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GC-MS analysis and Synergistic Inhibition of *Staphylococcus aureus*, *Streptococcus pyogenes* and Dermatophytes by Novel Plant Oil Blends Developed for Skin and Hair Therapy

Saadiya Hamisu and *Baha'uddeen Salisu

Department of Microbiology, Umaru Musa Yar'adua University, Katsina, Nigeria

*Correspondence: bahauddeen.salisu@umyu.edu.ng

Abstract

Skin and hair infections caused by bacterial and fungal pathogens pose significant health risks exacerbated by the rising prevalence of antimicrobial resistance (AMR). This study formulated and evaluated the antimicrobial potential of plant-based oil blends against selected pathogens that affect the skin and hair. Initially, phytochemical screening of individual plant oils, including garlic, tea tree, black seed, rosemary, moringa, lavender, sesame, coconut, and palm kernel oils was conducted, which revealed the presence of bioactive compounds such as alkaloids, saponins, steroids, and cardiac glycosides, which contribute to their antimicrobial properties. Thereafter, three oil formulations (A, B, and C) were developed and assessed for antimicrobial activity against Staphylococcus aureus, Streptococcus pyogenes, Trichophyton tonsurans, ***and*** Malassezia globosa ***using the disk diffusion assay and the 96-well plate method, in duplicate. The results showed that all formulations exhibited varying antimicrobial activity, with Formulation A demonstrating the highest inhibition against*** Staphylococcus aureus ***(23.3±7.8 mm), while Formulation B showed the strongest activity against*** Streptococcus pyogenes ***(10.9±12.7 mm),*** Trichophyton tonsurans ***(28.1±9.8 mm), and*** Malassezia globosa ***(12.7±0.6 mm). However, none of the formulations exhibited a bactericidal or fungicidal effect at the tested concentration. GC-MS analysis of Formulation B, which showed the broadest antimicrobial spectrum, identified 21 bioactive constituents, including terpenes, esters, aldehydes, and fatty acids, which may contribute to its antimicrobial properties. These findings suggest that the formulated plant-based oil blends have potential as natural antimicrobial agents, capable of inhibiting bacterial and fungal growth, in addition to their use in skin and hair treatments. However, further optimization and in vivo studies are necessary to assess their therapeutic efficacy and potential application in skin and hair care formulations.***

Keywords: Antimicrobial resistance (AMR), Dematophytoses, Hair pathogens, Oil blends, Skin pathogens

INTRODUCTION

Human skin and hair infections are caused by microorganisms such as *Staphylococcus aureus, Escherichia coli,* and *Candida albicans*. Individuals are susceptible to these infections, which may range in severity from irritations to severe health problems (Nogueira *et al.*, 2020). The therapeutic effects of natural plant-based oils against infections have drawn significant attention. These oils contain abundant bioactive compounds with antibacterial, antifungal, and anti-inflammatory properties. Natural oils such as tea tree oil, neem oil, and lavender oil serve as alternatives to synthetic antimicrobial drugs due to their considerable effectiveness against certain skin and hair infections (Elnakady *et al.*, 2021; Gull *et al.*, 2022). The phytochemical compounds present in these oils, including terpenes, phenolics, flavonoids, and alkaloids, confer them with antimicrobial and antioxidant properties that contribute to their effectiveness (Tan *et al.*, 2020). There are significant health threats globally due to skin and hair infections caused by bacterial and fungal pathogens; therefore, the need for alternative treatments is emphasized due to the increasing prevalence of antimicrobial resistance. Natural plant-based oils offer a promising solution due to their effectiveness, safety, antioxidant properties, and antimicrobial capabilities (Gupta *et al.*, 2019). The rise in antimicrobial resistance (AMR) necessitates the use of traditional antimicrobial agents as alternatives to synthetic drugs.

Plant-based oils have been proposed as suitable alternatives to antimicrobial drugs, given their broad-spectrum antimicrobial properties and minimal potential for resistance development. Unlike synthetic antimicrobial drugs, which are often associated with adverse effects and the emergence of resistant strains, natural oils are considered safe and have fewer side effects (Mittal *et al.*, 2019). This positions them as viable options for use in skin and hair care products. We hypothesized that combining plant-based oils would create a potent blend and potentially enhance their antimicrobial activity, thereby fostering a synergistic effect that could aid in the treatment of skin and hair infections.

MATERIALS AND METHODS

Selection of Individual Plant-Based Oils for Blend

A literature review was conducted to select individual oils with known antimicrobial activity. The selected oils include; Tea tree oil (Nascimento *et al.*, 2023; Jain *et al.*, 2022) moringa seed oil (Gharsallah *et al.*, 2021), garlic oil (Mahmoud *et al.*, 2020), black seed oil (Zouirech *et al.*, 2022), rosemary oil (Ciotea *et al.*, 2021; Donato *et al.*, 2020) palm kernel oil (Akpomie *et al.*, 2020), coconut oil (Akpomie *et al.*, 2020), sesame oil (Baqer, 2020) and lavender oil (AbdRashed *et al.*, 2021).

Preliminary Phytochemical Screening of Selected Plant-Based Oils

Phytochemical screening was conducted for the selected oils: tea tree oil, moringa seed oil, garlic oil, black seed oil, rosemary oil, palm kernel oil, coconut oil, sesame oil, and lavender oil, using analytical grade reagents to detect phytochemical compounds with potential therapeutic effects. The following procedures were employed to perform the screening in clean test tubes. All procedures were carried out at room temperature.

Saponins: 0.5 mL of oil was added to 10 mL of distilled water. The solution was shaken, and a layer of foam was observed within 15 minutes at room temperature.

Alkaloids: 0.15mL of Mayer's reagent was added to 1 mL of oil and allowed to sit for 10 minutes. The formation of a white creamy precipitate indicates the presence of alkaloids (Shaik and Patil, 2020).

Phenolic compounds: 1 mL of the oil blend was diluted in 5 mL of distilled water, followed by the addition of 0.15mL of 5% $FeCl_3$. The presence of a dark green or blue precipitate indicates a positive result (Shaik and Patil, 2020).

Tannins: 0.15mL of $FeCl_3$ was added to 1 mL of the oil blend and allowed to sit for 2 minutes (Shaik and Patil, 2020).

Cardiac glycosides: 0.05 mL of 5% $FeCl_3$ and 1 mL of H_2SO_4 were added to a test tube containing 0.5 mL of the oil formulation (Olasupo *et al.*, 2017).

Steroids: 2 mL of acetic anhydride was added to 0.5 mL of the oil formulation, followed by 2 mL of H_2SO_4 (Olasupo *et al.*, 2017).

Formulation of the blends based on the Phytochemical Distributions

The selected individual plant-based oils were used to create three different oil blends, based on their phytochemical content, to ensure that each formulation contained a sufficient number of phytochemical compounds. Early studies have shown that synergy is evident in 1:1 combination of oil blends (Bag and Chattopadhyay, 2015; Orchard and van Vuuren, 2017; Katiki *et al.*, 2017). According to this, formulations A and B were prepared as follows: Formulation A consisted of 1 mL each of garlic oil, tea tree oil, and black seed oil, resulting in a 1:1 combination.

Formulation B: 1 mL each of garlic oil, tea tree oil, black seed oil, rosemary, and moringa seed oil was mixed.

Formulation C was made with minimal modifications to A and B. Lavender oil, palm kernel oil, and sesame oil were mixed in equal ratios of 1 mL each, and 5 mL of coconut oil was added.

Screening of the Formulated Oil Blends for Antimicrobial Activity Against Common Skin and Hair Pathogens

Bacterial Isolates

Two bacterial isolates (*Staphylococcus aureus and Streptococcus pyogenes*) used for this study were obtained from the stock cultures of the Microbiology laboratory at the Department of Microbiology, Umaru Musa Yar'Adua University, Katsina, Nigeria. These isolates were subcultured on nutrient agar (Murray *et al.*, 2015; Siddiquee and Siddiquee, 2017) and then reconfirmed using Gram staining (Taylor and Unakal, 2021), as well as appropriate biochemical tests as described by Cheesbrough (2007).

Isolation and Identification of the Fungal Pathogens

Two fungal hair pathogens (*Trichophyton tonsurans* and *Malassezia globosa*) were used in this study. These isolates were isolated from volunteers as follows:

For *Trichophyton tonsurans*, a sterile swab stick was used to swab the affected area of a volunteer with *Tinea capitis* (Rasheed *et al.*, 2024). A liquid suspension of the collected sample was prepared with sterile normal saline. 1.5 g of Sabouraud dextrose broth was prepared in a conical flask with 50 mL of distilled water. The neck of the flask was flamed (Siddiquee, 2017), and the media was poured into test tubes. A small amount of the liquid sample was added to sterile Sabouraud dextrose broth and incubated at 37°C for 48 hours before being subjected to microscopic identification.

For *Malassezia globosa*, a sterile swab stick was used to scrape the infected area from a volunteer with dandruff. A liquid suspension of the sample was prepared with sterile normal saline. A small amount of the sample was added to sterile Sabouraud dextrose broth, and 2 drops of olive oil were added, as *Malassezia globosa* is lipid-dependent. It was then incubated at 37°C for 48 hours before being subjected to microscopic identification.

To identify the isolates, fungal structures were mounted on a sterile glass slide using a sterile wire loop (Senanayake *et al.*, 2020). Lactophenol cotton blue was added to stain the fungal structures, and then a cover slip was applied (Senanayake *et al.*, 2020). The slide was observed under a compound microscope using a 40x objective lens. Isolates were then identified based on their microscopic characteristics (types and arrangement of hyphae, conidiophore vesicle, conidia heads, ornamentation, phialides, and fruiting body) and macroscopic/cultural appearance on SDA and AFPA (colony colors, texture, reverse color, hyphae arrangement, conidia shape, and nature of spores) as described by Salisu *et al.*, (2020, 2022, 2024).

Antimicrobial sensitivity Test

Antimicrobial sensitivity tests (using disk diffusion assay and 96-well plate method) were carried out to check the effectiveness of the oil blends as follows:

Disk diffusion assay

Staphylococcus aureus and *Streptococcus pyogenes*

5.6 g of nutrient agar was prepared using 200 mL of distilled water, sterilized, and poured into petri dishes. 0.5 McFarland of each bacterial isolate was prepared, and 0.1 mL of the organism was spread on labeled plates using a sterile glass rod. Sterilized 6mm diameter of Whatman disc (Arbab *et al.*, 2022) soaked in the various 100% concentrations of the formulations, and controls were impregnated on the plates. The plates were incubated at 37ºC for 24 hours (Arbab et al., 2022). A vernier caliper was used to measure the zone of inhibition, extending from one edge of the clear zone to the other, both horizontally and vertically. Ampicillin was used as a positive control, and distilled water served as a negative control. The test was conducted twice to ensure its effectiveness.

Trichophyton tonsurans and *Malassezia globosa*

Sabouraud dextrose agar was prepared according to the manufacturer's instructions, followed by the preparation of 0.5 McFarland suspensions of each fungus. 0.1 mL of each organism was spread on plates using a sterile glass rod, with one plate for each oil formulation, as well as a positive and negative control. Sterilized 6mm diameter Whatman discs (Arbab et al., 2022), already soaked in the formulations and controls, were placed on labeled plates (Orchard and Van Vuuren, 2017). Plates were incubated at 37°C for 48 hours. Ketoconazole was used as the positive control, and distilled water served as the negative control. The formulations were used at 100% concentration, that is, they were undiluted. A vernier caliper was used to measure the zone of inhibition, extending from one edge of the clear zone to the other, both horizontally and vertically. The test was carried out twice.

96-well plate method

Staphylococcus aureus and *Streptococcus pyogenes*

96 well plates were used to carry out tests for both *Staphylococcus aureus* and *Streptococcus pyogenes*. Each well was inoculated with 0.1 mL nutrient broth, 0.1 mL of any of the three oil formulations, and 0.1 mL of the organism suspension. Three wells were used as control for each organism. One contained 0.1 mL each of nutrient broth, bacterial sample, and ampicillin, another contained 0.1 mL each of nutrient broth, bacterial sample, and the formulation, and the last contained nutrient broth only. The inoculated plate was incubated at 37°C for 24 hours. After 24 hours, a sterile wire loop was dipped into each well and placed on a labeled petri dish containing nutrient agar. The plates were incubated at 37°C, and growth was observed after 24 hours. This process was used due to the color of the essential oils as it may interfere with turbidimetric readings (Borman *et al.*, 2017).

Trichophyton tonsurans and *Malassezia globosa* Sabouraud dextrose broth was prepared according to manufacturer's instruction. 96 well plates were used to carry out MBC for both *Trichophyton tonsurans* and *Malassezia globosa*. Each well was inoculated with 0.1 mL of Sabouraud dextrose broth, 0.1 mL of one of the three oil formulations, and 0.1 mL of the organism suspension. Three wells were used as controls for each organism; one contained 0.1 mL each of Sabouraud dextrose broth, fungal sample, and ketoconazole, another contained 0.1 mL each of Sabouraud dextrose broth, fungal sample, and the formulations, and the last contained Sabouraud dextrose broth only. The inoculated plate was incubated at 37°C for 48 hours. After 48 hours, a small amount of content from each well was inoculated onto a labeled petri dish containing Sabouraud dextrose agar. The plates were incubated at 37°C, and growth was observed after 48 hours (Borman *et al.*, 2017).

Identification of the compounds in the formulation with the highest antimicrobial activity by gas chromatography-mass spectrometry

Formulation B, which exhibited the highest antimicrobial activity against all the test isolates, was selected as the successful formulation and analyzed by gas chromatography-mass spectrometry, as it most effectively inhibited the growth of the pathogens. The sample was dried in an oven at 105°C, ground, and extracted using N-Hexane (Anh *et al.*, 2019). GC-MS analysis was conducted on the GCMS-QP2010 PLUS SHIMADZU. The column employed was the PerkinElmer Elite-5 capillary column, measuring 30 m × 0.25 mm, with a film thickness of 0.25 µm, composed of 95% dimethyl polysiloxane. The carrier gas used was helium, at a flow rate of 0.5 mL/min. A 1 µL sample injection volume was utilized. The inlet temperature was maintained at 250°C. The oven temperature was initially programmed to 80°C for 4 minutes, and then increased to 200°C. The temperature was subsequently programmed to rise to 280°C at a rate of 20°C per minute, reaching this temperature within 5 minutes. The total run time was 25 minutes. The MS transfer line was maintained at a temperature of 200°C, while the source temperature was kept at 180°C. GC-MS analysis employed electron impact ionization at 70 eV (Jaji *et al.*, 2024), and the data were evaluated using total ion count (TIC) for compound identification and quantification. The spectra of the components were compared with the database of known components stored in the GC-MS library to identify the compounds in the formulation (Salisu and Shema, 2019).

RESULTS AND DISCUSSION

Phytochemical screening

Table 1 presents the results of screening for phytochemical compounds in the individual oils. The presence of compounds such as alkaloids, Saponins, and cardiac glycosides shows the potency of these oils to exhibit antimicrobial properties. A change of color or appearance of precipitate indicated a positive result.

Table 1: Phytochemical screening of selected individual plant-based oils

Phytochemical Compound	Inference	G	T	R	B	M	L	S	C	P
Saponin	Produce foam	+	+	+	+	+	-	-	-	+
Alkaloid	White Creamy precipitate	+	+	+	+	+	-	-	-	+
Phenolic Compounds	Bluish-green precipitate	-	-	+	-	-	-	-	-	-
Tannins	Dark green	-	-	-	-	-	+	-	-	+
Cardiac Glycoside	Brown at interface	-	+	-	-	+	+	+	+	-
Steroid	Reddish brown	+	+	+	+	+	+	+	+	-

Key: (+) indicates positive, (-) indicates negative, G= Garlic oil, T= Tea tree oil, R= Rosemary oil, B= Black seed oil, M= Moringa seed oil, L= Lavender oil, S= Sesame oil, C= Coconut oil, P= Palm kernel oil.

Formulation of oil blends

Individual oils were mixed together to form each oil blend (Table 2). Formulation A was created from a mixture of three oils, formulation B was created from a mixture of five oils, and formulation C was created from a mixture of four oils. These formulations contain a mixture of the individual oils as discussed and are thought to exhibit antimicrobial activity against the selected pathogens.

Table 2: Formulation of oil blends

Formulation A	Formulation B	Formulation C
Garlic oil	Garlic oil	Coconut oil
Tea tree oil	Rosemary oil	Lavender oil
Black seed oil	Tea tree oil	Sesame oil
	Black seed oil	Palm kernel oil
	Moringa seed oil	

Identification of the isolates

Table 3 describes the appearances of the different bacterial and fungal pathogens used in this research under a compound microscope. It includes columns that show the appearance of various organisms based on the ability of the bacterial samples to retain the color of crystal violet, their shapes, arrangement, and the shape of conidia/microconidia and hyphae of the fungal samples. Biochemical tests (catalase, coagulase, H2S, and pyrrolidonyl) were carried out to identify these organisms, although the tests performed were not applicable to fungi. Positive results for the catalase and coagulase tests were observed in one organism, confirming it to be *Staphylococcus aureus*. However, *Streptococcus pyogenes* turned out to be catalase and coagulase-negative; however, being ß-hemolytic further differentiated the organism from *Staphylococcus aureus* and *Streptococcus pneumoniae*.

Table 3: Microscopic identification of samples

Isolates	Isolate type	Microscopic appearance	Biochemical Tests			
			Cat	Coa	Hea	Pyr
Staphylococcus aureus	Bacteria	Gram-positive cocci in clusters	+	+	-	-
Streptococcus pyogenes	Bacteria	Gram-positive cocci in chains	-	-	ß-hea	+
Trichophyton tonsurans	Fungi	Tear drop shaped micro-conidia that protrude from hyphae	NA	NA	NA	NA
Malassezia globosa	Fungi	A cluster of yeast cells attached to a long hyphae	NA	NA	NA	NA

Key: (+) = positive, (-) = Negative, NA = Not applicable, Cat = Catalase, Coa = Coagulase, Hea = Heamolysis on blood agar, Pyr =L-pyrrolidonyl-ß-naphthylamide

Antimicrobial sensitivity test

Table 4 shows the level of activity of formulations A, B and C in inhibiting the growth of bacterial and fungal pathogens responsible for causing the following infections: *Staphylococcus aureus* (folliculitis, eczema, carbuncles), *Streptococcus pyogenes* (impetigo), *Trichophyton tonsurans* (Tinea capitis), and *Malassezia globosa* (dandruff). All formulations and controls were tested at 100% concentration only. The mean and standard deviations of the zone of inhibition were calculated for each formulation against the respective organisms. All formulations showed a level of activity, but formulation A exhibited greater activity against *Staphylococcus aureus*. Formulation B showed strong inhibition against *Streptococcus pyogenes*, *Trichophyton tonsurans*, and *Malassezia globosa* to a great extent. Formulation C showed moderate activity against all organisms, with the lowest activity observed in *Staphylococcus aureus*. The positive controls, which included ampicillin for bacteria and ketoconazole for fungi, also showed activity. No zone of inhibition was observed for the negative control.

Table 4: Antimicrobial sensitivity test- Disk diffusion assay

Oil blends	Concentration (%)	Zone of Inhibition (mm)			
		Staphylococcus aureus	*Streptococcus pyogenes*	*Trichophyton tonsurans*	*Malassezia Globosa*
Formulation A	100	23.3±7.8	7.3±8.9	19.7±3.4	8.5±1.7
Formulation B	100	3.8±4.4	10.9±12.7	28.1±9.8	12.7±0.6
Formulation C	100	17.2±10.6	5.0±6.2	11.2±9.1	7.2±0
Positive control	10µg	19.9±1.4	16.4±4.7	18.7±1.9	13±1.5
Negative control	Distilled water	N.I	N.I	N.I	N.I

Key: (N.I) indicates no inhibition. All figures were approximated to two decimal places.

The antimicrobial effect of all three formulations was compared statistically using one-way ANOVA to determine the significant effect of the formulations, that is, to see which formulation is more or less effective than the others. However, it revealed that there is no significant difference between the zones of inhibition exhibited by the formulations (Table 5).

Table 5: Analysis of Variance for Formulations

Formulations	*Streptococcus pyogenes*	*Trichophyton tonsurans*	*Malassezia globosa*	*Staphylococcus aureus*
Formulation A	11.975±16.83[a]	15.10±6.74[a]	17.95±2.30[a]	15.7±13.23[a]
Formulation B	9.4±11.81[a]	13.93±12.85[a]	17.6±4.00[a]	13.93±13.40[a]
Formulation C	20±13.0[a]	7.3±0.34[a]	17.10±4.00[a]	15.30±4.41[a]
Control	14.05±4.80[a]	4.23±5.0[a]	18.93±3.80[a]	13.83±5.92[a]
Total	13.86±11.80	10.13±8.30	17.90±3.3	14.70±9.10

Key: (a) indicate no significant differences at P<0.05

96 well plate method

Table 6 shows the ability of the oil blends to kill 99.9% of *Staphylococcus aureus, Streptococcus pyogenes, Trichophyton tonsurans,* and *Malassezia globosa,* demonstrating bactericidal and fungicidal activity. The antibiotics ampicillin and ketoconazole were used against bacteria and fungi, respectively. It is observed that all three oil blends and ampicillin were unable to kill the organisms at a 100% concentration. Ketoconazole exhibited fungicidal activity against *Trichophyton tonsurans*.

Table 6: Antimicrobial sensitivity test- 96 well plate method

Oil blends	Concentration (%)	*Staphylococcus aureus*	*Streptococcus pyogenes*	*Trichophyton tonsurans*	*Malassezia globosa*
Formulation A	100	-	-	-	-
Formulation B	100	-	-	-	-
Formulation C	100	-	-	-	-
Antibiotic	100	-	-	+	-

Key: (-) indicates negative result, (+) indicate positive result

Identification of the compounds in the formulation with the highest antimicrobial activity by gas chromatography-mass spectrometry

The Total Ion Chromatogram for the 100% concentration of Formulation Boil blend is shown in Figure 1. The chromatogram peaks were compared with the data spectrum of known components in the GC-MS NIST library to identify the compounds. This analysis revealed 21 constituents present in the oil blend (Table 7). The table displays the phytochemical compounds detected in the blend, along with their % peak area, IUPAC names, molecular formula, and structure. Twenty-one compounds were matched and identified in this analysis.

The major constituents were at peak 16 (Isobutylene bromide with a peak area of 16.68%), peak 21 (linoleoyl chloride with a peak area of 10.79%), peak 9 (Vanillylacetone with a peak area of 9.69%), peak 15 (3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)- with a peak area of 9.48%), peak 17 (Ethyl 3-(4-hydroxy-3-methoxyphenyl)propionate with a peak area of 8.34%), peak 4 (Humulene with a peak area of 8.22%), peak 19 (1-Bromo-3-(2-bromoethyl)heptane with a peak area of 7.83%), peak 18 (Cyclododecene epoxide with a peak area of 7.74%), and peak 14 (Z-10-Pentadecen-1-ol with a peak area of 5.24%). The remaining constituents made up a 16% composition by peak area.

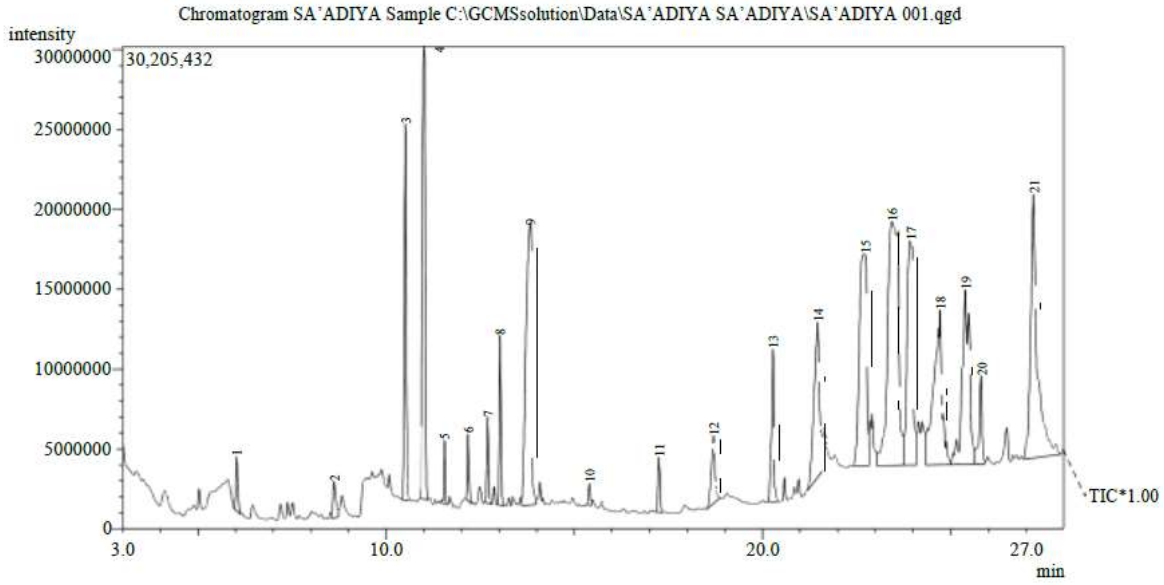
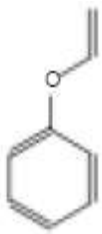
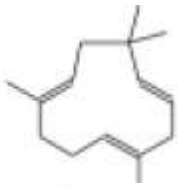
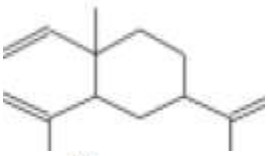
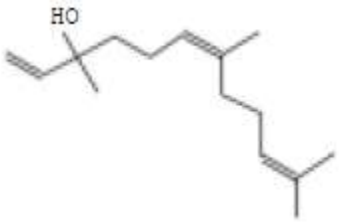
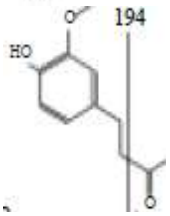
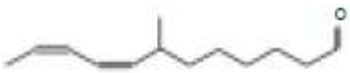
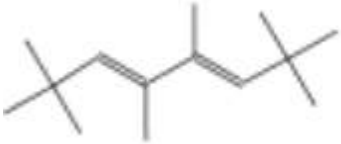
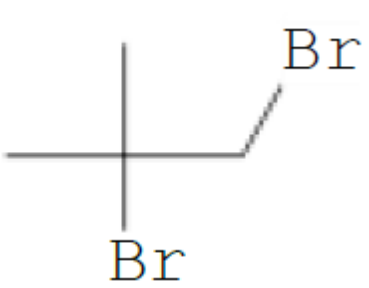
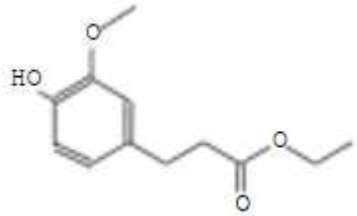
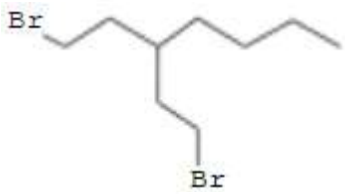
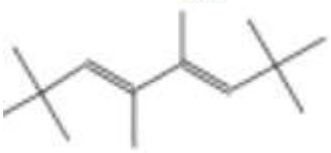


Figure 1: Total Ion Chromatogram of 100% concentration of Formulation B oil blend

Table 7: Total ions chromatogram (TIC)

Peak number	Retention Time	%Area	IUPAC Name	Molecular Formular	Structure
1	6.03	0.66	Linalol	C10H18O	
2	8.62	0.68	Benzene (etheynyloxy)	C8H8O	
3	10.51	4.41	Trans-alpha-Bergamotene	C15H24	

4	11.01	8.22	Humulene	C15H24	
5	11.55	0.59	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-	C15H24	
6	12.17	0.67	(+)-Nerolidol	C15H26O	
7	12.69	0.87	Z,Z,Z-4,6,9-Nonadecatriene	C19H34	
8	13.03	1.75	Pinane, 2,3-epoxy-	C10H16O	
9	13.83	9.69	Vanillylacetone	C11H14O3	
10	15.40	0.27	7-Methyl-Z-8,10-dodecadienal	C13H22O	
11	17.25	0.71	Methyl 14-methylpentadecanoate	C17H34O2	
12	18.70	1.55	Hexadecanoic acid	C16H32O2	
13	20.28	2.23	11-Octadecenoic acid, methyl ester	C19H36O2	
14	21.47	5.24	Z-10-Pentadecen-1-ol	C15H30O	
15	22.72	9.48	3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)-	C14H26	

16	23.43	16.68	Isobutylene bromide	C4H8Br2	
17	23.92	8.34	Ethyl 3-(4-hydroxy-3-methoxyphenyl)propionate	C12H16O4	
18	24.72	7.74	Cyclododecene epoxide	C12H22O	
19	25.38	7.83	1-Bromo-3-(2-bromoethyl)heptane	C9H18Br2	
20	25.80	1.62	3,5-Octadiene, 2,2,4,5,7,7-hexamethyl-, (E,Z)	C14H26	
21	27.20	10.79	linoleoyl chloride	C18H31ClO	

DISCUSSION

The findings of this study demonstrate the antimicrobial potential of plant-based oil blends against common bacterial and fungal pathogens associated with skin and hair infections. The observed antimicrobial activity across all formulations, though varying in potency, supports the hypothesis that natural oils, particularly when used in combination, possess promising therapeutic properties.

The phytochemical screening of the individual oils confirmed the presence of several bioactive compounds, including saponins, alkaloids, steroids, and cardiac glycosides, which are compounds known to exhibit antimicrobial properties (Shaikh & Patil, 2020; Olasupo et al., 2017). For instance, alkaloids and saponins are reported to disrupt microbial membranes, inhibit enzyme function, and modulate host immune responses (Tan et al., 2020). The positive results across most oils suggest a broad phytochemical base that may be harnessed for therapeutic use. Notably, oils like garlic, tea tree, rosemary, and black seed demonstrated strong profiles, which is consistent with prior reports of their antimicrobial efficacy (Mahmoud et al., 2020; Donato et al., 2020; Zouirech et al., 2022).

The disk diffusion assay revealed that all three formulations showed inhibitory effects on the test organisms, with Formulation A demonstrating the strongest inhibition against *Staphylococcus aureus* (23.3±7.8 mm), a common cause of folliculitis and eczema.

Conversely, Formulation B displayed the broadest spectrum of activity, especially against *Streptococcus pyogenes*, *Trichophyton tonsurans*, and *Malassezia globosa*, suggesting that the inclusion of rosemary and moringa oil may have enhanced its antifungal and antibacterial efficacy (Gharsallah et al., 2021; Ciotea et al., 2021). The variability in inhibitory zones between the formulations may be attributed to the different chemical profiles and synergistic interactions between the constituents (Bag & Chattopadhyay, 2015; Orchard & van Vuuren, 2017).

Despite the evident antimicrobial activity, the 96-well plate method revealed no bactericidal or fungicidal effects of the oil formulations at 100% concentration. This finding indicates that while the oils can inhibit microbial growth, they may not completely eradicate the pathogens, especially in vitro. The absence of microbial kill at full strength underscores the complexity of essential oil-based formulations and highlights the potential need for formulation refinement, such as the inclusion of permeation enhancers or the use in combination with standard antimicrobials (Mittal et al., 2019).

The GC-MS analysis of Formulation B, the most active blend, identified 21 bioactive compounds, including isobutylene bromide, linoleoyl chloride, vanillylacetone, humulene, and ethyl 3-(4-hydroxy-3-methoxyphenyl)propionate.

These compounds, particularly terpenes and phenolic esters, are known to have antimicrobial, anti-inflammatory, and antioxidant properties (Elnakady et al., 2021; Tan et al., 2020). The significant presence of compounds such as humulene and vanillylacetone, previously documented for membrane-disruptive and enzyme-inhibitory actions, likely contributed to the observed inhibition (Jaji et al., 2024).

While this study confirms the antimicrobial potential of plant-based oil blends, it also underscores several limitations. The lack of bactericidal and fungicidal activity may point to issues such as poor bioavailability, volatility, or insufficient contact time. Moreover, the formulations were not tested against resistant strains or under simulated skin conditions. Future studies should explore formulation optimization, including microencapsulation or nanoemulsion techniques, and assess in vivo efficacy in clinical settings. Additionally, exploring synergistic interactions with conventional antibiotics could offer a dual-action strategy against multidrug-resistant pathogens.

CONCLUSION

Phytochemical screening and GC-MS analysis is important to assess the bioactive compounds in oil blends and determine which is responsible for observed antimicrobial effects. This study concludes that the developed oil blends have the potential to inhibit bacterial and fungal growth, but they lack bactericidal and fungicidal efficacy. However, the vast array of bioactive compounds present in the oils, as revealed by GC-MS analysis, potentially contribute to the activity of the oil. Therefore, this study provides a strong foundation for the development of natural oil-based antimicrobial agents for skin and hair care. While promising, further investigation is needed to translate these findings into effective, clinically relevant products.

Recommendations for further research

A. Further research should focus on analyzing the potency of combined plant-based oils.
B. Oil formulations should be refined to provide *in vivo* efficacy

Further studies should provide an understanding of the therapeutic effects of oil blends and the actual concentration of oil blends required for therapeutic use.

REFERENCES

AbdRashed, A., Rathi, D. N. G., Ahmad Nasir, N. A. H., & AbdRahman, A. Z. (2021). Antifungal properties of essential oils and their compounds for application in skin fungal infections: Conventional and nonconventional approaches. *Molecules*, 26(4), 1093. [Crossref]

Akpomie, O. O., Akponah, E., Ehwarieme, A. D., & Paul, R. E. (2020). Antimicrobial activity of coconut water, oil and palm kernel oils extracted from coconut and palm kernel on some plasmid-mediated multi-drug resistant organisms associated with food spoilage. *African Journal of Microbiology Research*, 14(7), 366–373.

Anh, P. N. Q., Ngan, T. T. K., Anh, N. Q., Phuong, L. T. B., Quyen, N. T. C., Thi, N. T. N., ... & Nhan, L. T. H. (2019). Essential oil hydro-distillation process from Vietnamese calamondin (*Citrus microcarpa*) peels and GC/MS analysis of essential oils components. *Asian Journal of Chemistry*, 31, 2585–2588. [Crossref]

Arbab, S., Ullah, H., Bano, I., Li, K., Ul Hassan, I., Wang, W., ... & Zhang, J. (2022).

Evaluation of in vitro antibacterial effect of essential oil and some herbal plant extract used against mastitis pathogens. *Veterinary Medicine and Science*, 8(6), 2655–2661. [Crossref]

Bag, A., & Chattopadhyay, R. R. (2015). Evaluation of synergistic antibacterial and antioxidant efficacy of essential oils of spices and herbs in combination. *PloS One*, 10(7), e0131321. [Crossref]

Baqer, L. K. (2020). Antibacterial activity of sesame oil and coconut oil against the cariogenic *Streptococcus mutans* and *Lactobacillus* species-an in vitro study. *Biochemical and Cellular Archives*, 20(1), 1961–1964.

Borman, A. M., Fraser, M., Palmer, M. D., Szekely, A., Houldsworth, M., Patterson, Z., & Johnson, E. M. (2017). MIC distributions and evaluation of fungicidal activity for amphotericin B, itraconazole, voriconazole, posaconazole and caspofungin and 20 species of pathogenic filamentous fungi determined using the CLSI broth microdilution method. *Journal of Fungi*, 3(2), 27. [Crossref]

Cheesbrough, M. (2007) District Laboratory Practice in Tropical Countries, 2nd Edition. Cambridge University Press. Cambridge.

Ciotea, D., Shamtsyan, M., & Popa, M. E. (2021). Antibacterial activity of peppermint, basil and rosemary essential oils obtained by steam distillation. *Agro Life Scientific Journal*, 10(1), 125-130. [Crossref]

Donato, R., Sacco, C., Pini, G., & Bilia, A. R. (2020). Antifungal activity of different essential oils against *Malassezia* pathogenic species. *Journal of Ethnopharmacology*, 249, 112376. [Crossref]

Elnakady, Y. A., Al-Sayed, E., & Abo-Elmagd, R. (2021). Antimicrobial activity of plant essential oils: A review. *Journal of Medicinal Plants Research*, 15(5), 174-185. [Crossref]

Gharsallah, K., Rezig, L., Msaada, K., Chalh, A., & Soltani, T. (2021). Chemical composition and profile characterization of *Moringa oleifera* seed oil. *South African Journal of Botany*, 137, 475-482. [Crossref]

Gull, I., Iqbal, Z., & Khan, S. U. (2022). Phytochemical composition and antimicrobial activity of essential oils from medicinal plants. *Journal of Essential Oil Research*, 34(3), 181-198. [Crossref]

Gupta, R. K., & Dai, F. (2019). Plant-based oils as an alternative to conventional antimicrobial agents: Safety, efficacy, and future prospects. *International Journal of Molecular Sciences*, 20(5), 1075. [Crossref]

Jain, S., Arora, P., & Nainwal, L. M. (2022). Essential oils as potential source of anti-dandruff agents: A review. *Combinatorial Chemistry & High Throughput Screening*, 25(9), 1411-1426. [Crossref]

Jaji, B. A., Oloruntele, I. O., Sekoni, H. A., Ibrahim, M. M., & Adeoye, S. O. (2024). Characterization and evaluation of physicochemical properties, phytochemicals and antimicrobial analysis of coconut oil. *Dutse Journal of Pure and Applied Sciences*, 10(3b), 124-137. [Crossref]

Katiki, L. M., Barbieri, A. M. E., Araujo, R. C., Veríssimo, C. J., Louvandini, H., & Ferreira, J. F. S. (2017). Synergistic interaction of ten essential oils against *Haemonchus contortus* in vitro. *Veterinary Parasitology*, 243, 47-51. [Crossref]

Mahmoud, N. M., Rahma, E. H., Osheba, A. S., El-Bedawey, A. A., & Saad, M. M. (2020). Chemical components, antioxidant and antimicrobial activities of garlic, cumin and parsley volatile oils. *Menoufia Journal of Food and Dairy Sciences*, 5, 53-63. [Crossref]

Mittal, R. P., Rana, A., & Jaitak, V. (2019). Essential oils: An impending substitute of synthetic antimicrobial agents to overcome antimicrobial resistance. *Current Drug Targets*, 20(6), 605-624. [Crossref]

Murray, P. R., Rosenthal, K. S., & Pfaller, M. A. (2015). *Medical microbiology*. Elsevier Health Sciences.

Nascimento, T., Gomes, D., Simões, R., & da Graça Miguel, M. (2023). Tea tree oil: Properties and the therapeutic approach to acne-A review. *Antioxidants*, 12(6), 1264. [Crossref]

Nogueira, S. F., Carvalho, A. S., & Pereira, P. M. (2020). Skin infections caused by bacteria: A review of the epidemiology, diagnosis, and treatment. *Journal of Dermatological Treatment*, 31(4), 351-362. [Crossref]

Olasupo, A. D., Olagoke, O. V., & Aborisade, A. B. (2017). Qualitative phytochemical screening of bologi (*Senecio biafrae*) and bitter leaf (*Vernonia amygdalina*) leaves. *Chemical Science International Journal*, 20(3), 1–6. [Crossref]

Orchard, A., & van Vuuren, S. (2017). Commercial essential oils as potential antimicrobials to treat skin diseases. *Evidence-Based Complementary and Alternative Medicine*, 2017, 4517971. [Crossref]

Rasheed, S. O., Farhan, M. S., & Numan, R. S. (2024). Isolation and identification of certain types of skin fungi and their ability to produce virulence factors. *World of Medicine: Journal of Biomedical Sciences*, 1(6), 1–9.

Salisu, B. D., & Shema, M. (2019). Phytochemical screening and antimicrobial activity of aqueous stem extract of *Aloe vera* on some common pathogenic bacteria. *UMYU Journal of Microbiology Research (UJMR)*, 4(2), 49–56. [Crossref]

Salisu, B., Anua, M. S., Wan Ishak, W. R., & Mazlan, N. (2020). Incidence, distribution and phenotypic characterisation of aflatoxigenic fungi contaminating commonly consumed food grains in Katsina State, Nigeria. *Malaysian Journal of Medicine and Health Sciences*, 16(SUPP11), 2636-9346.

Salisu, B., Anua, M. S., Wan Ishak, W. R., & Mazlan, N. (2022). Mycotoxigenic fungi contamination of grains and peanuts from open markets in Kelantan, Malaysia. *Food Research*, 6(1), 69-77. [Crossref]

Salisu, B., Anua, M. S., Wan Ishak, W. R., & Mazlan, N. (2024). Bioburden, phenotypic and spectroscopic characterisation of toxigenic and atoxigenic *Aspergillus* section *Flavi* from poultry feeds in Kelantan, Malaysia and Katsina, Nigeria. *Malaysian Journal of Microbiology*, 20(2), 120–131. [Crossref]

Senanayake, I. C., Rathnayaka, A. R., Marasinghe, D. S., Calabon, M. S., Gentekaki, E., Lee, H. B., ... & Xiang, M. M. (2020). Morphological approaches in studying fungi: Collection, examination, isolation, sporulation and preservation. *Mycosphere*, 11(1), 2678-2754. [Crossref]

Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603-608. [Crossref]

Siddiquee, S., & Siddiquee, S. (2017). The basic concept of microbiology. In S. Siddiquee (Ed.), *Practical handbook of the biology and molecular diversity of* Trichoderma *species from tropical regions* (pp. 1–15). Springer. [Crossref]

Tan, R. P., Lim, S. W., & Lim, J. M. (2020). The role of phytochemicals in natural plant-based oils: Antioxidant and antimicrobial properties. *Phytotherapy Research*, 34(10), 2589–2599. [Crossref]

Taylor, T. A., & Unakal, C. G. (2021). *Staphylococcus aureus*. StatPearls Publishing.

Zouirech, O., Alyousef, A. A., El Barnossi, A., El Moussaoui, A., Bourhia, M., Salamatullah, A. M., ... & Derwich, E. (2022). Phytochemical analysis and antioxidant, antibacterial, and antifungal effects of essential oil of black caraway (*Nigella sativa* L.) seeds against drug-resistant clinically pathogenic microorganisms. *BioMed Research International*, 2022, 5218950. [Crossref]