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Antibacterial Activity of Eucalyptus (*Melaleuca leucadendron*) Leaf Extracts Against Acne-Causing Bacteria

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ABSTRACT

Melaleuca leucadendron (Ecalyptus) plant has been widely utilized in both traditional and modern medicine due to its diverse pharmacological properties. The plant contains various bioactive compounds contributing to its medicinal properties, such as anti-inflammatory, analgesic, antidiabetic, and anticancer effects. This study aimed to evaluate the antibacterial activity of *M. leucadendron* leaf extracts against acne-related bacteria. Dried *M. leucadendron* leaf powder was sequentially macerated with n-hexane, dichloromethane (DCM), and methanol to yield MLEH, MLED, and MLEM extracts, respectively. The antibacterial activity of *M. leucadendron* leaf extracts (MLEH, MLED, and MLEM) was assessed via disc diffusion against *Staphylococcus epidermidis* and *Propionibacterium acnes* at various concentrations (1%–20%). Additionally, gas chromatography–mass spectrometry (GC-MS) analysis was conducted to identify the phytochemical constituents. Among the extracts, MLEM yielded the highest extraction efficiency (11.41%) and tested positive for flavonoids, phenolics, saponins, and terpenoids. The MLEH exhibited antibacterial activity against *S. epidermidis* and *P. acnes* at a 20% concentration, with inhibition zones of 11.56 mm and 10.36 mm, respectively. MLED showed stronger antibacterial activity, with an inhibition zone of 17.41 mm against *S. epidermidis* at 20% concentration, and 12.29 mm against *P. acnes* at 10% concentration. Meanwhile, MLEM displayed antibacterial activity only against *P. acnes* at 20% concentration (10.47 mm), with no observed inhibition against *S. epidermidis*. These findings suggest that *M. leucadendron* leaf extracts, particularly the MLEH and MLED, possess notable antibacterial activity against acne-associated bacteria and hold potential for development in topical antimicrobial applications.

Keywords: Acne, antibacterial, *Melaleuca leucadendron*, *Staphylococcus epidermidis*, *Propionibacterium acnes*

Introduction

The skin is a complex organ composed of various components that play critical roles in maintaining its structure and function. One of the most common skin problems among adolescents is acne, which arises from multiple contributing factors, including excessive sebum production, abnormal desquamation of the follicular epithelium, and inflammation triggered by bacterial infections such as *Staphylococcus epidermidis* and *Propionibacterium acnes*. These acne-causing bacteria are typically treated with antibiotics such as clindamycin, doxycycline, and tetracycline. However, prolonged use of antibiotics may lead to side effects, including skin irritation, headaches, and the development of antibiotic-resistant bacterial strains.[1] To minimize these adverse effects, there is a growing interest in utilizing naturally derived compounds that are considered safer alternatives. One such plant with potential antibacterial properties is eucalyptus (*Melaleuca leucadendron*). Eucalyptus (*Melaleuca leucadendron*) is a plant native to Indonesia that plays an important role across various industries. It typically thrives in regions with annual rainfall ranging from 1,300 to 1,750 mm and a hot, humid climate.

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Several parts of the eucalyptus plant have been utilized in traditional medicine, with the leaves being the most extensively studied.[2] Previous research has shown that the essential oil extracted from *M. leucadendron* leaves contains major components such as 1,8-cineole (66.7%), α-pinene (2.45%), and L-α-terpineol (7.56%).[3] These compounds are known for their antibacterial properties. For instance, 1,8-cineole and α-pinene disrupt bacterial cell membranes, leading to increased permeability and leakage of intracellular contents.[4] α-Terpineol, in addition to membrane disruption, can interfere with bacterial enzyme systems and energy metabolism, impairing cell function and viability.[4]

Phytochemical screening of the methanol extract of *M. leucadendron* leaves has identified the presence of terpenoids, steroids, alkaloids, flavonoids, saponins, and anthraquinones, many of which also contribute to antimicrobial activity through various mechanisms such as inhibition of nucleic acid synthesis, protein synthesis, or disruption of microbial adhesion.[5] Notably, 1,8-cineole has been reported to inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) with a minimum inhibitory concentration (MIC) of 7.23 mg/mL.[6] Additionally, essential oils from *M. leucadendron* leaves have demonstrated antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, with MIC and minimum bactericidal concentration (MBC) values ranging from 4 to 8 µg/mL.[7] Nevertheless, studies investigating the antibacterial activity of *M. leucadendron* leaf extract specifically against *S. epidermidis* and *P. acnes*—two key acne-causing bacteria—have not yet been reported. Therefore, the aim of this study was to evaluate the antibacterial potential of *M. leucadendron* leaf

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extracts against these organisms.

Materials and Methods

Materials

The materials used in this study included analytical-grade solvents and reagents: distilled water, n-hexane, dichloromethane (DCM), methanol, 10% ammonia, magnesium powder, 5% hydrochloric acid (HCl), Mayer's reagent, Wagner's reagent, Dragendorff's reagent, Liebermann–Burchard reagent, 0.9% sodium chloride (NaCl), and 70% ethanol. Antibiotics used as controls were chloramphenicol (Oxoid) and vancomycin (Oxoid). Culture media included Mueller Hinton Agar (MHA), Nutrient Agar (NA), and other components supplied by Oxoid. The bacterial strains used were *P. acnes* ATCC 11827 and *S. epidermidis* ATCC 12228. All chemicals and reagents were of analytical grade and used without any further purification. Bacterial cultures were obtained from the laboratory and subcultured on appropriate media prior to use in the experiments.

Plant collection and identification

Eucalyptus (*M. leucadendron*) leaf samples were collected on May 15 2023 from Blang Village, Blang Bintang District, Aceh Besar Regency, Indonesia (GIS coordinates: 5.5168400, 95.4409320). Sample selection followed specific criteria to ensure optimal quality: the leaves had to be fresh and healthy, neither too young nor overly mature. The collected specimens were identified and verified at the Herbarium Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, and were assigned the voucher specimen number 408/UN11.1.8.1/TA.00.03/2023.

A total of 3 kg of selected eucalyptus leaves were air-dried indoors for two weeks to reduce the moisture content.

Extract preparation

Dried *M. leucadendron* leaf samples weighing 1 kg were reduce to fine powder. The powdered material was then subjected to sequential maceration using three solvents of increasing polarity: n-hexane, dichloromethane (DCM), and methanol. For each solvent, the maceration was carried out by soaking the plant material in 3 L of solvent in a sealed glass container for 72 hours at room temperature (±28°C), with occasional stirring to enhance the extraction process. After each maceration cycle, the mixture was filtered using Whatman No. 1 filter paper, and the filtrate was collected. The residue was subsequently re-macerated twice with fresh solvent of the same type to ensure maximum extraction. All filtrates for each solvent were pooled and concentrated using a rotary evaporator (Büchi Rotavapor®) under reduced pressure at temperatures not exceeding 40°C to prevent thermal degradation of bioactive compounds. The resultant extracts were labeled according to their respective solvents: MLEH for the n-hexane extract, MLED for the dichloromethane (DCM) extract, and MLEM for the methanol extract. The concentrated extracts were then placed in pre-weighed containers and stored at 4°C until further use in antibacterial and phytochemical testing. The percentage yield of each extract was then calculated using Equation 1, as suggested by a previous study.[9]

$$Yield = \frac{Weight\ of\ extract}{Weight\ of\ M.leucadendron\ leaf\ powder} x\ 100\% \qquad \text{(Eq. 1)}$$

Qualitative phytochemical screenings

Qualitative phytochemical screening was performed to identify the presence of major secondary metabolites, including flavonoids, saponins, terpenoids, steroids, and alkaloids in the MLEH (n-hexane extract), MLED (dichloromethane extract), and MLEM (methanol extract) of *Melaleuca leucadendron* leaves, following the protocols reported previously.[8]

Disc diffusion assays

Two common pathogenic microorganisms were used in this study: *S. epidermidis* (ATCC 12228) and *P. acnes* (ATCC 11827). The antibacterial activity of MLEH, MLED, and MLEM was evaluated using the disc diffusion method. A suspension of each microorganism was evenly spread onto the surface of Mueller Hinton Agar (MHA) plates using sterile cotton swabs. Sterile blank paper discs were individually impregnated with 20 µL of each extract at concentrations of 20%, 10%, 5%, and 1%, then placed on the inoculated agar surfaces. Plates were subsequently incubated at 37°C for 24 hours under aerobic conditions for *S. epidermidis*. For *P. acnes*, incubation was performed under anaerobic conditions using anaerobic jar tubes. Antibacterial activity was assessed by measuring the diameter of the inhibition zone surrounding each disc, recorded in millimeters. This procedure followed the protocol described in a previous report.[9]

Gas chromatography-mass spectrometry analysis

The gas chromatography mass spectrometry (GC-MS) analysis was conducted on the MLEH, MLED, and MLEM, following a previously described protocol.[3] The analysis was performed using a Shimadzu GC-2010 Plus gas chromatography instrument. A TG-5MS capillary column (30 m length, 0.2 mm internal diameter, 0.25 µm film thickness) was used, with a total run time of 50 minutes. The MLED sample was dissolved in an appropriate solvent, and 2 µL of the solution was injected into the GC system. The temperature was initially set at 60°C and maintained for 4 minutes, increased to 150°C for another 4 minutes, and finally raised to 250°C. Helium (He) served as the carrier gas. Mass spectrometric detection was conducted under electron ionization (EI) mode at 70 eV. The resulting peaks were compared with known spectra from the database using Chromeleon software to identify the chemical constituents.

Results and Discussion

Extraction yields

MLEH, MLED, and MLEM were obtained from solvents with different polarities, aiming to isolate secondary metabolites based on their polarity. Solvent polarity influences the extraction efficiency, with non-polar solvents like n-hexane primarily extracting lipophilic compounds, while polar solvents like methanol can penetrate vacuoles and dissolve a wider range of bioactive constituents. Herein, the extract yields were 3.919% for MLEH, 2.620% for MLED, and 11.411% for MLEM. Methanol yielded the highest extract amount. This is consistent with previous findings reporting yields of 3.797% and 22.762% for n-hexane and methanol extracts of *Eucalyptus globulus*, respectively.[12] Another study reported yields of 7.32%, 25.90%, and 30.34% for DCM, ethanol, and methanol extracts of *E. globulus*, respectively.[13] The high yield from methanol extraction is attributed to its strong polarity, which enables deeper penetration into plant tissues and better solubility of diverse secondary metabolites.[14]

Phytochemical groups contained in the leaf extracts

The screening assessed the presence of phenolics, flavonoids, saponins, terpenoids, steroids, and alkaloids, where the result summary is presented in Table 1.

Table 1: Phytochemical constituents served in eucalyptus leaf extracts

Sample	Alkaloids	Flavonoids	Saponins	Terpenoids	Steroids	Phenolics
MLEH	-	-	-	+	+	-
MLED	-	-	-	+	+	+
MLEM	-	+	+	+	+	+

Note: MLEH : *Melaleuca leucadendron* leaves n-hexane extract, MLED: *Melaleuca leucadendron* leaves dichloromethane extract, MLEH: *Melaleuca leucadendron* leaves methanol extract, (+): Presence of compound, (-) : Absence of component

MLEH was positive for terpenoids and steroids, indicated by a greenish-red color change. However, it did not show the presence of saponins, phenolics, or flavonoids. Flavonoids, which consist of two aromatic rings and multiple hydroxyl (OH) groups, are highly polar; their absence in the MLEH may be due to the non-polar nature of n-hexane, which is ineffective in extracting polar compounds.[15] MLED was

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positive for terpenoids, steroids, and phenolics, with the latter confirmed by a dark color change upon addition of $FeCl_3$. However, saponins, flavonoids, or alkaloids were not detected in this sample. As a moderately polar solvent, DCM can extract compounds with intermediate polarity, which may explain the presence of both terpenoids and phenolics. As for MLEM, it exhibited the broadest phytochemical profile, testing positive for flavonoids, phenolics, saponins, and terpenoids. This is due to the fact that methanol has a high polarity that facilitates the extraction of a wide range of polar secondary metabolites. These findings support the role of solvent polarity in influencing the phytochemical content of plant extracts and provide a basis for further bioactivity analysis.

Antibacterial Activity of Eucalyptus Leaf Extracts

The antibacterial activity of eucalyptus leaf extracts against *Staphylococcus epidermidis* and *Propionibacterium acnes* varied depending on the solvent used, as shown in Fig. 1. MLEH (n-hexane extract) at concentrations of 10% and 20%, and MLED (DCM extract) at 5%, 10%, and 20% demonstrated inhibitory effects against *S. epidermidis*. In contrast, MLEM (methanol extract) did not exhibit any inhibition against *S. epidermidis*. All three extracts, namely MLEH, MLED, and MLEM, showed antibacterial activity against *P. acnes*. According to a published classification, inhibition zones ≤14 mm indicate bacterial resistance, 15–19 mm indicate intermediate susceptibility, and ≥20 mm indicate sensitivity.[16]

Among the tested extracts, MLED showed the strongest antibacterial activity, with inhibition zones of 17.41 mm against *S. epidermidis* and 17.02 mm against *P. acnes* at 20% concentration, placing them in the intermediate category. These results are comparable to previous findings, which reported that *Melaleuca alternifolia* essential oil produced inhibition zones of 21.02 mm against *S. epidermidis* and 20.05 mm against *Cutibacterium acnes*.[17] Additionally, methanol extracts of *M. alternifolia* prepared via maceration and soxhlet methods showed inhibition zones of 13.47 mm and 13.50 mm, respectively, against *E. coli* and *S. aureus* at 50% concentration.[18] The results suggest that MLED has promising antibacterial potential, particularly against *S. epidermidis* and *P. acnes*, likely due to the intermediate polarity of DCM, which enables efficient extraction of antibacterial secondary metabolites such as terpenoids, phenolics, and steroids. These compounds are known to disrupt bacterial cell membranes, increase membrane permeability, and interfere with essential enzymatic processes. Terpenoids, in particular, can integrate into lipid bilayers and alter membrane fluidity, while phenolic compounds may induce oxidative stress through the generation of reactive oxygen species, leading to bacterial cell death. The absence of activity in MLEM against *S. epidermidis* further supports the role of compound polarity and solvent affinity in determining antibacterial efficacy.

Phytochemical profile of eucalyptus leaf extracts

GC-MS analysis identified a total of 37 compounds in MLED, 42 in MLEH, and 29 in MLEM, with the detailed profiles shown in Table 2. The chemical constituents varied significantly across extracts, depending on solvent polarity, affecting both the yield and types of metabolites isolated. The major constituents in MLEH were eucalyptol (9.69%), *α*-terpinyl acetate (8.24%), phytol (5.65%), *β*-sitosterol (10.57%), and ursolic aldehyde (10.34%). These are largely terpenoids, phytosterols, and fatty acid derivatives, consistent with the non-polar nature of n-hexane, which favors the extraction of lipophilic and volatile compounds. In MLED, the dominant compounds were eucalyptol (35.56%) and *α*-terpinyl acetate (18.48%), followed by terpinene, phytol, fenchol, endo-borneol, and *β*-sitosterol (0.81%). These include oxygenated monoterpenes, sesquiterpenes, and triterpenoids, representing a broad range of moderately polar metabolites. MLEM showed a markedly different profile, rich in fatty acids, polyphenols, and triterpenes. The most abundant compounds included n-hexadecanoic acid (29.11%), 9,12,15-octadecatrienoic acid (19.85%), *β*-sitosterol (5.28%), *α*-amyrin (1.97%), and several polyhydroxylated flavonoid-like structures such as 4H-pyran-4-one derivatives and benzopyranones. The strong polarity of methanol enables efficient extraction of these high molecular weight and polar compounds.

Table 2: Inhibition zones yielded by (A) MLEH, MLED, and MLEM against *Staphylococcus epidermidis* and (B) *Propionibacterium acnes* bacteria

Sample	Zone of Inhibition (mm) ± SD							
	Staphylococcus epidermidis				***Propionibacterium acnes***			
	1%	5%	10%	20%	1%	5%	10%	20%
MLEH	-	-	9.56±0.05	11.56±0.24	7.46±0.16	8.46±0.31	9.24±0.19	10.36±0.24
MLED	6.91±0.20	10.93±0.19	17.11±0.05	17.41±0.24	8.50±0.21	12.22±0.28	17.29±0.10	17.02±0.04
MLEM	-	-	-	-	-	8.08±0.08	9.14±0.07	10.47±0.28
Control*	26.56±0.14				31.33±0.39**			

Note: MLEH: *Melaleuca leucadendron* leaves n-hexane extract, MLED : *Melaleuca leucadendron* leaves dichloromethane extract, MLEH: Melaleuca leucadendron leaves methanol extract, *:Chloramphenicol 30 µg/mL, **: Vancomycin 30 µg/mL

Eucalyptol (1,8-cineole), the dominant compound, has been reported to inhibit *Staphylococcus aureus* and *Escherichia coli*, with a minimum inhibitory concentration (MIC) of 1250 µg/mL.[21] Its antibacterial action is thought to involve disruption of membrane integrity and inhibition of bacterial respiration. *α*-terpinyl acetate, the second most abundant compound, has demonstrated antimicrobial effects against *S. aureus* and *E. coli*, with MIC values of 31.30 mg/mL and 125 mg/mL, respectively.[19] Its mechanism may involve interference with bacterial lipid structures and enzyme systems. Other detected compounds, such as *α*-terpineol, have also shown potent activity against a range of oral pathogens including *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Fusobacterium nucleatum*, with MIC values ranging from 0.2 to 0.8 mg/mL and minimum bactericidal concentration (MBC) values between 0.2 and 0.4 mg/mL.[20] Borneol compounds, present in both exo- and endo-forms, have been shown to inhibit *S. aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *E. coli*, with MIC values as low as 6.49 µg/mL.[22] These compounds may act by increasing cell membrane permeability and inducing leakage of intracellular contents.

Overall, the strong antibacterial activity of MLED can be attributed to the combined effect of its major constituents—eucalyptol (1,8-cineole), *α*-terpinyl acetate, and borneol derivatives—which are known to act synergistically in disrupting bacterial physiology. Eucalyptol, a cyclic ether monoterpenoid, exerts its antibacterial action primarily by penetrating lipid bilayers, causing increased membrane permeability, cytoplasmic leakage, and ultimately bacterial cell lysis. *α*-terpinyl acetate, another oxygenated monoterpene, has demonstrated the ability to alter membrane integrity and inhibit efflux mechanisms, which may enhance the intracellular accumulation of antibacterial agents. Similarly, borneol and fenchol are bicyclic monoterpenes known to compromise bacterial membrane stability and energy production by disrupting proton gradients and respiratory enzymes.

This mechanism aligns with what has been previously proposed in Annonaceae spp. plants, where terpenoids and other secondary metabolites inhibit bacterial growth through multiple modes of action, including disruption of cell wall synthesis, interference with membrane-

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bound enzymes, and induction of oxidative stress via reactive oxygen species (ROS) production.[23] The distribution of bioactive compounds in MLED in this present study, particularly those with intermediate polarity, supports these mechanisms. The co-existence of eucalyptol, *α*-terpinyl acetate, and borneol-type molecules in a single extract likely amplifies membrane-targeting effects and may explain the superior inhibition zones observed against *S. epidermidis* and *P. acnes*. Such synergistic interactions among terpenoids and other secondary metabolites have been previously shown to result in enhanced bacteriostatic and bactericidal outcomes, including against multidrug-resistant strains (Table 3).

Table 3: Identified secondary metabolite compounds from eucalyptus leaf extracts using GC-MS

Identified compound	Retention time (min)	Area (%)		
		MLEH	MLED	MLEM
α-Pinene	7.03	0.9	1.09	-
Benzene, 1-ethyl-3methyl	7.779	0.39	-	-
Benzene, 1,2,4-trimethyl	8.667	0.57	-	-
o-cymene	9.554	1.27	0.92	-
Eucalyptol	9.775	9.69	35.56	-
Terpinene	10.554	1.85	2.46	-
3-cyclohexen-1-ol, 4-methyl-1--(1-methylethyl)-,(R)	14.003	0.58	1.35	-
3-cyclohexen-1-methanol, *α, α*,4-trimethyl-,(R)-	14.397	0.9	2.11	-
α- terpinyl acetate	18.761	8.24	18.48	-
Caryophyllene	20.625	3.92	1.79	-
1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-	21.101	0.78	-	-
Humulen	21.468	0.37	-	-
(1R,9R,E)-4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene	21.649	0.35	-	-
1-Bromo-3-(2-bromoethyl)-nonane	22.22	0.45	-	-
1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8α-hexahydronaphthalene	23.159	1.78	-	-
Cubenene	23.376	0.31	0.68	-
1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-	24.063	0.64	-	-
(-)-Spathulenol	24.506	2.37	-	-
(-)-Globulol	24.638	2.25	-	-
1H-Cycloprop[e]azulene, decahydro-1,1,4,7-tetramethyl-,[1aR-(1*αα,* 4*ß*, 4*αβ*, 7*α*, 7*αβ*, 7*αα*)]	24.835	0.49	-	-
4*α*(2H)-Napthalenol, 1,3,4,5,6,8*α*-hexahydro-4,7-dimethyl-1-(1-methylethyl)-,(1*S*, 4*R*, 4*αS*, 8*αR*)-	25.635	0,85	-	-
tau-Muuralol	25,951	0.41	-	-
Decane,5,6-bis(2,2-dimethylpropylidene)-,(*E,Z*)-	27.213	2.7	-	-
1,1,4,7-Tetramethyldecahydro-1H-Cyclopropa[e]azulene-4,7-diol	27.655	0.4	-	-
Neophytadiene	30.036	0.5	0.45	
n-hexadecanoic acid	32.665	0.82	0.76	29.11
Phytol	35.352	5.65	3.02	5.68
17-Octadecynoic acid	36.001	2.81	-	-
Hexanedioic acid, bis(2-ethylhexyl)ester	40.171	1.23	-	-
Phythalic acid, di(2-propylpentyl) ester	42.518	0.83	-	-
Tetradecane, 2,6,10-trimethyl-	44.698	0.74	-	-
1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	45.419	0.79	-	-
6,9,12,15-Docosatetraenoic acid, methyl ester	45.575	0.5	2.31	-
Di-isononyl phtlate	45.844	0.94	-	-
Squalene	46.575	3.93	-	-
Phthalic acid, nonyl pentadecyl ester	46.973	0.35	-	-
Carbonic acid, eicosyl vinyl ester	47.487	1.42	-	-
1,6,10,14,18,22-Tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-,(all-*E*)-(±)-	47.987	0.35	-	-
Octacosanal	49.341	0.62	-	-
4H-1-Benzopyran-4-one, 3,5,7-trimethoxy-2- phenyl-	49.749	0.43	-	-
4H-1-Benzopyran-4-one, 5-hydroxy-7-methoxy-2-(4-methoxyphenyl)-6,8-dimethyl	50.113	0.95	1.68	-
Vitamin E	50.64	3.36	-	-
ß-Sitosterol	53.085	10.57	0.81	5.28
ß-Amyrin	53.497	0.77	-	-
α-Amyrin	54.184	5.07	-	1.97
Stigmast-4-en-3-one	54.973	0.93	-	-
Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3*ß*)-	56.133	0.32	-	-
1-Heptatriacotanol	56.49	1.4	1.83	-
Ursolic aldehyde	58.561	10.34	-	-
Betulinaldehyde	57.629	0.76	-	-
Olean-12-ene-3,28-diol, (3*ß*)-	59.105	0.32	-	-
Urs-12-en-28-ol	60.289	0.69	-	-
Cyclohexene, 4-methylene-1-(1-Methylethyl)-	8.231	-	0.51	-
Bicyclo[3.1.0] hex-2-ene, 4-methyl-1-(1-methylethyl)-	9.007	-	0.75	-
Fenchol, exo-	12.176	-	0.51	-
Endo-borneol	13.693	-	1.05	-

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Identified compound	Retention time (min)	Area (%)		
		MLEH	MLED	MLEM
2-oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl,-acetate	18.53	-	0.57	-
1-methyl-4-(1-acetoxy-1-methylethyl)-cyclohex-2-enol	20.897	-	0.43	-
ß-Acorenol	22.601	-	0.59	-
Cis-calamenene	23.159	-	0.88	-
Epoxy-4-Terpinyl acetate	23.536	-	0.75	-
1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl	24.492	-	1.28	-
Caryophyllene oxide	24.625	-	3.44	-
1H-Cycloprop[e]azulene-4-ol, decahydro-1,1,4,7-tetramethyl-,[1*αR*-(1*αα*, 4*ß*, 4*αβ*, 7*α*, 7*αβ*, 7*βα*)]	24.835	-	0.68	-
cubenol	25.638	-	1.73	-
11,11-dimethyl, 4,8-dimethylene bicyclo[7.2.0]undecan-3-ol	25.839	-	0.76	-
trans-*Z*-*α*-Bisabolene epoxide	26.611	-	0.51	-
6-hydroxy-4,4,7 *α* -trimethyl-5,6,7,7*α*-tetrahydrobenzofuran-2(4H)-one	26.692	-	0.51	-
5,5,8*a*-trimethyl-3,5,6,7,8,8*α*-hexahydro-2H-chromene	29.185	-	2.91	-
Cis-*Z*- *α*-Bisabolene epoxide	29.185	-	0.45	-
Thiophene-2-carboxylic acid, 5-tert-buthyl-3-chloromethyl-, methyl ester	31.808	-	0.8	-
1H-2,8*a*-methanocyclopenta[a]cyclopropa[e]cyclodecen-11-one, 1*α*,2,5,5*α*,6,9,10,10α-octahydro	46.49	-	3.16	-
2,3-dimethoxy-5-methyl-6-dekaisoprenyl-chinon	47.364	-	1.37	-
Z,E-3,13-octadecadien-1-ol	49.344	-	0.79	-
(E)-3,7-Dimethyl-2,6-octadien-1-yl propionate	7.068	-	-	0.52
Stigmasta-3,5-diene	50.973	-	-	0.91
10-methyl-8-tetradecan-1-1-ol-acetate	26.621	-	-	3.67
hexadecanoid acid, Methyl ester	31.774	-	-	2.64
5-cis-methyl- 1*R*,3-cis-cyclohexanediol	9.514	-	-	1.1
4H-pyran-4-one, 2,3 dihydro-3,5-dehydroxy-6-methyl	13.173	-	-	4.15
Cyclohexan-1,4,5-triol-3-one-1-carboxylic acid	15.35	-	-	0.55
2H-indol-2-one, 1,4,5,6,7,7*α*-hexahydro-7*α*-methyl-,(*S*)	17.857	-	-	0.98
1-Gala-1-ido-octose	23.104	-	-	1.45
D-Streptamine-*O*-6-amino-6-deody-*α*-D-glucopyranosyl-(1-4)-o-(3-deoxy-4-c-methyl-3-(methylamino)-*β*-L-arabinopyranosyl-(1-6))-2-deoxy	26.322	-	-	1.37
Acetamide, N-methyl-N-[4-(3-hydroxypyrolidinyl)-2-butynyl]-	29.209	-	-	1.1
3,7,11,15-tetramethyl-2-hexadecan-1-ol	30.039	-	-	1.49
E-2-Tetradecan-1-ol	30.91	-	-	0.73
[1,1-Bicyclopropyl]-2-octanoic acid,2-hexyl, methyl ester	34.971	-	-	0.81
9[*E*],11[*E*]-conjugated linoleic acid	35.774	-	-	4.2
9,12,15-octadecatrienoic acid,(*Z, Z, Z*)-	35.899	-	-	19.85
Hexadecanoic acid, 2-hidroxy-1-(hydroxymethyl), ethyl ester	41.926	-	-	3.24
9,10-secocholesta-5,7,10(19)-triena-3,24,25-triol (3*β*, 5*Z*, 7*E*)	49.888	-	-	1.23
α-Tocopherylacetate	50.602	-	-	0.54
Curcubitacin b, 25-desacetoxy	53.609	-	-	0.77

Note: MLEH: *Melaleuca leucadendron* leaves n-hexane extract, MLED: *Melaleuca leucadendron* leaves dichloromethane extract, MLEH: Melaleuca leucadendron leaves methanol extract, (-): not detected

Conclusion

Eucalyptus leaf extracts (*M. leucadendron*) exhibited varying degrees of antibacterial activity against acne-causing bacteria. Among the tested extracts, the MLED showed the most potent inhibitory effect against *S. epidermidis* and *P. acnes*, two key bacteria involved in acne pathogenesis. GC-MS analysis of MLED revealed major bioactive compounds including eucalyptol (1,8-cineole), *α*-terpinyl acetate, *α*-terpineol, fenchol, and endo-borneol—compounds known to disrupt bacterial membranes and interfere with essential cellular functions. These findings suggest that MLED has potential as a topical antibacterial agent for the treatment of acne caused by bacterial infection. Further research is recommended to isolate and characterize the active constituents, elucidate their mechanisms of action, and evaluate their ability to enhance antibiotic efficacy through sensitizing activity. *In vivo* studies and safety evaluations should also be conducted to support the therapeutic application of MLED in acne management.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Agustin V, Ismiyati N, Sulistyawati R. Formulation of acne spot gel preparation of jasmin flower extract (*Jasminum sambac* L). Indones J Med Sci .2023; 10(1): 31–36.
2. Sadono R, Soeprijadi D, Wirabuana PYAP. Land suitability for eucalyptus development and implications for silvicultural techniques. J Nat Res Env Man. 2019; 10(1): 43–51.
3. Ginting B, Sufriadi E, Harnelly E, Nadia I, Farid M, Suparto IH, Ilmiawati, A, Ernawati E, Syaifullah M, Syakira M, Riski CD. Identification of volatile compounds contained in the therapeutic essential oils from *Pogostemon cablin*, *Melaleuca leucadendra*, and *Mentha piperita* and their purified fractions. J Adv Pharm Technol Res. 2023; 14(3): 208–212.
4. Joen ST. Effectiveness of eucalyptus leaf extract (*Melaleuca leucadendron* L) as antibacterial in vitro. Medic J Lampung Univ. 2020; 9(2): 45–48.
5. Aini DM, Hadi S. Phytochemical screening and chemical compotition of eucalyptus leaves (*Melaleuca leucadendron* Linn) by GC-MS. Ind E J App Chem. 2022; 10(1): 52–60.
6. Merghni A, Belmamoun AR, Urcan AC, Bobiş O, Lassoued MA. 1,8-Cineol (Eucalyptol) disrupts membrane integrity and induces oxidative stress in Methicillin-Resistant *Staphylococcus aureus*. Antioxidants. 2023; 12(7): 1338.
7. Siddique S, Parveen Z, Firdaus-e-Bareen, Mazhar S. Chemical composition, antibacterial and antioxidant activities of essential oils from leaves of three Melaleuca species of Pakistani flora. Arab J Chem. 2020; 13(1): 67–74.
8. Kemenkes RI, Indonesian Herbal Pharmacopoeia. Indonesia: Department of health of the Republic of Indonesia, 2008.
9. Fajrin FA, Holidah D, Dewi IP, Christianty FM, Pratoko DK, Kinanti MDP, Fadhilah NA, Kusuma RA, Haq A, Istidamah R. Analgesic and anti-inflammatory activity of ethanol extract from *Cocoa pod* Husk. Trop J Nat Prod Res. 2025; 9(5): 1909–1917.
10. Saidi N, Awang K, Yahya M. Isolation of oxoaphorpine alkaloid from bark of *Cryptocarya ferrea*. Pharmacogn J. 2019; 11(4): 782–786.
11. Khairan K, Astuti Y, First IS. Gel formulation of ethyl acetate garlic extraction and its activity against *Staphylococcus epidermidis*. J Chem Nat Resour. 2019; 1(2): 69–78.
12. Vera ZM, Dutta B, Mercer DG, MacLean HL, Touchie MF. Assessment of moisture content measurement methods of dried food products in small-scale operations in developing countries: A review. Trends Food Sci Technol. 2019; 88: 484–496.
13. Nortjie E, Basitere M, Moyo D, Nyamukamba P. Assessing the efficiency of antimicrobial plant extracts from *Artemisia afra* and *Eucalyptus globulus* as coatings for textiles. Plants. 2024; 13(4): 514.
14. Rodrigues VH, Demelo MMR, Portugal I, Silva CM. Extraction of eucalyptus leaves using solvents of distinct polarity. Cluster analysis and extracts characterization. J Supercrit Fluids. 2018; 135: 263–274.
15. Truong DH, Nguyen DH, Ta NTA, Bui AV, Do TH, Nguyen HC. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. J Food Qual. 2019; 2019 ;1-9.
16. Burhana NA, Akbar MF, Putri AS. Comparison of pandan leaf extract (*Pandanus amaryllifolius*) using ethanol and n-hexane to the content of bioactive compounds. Int Conf Sustain Heal Promot. 2023; 3(1): 30–31.
17. Cockerill FR, Wikler MA, Alder J, Dudley MN, Eliopoulus GM, Ferraro MJ, Hardy DJ, Hecht DW, Hindler JA, Patel JB, Power M, Swenson JM, Thomson RB, Traczewski MM, Turnidge J D, Weinstein MP, Zimmer, BL. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically ; Approved Standard — Ninth Edition*. 2012; 32(2): 1-88.
18. Esmael A, Hassan MG, Amer MM, Abdelrahman S, Hamed AM, Abd-raboh HA, Foda MF. Antimicrobial activity of certain natural-based plant oils against the antibiotic-resistant acne bacteria. Saudi J Biol Sci. 2020; 27(1): 448–455.
19. Widiyastuti Y, Matoha HK, Fitriana F. Antibacterial activity of *Melaleuca alternifolia* extract from different extraction method. Biosaintifika. 2022; 14(2): 285–292.
20. Vaičiulytė V, Ložienė K, Švedienė J, Raudonienė J, Paškevičius A. α-terpinyl acetate: Occurrence in essential oils bearing *Thymus pulegioides*, phytotoxicity, and antimicrobial effects. Molecules. 2021; 26(4): 1065.
21. Park SN, Lim YK, Freire MO, Cho E, Jin D, Kook JK. Antimicrobial effect of linalool and α-terpineol against periodontopathic and cariogenic bacteria. Anaerobe. 2012; 18(3): 369–372.
22. Göger G, Karaca N, Altınbaşak BB, Demirci B, Demirci F. In vitro antimicrobial, antioxidant and anti-inflammatory evaluation of *Eucalyptus globulus* essential oil. Nat Volatiles Essent Oils. 2020; 7(3): 1–11.
23. Pham TV, Hoang HNT, Nguyen HT, Nguyen HM, Huynh CT, Vu TY, Do AT, Nguyen NH, Do BH. Anti-Inflammatory and antimicrobial activities of compounds isolated from *Distichochlamys benenica.* Biomed Res Int. 2021; 20(21): 1-10.
24. Harahap D, Niaci S, Mardina V, Zaura B, Qanita I, Purnama A, Puspita K, Riski DR, Iqrammullah, M. Antibacterial activities of seven ethnomedical plants from family *Annonaceae*. J Adv Pharm Technol Res. 2022: 5(13): 148-153.

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