Trop J Nat Prod Res, September 2025; 9(9): 4336 - 4342 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Antibacterial Activity of *Melastoma malabathricum* L. Leaves and Efficacy in Liquid Spray Formulation against Acne-Causing Bacteria (*Staphylococcus epidermidis*)

Rizky Y. Putra[1], Gesit Ayuningtyas[1], Ruri P. Mariska[1], Santi Perawati[2], Lili Andriani[3], Priyambodo[4], Asep Muhamad Ridwanuloh[5], Dwi Wulandari[5], Rina Isnawati[5], Siti Hamidatul Aliyah[5]*

[1]*Departement of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi, 36122, Jambi, Indonesia*
[2]*Departement of Pharmacy, Faculty of Medicine and Health Sciences, Universitas Jambi, 36361, Jambi, Indonesia;*
[3]*Chemistry Doctoral Program, Faculty of Mathematics and Natural Sciences, Universitas Andalas, 25163, Padang, West Sumatera, Indonesia;*
[4]*Department of Biology, Faculty of Mathematics and Sciences, Universitas Lampung, Bandar Lampung, 35141, Lampung, Indonesia;*
[5]*Center for Biomedical Research, Research Organisation for Health, National Research and Innovation Agency (BRIN), Cibinong Science Centre, Cibinong- Bogor, 16912, West Java, Indonesia.*

ARTICLE INFO

Article history:
Received 07 July 2025
Revised 31 July 2025
Accepted 05 August 2025
Published online 01 October 2025

ABSTRACT

Melastoma malabathricum L. *(M. malabathricum L)* is traditionally used by the Anak Dalam tribes in Jambi Province to treat diarrhea and is empirically recognized for antibacterial properties, particularly as an anti-acne agent. Therefore, this study aimed to evaluate the antibacterial activity of crude extracts, fractions, and the physical stability of the liquid spray formulation derived from *M. malabathricum* L. leaves. For the experiment, leaves were extracted using the maceration method, and the crude extract was fractionated with liquid-liquid extraction. Antibacterial activity of crude extracts and fractions of *M. malabathricum* L. was tested using the disc diffusion method at concentrations of 5%, 10%, and 20% against *Staphylococcus epidermidis* (*S. epidermidis*) ATCC 12228. The results showed that all samples had antibacterial activity, namely crude extract (10.4 mm, 16.2 mm, 20.4 mm), n-hexane fraction (10.2 mm, 15 mm, 17.5 mm), ethyl acetate fraction (10.5 mm, 16.4 mm, 18.3 mm), and aqueous residue (10.3 mm, 12 mm, 16.8 mm). Ethyl acetate fraction (10%) showed the highest antibacterial effect (16.4 mm ± 0.20), while liquid spray formulation of the fraction produced a 15.7 mm ± 0.88 inhibition zone. F1 comprising ethyl acetate fraction (10%) was successfully incorporated into liquid spray formulation that showed good stability and was non-irritating. In conclusion, ethyl acetate fraction of *M. malabathricum* L. leaves showed the most potent antibacterial activity against *S. epidermidis* and remained effective after being formulated into a stable liquid spray.

Keywords: Anti-acne, *Melastoma malabathricum* l., Liquid Spray, *Staphylococcus epidermidis.*

Introduction

Acne (Acne vulgaris) is a common skin infection experienced by male and female adolescents aged 15–18 years, with a prevalence of 80–85%.[1] The prevalence of acne reduces progressively with advancing age following adolescence, although the disease burden remains substantial among young adults.[2] Acne has a complex etiology that includes follicular hyperkeratinization, microbial colonization, local immunological responses, and increased sebum production. *Staphylococcus aureus* and *Propionibacterium acnes* (*Cutibacterium acnes*) are believed to be the main bacteria causing acne, capable of turning sebum lipids into substances that trigger inflammation and activate the body's immune response.[3] However, growing evidence suggests that *S. epidermidis* is commonly considered a harmless skin bacteria that plays an important role in microbial homeostasis in skin. Under healthy conditions, *S. epidermidis* remains non-pathogenic and supports skin defense mechanisms without triggering host immune activation.

*Corresponding author. Email: siti.hamidatul.aliyah@brin.go.id
Tel: +62-852-5828-9670

Citation: Putra RY, Ayuningtyas G, Mariska RP, Perawati S, Andriani L, Priyambodo, Ridwanuloh AM, Wulandari D, Isnawati R, Aliyah SH. Antibacterial Activity of *Melastoma malabathricum* L. Leaves and Efficacy in Liquid Spray Formulation against Acne-Causing Bacteria (*Staphylococcus epidermidis*). Trop J Nat Prod Res. 2025; 9(9): 4336 – 4342
https://doi.org/10.26538/tjnpr/v9i9.32

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

These bacteria, associated with acne, can lead to opportunistic infections, particularly during puberty, due to elevated androgen levels that stimulate sebaceous gland enlargement and increased sebum production.[4] In this study, *S. epidermidis* was selected for antimicrobial testing due to the role as both a commensal and an opportunistic pathogen in acne. *S. epidermidis* can compete with *C. acnes*, form biofilms, and produce modulins as well as proteases that contribute to inflammation and damage skin barrier. However, excessive *S. epidermidis* has the potential to cause blocked hair follicles and problems with the immune system, particularly in skin prone to acne. It also has the ability to ferment glycerol into succinic acid, which can inhibit *C. acnes*, suggesting a dualistic role in acne pathogenesis.[5] Compared to *S. aureus*, which is more commonly associated with secondary infections and abscesses, *S. epidermidis* is more frequently isolated from early acne lesions and reflects the dynamic microbial interactions in the pilosebaceous unit. Although *C. Acnes* remains a key contributor to acne, it is a strict anaerobe that presents greater challenges for in vitro culturing and formulation testing.[6] This shows that *S. epidermidis* serves as a relevant and practical model for preliminary screening of anti-acne antibacterial agents.

Conventional treatment of acne often includes the administration of topical or systemic antibiotics, such as erythromycin, doxycycline, and clindamycin. Although these therapies effectively reduce bacteria load and inflammation, prolonged or inappropriate use can lead to undesirable outcomes such as cutaneous irritation, antibiotic resistance, and immune hypersensitivity reactions. Synthetic agents commonly used in acne formulation, such as benzoyl peroxide and azelaic acid, are frequently associated with adverse dermatological effects.[7] Given the limitations and safety concerns associated with conventional acne treatments, there is growing interest in developing alternative

therapeutic agents derived from natural sources. Plant-based compounds with inherent antibacterial and anti-inflammatory properties offer promising potential as safer, more biocompatible options. A widely known candidate is *M. malabathricum* L., a medicinal plant traditionally used in various ethnobotanical practices and reported to possess broad-spectrum antimicrobial activity.

M. malabathricum L. is a medicinal plant commonly found in shrublands and agricultural environments, including rubber and oil palm plantations. This plant, particularly the leaves, is known for various pharmacological activities, such as antibacterial, antioxidant, anti-inflammatory, anticancer, antihepatotoxic, antidiabetic, and antiseptic effects.[8] Traditionally, the leaves of *M. malabathricum* L. have been used to treat various ailments, including diarrhea, dysentery, leucorrhea, hemorrhoids, wounds, infections, toothaches, stomachaches, and cancer sores.[9] Phytochemical analyses have identified several major bioactive compounds in plant, including flavonoids, saponins, steroids, terpenoids, and tannins.[10] As a natural remedy widely used in traditional medicine, *M. malabathricum* L. is considered relatively safe and associated with minimal side effects. Regarding acne treatment, there is a need to develop a practical and user-friendly dosage form of *M. malabathricum* L. leaves extract. One promising formulation is liquid spray, which offers several advantages, including ease of application, reduced risk of microbial contamination due to sealed packaging, and minimized skin irritation during use.

A study conducted by Novasella et al. (2022) showed antibacterial activity of an ethanol-based ointment formulated from *M. malabathricum* L. leaves extract against *S. aureus*, with notable inhibition zones observed at concentrations of 6% (11.04 mm), 8% (11.24 mm), and 10% (11.47 mm).[11] Phytochemical screening of the extract confirmed the presence of flavonoids, saponins, tannins, and steroids, which are known for antimicrobial properties.[12] Based on the results, further investigation is recommended to explore antibacterial potential of *M. malabathricum* L. in alternative dosage forms. Therefore, this study aimed to evaluate antibacterial activity of crude extracts, fractions, and the physical stability of liquid spray formulation derived from *M. malabathricum* L. leaves.

Materials and Methods

Plant Collection and Identification

M. malabathricum L. leaves were collected from Bagan Pete Subdistrict, Jambi City, in June 2024, with the coordinates of 1°40'05.2"S and 103°32'50.9"E. The plant was authenticated by the Plant Systematics Laboratory, Faculty of Biology, Universitas Gadjah Mada. No. 00532/S.Tb./I/2024. A total of 17,296 grams of *M.malabathricum* L. leaves were cut into small pieces, dried, ground, and powdered using a blender, then sieved through a 60-mesh sieve to obtain a uniform fine powder suitable for extraction (Figure 1).

Figure 1: *Melastoma malabathricum* L. (A) leave and flower; (B) dried leave; (C) dried leave powder before extraction; and (D) crude extract.

Extraction and Fractination of Plant Material

A total of 4,678 grams of the *M.malabathricum* L. powder was extracted using 96% ethanol as the solvent for 24 hours at a 1:10 (w/v) ratio. The collected filtrate was evaporated using a rotary evaporator at at a temperature of 50℃ and a pressure of 175 mbar to yield a thick and semi-solid crude extract. Subsequently, this crude ethanol extract was fractionated with n-hexane and ethyl acetate solvents in a 1:2 ratio. The fractions were then concentrated and prepared as test samples, while dried extracts were weighed and stored at -4℃. The percentage yield was obtained using this formula:

$$\text{The percentage yield } = \frac{W2 - W1}{W0} \text{ x } 100\%$$

where W2 is the weight of the fraction and the container, W1 is the weight of the container alone, and W0 is the weight of the total raw material.

Antibacterial activity of extracts and fractions

Microorganism and culture

A total of 2 grams of Nutrient Agar (NA) was dissolved in 100 mL of distilled water, heated to 120℃ until completely dissolved, and sterilized in an autoclave at 121℃ for 15 min. The sterile NA solution was poured into 10 mL test tubes and solidified in a slanted position at a 45° angle. A colony of *S. epidermidis* ATCC 12228 from a pure culture was obtained using an inoculating needle and streaked onto the surface of the solidified NA, followed by incubation at 35℃ for 24 h. The rejuvenated bacteria were suspended in 0.9% NaCl solution and adjusted using a UV-Vis spectrophotometer at a wavelength of 580 nm to reach a 25% transmittance. This served as the inoculum standard before testing antibacterial activity of the crude extract, fractions, and ethyl acetate liquid spray of *M. malabathricum* L.[13]

Agar disk diffusion method

Antibacterial activity assay was performed using the disk diffusion method. Initially, 1 mL of bacteria suspension (*S. epidermidis* ATCC 12228) was inoculated onto MHA medium in petri dishes containing 20 mL of agar. The suspension was evenly spread using a hockey stick or cotton swab and allowed to dry. Sterile paper discs (6 mm in diameter) were immersed in the crude extract, n-hexane, and ethyl acetate fractions solutions at concentrations of 5%, 10%, and 20%. The positive (Clindamycin disc, 2 µg) and negative controls (10% DMSO) were placed on the agar surface aseptically using sterile forceps, ensuring a 2–3 cm distance from the petri dish edge. DMSO served as the solvent for both the crude extract and fractions of *M. malabathricum* leaves. At a concentration of 10% (or lower, often 1-5%), DMSO was considered to have no inhibitory effect on bacteria growth, serving as non-cytotoxic to specific cells. Subsequently, the plates were incubated at 37℃ for 24 hs, and all treatments were performed in triplicate. Antibacterial activity of *M. malabathricum* L. leaves extract against *S. epidermidis* was evaluated by observing and measuring the inhibition zones formed around the paper discs after incubation. The diameter of the clear zones was measured using a caliper. The inhibition zone diameter was categorized as follows, weak (5 mm), moderate (5–10 mm), strong (10–20 mm), and very strong (20–30 mm). [14]

Development of liquid spray

The liquid spray formulation was developed using acceptable pharmaceutical-grade ingredients, as shown in Table 1. The ethyl acetate fraction of *M. malabathricum* L. at a concentration of 10%, which showed the largest inhibition zone among other concentrations and fractions, was selected as the active antibacterial agent. A combination of propylene glycol as a co-solvent, dimethyldimethyl hydantoin (DMDM hydantoin), which was the preservative, and distilled water as a solvent produced a stable and homogenous liquid formulation[15]. All components were thoroughly mixed until a uniform solution was obtained. A total of 2 formulations were prepared, namely F0 (control, without extract) and F1 (containing 10% ethyl acetate fraction). The final volume of each formulation was adjusted to 150 mL with distilled water.

Evaluation and physical stability testing of liquid spray formulation

The physical characteristics of liquid spray formulation were assessed

visually to observe any changes in colour, odour, and texture during storage. Homogeneity testing was conducted by placing a small amount of formulation onto a glass slide and visually inspecting for coarse particles or clumps, showing incomplete mixing. Viscosity was evaluated using a Brookfield viscometer. A total volume of 100 mL of formulation was placed into a beaker, and viscosity was measured using spindle No. 1 at 6 rpm. An ideal spray formulation should have a viscosity of less than 150 cps. The pH values were measured using a digital pH meter. The electrode was immersed in liquid spray until a stable reading was obtained. The target pH range was 4.5–7 to ensure skin compatibility and minimize the risk of irritation. Spray pattern testing was performed by spraying formulation onto a plastic mica sheet from distances of 5, 10, and 20 cm.[16] Drying time was recorded using a stopwatch, and the diameter of spray pattern was measured. An optimal spray pattern was characterized by forming fine, evenly dispersed particles. Furthermore, a stability test was performed to examine organoleptic properties, pH, homogeneity, and viscosity over a four-cycle storage period.[17] One cycle included storing liquid spray at 4°C for 24 h and 40°C for 24 h. Physical properties were compared throughout the study period to assess any changes in formulation.

Table 1: Liquid Spray Formulation of *M. malabathricum* L. Leaves

Ingredients	Function	F0	F1
Ethyl acetate fraction	Antibacterial active ingredient	0%	10%
Propylene glycol	Co-solvent	5%	5%
Dimethyldimethyl Hydantoin (DMDM hydantoin)	Preservative	0.18%	0.18 %
Distilled water	Solvent	ad 150 mL	ad 150 mL

Note: **F0**: Without extract and fraction, **F1**: Ethyl acetate fraction with concentration 10%.

Skin irritation testing by the open patch test

The volunteer skin-irritation test and satisfaction evaluation were obtained from the Health Research Ethics Committee of Poltekkes Kemenkes Jambi with No. LB.02.06/2/620/2024. Skin irritation testing was conducted on 20 healthy volunteers aged 20 to 30 using the open patch method. The skin irritation testing included the application of 0.5 mL of F1 liquid spray formulation onto a patch, which was placed on the inner forearm before covering for four hs. The first observations were made after 15 minutes to detect any signs of irritation, such as redness, itching, or swelling. [18]

Preference test

The preference test evaluated user satisfaction with the F1 liquid spray formulation. A cohort of 20 participants was recruited to assess specific sensory parameters, such as colour, odour, texture, and ease of spraying. Responses were collected using a questionnaire comprising a like and dislike scale represented by 1 and 0, respectively. [19]

Antibacterial activity of ethyl acetate liquid spray

Ethyl acetate liquid spray was evaluated for its antibacterial activity on *S. epidermidis* using the disc diffusion method. Bacteria suspension was adjusted using a UV-Vis spectrophotometer at a wavelength of 580 nm to reach a 25% transmittance, serving as the inoculum standard before testing antibacterial activity of the F0 and F1 liquid spray formulation. Sterile paper discs (6 mm in diameter) were immersed in the F0 and F1. Furthermore, positive (Commercial anti-acne product) (FP) and negative (10% DMSO) controls were placed on the agar surface aseptically using sterile forceps, ensuring a 2–3 cm distance from the petri dish edge. The plates were incubated at 37°C for 24 h, and antibacterial activity of liquid spray formulation against *S. epidermidis* was evaluated by observing and measuring the inhibition zone formed around the paper discs after incubation with a caliper.[18]

Data analysis

All data analyses used were descriptive, with results presented as tables or diagrams. In this study, the data to be obtained were the inhibition values of the extract, fractions, and liquid spray formulation of *M. malabathricum* L. leaves, as well as the evaluation and physical stability expressed as mean ± standard deviation calculated using Microsoft Excel.

Results and Discussion

Yield of the extracts and fractions

A total of 4,678 grams of dried leaves of *M. malabathricum* L. was extracted using ethanol as the solvent, yielding 482 grams of thick crude extract. The extraction yield was determined to be 10.3% (w/w). The physical appearance of *M. malabathricum* L. leaves extract was a thick, dark green extract with a characteristic odour. The ethanol extract of *M. malabathricum* L. contains flavonoids, phenols, tannins, saponins, and terpenoid compounds.[20] The fractionation process used 231.66 grams of crude extract *M. malabathricum* L., yielding fraction percentages of 52.4%, 29.4%, and 16.3% for n-hexane, ethyl acetate, and aqueous residue. The n-hexane fraction was found to contain alkaloids and steroids,[21] while ethyl acetate consisted of phenol, tannin, and flavonoids.[22]

The extraction process of natural materials is usually used to obtain the desired active compounds. In this study, extraction was carried out from *M. malabathricum* for the application as antibacterial against acne-causing bacteria (*S. epidermidis*). Current extraction methods are categorized into conventional which includes maceration, percolation, and reflux extraction, while modern extraction consists of Ultrasound Assisted Extraction (UAE), Microwave Assisted Extraction (MAE), Pressurized Liquid Extraction (PLE), Supercritical fluid extraction (SFE), Pulsed electric field extraction (PFE), and Enzyme Assisted Extraction (EAE). The maceration method was selected in this study due to the lack of changing the solvent during soaking, simplicity (no special tools required), and absence of heating to protect the active compounds from the risk of degradation.[23]

A total of 4,678 grams of dried *M. malabathricum* leaves were mixed to reduce particle size, thereby increasing extraction efficiency due to high interaction between the sample and the solvent. The crude extract yield was 10.3%, which met the minimum yield requirement of 10% as specified in the Indonesian Herbal Pharmacopoeia, Edition II.[24] Ethanol was used as the solvent due to the numerous advantages, including high absorption capacity, antifungal and antibacterial properties, and the ability to dissolve a wide range of active compounds with varying polarities.[25]

The fractionation process used non-polar (n-hexane) and semi-polar (ethyl acetate) solvents. The n-hexane solvent selectively extracted non-polar compounds, while ethyl acetate extracted semi-polar compounds. Based on the results, fractionation yields were 54.2%, 29.4%, and 16.3% for n-hexane, ethyl acetate, and aqueous residue. The differences in fractionation yields were due to variations in the polarity of the chemical compounds.[26] The n-hexane fraction had the highest yield, showing the role in extracting non-polar compounds. Solvent separation from the macerate was performed using a rotary evaporator with a chiller water temperature of 15°C, which was considered optimal for maintaining a stable sublimation flow rate.

Antibacterial Activity

The results of antibacterial activity test of *M. malabathricum* L. leaves extract and fractions against *S. epidermidis* and the inhibition zone diameter of the extract and fractions are presented in Table 2. Antibacterial activity test results of crude extract and fractions at three different concentrations (5%, 10%, and 20%) showed that the highest Minimum Inhibitory Concentration (MIC) from the lowest concentration was observed in the ethyl acetate fraction at 10%, with a substantial inhibition zone diameter of 16.4 mm ± 0.20 against *S. epidermidis*.

Table 2: Antibacterial activity of *M. malabathricum* L. leaves extract against *S. epidermidis* by the agar disc diffusion method

Conc. (%)	Average (mm) ± standard deviation			
	Crude Extract	n-Hexane Fraction	Ethyl Acetate Fraction	Aqueous Residue
5	10.4 ± 0.49	10.2 ± 0.81	10.5 ± 3.28	10.3 ± 0.79
10	16.2 ± 0.20	15 ± 0.47	16.4 ± 0.20	12 ± 0.32
20	20.4 ± 0.20	17.5 ± 0.35	18.3 ± 0.45	16.8 ± 0.88
Positive control	24.7 ± 0.25	25.6 ± 0.30	27.5 ± 0.32	26.3 ± 0.11
Negative control	0	0	0	0

Antibacterial activity of *M. malabathricum* L. crude extract and the fractions was evaluated using the disc diffusion method against *S. epidermidis*. This method was selected due to simplicity and the absence of creating agar wells, which posed a higher risk of damaging the agar medium.[27] *S. epidermidis* was selected as the test bacteria due to the potential to cause acne. Other acne-associated bacteria include *S. aureus* and *Propionibacterium acnes*, which can hydrolyze fats, breaking down free fatty acids from skin lipids. This process triggers inflammation, leading to bacterial proliferation and exacerbation of acne lesions. [3]

The results showed that ethyl acetate fraction at a 10% concentration had the highest MIC value, with the inhibition zone diameter of 16.4 mm ± 0.20, categorized as strong inhibition. At a 5% concentration, the inhibition zone fell into the moderate category. Previous studies reported that ethyl acetate fraction was the most effective in inhibiting *S. epidermidis*. Clindamycin was used as a positive control due to its high sensitivity against *S. epidermidis.* The ethyl acetate fraction of *M. malabathricum* L. is assumed to contain phenol, tannin, and flavonoid, contributing to antibacterial activity. Flavonoid is a potential compound with antibacterial activity, although the mechanism of action is not fully understood.[28,29] Additionally, flavonoids can reduce biofilm formation, membrane permeability, and reverse antibiotic resistance.[30] These results support the potential of ethyl acetate fraction as antibacterial agent, particularly against *S. epidermidis.*

A previous report by Novasella used *M. malabathricum L.* leaves extract against *S. aureus*, showing antibacterial activity categorized as strong (range 11 mm). Meanwhile, this study used *S. epidermidis* and showed strong antibacterial activity, supporting the hypothesis that *M. malabathricum L.* could serve as antibacterial agent.

Characterization of liquid spray formulation

F0 (control, without extract) and F1 (containing 10% ethyl acetate fraction of *M. malabathricum* L.), were evaluated based on organoleptic properties, homogeneity, pH, viscosity, spray pattern, and stability as shown in Figure 2 and Table 3. Formula F0 showed a clear colour, and F1 had a clear green colour and a distinctive herbal aroma. The homogeneity test showed that F0 and F1 were homogeneous and free from lumps or coarse particles, showing an even mix of components. The viscosity test was used to measure the ease of spraying using a Brookfield viscometer. Both formulations had viscosity values below 150 cps (19.3 cps for F0 and 46.5 cps for F1), showing suitability for spray products. The pH value of liquid spray must be within the skin pH range, which is 4.5-7. Based on the results, pH measurement at F0 was 5, and F1 showed a slightly acidic of 4.7, which was within the safe range for topical products (4.5–7), showing compatibility with skin. Spray pattern test was observed in terms of drying time and distribution diameter at test distances of 5 cm, 10 cm, and 20 cm. F1 spray pattern produced fine particles evenly distributed at all test distances, and the drying time was less than 30 seconds, which was expected to provide comfort and practicality for users. During four cycles of stability testing with alternating storage at 4°C and 40°C, F1 maintained organoleptic characteristics, viscosity, homogeneity, and pH without significant changes, showing good physical stability (Table 4). These results suggested that the F1 was considered optimal for further antibacterial activity testing and user preference testing.

Table 3: Characteristic of liquid spray formulation of *M. malabathricum* L. Leaves

Formulation	Colour	Odour	pH
F0	clear	distinctive aroma	5 ± 0
F1	clear green	herbal-like smell	4,7 ± 0,2
FP (control)	clear	distinctive aroma	5 ± 0,1

Note: **F0**: Without extract and fraction, **F1**: Ethyl acetate fraction with concentration 10%, **FP:** positive control (commercial anti-acne).

Figure 2: The appearance of liquid spray formulation. (F0) liquid spray base, (F1) containing 10% ethyl acetate fraction (F1), (FP) positive control (commercial anti-acne)

The liquid spray formulation was developed due to several advantages, including lower microbial contamination risk, rapid effect, and hands-free application. Physical evaluation of liquid spray included organoleptic tests for colour, odour, and texture, which were influenced by the ethyl acetate fraction. Homogeneity testing confirmed uniform composition across all formulations (F0, F1, FP) without coarse particles or aggregates. pH testing ensured safety, with values of 5 ± 0 for F0, 4.7 ± 0.2 for F1, and 5 ± 0.1 for FP, all within the acceptable skin range (4.5–7). Viscosity measurements, conducted using a Brookfield viscometer, showed compliance with spray formulation requirements (<150 cps). Spray pattern evaluation showed effective dispersion, with the best distribution observed in F0 formula. Drying time analysis showed that a 20 cm spraying distance obtained the fastest drying time, with the best results observed in the 10% ethyl acetate fraction formulation. Stability testing over four cycles showed consistent organoleptic properties and homogeneity. Viscosity increased over time due to storage temperature variations. Changes in pH were within acceptable limits, although prolonged storage could promote microbial growth and degradation [31].

Table 4: Stability of liquid spray formulation after four cycles

Parameters	Formulation	Cycle 1	Cycle 2	Cycle 3	Cycle 4
Colour	F0	clear	clear	clear	clear
	F1	clear green	clear green	clear green	clear green
Odour	F0	distinctive aroma	distinctive aroma	distinctive aroma	distinctive aroma
	F1	herbal-like smell	herbal-like smell	herbal-like smell	herbal-like smell
Homogeneity	F0	homogen	homogen	homogen	homogen
	F1	homogen	homogen	homogen	homogen
pH	F0	5 ± 0	4.85 ± 0.25	5.07 ± 0.28	5.1 ± 0.24
	F1	4.8 ± 0.1	5.3 ± 0.40	5.3 ± 0.36	5.13 ± 0.23
Viscosity	F0	18.4 ± 0.34	58.6 ± 0.05	112.4 ± 0.32	144 ± 0.88
	F1	43 ± 0.72	72.3 ± 0.28	76.03 ± 0.80	72.3 ± 0.28

Irritation testing

The liquid spray formulation containing 10% w/v ethyl acetate fraction of *M. malabathricum* L. was evaluated for skin irritation by patch testing with an observation period of 4 h. Following the application, the test areas were monitored for any signs of erythema or edema. All subjects included in the test (n = 20) showed no itching, redness, or swelling response to all formulations.

Satisfaction test

The assessments of a sample of 20 subjects showed the implications related to participant approval of liquid spray formulation. All subjects generally liked formulation containing ethyl acetate fraction in terms of colour, odour, texture, and ease of spraying, with a preference level reaching 100%. However, regarding aroma, the majority did not like the smell of F1 compared to F0, with a dislike level reaching 90%. Preference testing showed that 100% of subjects favoured all formulations' colour, texture, and spray ability. However, 90% found the odour of F1 unpleasant due to the herbal scent.

Antibacterial activity of liquid spray formulation

Antibacterial activity of the liquid spray preparation was evaluated based on the diameter of the inhibition zone against bacterial growth. The results showed that all formulations, namely F0, F1, and FP (positive control), showed relatively potent inhibition (Figure 3). F1 showed the highest antibacterial activity with an average inhibition zone diameter of 15.7 ± 0.88 mm, followed by FP (12.9 ± 0.3 mm) and F0 (11.5 ± 0.23 mm). Meanwhile, the negative control (DMSO) showed no inhibition zone (0 mm) (Table 5). These data showed that adding active ingredients, particularly F1, increased the antibacterial effectiveness of the liquid spray preparation, compared to the positive control.

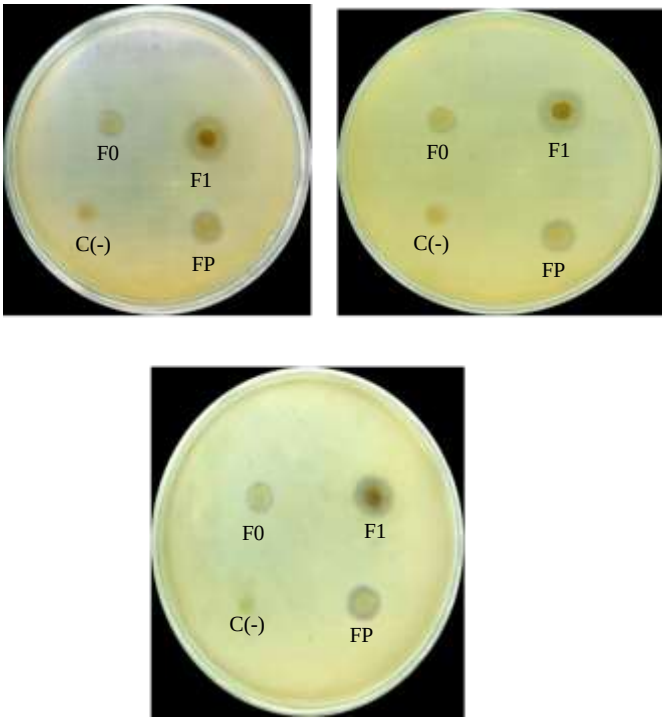

Figure 3: Antibacterial activity test result of liquid spray containing 10% ethyl acetate fraction of *M. malabathricum* L (F1), liquid spray base (F0), positive control (commercial anti-acne) (FP), Negative control (10% DMSO) (C(-)) against *S. epidermidis*.

Antibacterial efficacy of liquid spray was highest in ethyl acetate fraction at 10%, showing potent inhibition (16.4 mm ± 0.20), while the 5% concentration was moderate. The average inhibition zone diameter of liquid spray against *S. epidermidis* was 11.5 mm ± 0.23 for F0 (base formula), 15.7 mm ± 0.88 for F1 (ethyl acetate fraction), and 12.9 mm ± 0.3 for FP (commercial anti-acne product).

Table 5: Antibacterial activity of formulation containing *M. malabathricum* L. ethyl acetate fraction against *S. epidermidis* by the agar disc diffusion method

Formulation	Zone of Inhibition (mm) mean ± SD	Inhibitory activity category
negative control (10% DMSO) (C (-))	0	No activity
Liquid spray base (F0)	11.5 ± 0.23	Strong
Liquid spray containing 10% ethyl acetate fraction (F1)	15.7 ± 0.88	Strong
positive control (FP)	12.9 ± 0.3	Strong

The F0 formulation showed potent antibacterial activity (11.5 mm ± 0.23), while F1 (10% ethyl acetate fraction) had even more vigorous activity (15.7 mm ± 0.88). The minor difference in the inhibition zone between formulation was attributed to the co-solvent concentration

(5%) and active ingredient concentration (10%). Propylene glycol served dual functions as a cosolvent and a humectant, contributing to water retention and overall formulation stability.[32] Additionally, DMDM hydantoin was incorporated as a preservative to prevent microbial contamination due to the high water content of spray.[33] Ethyl acetate fraction (10%) had the highest inhibition zone diameter compared to the commercial anti-acne product and base formula, showing superior antibacterial efficacy for liquid spray formulation. F0 (without plant extracts) showed moderate antibacterial activity, which was due to the presence of propylene glycol. [34] Besides serving as a cosolvent and humectant, propylene glycol has mild antibacterial activity, particularly against Gram-positive bacteria such as *S. epidermidis*. DMDM hydantoin, used as a preservative, caused cessation of microbial growth and played a small role in the inhibition zone of F0.

Further studies are recommended to identify the active compounds in the ethyl acetate fraction using the LC-MS/MS method and determine the specific components that play a role in antibacterial activity. In the development of a liquid spray formulation based on the ethyl acetate fraction of *M. malabathricum* L. leaves, it is recommended to add perfume to increase users' acceptance of the aroma, considering that most respondents thought the current aroma less desirable.

Conclusion

In conclusion, extracts and fractions of *M. malabathricum* L. leaves show antibacterial activity against *S. epidermidis*, which is associated with acne. Furthermore, ethyl acetate fraction (10%) liquid spray formulation shows vigorous antibacterial activity against *S. epidermidis*, with inhibition zone of 16.4 mm ± 0.20. This formulation also maintains good physical stability, as shown by organoleptic evaluation, homogeneity, pH, and viscosity tests throughout the storage period. The results provide a valuable contribution for in vivo test using an established formulation in an animal model with acne-associated bacteria.

Conflict of Interest

The author`s 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.

References

1. Bhate K, Williams HC. Epidemiology of acne vulgaris. Br J Dermatol. 2013; 168(3): 474-485. Doi: 10.1111/bjd.12149.
2. Knutsen-Larson S, Dawson AL, Dunnick CA, Dellavalle RP. *Acne vulgaris*: pathogenesis, treatment, and needs assessment. Dermatol Clin. 2012; 30(1): 99-106, viii-ix. Doi: 10.1016/j.det.2011.09.001.
3. McLaughlin J, Watterson S, Layton AM, Bjourson AJ, Barnard E, McDowell A. *Propionibacterium acnes* and Acne Vulgaris: New Insights from the Integration of Population Genetic, Multi-Omic, Biochemical and Host-Microbe Studies. Microorganisms. 2019; 7(5):128: 2-29. Doi: 10.3390/microorganisms7050128.
4. Foster TJ, Geoghegan JA, Ganesh VK, Höök M. Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. Nat Rev Microbiol. 2014; 12(1): 49-62. Doi: 10.1038/nrmicro3161.
5. Severn MM, Horswill AR. *Staphylococcus epidermidis* and its dual lifestyle in skin health and infection. Nat Rev Microbiol. 2023; 21(2): 97-111. Doi: 10.1038/s41579-022-00780-3.
6. Jeverica S, El Sayed F, Čamernik P, Kocjančič B, Sluga B, Rottman M, Papst L. Growth detection of *Cutibacterium acnes* from orthopaedic implant-associated infections in anaerobic bottles from BACTEC and BacT/ALERT blood culture systems and comparison with conventional culture media. Anaerobe. 2020; 61:102133: 1-5. Doi: 10.1016/j.anaerobe.2019.102133.
7. Vasam M, Korutla S, Bohara RA. Acne vulgaris: A review of the pathophysiology, treatment, and recent nanotechnology based advances. Biochem Biophys Rep. 2023; 36:101578: 1-8. Doi: 10.1016/j.bbrep.2023.101578.
8. Joffry SM, Yob NJ, Rofiee MS, Affandi MM, Suhaili Z, Othman F, Akim AM, Desa MN, Zakaria ZA. *Melastoma malabathricum* (L.) Smith Ethnomedicinal Uses, Chemical Constituents, and Pharmacological Properties: A Review. Evid Based Complement Alternat Med. 2012; 2012:258434: 1-48. Doi: 10.1155/2012/258434.
9. Suleiman D, Idris AM, Ibrahim UI. Review of Pharmacognostic Features, Phytochemical Constituents and Pharmacological Actions of *Melastoma malabathricum* LINN (Melastomaceae). Niger J Pharm Appl Sci Res. 2018; 7(2): 7-18.
10. Anggraini T, Lewandowsky P. The exotic plants of Indonesia: Mahkota dewa (*Phaleria macrocarpa*), sikaduduak (*Melastoma malabathricum* Linn), and mengkudu (*Morinda citrifolia*) as potent antioxidant sources. Int J Adv Sci Eng Inf Technol. 2015; 5(2): 115-118. Doi: 10.18517/ijaseit.5.2.496.
11. Novasella N, Rahmatullah S, Wirasti W, Pambudi DB. Antibacterial Activity Test of Ointment Formulation of Ethanol Extract of Senggani Leaves (*Melastoma malabathricum* L.) Against *Staphylococcus aureus*. J Ilm JOPHUS J Pharm UMUS. 2022; 3(02):104-110. Doi: 10.46772/jophus.v3i02.527.
12. Hainil S, Rachdiati H, Prawita D. Phytochemical Screening and Antibacterial Activity of Senduduk Leaves (*Melastoma malabathricum* L.). In: Proceedings of the 2nd International Conference on Contemporary Science and Clinical Pharmacy 2021 (ICCSCP 2021). 2022: 211-213. Doi: 10.2991/ahsr.k.211105.031.
13. Halimah; Maryani, Y.; Krisyudhanti, E.; Wardoyo, S. Effectiveness of Secang Wood (*Caesalpinia Sappan* l.) in Inhibiting the Growth of Streptococcus Mutans. Trop J Nat Prod Res. 2024; 8 (6): 7504-7508. Doi: 10.26538/tjnpr/v8i6.26.
14. Sanam MUE, Detha AIR, Rohi NK. Detection of antibacterial activity of lactic acid bacteria, isolated from Sumba mare's milk, against *Bacillus cereus, Staphylococcus aureus*, and *Escherichia coli*. J Adv Vet Anim Res. 2022; 9(1): 53-58. Doi: 10.5455/javar.2022.i568.
15. Fiume MM, Bergfeld WF, Belsito DV, Hill RA, Klaassen CD, Liebler D, Marks JG Jr, Shank RC, Slaga TJ, Snyder PW, Andersen FA. Safety assessment of propylene glycol, tripropylene glycol, and PPGs as used in cosmetics. Int J Toxicol. 2012; 31(5 Suppl): 245-260. Doi: 10.1177/1091581812461381.
16. Martihandini N, Denada D., & Sutiswa, S. I. Formulation of Spray Gel Hand Sanitizer from Citronella Oil Using Gelling Agent Carbopol 940 and Humectant Propylene Glycol. Indones. J. Pharm. Educ. 2023: 447–455. Doi: 10.37311/ijpe.v3i3.22166.
17. Leelapornpisid P., Mungmai L., Sirithunyalug B., Jiranusornkul S., and Peerapornpisal Y. A novel moisturizer extracted from freshwater macroalga [*Rhizoclonium hieroglyphicum* (C.agardh) K. Tzing] for skin care cosmetic. Chiang Mai J. Sci. 2014; 41(5.2): 1195-1207.
18. Chaiwaree S, Khoothiam K, Thepmalee C, Thephinlap C, Suwannasom N. Antibacterial Activity of *Acorus calamus* Linn. and *Litsea cubeba* (Lour.) Pers. and Their Efficacy in Shower Gel Formulation. Trop. J. Nat. Prod. Res. 2021; 5(12): 2085-2091. Doi: 10.26538/tjnpr/v5i12.7.
19. Suena NM, Rahayu LR, Wijaya SC, Antari NP, Adrianta KA. Formulation and Antioxidant Activity Test of *Centella asiatica* Herba Extract and *Moringa oleifera* Leaves Extract as an Anti-Aging Emulgel. Trop. J. Nat. Prod. Res. 2024; 8(3): 6525-6536. Doi:10.26538/tjnpr/v8i3.9.

20. Apridamayanti P, Sari R, Rachmaningtyas A, Aranthi V. Antioxidant, antibacterial activity and FICI (Fractional Inhibitory Concentration Index) of ethanolic extract of *Melastoma malabathricum* leaves with amoxicillin against pathogenic bacteria. Nusant Biosci. 2021; 13(2): 140-147. Doi: 10.13057/nusbiosci/n130202.
21. Apridamayanti P, Pratiwi L, Sari R. Gas chromatography study of n-hexane and chloroform fractions of ethanol extract of *Melastoma malabathricum* L. Leaves: an in vitro study of antioxidant and SPF values. Int J Appl Pharm. 2022; 14(3): 40-46. Doi: 10.22159/ijpps.2022v14i3.43801.
22. Mayasari D, Murti YB, Sudarsono S, Pratiwi SUT. Phytochemical, antioxidant, and antibacterial evaluation of *Melastoma malabathricum* L.: An Indonesian traditional medicinal plant. Trop J Nat Prod Res. 2021; 5(5): 819–24. Doi: 10.26538/tjnpr/v5i5.5.
23. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: a comprehensive review. Chin Med. 2018; 13:20: 2-26. doi: 10.1186/s13020-018-0177-x.
24. Ministry of Health of the Republic of Indonesia. Indonesia Herbal Pharmacopedia. Edisi II, 2017: 4-8.
25. Lee J-E, Jayakody JTM, Kim J-I, Jeong J-W, Choi K-M, Kim T-S, Seo C, Azimi I, Hyun J, Ryu B. The Influence of Solvent Choice on the Extraction of Bioactive Compounds from Asteraceae: A Comparative Review. Foods. 2024; 13(19): 3151: 2-21. Doi: 10.3390/foods13193151.
26. Haslina H, Eva M. Extract Corn Silk with Variation of Solvents on Yield, Total Phenolics, Total Flavonoids and Antioxidant Activity. Indones Food Nutr Prog. 2017; 14(1): 21-28. Doi: 10.22146/ifnp.24280.
27. Jenkins SG, Schuetz AN. Current concepts in laboratory testing to guide antimicrobial therapy. Mayo Clin Proc. 2012; 87(3): 290-308. Doi: 10.1016/j.mayocp.2012.01.007.
28. Periferakis A, Periferakis K, Badarau IA, Petran EM, Popa DC, Caruntu A, Costache RS, Scheau C, Caruntu C, Costache DO. Kaempferol: Antimicrobial Properties, Sources, Clinical, and Traditional Applications. International Journal of Molecular Sciences. 2022; 23(23):15054: 2-39. Doi: 10.3390/ijms232315054.
29. Iranshahi M, Rezaee R, Parhiz H, Roohbakhsh A, Soltani F. Protective effects of flavonoids against microbes and toxins: The cases of hesperidin and hesperetin. Life Sci. 2015; 137: 125-32. Doi: 10.1016/j.lfs.2015.07.014.
30. Shamsudin NF, Ahmed QU, Mahmood S, Ali Shah SA, Khatib A, Mukhtar S, Alsharif MA, Parveen H, Zakaria ZA. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. Molecules. 2022; 9;27(4):1149: 2-43. Doi: 10.3390/molecules27041149.
31. Ardhany SD, Putra CD, Novaryatiin S. Modification of Anti-acne Bawang Dayak (*Eleutherine bulbosa* (Mill.)Urb.) Cream to *Propionibacterium acnes*. J Adv Pharm Technol Res. 2021; 12(1): 94-98. Doi: 10.4103/japtr.JAPTR_107_20
32. Preedalikit W, Chittasupho C, Leelapornpisid P, Qi S, Kiattisin K. Development and Evaluation of Anti-Pollution Film-Forming Facial Spray Containing Coffee Cherry Pulp Extract. Pharmaceutics. 2025; 17(3):360: 2-25. Doi: 10.3390/pharmaceutics17030360.
33. Van Dam A, Van Dam AJ. DMDM-Hydantoin: The Promising Result of a Search for an Alternative in Fluid Preservation of Biological Specimens. Collect Forum. 2023; 18(2):104–15.
34. Mosiichuk N, Enggren G, Da Silva ZL, Morén AK, Hansson H, Engblom J, Ruzgas T. Antibacterial activity of propylene glycol against *Staphylococcus aureus* and *Staphylococcus epidermidis* in neutral and mild acidic conditions. J Antimicrob Chemother. 2025; 80(7):1947-1950. Doi: 10.1093/jac/dkaf153.