The inhibition zones recorded at 500mg/ml were 41, 38, 21, and 23 mm in diameter, respectively (Tables 1, 2, 3, and 4).

Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC), and Minimum Bactericidal Concentration MBC of the Extract of *E. aromatica*

The MIC of *E. aromatica* ethanolic extract against the test organisms, *M. restricta*, *M. globossa*, *S. epidermidis,* and *P. acne* was 50mg/ml, 50mg/ml, 5mg/ml, and 50mg/ ml, respectively (Table 3.1, 3.3, 3.5, and 3.7). The MBC and MFC of all the test strains were observed at 500mg/ml except for *M. restricta,* which was observed at 50mg/ml. (Tables: 5, 6, 7, and 8). MIC of methanolic extract of clove against test organisms was found to be 50mg/ml for both *M. restricta M globossa,* and that of *S. epidermidis* and *P. acne* was 5mg/ml. The MBC and MFC of all the test strains happened to be 500mg/ml, except for *M. restricta,* which was found to be 50mg/ml. (Tables: 5, 6, 7, and 8).

Table 6: Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC) of the Extracts of *E. aromatica* Against the Growth of *M. restricta*

Extracts		Concentration (mg/ml)							
		500	50	5	0.5	0.05	0.005	0.0005	0.00005
Aqueous	MIC	-	-	+	+	+	+	+	+
	MFC	-	+	+	+	+	+	+	+
Methanolic	MIC	-	-	+	+	+	+	+	+
	MFC	-	+	+	+	+	+	+	+
Ethanolic	MIC	-	-	+	+	+	+	+	+
	MFC	-	-	+	+	+	+	+	+
n-Hexane	MIC	-	-	-	+	+	+	+	+
	MFC	-	+	+	+	+	+	+	+
Chloroform	MIC	-	-	+	+	+	+	+	+
	MFC	-	-	+	+	+	+	+	+

+ = Growth; - = no growth.

Table 7: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) of the Extracts of *E. aromatica* Against the Growth of *P. acne*

Extracts		Concentration (mg/ml)							
		500	50	5	0.5	0.05	0.005	0.0005	0.00005
Aqueous	MIC	-	-	-	-	+	+	+	+
	MBC	-	+	+	+	+	+	+	+
Methanolic	MIC	-	-	-	+	+	+	+	+
	MBC	-	-	+	+	+	+	+	+
Ethanolic	MIC	-	-	-	+	+	+	+	+
	MBC	-	+	+	+	+	+	+	+
n-Hexane	MIC	-	-	-	-	+	+	+	+
	MBC	-	-	+	+	+	+	+	+
Chloroform	MIC	-	-	-	+	+	+	+	+
	MFC	-	-	+	+	+	+	+	+

+ = Growth; - = no growth.

Table 8: Minimum Inhibitory Concentration (MIC), Minimum Fungicidal Concentration (MFC)of the Extracts of *E. aromatica* Against the Growth of *M. globossa*

Extracts		Concentration (mg/ml)							
		500	50	5	0.5	0.05	0.005	0.0005	0.00005
Aqueous	MIC	-	-	+	+	+	+	+	+
	MFC	-	+	+	+	+	+	+	+
Methanolic	MIC	-	-	+	+	+	+	+	+
	MFC	-	-	+	+	+	+	+	+
Ethanolic	MIC	-	-	+	+	+	+	+	+
	MFC	-	+	+	+	+	+	+	+
n-Hexane	MIC	-	-	+	+	+	+	+	+
	MFC	-	+	+	+	+	+	+	+
`Chloroform	MIC	-	-	+	+	+	+	+	+
	MFC	-	+	+	+	+	+	+	+

+ = Growth; - = no growth.

Table 9: .GC-MS Chromatogram of the Bioactive Compounds Present in *Eugenia aromatica*

Peak	RT (min)	Area (%)	Name of Compound	Molecular Weight	Molecular Formula
1	7.482	45.90	Phenol, 2-methoxy-3-(2-propenyl)	164	$C_{10}H_{12}O$
2	7.873	18.05	Eugenol	164	$C_{10}H_{12}O_2$
3	12.185	0.14	Caryophyllene oxide	220	$C_{15}H_{24}O$
4	13.608	0.58	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecan-5-β-ol	220	$C_{15}H_{24}O$
5	13.902	0.01	2-Isopropylidene-3-methylhexa-3,5-dienal	150	$C_{10}H_{14}O$
6	13.947	0.01	cis-p-mentha-1(7),8-dien-2-ol	150	$C_{10}H_{16}O$
7	17.300*	0.09	2H-Benzocyclohepten-2-one, octahydro-4a-methyl-(S)-	178	$C_{12}H_{18}O$
8	14.211	0.03	Ar-tumerone	216	$C_{15}H_{20}O$
9	14.244	0.08	2-Methyl-2-vinyloxirane	216	C_5H_8O
10	14.526	0.08	7'-Oxaspiro[...]nonan-2'-one	164	$C_8H_{13}NO_2$
11	17.484	0.00	1-Methylbicyclo[3.2.1]octane	208	C_9H_{16}
12	17.567	0.01	cis-Z-α-Bisabolene epoxide	220	$C_{15}H_{24}O$
13	18.858	0.04	1,1,7-Trimethyl-[...]-3,6-diol	238	$C_{15}H_{26}O_2$
14	18.878	0.01	Culmorin	238	$C_{15}H_{26}O_2$
15	21.561	2.55	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
16	22.075	0.03	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
17	23.219	0.05	Oleic acid	284	$C_{18}H_{34}O_2$
18	24.746	16.69	cis-13-Octadecenoic acid	284	$C_{18}H_{34}O_2$
19	25.139	6.93	Octadecanoic acid	284	$C_{18}H_{36}O_2$
20	29.905	0.07	Octacosyl propyl ether	452	$C_{31}H_{64}O$
21	30.089	0.41	Tetracosyl heptafluorobutyrate	180	$C_{28}H_{49}F_7O_2$
22	30.112	0.19	10-Heneicosene (c,t)	294	$C_{21}H_{42}$
23	30.178	0.09	2-Methyl-Z,Z-3,13-octadecadienol	280	$C_{19}H_{36}O$
24	30.235	0.25	1-Octadecene	252	$C_{18}H_{36}$
25	30.301	0.18	Tetrapentacontane	835	$C_{54}H_{110}$
26	30.391	0.13	1-Nonadecene	266	$C_{19}H_{38}$
27	30.453	0.24	2-Methyl-Z,Z-3,13-octadecadienol	280	$C_{19}H_{36}O$
28	30.548	0.29	Octadecanal	250	$C_{18}H_{36}O$
29	30.586	0.22	Methyl-Z-10-tetradecen-1-ol acetate	268	$C_{17}H_{32}O_2$
30	30.655	0.23	cis-10-Nonadecenoic acid	278	$C_{19}H_{32}O_2$
31	30.813	0.43	Z-8-Methyl-9-tetradecenoic acid	240	$C_{15}H_{28}O_2$
32	30.862	0.08	Pentadecafluorooctanoic acid, octadecyl ester	648	$C_{26}H_{37}F_{15}O_2$
33	31.101	0.47	Tetrapentacontane, 1,54-dibromo-	835	$C_{54}H_{108}Br$
34	31.136	0.28	Tricosane	324	$C_{23}H_{48}$
35	31.184	0.32	Cyclotetradecane[...]	280	$C_{20}H_{40}$
36	31.253	0.27	1-Docosene	308	$C_{22}H_{44}$
37	31.301	0.22	1-Docosene	308	$C_{22}H_{44}$
38	31.344	0.17	cis-Vaccenic acid	284	$C_{18}H_{34}O_2$
39	31.414	0.33	Tetratriacontyl heptafluorobutyrate	672	$C_{34}H_{61}F_7O_2$
40	31.524	0.59	3-Eicosene (E)	280	$C_{20}H_{40}$
41	31.553	0.16	Oleic acid	264	$C_{18}H_{34}O_2$
42	31.610	0.42	Octadecanal	250	$C_{18}H_{36}O$
43	31.678	0.38	1-Docosene	308	$C_{22}H_{44}$
44	31.736	0.29	Erucic acid	320	$C_{22}H_{42}O_2$
45	31.785	0.43	Octadecanal	250	$C_{18}H_{36}O$
46	31.829	0.52	Oleic acid	264	$C_{18}H_{34}O_2$
47	31.907	0.65	Pentadecafluorooctanoic acid, heptadecyl ester	250	$C_{26}H_{37}F_{15}O_2$
48	33.288	0.20	Pentadecafluorooctanoic acid, octadecyl ester	648	$C_{26}H_{37}F_{15}O_2$
49	36.098	0.08	Tritetracontane	604	$C_{43}H_{88}$
50	38.266	0.12	Hentriacontane	436	$C_{31}H_{64}$
51	38.513	0.02	3-Eicosene (E)	280	$C_{20}H_{40}$

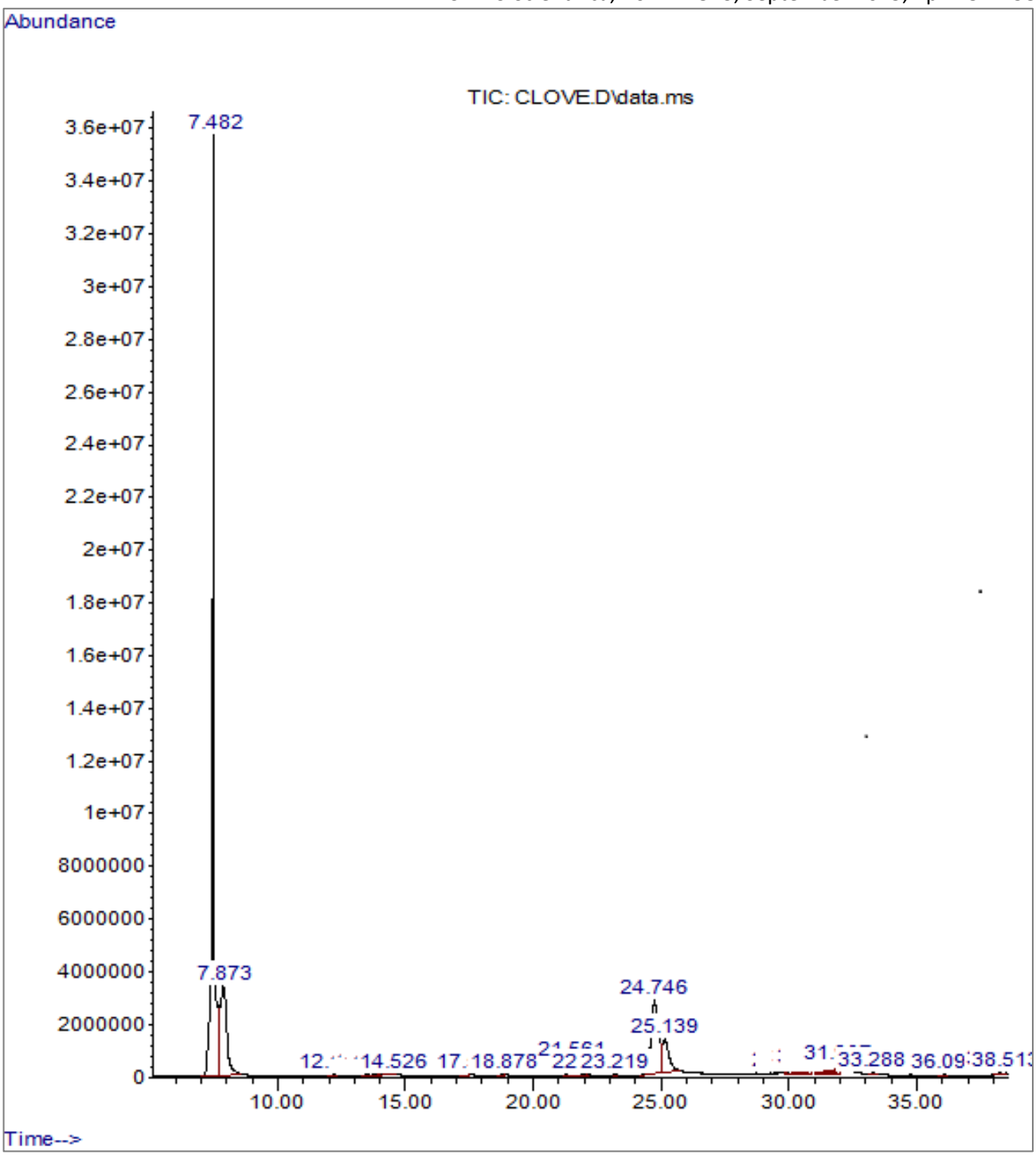

Figure. 1 .GC-MS chromatogram of the bioactive compounds present in *Eugenia aromatica*

The MIC of *E. aromatica* aqueous extract against *S. epidermidis* and *P. acne* was lower than that against both *M. restricta* and *M globossa.* Hence, *M. restricta* and *M globossa* were the most sensitive to aqueous *E. aromaica* extract. The MIC of aqueous extract of clove against test organisms was found to be 50mg/ml for both *M. restricta* and *M. globossa,* and that of *S. epidermidis* and *P. acne* was 5mg/ml (Tables: 5, 6, 7, and 8). The MFC and MBC of all the isolates were shown to be 500mg/ml.

The MIC of chloroform extracts of *E. aromatica* against *M. restricta* and *M.globossa* were 50mg/ml both while S. *epidermidis* and *P. acne* demonstrated 5mg/ml. High MBCs of 500mg/ml were observed against *S. epidermidis* and *P. acne* with chloroform extract, and 50mg/ml MFC was observed in M. *restricta* and *M. globossa* (Tables: 5, 6, 7, and 8).

The MIC and MBC of both *S. epidermidis* and *P. acnes* with hexane extract were observed to be 0.5mg/ml and 50mg/ml, respectively. While that of MFC for M. *restricta* and *M.globossa* was shown to be the same at 50mg/ml, the MIC was seen at 5mg/ml and 50mg/ml, respectively (Tables: 5, 6, 7, and 8).

Gas Chromatography Mass Spectroscopy (GC-MS) Analysis of *Eugenia aromatica*

Analysis of the chemical composition of the *Eugenia aromatica* revealed fifty-one (51) chemical constituents as presented in Table 9. The partial chromatogram scan is presented in Figure 1. Among the major bioactive components analyzed by GC-MS were found to be Eugenol (45.90%), Eugenol (18.05%), cis-13-Octadecenoic acid (16.69%), Octadecanoic acid (6.93%), and n-Hexadecanoic acid (2.55%).

DISCUSSION

This study evaluated the antimicrobial efficacy and phytochemical composition of Eugenia aromatica (clove) extracts against dandruff-associated fungi (Malassezia restricta and M. globosa) and bacteria (Staphylococcus epidermidis and Propionibacterium acnes), with supporting GC–MS profiling.

Overall, all solvent extracts exhibited antimicrobial activity, though with varying intensities depending on solvent polarity, concentration, and organism type. The ethanolic extract demonstrated the strongest activity, producing inhibition zones of 60 mm and 59 mm against *M. restricta* and *M. globosa*, respectively, at 1000 mg/mL. Comparable activity was observed against bacterial strains, albeit with smaller inhibition zones. The ethanolic extract also yielded the lowest MIC (5–50 mg/ml) and MBC/MFC (50–500 mg/ml) values, indicating strong fungicidal potential, particularly against M. restricta. Methanolic extracts followed a similar pattern, confirming that ethanol and methanol efficiently extract key antimicrobial compounds, especially phenolics.

Aqueous extracts also inhibited both fungi and bacteria, though higher concentrations were required to achieve complete microbial killing. In contrast, chloroform and hexane extracts showed comparatively weaker effects. Despite the hexane extract showing low MICs against bacterial isolates, its high MBC/MFC values suggested limited bactericidal activity. Collectively, these results affirm that polar solvents enhance the recovery of bioactive agents from *E. aromatica* buds.

The observed antimicrobial trends align with previous studies demonstrating the inhibitory effects of clove extracts on Malassezia, Candida albicans, P. acnes, and S. epidermidis (Gonelimali et al., 2018; Mansourian et al., 2014; Fu et al., 2009). Earlier findings have attributed these effects to the phenolic constituents, especially eugenol and carvacrol, which disrupt fungal ergosterol biosynthesis and compromise microbial membranes (Pinto et al., 2009; Chami et al., 2005). Similar antibacterial and antifungal properties of clove against diverse Gram-positive and Gram-negative pathogens have also been reported (Duraipandiyan et al., 2006; Gupta et al., 2013; Kumar et al., 2012).

GC–MS analysis of the ethanolic extract revealed 51 phytoconstituents, dominated by eugenol (45.90% and 18.05%), cis-13-octadecenoic acid (16.69%), octadecanoic acid (6.93%), and n-hexadecanoic acid (2.55%). The abundance of eugenol—renowned for its antimicrobial, antifungal, and anti-inflammatory actions—explains the high efficacy of the ethanolic and methanolic extracts. Fatty acids such as oleic, stearic, and palmitic acids likely act synergistically by perturbing microbial membranes, thereby enhancing the overall antimicrobial potency. This pattern of validating traditional use through phytochemical and bioactivity analysis is well-established, as seen in studies on *Carica papaya*, where its traditional use for treating ulcers was corroborated by identifying cytoprotective compounds like flavonoids and tannins in its extracts (Aliyu et al., 2023)

The findings corroborate earlier reports highlighting the multifaceted biological roles of eugenol and long-chain fatty acids, including antibacterial, antifungal, antioxidant, and anti-inflammatory activities (Park et al., 2007; Li et al., 2005; Adeniyi et al., 2019; Sunita et al., 2017). Thus, the combined phytochemical richness and strong antimicrobial efficacy of *E. aromatica* make it a promising natural candidate for developing antifungal and antidandruff formulations.

CONCLUSION

This study demonstrated that *Eugenia aromatica* (clove) extracts possess significant antimicrobial activity against both fungal (*Malassezia restricta*, *M. globossa*) and bacterial (*Staphylococcus epidermidis*, *Propionibacterium acnes*) pathogens commonly associated with skin and scalp infections. Among the tested solvents, the ethanolic extract consistently showed the highest antimicrobial potency, likely due to its superior extraction efficiency of key bioactive compounds, particularly *eugenol.*

The GC-MS analysis confirmed the presence of fifty-one (51) chemical constituents in *E. aromatica*, with *eugenol* being the dominant compound. The results from the MIC and MBC/MFC tests further supported the inhibition zone data, demonstrating a clear dose-dependent antimicrobial effect and highlighting the extracts' fungistatic, fungicidal, and bactericidal potentials.

Overall, these findings validate the traditional use of clove for treating microbial infections and emphasize its promise as a source of natural antimicrobial agents. Further research is recommended to isolate, purify, and characterize the individual bioactive compounds, assess their synergistic effects, and evaluate their safety and efficacy in clinical applications or as potential ingredients in antimicrobial formulations for skin and scalp care.

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