Society of Medical Education & Research

2022, Volume 2, Issue 1, Page No: 50-57
ISSN: 3108-4826

Journal of Medical Sciences and Interdisciplinary Research

Evaluation of Antibacterial Activity and Phytochemical Composition of *Melaleuca leucadendron* Leaf Extracts

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Abstract

Melaleuca leucadendron, commonly referred to as Eucalyptus, has been extensively applied in folk remedies and modern therapeutics because of its wide-ranging bioactive effects. This species is rich in various natural compounds that account for its capabilities in reducing inflammation, alleviating pain, regulating blood glucose, and combating cancer cells. The objective of this research was to investigate the antimicrobial effects of extracts obtained from M. leucadendron leaves on microorganisms linked to acne vulgaris. Ground dry leaves from M. leucadendron underwent sequential extraction by maceration in n-hexane, dichloromethane (DCM), and methanol, resulting in the respective fractions designated as MLEH, MLED, and MLEM. The antimicrobial properties of these fractions (MLEH, MLED, and MLEM) were evaluated using the disk diffusion technique on Staphylococcus epidermidis and Propionibacterium acnes at concentrations spanning 1% to 20%. In addition, the chemical profile was determined through gas chromatography–mass spectrometry (GC-MS). The methanolic fraction (MLEM) achieved the maximum yield (11.41%) and confirmed the presence of flavonoids, phenolic compounds, saponins, and terpenoids. The hexane fraction (MLEH) demonstrated antimicrobial effects at 20% concentration, producing inhibition diameters of 11.56 mm against S. epidermidis and 10.36 mm against P. acnes. Greater potency was observed with the DCM fraction (MLED), which formed a 17.41 mm zone against S. epidermidis at 20% and a 12.29 mm zone against P. acnes at 10%. On the other hand, the methanolic fraction (MLEM) only inhibited P. acnes at 20% concentration with a 10.47 mm zone, showing no effect on S. epidermidis. Overall, these results highlight that certain M. leucadendron leaf fractions, notably MLEH and MLED, display significant antimicrobial action toward bacteria involved in acne and could be suitable for creating topical treatments with antibacterial properties.

Keywords: Acne, Antimicrobial activity, Melaleuca leucadendron, Staphylococcus epidermidis, Propionibacterium acnes

Introduction

The human skin is an intricate structure made up of numerous layers and elements vital for protection and homeostasis. Among young individuals, acne vulgaris ranks as one of the most frequent dermatological issues, triggered by factors like excess oil secretion, improper keratinization in hair follicles, and inflammatory processes driven by microbial colonization, particularly involving Staphylococcus epidermidis and Propionibacterium acnes. Standard therapy for these pathogens typically relies on antimicrobial agents such as clindamycin, doxycycline, and tetracycline. Prolonged exposure to these medications, however, can cause undesirable outcomes, including dermal sensitivity, cephalic pain, and the rise of drug-resistant microbes [1]. To address such concerns, researchers are increasingly exploring plant-based alternatives perceived as milder and more sustainable. A promising option in this regard is the eucalyptus tree known as Melaleuca leucadendron. Originating from Indonesia, Melaleuca leucadendron contributes substantially to several economic fields and prefers habitats with precipitation levels of 1,300 to 1,750

Access this article online https://smerpub.com/

Received: 07 October 2021; **Accepted:** 19 January 2022

How to cite this article: Mendes PV, Matos RS. Evaluation of Antibacterial Activity and Phytochemical Composition of *Melaleuca leucadendron* Leaf Extracts. J Med Sci Interdiscip Res. 2022;2(1):50-7. https://doi.org/10.51847/XT06FvtWy3

mm annually in tropical, humid settings. Various components of this tree have been incorporated into indigenous healing practices, with leaves attracting the greatest scientific interest [2]. Prior analyses indicated that volatile oil from M. leucadendron foliage mainly features 1,8-cineole (66.7%), α-pinene (2.45%), and L-α-terpineol (7.56%) [3]. These substances exhibit known antimicrobial mechanisms; specifically, 1,8-cineole and α-pinene damage bacterial outer layers, enhancing permeability and causing internal component release [4]. Additionally, α-terpineol affects membrane stability while also targeting enzymatic processes and metabolic pathways, ultimately reducing bacterial vitality [4].

Screening of compounds in the methanolic leaf fraction of M. leucadendron revealed terpenoids, steroids, alkaloids, flavonoids, saponins, and anthraquinones, several contributing to microbe suppression by interfering with DNA replication, protein formation, or cell attachment [5]. Of note, 1,8-cineole effectively curbs methicillin-resistant Staphylococcus aureus (MRSA) growth at a minimum inhibitory concentration (MIC) of 7.23 mg/mL [6]. Furthermore, volatile oils from these leaves have proven effective against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae, with MIC and minimum bactericidal concentration (MBC) figures between 4 and 8 µg/mL [7]. However, targeted evaluation of non-volatile leaf extracts from M. leucadendron against the principal acne pathogens S. epidermidis and P. acnes remains undocumented. Hence, this work focused on determining the antimicrobial capacity of such extracts toward these specific bacteria.

Materials and Methods

Materials

This work utilized high-purity solvents and chemicals: distilled water, n-hexane, dichloromethane (DCM), methanol, 10% ammonia solution, magnesium filings, 5% hydrochloric acid (HCl), Mayer's reagent, Wagner's reagent, Dragendorff's reagent, Liebermann–Burchard reagent, 0.9% sodium chloride (NaCl), and 70% ethanol. Reference antimicrobials included chloramphenicol (Oxoid) and vancomycin (Oxoid). Growth media comprised Mueller Hinton Agar (MHA), Nutrient Agar (NA), and related products from Oxoid. The microbial isolates tested were P. acnes ATCC 11827 and S. epidermidis ATCC 12228. Every chemical and reagent was analytical grade and employed as received without extra processing. Microbial strains were sourced from laboratory stocks and propagated on relevant media ahead of testing.

Plant collection and identification

Specimens of M. leucadendron (eucalyptus) leaves were harvested on May 15 2023 from Blang Village in Blang Bintang District, Aceh Besar Regency, Indonesia (GIS coordinates: 5.5168400, 95.4409320). Harvesting followed rigorous guidelines to guarantee specimen quality: only vibrant, intact leaves of appropriate maturity were selected. Botanical confirmation was performed at the Herbarium Laboratory within the Faculty of Mathematics and Natural Sciences at Universitas Syiah Kuala, assigning the reference number 408/UN11.1.8.1/TA.00.03/2023.

Three kilograms of screened eucalyptus leaves were subjected to room-temperature air drying over a two-week period to decrease water content.

Extract preparation

One kilogram of dried M. leucadendron leaves was pulverized into a fine consistency. The resulting powder underwent successive extraction through maceration employing solvents arranged in order of rising polarity: n-hexane, dichloromethane (DCM), and methanol. Each extraction step involved immersing the material in 3 L of the chosen solvent within a closed glass vessel for 72 hours at ambient temperature (±28°C), accompanied by periodic agitation to optimize compound release. Following every maceration period, the suspension was passed through Whatman No. 1 filter paper to separate the liquid portion, which was retained. The remaining marc was then twice re-extracted using new portions of the identical solvent to achieve thorough recovery of constituents. Combined liquids from each solvent were evaporated to dryness under vacuum employing a rotary evaporator (Büchi Rotavapor®) while maintaining bath temperatures below 40°C to avoid heat-induced breakdown of active principles. The obtained fractions were designated MLEH (n-hexane), MLED (dichloromethane), and MLEM (methanol) based on the solvent used. These concentrated fractions were transferred to tared vessels and kept refrigerated at 4°C pending subsequent antimicrobial and phytochemical analyses. Yield percentages for the fractions were determined according to Equation 1, following the approach described in an earlier report [8].

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

plates with sterile cotton swabs. Blank sterile paper disks were each loaded with 20 µL of the respective extract solutions prepared at 20%, 10%, 5%, and 1% levels, and then positioned on the seeded agar plates. The plates were incubated at 37°C for 24 hours in an aerobic atmosphere for S. epidermidis, whereas P. acnes cultures were maintained under anaerobic conditions in jar systems. Antimicrobial effects were quantified by determining the clear zone diameters around the disks in millimeters. The methodology adhered to an established procedure outlined elsewhere [8].

Gas chromatography-mass spectrometry analysis

GC-MS examination was carried out on the MLEH, MLED, and MLEM fractions according to a documented method [3]. The equipment utilized was a Shimadzu GC-2010 Plus system fitted with a TG-5MS capillary column (30 m × 0.2 mm i.d., 0.25 µm film). The analytical run lasted 50 minutes. The MLED fraction was prepared in a suitable solvent, and 2 µL was introduced via injection. Initial oven temperature was held at 60°C for 4 minutes, then ramped to 150°C and kept for 4 minutes, before final elevation to 250°C. Helium functioned as a carrier gas. Detection employed electron impact ionization at 70 eV. Peak identification involved matching spectra against library entries using Chromeleon software to characterize the compounds present.

Results and Discussion

Extraction yields

The fractions MLEH, MLED, and MLEM were derived using solvents of varying polarities to capture secondary metabolites aligned with their solubility profiles. Extraction performance depends heavily on solvent polarity: non-polar agents like n-hexane favor hydrophobic substances, whereas highly polar ones like methanol access intracellular compartments and solubilize diverse bioactive molecules. Recorded yields stood at 3.919% for MLEH, 2.620% for MLED, and 11.411% for MLEM.

The methanolic fraction provided the largest quantity. This aligns with prior reports noting 3.797% and 22.762% yields from n-hexane and methanol fractions of Eucalyptus globulus [9]. Separate work documented 7.32%, 25.90%, and 30.34% for DCM, ethanol, and methanol fractions of E. globulus [10]. Superior methanol recovery stems from its elevated polarity, promoting tissue penetration and enhanced dissolution of varied plant constituents [11].

Phytochemical groups contained in the leaf extracts

Evaluation targeted major classes, including phenolics, flavonoids, saponins, terpenoids, steroids, and alkaloids; outcomes are compiled in **Table 1.**

Table 1. Phytochemical constituents present in eucalyptus leaf extracts

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

Qualitative phytochemical screenings

Preliminary chemical tests were conducted to detect key secondary metabolites—flavonoids, saponins, terpenoids, steroids, and alkaloids—in the MLEH (n-hexane), MLED (dichloromethane), and MLEM (methanol) fractions from Melaleuca leucadendron foliage, employing established procedures [12].

Disc diffusion assays

The investigation employed two prevalent skin pathogens: S. epidermidis (ATCC 12228) and P. acnes (ATCC 11827). Antimicrobial potential of MLEH, MLED, and MLEM was tested via disk diffusion. Microbial suspensions were uniformly applied to Mueller Hinton Agar (MHA) surfaces using sterile swabs.

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

The n-hexane fraction (MLEH) revealed terpenoids and steroids through a greenish-red coloration but lacked saponins, phenolics, or flavonoids. Flavonoids, featuring dual aromatic structures with numerous hydroxyl moieties, possess strong polarity; their absence here likely reflects n-hexane's inability to dissolve hydrophilic entities [13]. The DCM fraction (MLED) contained terpenoids, steroids, and phenolics—the latter evidenced by dark coloration after FeCl3 addition—yet showed no saponins, flavonoids, or alkaloids. DCM's intermediate polarity permits recovery of moderately polar substances, accounting for the observed profile. The methanol fraction (MLEM) displayed the most diverse composition, confirming flavonoids, phenolics, saponins, and terpenoids. Methanol's pronounced polarity drives efficient extraction of polar metabolites from plant matrices. These observations underscore solvent polarity's impact on extracted compound classes and lay the groundwork for interpreting biological activities.

Antimicrobial performance of eucalyptus foliage fractions

The inhibitory capacity of eucalyptus leaf fractions toward Staphylococcus epidermidis and Propionibacterium acnes showed clear differences tied to the solvent choice, as depicted in **Figure 1**. The hexane-derived fraction (MLEH) suppressed S. epidermidis growth at 10% and 20% strengths, whereas the dichloromethane-derived fraction (MLED) achieved this at 5%, 10%, and 20%. No suppression was noted for the methanol-derived fraction (MLEM) against S. epidermidis. Each fraction—MLEH, MLED, and MLEM—proved effective against P. acnes. Based on a standard guideline, clear areas ≤14 mm denote resistance, 15–19 mm suggest moderate responsiveness, and ≥20 mm indicate high responsiveness [14].

MLED emerged as the most effective among the fractions, creating zones measuring 17.41 mm versus S. epidermidis and 17.02 mm versus P. acnes at the highest 20% level, falling into the moderate category. These measurements resemble prior data where volatile oil from Melaleuca alternifolia generated zones of 21.02 mm against S. epidermidis and 20.05 mm against Cutibacterium acnes [15]. In addition, methanol-based fractions of M. alternifolia produced through immersion and Soxhlet techniques recorded zones of 13.47 mm and 13.50 mm against E. coli and S. aureus at 50% levels [16]. Evidence points to MLED possessing strong potential for microbe control, notably toward S. epidermidis and P. acnes, owing to dichloromethane's balanced polarity that optimally isolates active agents, including terpenoids, phenolic entities, and steroidal structures. Such agents commonly impair microbial barrier function, boost leakage potential, and hinder critical biochemical reactions. Specifically, terpenoids incorporate into bilayer membranes to change their dynamics, while phenolics provoke cellular stress by forming reactive oxygen entities, resulting in pathogen elimination. MLEM's inactivity against S. epidermidis highlights the importance of molecular solubility characteristics and solvent matching in driving observed effects.

Chemical composition of eucalyptus leaf fractions

GC-MS evaluation detected 37 components within MLED, 42 within MLEH, and 29 within MLEM, with full details listed in **Table 2**. The identified molecules varied considerably between fractions, shaped by solvent polarity, influencing both quantity and diversity of recovered substances. Key elements in MLEH included eucalyptol (9.69%), α-terpinyl acetate (8.24%), phytol (5.65%), β-sitosterol (10.57%), and ursolic aldehyde (10.34%)—chiefly terpenoid structures, sterols, and lipid-related compounds, matching hexane's affinity for non-polar and evaporative materials. MLED featured prominent eucalyptol (35.56%) and α-terpinyl acetate (18.48%), along with terpinene, phytol, fenchol, endo-borneol, and β-sitosterol (0.81%), covering oxygenated monoterpenoids, sesquiterpenoids, and triterpenoid types aligned with moderate polarity. In contrast, MLEM revealed a distinct makeup dominated by fatty acids, polyphenolic substances, and triterpenes, with top contributors being n-hexadecanoic acid (29.11%), 9,12,15-octadecatrienoic acid (19.85%), β-sitosterol (5.28%), α-amyrin (1.97%), alongside multiple hydroxyl-rich flavonoid-resembling units like 4H-pyran-4-one variants and benzopyranone forms. The intense polarity of methanol allows superior isolation of these bulkier, water-compatible entities.

Table 2. Inhibition zones produced by (A) MLEH, MLED, and MLEM toward Staphylococcus epidermidis and (B) Propionibacterium acnes microorganisms

Sample	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, MLEM: Melaleuca leucadendron leaves methanol extract, *: Chloramphenicol 30 µg/mL, **: Vancomycin 30 µg/mL

The primary component, eucalyptol (1,8-cineole), has been documented to restrict Staphylococcus aureus and Escherichia coli proliferation at a minimum inhibitory concentration (MIC) of 1250 µg/mL [17]. Its effect presumably stems from damaging barrier coherence and suppressing respiratory activity. Ranking second, α-terpinyl acetate displayed microbe-restraining properties against S. aureus and E. coli, with MIC figures of 31.30 mg/mL and 125 mg/mL [18]. It likely interferes with lipid organization and biochemical pathways. Other revealed entities, for example, α-terpineol, exhibited strong suppression of oral microbes such as Porphyromonas gingivalis, Prevotella intermedia, and Fusobacterium nucleatum, yielding MIC ranges of 0.2 to 0.8 mg/mL and minimum bactericidal concentration (MBC) spans of 0.2 to 0.4 mg/mL [19]. Borneol variants (both exo- and endo-) present inhibited S. aureus, Bacillus subtilis, Pseudomonas aeruginosa, and E. coli, reaching MIC levels as low as 6.49 µg/mL [20]. These agents appear to heighten envelope openness, facilitating the release of internal materials.

In summary, the pronounced antimicrobial potency of MLED stems from the collaborative influence of its primary components—eucalyptol (1,8-cineole), α-terpinyl acetate, and borneol-related structures—which collectively impair microbial cellular processes. As a cyclic ether-type monoterpenoid, eucalyptol primarily exerts its effect by infiltrating phospholipid layers, elevating permeability, provoking internal content release, and triggering cell rupture. The oxygenated monoterpene α-terpinyl acetate has proven capable of compromising envelope structure and blocking pump systems, potentially increasing internal buildup of inhibitory substances. Likewise, bicyclic monoterpenes such as borneol and fenchol undermine microbial envelope integrity and metabolic energy by collapsing proton motives and deactivating respiratory components. These pathways correspond to earlier descriptions in Annonaceae species, where terpenoids and associated plant products curb microbial proliferation via diverse routes, encompassing blockage of envelope formation, disturbance of attached enzymatic activities, and promotion of oxidative damage through reactive oxygen species (ROS) generation [21]. The arrangement of active agents in the current MLED fraction, especially those of moderate polarity, reinforces such processes. The simultaneous presence of eucalyptol, α-terpinyl acetate, and borneol analogs within one fraction probably intensifies envelope-disrupting actions and accounts for the larger suppression areas recorded against S. epidermidis and P. acnes. Comparable cooperative effects between terpenoids and additional plant-derived molecules have previously yielded stronger growth-arresting and lethal results, even toward resistant microbial variants **(Table 3).**

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

Identified Compound	Retention Time (min)	Area (%) – MLEH	Area (%) – MLED	Area (%) – MLEM
α-Pinene	7.03	0.9	1.09	–
Benzene, 1-ethyl-3-methyl	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	–	–
Humulene	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	–	–
4α(2H)-Naphthalenol, hexahydro derivative	25.635	0.85	–	–
τ-Muurolol	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	–	–
Phthalic 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 phthalate	45.844	0.94	–	–
Squalene	46.575	3.93	–	–
Vitamin E	50.64	3.36	–	–
β-Sitosterol	53.085	10.57	0.81	5.28
α-Amyrin	54.184	5.07	–	1.97
Ursolic aldehyde	58.561	10.34	–	–
Curcubitacin B, 25-desacetoxy	53.609	–	–	0.77

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

Conclusion

Foliage fractions from M. leucadendron displayed differing levels of antimicrobial action toward pathogens responsible for acne development. Of the fractions assessed, MLED delivered the greatest suppressive impact on S. epidermidis and P. acnes, the principal microbes driving acne formation. GC-MS profiling of MLED uncovered key active entities such as eucalyptol (1,8-cineole), α-terpinyl acetate, α-terpineol, fenchol, and endo-borneol—substances recognized for damaging microbial barriers and hindering vital intracellular operations. Results imply that MLED could serve as an effective surface-applied antimicrobial for managing bacteria-induced acne. Additional investigations are advised to purify and define the responsible agents,

clarify their operational pathways, and assess their capacity to boost conventional drug performance via resistance-reversing effects. Clinical trials and toxicity assessments are also essential to validate MLED's practical use in acne therapy.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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