



Antibacterial and anti-tyrosinase activity of eucalyptus leaf [*Melaleuca leucadendra* (L.) L.] acne spot emulgel: Formulation and evaluation

[Actividad antibacteriana y antitirosinasa del emulgel para el acné de hoja de eucalipto [*Melaleuca leucadendra* (L.) L.]:
formulación y evaluación]

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Abstract

Context: The eucalyptus plant [*Melaleuca leucadendra* (L.) L.] is one of the plants that has long been used in various traditional and modern medicines. This plant contains numerous active compounds and is beneficial for treating various conditions, including inflammation, pain, diabetes, and cancer.

Aims: To formulate an acne spot emulgel containing *M. leucadendra* leaf extract and to evaluate its antibacterial and anti-tyrosinase activities.

Methods: The extract was obtained by maceration using dichloromethane. Emulgels were prepared using Carbomer 940 as the gelling agent, with three extract concentrations tested (F1, F2, and F3). The formulations were assessed through organoleptic properties, pH, viscosity, spreadability, cycling stability, and irritation tests. Antibacterial activity was determined against *Staphylococcus epidermidis* and *Propionibacterium acnes* using the disc diffusion and dilution method. Anti-tyrosinase activity was evaluated based on its IC₅₀ value.

Results: The F3 formulation demonstrated the highest antibacterial activity, with inhibition zones of 19.5 mm (MIC 250 mg/mL and MBC 250 mg/mL) and 14.27 mm (MIC 125 mg/mL and MBC 125 mg/mL) against *S. epidermidis* and *P. acnes*, respectively. The IC₅₀ value of 271 µg/mL indicated weak inhibition of tyrosinase.

Conclusions: These findings suggest that *M. leucadendra*-based emulgel is a promising candidate for acne treatment. However, further studies, including *in vivo* assessments, are recommended.

Keywords: acne spot; antibacterial; anti-tyrosinase; *Melaleuca leucadendra*; *Propionibacterium acnes*; *Staphylococcus epidermidis*.

Resumen

Contexto: El eucalipto [*Melaleuca leucadendra* (L.) L.] es una planta que se ha utilizado durante mucho tiempo en diversas medicinas tradicionales y modernas. Esta planta contiene numerosos compuestos activos y es beneficiosa para el tratamiento de diversas afecciones, como la inflamación, el dolor, la diabetes y el cáncer.

Objetivos: Formular un emulgel para el acné con extracto de hoja de *M. leucadendra* y evaluar su actividad antibacteriana y antitirosinasa.

Métodos: El extracto se obtuvo mediante maceración con diclorometano. Los emulgeles se prepararon utilizando Carbómero 940 como gelificante, con tres concentraciones de extracto probadas (F1, F2 y F3). Las formulaciones se evaluaron mediante propiedades organolépticas, pH, viscosidad, extensibilidad, estabilidad cíclica y pruebas de irritación. Se determinó la actividad antibacteriana contra *Staphylococcus epidermidis* y *Propionibacterium acnes* mediante el método de difusión y dilución en disco. La actividad antitirosinasa se evaluó con base en su valor de CI₅₀.

Resultados: La formulación F3 demostró la mayor actividad antibacteriana, con zonas de inhibición de 19,5 mm (MIC 250 mg/mL y MBC 250 mg/mL) y 14,27 mm (MIC 125 mg/mL and MBC 125 mg/mL) contra *S. epidermidis* y *P. acnes*, respectivamente. El valor de CI₅₀ de 271 µg/mL indicó una inhibición débil de la tirosinasa.

Conclusiones: Estos hallazgos sugieren que el emulgel a base de *M. leucadendra* es un candidato prometedor para el tratamiento del acné. Sin embargo, se recomiendan estudios adicionales, incluyendo evaluaciones *in vivo*.

Palabras Clave: acné; antibacteriano; antitirosinasa; *Melaleuca leucadendra*; *Propionibacterium acnes*; *Staphylococcus epidermidis*.

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Abbreviations: DMSO: Dimethyl sulfoxide; ELISA: Enzyme-Linked Immunosorbent Assay; MHB: Mueller Hinton Broth; MIC₅₀: Minimum Inhibition Concentration for 50% of the isolate bacteria; TEA: Triethanolamine.

INTRODUCTION

Acne is a chronic inflammatory skin disorder associated with the obstruction and inflammation of the pilosebaceous units. This condition is prevalent among both men and women, affecting adolescents and adults alike. Globally, acne is reported to impact approximately 85% of individuals between the ages of 11 and 30 years. In Indonesia, the prevalence of acne among adolescents aged 15-18 years is estimated to be 80-85%, while 12% of women over the age of 25, and only 3% of individuals aged 35-44 years, are affected. The condition often arises due to increased sebum production, follicular hyperkeratinization, colonization by *Propionibacterium acnes* and *Staphylococcus epidermidis*, and subsequent immune responses. Current treatments include topical antibiotics and anti-inflammatory agents. However, prolonged use may lead to adverse effects and antibiotic resistance (Les-tari et al., 2020).

Acne affects approximately 80% of adolescents, presenting clinical features such as papules, blackheads, pustules, and nodules, which can significantly impact appearance (Sifatullah and Zulkarnain, 2021). Al-Qarqaz et al. (2021) reported that a common sequela of acne is hyperpigmentation, a condition characterized by increased melanin production resulting in the formation of lesions and dark spots on the face. Hyperpigmentation generally arises from two categories of factors: internal and external. Internal factors include genetics, ethnicity, hormonal influences, and post-inflammatory hyperpigmentation (PIH), while external factors encompass the use of certain cosmetics, drugs, chemicals, and exposure to ultraviolet (UV) radiation. Post-inflammatory PIH triggers melanogenesis, the process by which melanin is synthesized in the skin. This process is catalyzed by the enzyme tyrosinase through an oxidative pathway, leading to increased melanin production. Natural ingredients, particularly plant-derived extracts with antibacterial and antioxidant properties, are being increasingly investigated as alternative therapies for acne. One such plant is *Melaleuca leucadendra*, known for its essential oil content, rich in 1,8-cineole, α -pinene, and terpineol, which exhibit antimicrobial and anti-inflammatory activity.

Melaleuca leucadendra (L.) L. (family *Myrtaceae*), commonly known as eucalyptus, is a plant native to Indonesia that plays an important role in the industry. This plant typically grows in areas with rainfall of 1,300-1,750 mm/year, characterized by a hot, humid

climate. Some parts of the eucalyptus plant can be used in traditional medicine. According to Sadono et al. (2019), the most widely used part of the *M. leucadendra* plant in research is its leaves. The results of research by Ginting et al. (2023) show that the main content of essential oils in *M. leucadendra* leaves is 1,8-cineole (66.7%), α -pinene (2.45%), and L- α -terpineol (7.56%). Gunana et al. (2025) reported that the dichloromethane extract of *M. leucadendra* leaves exhibits antioxidant activity with an IC₅₀ value of 39.928 μ g/mL. Joen (2020) stated that compounds containing antibacterial activity in eucalyptus plants include 1,8-cineole, α -terpineol, α -pinene, and β -pinene. These four compounds are monoterpenes that inhibit bacterial growth by disrupting cell wall formation, damaging cell membranes, inhibiting bacterial enzyme activity, and destroying genetic material. The aim of this study was to formulate an acne spot emulgel containing *M. leucadendra* extract and evaluate its antibacterial and anti-tyrosinase activities.

MATERIAL AND METHODS

Material

The equipment used in this research was an electric balance, UV-Vis spectrophotometer (Shimadzu), vacuum rotary evaporator (Buchi R-300), pH meter, thermo scientific viscometer, glass tools, incubator (Mettler), vortex mixer Bransted International 50 Hz, Biosafety cabinet (BSC Scitex Class II A2), and ELISA reader (TECAN Infinix 200 pro M). The materials used in this study were distilled water, n-hexane, dichloromethane, methanol, *M. leucadendra* essential oil, *M. leucadendra* leaves, triethanolamine, methyl paraben, carbomer, patchouli oil, niacinamide, nutrient agar, chloramphenicol, vancomycin, Mueller Hinton agar, phenoxyethanol, glycerol, tween 80, span 80, *Propionibacterium acnes* bacteria (ATCC 11827), *Staphylococcus epidermidis* bacteria (ATCC 12228), tyrosinase enzyme (Sigma Aldrich), L-tyrosine (Sigma Aldrich), and kojic acid.

Plant determination

Melaleuca leucadendra (L.) L. leaf samples were obtained from Blang Village, Blang Bintang District, Aceh Besar Regency (5°31'00.6"N and 95°26'27.4"E). The selected samples had fresh criteria and were not too old. Samples were determined at the Herbarium Laboratory, Faculty of Mathematics and Natural Sciences, Syiah Kuala University (voucher 408/UN11.1.8.1/TA.00.03/2023).

Dried leaf powder preparation

After the samples were selected according to the criteria, the *M. leucadendra* leaf samples, which weighed up to 3 kg, were air-dried at room temperature. Drying was conducted for two weeks with the goal of reducing the water content in eucalyptus leaf samples.

Extract preparation

A sample of *M. leucadendra* leaf that had been dried and weighed as much as 1 kg was then mashed and macerated using n-hexane, dichloromethane, and methanol for 72 hours with occasional stirring. The macerate was filtered and concentrated under reduced pressure using a rotary evaporator until a thick extract was formed.

Preparation of *M. leucadendra* leaf extract acne spot emulgels

The preparation of acne spot emulgel followed the method of Khullar et al. (2012) with slight modifications. The emulgels preparation formulation was made by varying the concentration of active substances and *M. leucadendra* essential oil with the formulation F0 without *M. leucadendra* leaf extract or *M. leucadendra* essential oil, F+ is a positive control containing 0.5% *M. leucadendra* essential oil, F1 contains 5% *M. leucadendra* leaf extract and 0.5% *M. leucadendra* essential oil, F2 containing 10% *M. leucadendra* leaf extract and 0.5% *M. leucadendra* essential oil, F3 contains 15% *M. leucadendra* leaf extract and 0.5% *M. leucadendra* essential oil. The formulation of acne spot emulgel preparations is presented in Table 1.

Emulgels were prepared using Carbomer 940 as the gelling agent and Span 80/Tween 80 as emulsifying agents. The oil phase and water phase were heated separately to 55°C and mixed under continuous stirring. After cooling, the *M. leucadendra* extract was incorporated into the gel base at varying concentrations to yield three formulations: F1 (5%), F2 (10%), and F3 (15%).

Evaluation of preparations emulgels acne spot

Organoleptic test

Organoleptic evaluation was a physical test of the preparation, which includes color, odour, and shape, by storing the preparation at room temperature (Standar Nasional Indonesia, 2006).

pH test

The pH test was performed by dissolving 1 g of the preparation in 10 mL of distilled water. Then dip the pH into the solution until the pH value of the preparation was obtained (the pH of the preparation must be in accordance with the pH of the skin, 4.5-8.0) (Badan Standarisasi Nasional, 1996).

Viscosity test

Viscosity tested using a Thermo Scientific viscometer with a spindle size of L4. Measurements were taken by placing 10 g of the preparation on the roundabout of the Thermo Scientific viscometer tool, then setting the rpm to 60 and recording the viscosity obtained. According to the Badan Standarisasi Nasional (1996), the viscosity of a good preparation ranges from 2,000 to 50,000 cPs.

Table 1. Formulation of acne spot emulgel preparation with the addition of *M. leucadendra* leaf extract.

Ingredient	Concentration (%w/w)				
	F0	F+	F1	F2	F3
<i>M. leucadendra</i> extract	-	-	5	10	15
Carbomer 940	1	1	1	1	1
TEA (triethanolamine)	0.5	0.5	0.5	0.5	0.5
Liquid paraffin	7.5	7.5	7.5	7.5	7.5
Tween 80	0.5	0.5	0.5	0.5	0.5
Span 80	1	1	1	1	1
Propyleneglycol	5	5	5	5	5
Glycerol	5	5	5	5	5
Patchouli oil	-	0.5	0.5	0.5	0.5
Methyl paraben	0.03	0.03	0.03	0.03	0.03
Niacinamide	2	2	2	2	2
<i>M. leucadendra</i> essential oil	-	0.5	0.5	0.5	0.5
Distilled water	Ad 100	Ad 100	Ad 100	Ad 100	Ad 100

Homogeneity test

The sample, weighing up to 1 g, was smeared onto the glass object, and then it was observed visually. Good preparation produces a smooth surface evenly (Khairan et al., 2019).

Spreadability test

The preparation was taken in amounts of up to 0.5 g and then placed on a Petri dish. Then, the initial measurement of the diameter of the spread of the preparation was carried out without any load. Then, a load of 50 g was added every 1 minute until the total load weight reached 200 g, and the diameter of the spread of the preparation was recorded. Spreadability for a good preparation ranges from 5-7 cm (Agustin et al., 2023).

Cycling test

The stability test of the acne spot preparation was treated by placing 5 g of the preparation in a vial bottle and then placing the preparation at a temperature of $\pm 4^{\circ}\text{C}$ for 24 hours then heating the preparation at a temperature of $\pm 40^{\circ}\text{C}$ for 25 hours (1 cycle), testing for 6 cycle and observing changes that occurred in the preparation including color, odor, pH, viscosity and spreadability (Khairan et al., 2019).

Irritation test

The irritation test was followed by the method of Yasir et al. (2021), where each of 10 volunteers received one preparation of emulgel formulas that showed the best activity and underwent evaluation. The preparation was applied to the forearm and allowed to stand for 60 minutes. The change that occurred on the skin was then observed. Positive reactions are characterized by the appearance of itching, edema, and erythema.

Antibacterial activity of formulated emulgel

Test microorganisms

A panel of two common pathogenic microorganisms was involved in the study: *Staphylococcus epidermidis* and *Propionibacterium acnes*.

Disc diffusion method

The antibacterial activity of the formulated emulgels was assessed using the disc diffusion method. Microorganism suspensions were evenly spread on agar plates using sterile cotton swabs. Sterile blank disks were individually impregnated with the emulgel dosage formulations, which were applied to

0.5 g of sterile paper discs and placed into the inoculated agar plates. Incubation at 37°C for 24 h for *S. epidermidis* bacteria. For *P. acnes* bacteria, incubation was performed under anaerobic conditions using an anaerobic jar for 48 hours at 37°C . Positive controls used against *S. epidermidis* and *P. acnes* bacteria were chloramphenicol and vancomycin, respectively. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone produced against the test microorganisms in millimeters (Khairan et al., 2019).

Determination of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC)

In vitro susceptibility to the acne spot emulgel formula was determined using the microdilution method described by the Clinical and Laboratory Standards Institute (Cockerill et al., 2012). The microdilution method was performed in a 96-well microplate using a twofold dilution series ranging from 125 mg/mL to 1000 mg/mL. In brief, the bacteria were inoculated into Mueller-Hinton Broth (MHB) and incubated at 37°C for 24 hours for *S. epidermidis* and 48 hours for *P. acnes* (anaerobic conditions). Next, a bacterial suspension of 0.5 McFarland (1×10^8 CFU/mL) was prepared. The 1×10^8 CFU/mL suspension was diluted in MHB medium at a 1:20 ratio to produce a 5×10^6 CFU/mL suspension. The 5×10^6 CFU/mL suspension was added to each well at 100 μL , and varying concentrations of acne spot emulgel were added to each well at 100 μL . The samples were then incubated at 37°C for 24 hours for *S. epidermidis* bacteria and 48 hours for *P. acnes* bacteria. The MBC test was performed by transferring 100 μL from each concentration variation in the microplate wells onto the surface of Mueller-Hinton Agar medium. The inoculated plates were incubated at 37°C for 24 hours, after which the lowest concentration exhibiting no visible bacterial growth was recorded as the MBC value. Measurements were performed using an ELISA Reader (Enzyme-Linked Immunosorbent Assay Reader).

Antityrosinase activity of formulated emulgel

Antityrosinase activity tested follows the method of Batubara et al. (2010) with slight modifications.

Preparations of 50 mM phosphate buffer solution

The preparation of phosphate buffer solution was carried out by weighing KH_2PO_4 (molar mass = 136.09) to 3.402 g and then dissolving it in CO_2 -free distilled water to a final volume of 450 mL. Furthermore, the pH was adjusted to 6.8 by adding 1 M KOH solution.

Preparation of L-tyrosine 2 mM substrate solution

L-Tyrosine powder (BM = 181.19) was weighed at 3.94 mg and placed into a 10 mL volumetric flask. After that, add a 50 mM phosphate buffer solution with a pH of 6.8. L-Tyrosine solution was stored and protected from exposure to light.

Preparation of tyrosinase enzyme solution 333 units/mL

Tyrosinase enzyme was taken and weighed as much as 1 mg (50,000 Units/mg) of tyrosinase enzyme dissolved with 50 mM phosphate buffer solution, pH 6.8, as much as 1 mL (resulting in 50,000 Units/mL enzyme solution) as a stock solution. To prepare a tyrosinase enzyme solution of 333 Units/mL, 66.6 µL of stock solution was pipetted into a 10 mL volumetric flask, and then 50 mM phosphate buffer at pH 6.8 was added. Tyrosinase solution 333 Unit/mL and tyrosinase stock solution 50,000 Unit/mL were stored at -20°C.

Preparation of acne spot formula and kojic acid solution

The acne spot formula preparation was weighed up to 400 mg and then added to 5% DMSO. After that, 50 mM phosphate buffer solution, pH 6.8, was added in 10 mL (so that 4,000 µg/mL was obtained as a stock solution). Furthermore, variations in the concentration of the acne spot formula were carried out to 125, 250, 500, 1000, and 2000 µg/mL. As a positive control, kojic acid powder was weighed at 5 mg and added to a 10 mL volumetric flask, where it was then dissolved in 50 mM phosphate solution, pH 6.8, to obtain a kojic acid solution with a concentration of 500 µg/mL. Concentration variations of 31.25, 62.5, 125, and 250 µg/mL were carried out. Briefly, the antityrosinase activity test was performed by adding each concentration variation of the acne spot formula, up to 70 µL, to 96-well plates. Then, the substrate L-tyrosine (2 mM) was added in an amount of 110 µL, and the tyrosinase enzyme (333 Units/mL) was added in an amount of 30 µL. Absorbance values were measured using an ELISA Reader (Tecan Multimode Reader Infinite 200 Pro). The percent inhibition of antityrosinase was determined using the equation [1].

$$\text{Tyrosinase inhibition (\%)} = \frac{(A-B)}{(C-D)} \times 100\% \quad [1]$$

where A is the control absorbance value, B is the blank control absorbance value, C is the sample absorbance value, and D is the sample control absorbance value. Antityrosinase activity was expressed as IC₅₀ value (concentration in inhibiting 50% of tyrosinase enzyme, µg/mL).

Statistical analysis

All data were presented as the mean and standard deviation (SD) of each test, determined using Excel 2010 software (Microsoft Corp., Redmond, WA) (n =

3). All data were presented as means, and standard deviations were calculated using Graphpad Prism 9 software.

RESULTS AND DISCUSSION

Extraction of *M. leucadendra* leaf

Extraction was carried out using the maceration method on the leaves of *M. leucadendra* using n-hexane, dichloromethane, and methanol solvents. The purpose of the extraction process was to extract secondary metabolite compounds contained in the leaf cells. The yield obtained from the extraction process of eucalyptus leaves was obtained successively, namely n-hexane (3.919%), dichloromethane (2.620%), and methanol (11.411%). According to research by Nortjie et al. (2024), the yield obtained from n-hexane and methanol extracts of eucalyptus leaves (*Eucalyptus globulus*) was 3.797% and 22.762%, respectively. Research by Rodrigues et al. (2018) reported that the extraction of eucalyptus leaves (*E. globulus*) using dichloromethane, ethanol, and methanol solvents yielded 7.32%, 25.90%, and 30.34%, respectively. According to Truong et al. (2019), methanol extract has a greater yield because the solubility of secondary metabolite compounds contained in vacuolar cells is higher in methanol solvents.

Preparation of *M. leucadendra* leaf extract acne spot emulgels

Emulgel acne spot was formulated using ingredients consisting of carbomer, trietanolamine (TEA), liquid paraffin, tween 80, span 80, propylene glycol, patchouli oil, methyl paraben, niacinamide, *M. leucadendra* essential oil, *M. leucadendra* dichloromethane extract, and distilled water.

Evaluation of acne spot formula preparations

Organoleptic test

Organoleptic tests were carried out visually based on characteristics of color, odor, and dosage form. The results of the organoleptic acne spot emulgels preparation can be seen in Table 2. The five formulations exhibit differences in color and odor of the preparation. In the F0 formula preparation, namely the base, the green color is obtained. The F+ preparation, which contains *M. leucadendra* essential oil and patchouli oil, has a white color with a distinctive eucalyptus-like odor. In the F1 formula preparation, which is prepared with a concentration of 10% *M. leucadendra* leaf dichloromethane extract, the solution has a green color. In contrast, the F3 preparation, with a concentration of 15% *M. leucadendra* leaf dichloromethane extract, has a dark green color. In terms of shape and

odor, the F1, F2, and F3 preparation formulas showed similarities, exhibiting the distinctive odor of *M. leucadendra* extract and a semi-solid consistency. Organoleptic tests were conducted to determine the physical characteristics of acne spot emulgels containing *M. leucadendra* dichloromethane extract.

pH test

The pH test was conducted using a pH meter electrode. The measurement of pH in acne spot emulgel preparations was performed by weighing 1 g of the emulgel preparation and then adding 10 mL of water, followed by homogenization. Afterward, the pH meter electrode was immersed in the preparation (Khairan et al., 2019). The results of the pH test on the emulgel preparation are presented in Table 3.

Based on Table 3, it can be seen that the emulgel formula has a different pH value. This was due to the addition of *M. leucadendra* leaf dichloromethane extract and *M. leucadendra* essential oil. In theory, the addition of *M. leucadendra* leaf dichloromethane extract and *M. leucadendra* essential oil to the prepara-

tion is expected to cause a decrease in pH. The decrease in pH of the formula preparation upon the addition of the extract concentration has been demonstrated by Helsawati et al. (2023) and Marlina et al. (2023), who showed that the higher the extract concentration in the nanoemulgel formula, the lower the pH of the preparation. The decrease in pH was also attributed to the release of H⁺ ions that originated from secondary metabolite compounds contained in the extract (Auliya et al., 2022).

According to Auliya et al. (2022), the decrease in pH after the accelerated cycling test was caused by the ingredients contained in the preparation that can release H⁺ ions. Furthermore, temperature and environmental conditions also influenced the pH of the preparation. Nonci et al. (2017) stated that the pH test needs to be done, which aims to determine the safety of the preparation to be used. According to Badan Standarisasi Nasional (1996), the pH of skin preparations ranges from 4.5 to 8.0. If the pH of a preparation is too high (alkaline), it could cause the skin to be dry or scaly.

Table 2. Organoleptic test result of *M. leucadendra* extract acne spot emulgel.

Formulated	Observation	Observation day				
		1	3	6	9	12
F0	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Color	White	White	White	White	White
	Shape	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
	Odor	Typical preparation	Typical preparation	Typical preparation	Typical preparation	Typical preparation
F+	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Color	White	White	White	White	White
	Shape	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
	Odor	Typical eucalyptus	Typical eucalyptus	Typical eucalyptus	Typical eucalyptus	Typical eucalyptus
F1	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Color	Faint green	Faint green	Faint green	Faint green	Faint green
	Shape	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
	Odor	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract
F2	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Color	Green	Green	Green	Green	Green
	Shape	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
	Odor	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract
F3	Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
	Color	Intense green	Intense green	Intense green	Intense green	Intense green
	Shape	Semi solid	Semi solid	Semi solid	Semi solid	Semi solid
	Odor	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract	Typical eucalyptus extract

Table 3. pH test results of acne spot emulgel.

Formulation	Mean pH $\pm$ SD, n = 3		Standard SNI 2016-4399-1996
	Before Cycling test	After Cycling test	
F0	5.83 $\pm$ 0.072	5.70 $\pm$ 0.0379	4.5-8.0
F+	5.78 $\pm$ 0.010	5.66 $\pm$ 0.005	
F1	5.5 $\pm$ 0.005	5.04 $\pm$ 0.01	
F2	5.35 $\pm$ 0.005	4.85 $\pm$ 0.005	
F3	5.24 $\pm$ 0.005	4.67 $\pm$ 0.28	

N: Number of repetitions; F0: Base emulgel; F+: Emulgel with the addition of *M. leucadendra* essential oil; F1: Emulgel with 5% *M. leucadendra* dichloromethane extract; F2: Emulgel with 10% *M. leucadendra* dichloromethane extract; F3: Emulgel with 15% *M. leucadendra* dichloromethane extract.

Table 4. Viscosity test results of acne spot emulgel.

Formulation	Viscosity formulation		Standard SNI 2016-4399-1996
	Before Cycling test	After Cycling test	
F0	4039.0	3918.9	2,000-50,000
F+	3824.0	3612.2	
F1	4742.5	4519.5	
F2	5132.7	5037.8	
F3	5739.2	5583.8	

F0: Base emulgel; F+: Emulgel with the addition of *M. leucadendra* essential oil; F1: Emulgel with 5% *M. leucadendra* dichloromethane extract; F2: Emulgel with 10% *M. leucadendra* dichloromethane extract; F3: Emulgel with 15% *M. leucadendra* dichloromethane extract.

Viscosity test

The viscosity test of the preparation was carried out using a Thermo Scientific Viscometer equipped with a spindle size of L4 and a speed of 60 rpm. The results of the viscosity test are presented in Table 4.

Based on Table 4, it can be observed that the viscosity of the acne spot emulgel formula increases with the addition of *M. leucadendra* dichloromethane extract prior to the cycling test. The results of this study corresponded with those of Sitohang et al. (2020), which showed that the greater the concentration of *Barringtonia racemosa* extract, the greater the viscosity of the gel preparation. Syakri et al. (2021) reported that as the concentration of *Achillea millefolium* extract increased, the viscosity value of the gel preparation increased. According to Biglarian et al. (2022), viscosity is defined as a measure of a liquid's flow resistance; the higher the viscosity, the greater the resistance. The viscosity value was strongly influenced by the particle size, the proportion of the dispersed phase, and the surfactant thickening agent used. Based on Table 4, it can be observed that there was a change in viscosity value before and after the cycling test.

According to research, Jufri et al. (2018) reported that the viscosity value of emulgel preparations from tobacco leaf extract can decrease after the cycling test. The change in viscosity value after treatment with the cycling test was supported by the research results of Suzilla et al. (2020), which reported the preparation of gel formulations from *Quercus infectoria* extract after storage at 45°C for three weeks. Suzilla et al. (2020) reported that the change in viscosity value that occurs is influenced by storage temperature. The higher the temperature, the faster the acceleration of chemical reactions, as well as changes in the activity of active ingredients, color, and odor. However, the viscosity values both before and after the cycling test treatment remained within the standard limits set by SNI 2016-4399-1996, which range from 2,000 to 50,000 cPs.

Homogeneity test

Homogeneity was tested by applying each preparation to a glass object and visually observing the result. The results of the homogeneity test are presented in Table 5.

Based on Table 5, it was observed that the acne spot emulgel formula was evenly mixed and homo-

geneous. The homogeneity test aimed to determine whether the ingredients added to the acne spot emulgel preparation formula were homogeneous. Homogeneity was assessed by the absence of particles from any ingredient that had not been evenly mixed. The analysis of the homogeneity test results for the four emulgel preparations showed that all were quite good and stable.

Spreadability test

The spreadability test was performed by weighing a 0.5-g sample, placing it in a Petri dish, and then measuring its diameter. Initial measurements were taken with no load (0 g). After that, the preparation was closed and subjected to loads of 50 g, 100 g, 150 g, and 200 g. Each additional load was waited for 5 minutes, and the diameter of the spread was measured. The spreadability test results before and after the cycling test are presented in Table 6.

Based on Table 6, there are differences in the spreadability of each dosage formulation before the cycling test. This was also obtained by Ulfa et al. (2022), it was reported that increasing the concentration of *Colocasia esculenta* extract caused a decrease in the spreadability of emulgel formula preparation. Wuryandari et al. (2019) reported that the higher the concentration of *Moringa oleifera* extract, the less the spreadability of the emulgel formula preparation. This research is also supported by Muliasari et al.

(2022), who reported that the higher the concentration of *Brucea javanica* extract, the less the spreadability of the emulgel preparation.

Based on Table 6, it was observed that the acne spot emulgel preparation decreased during the cycling test. Previous research from Djajanti et al. (2021) reported that there were changes to the preparation of F1, F2, F3 gel formulas from varied *Apium graveolens* leaf extracts. The spreadability obtained by each of these formulas before the cycling test was 6.123; 6.297; and 6.403 cm. After the cycling test, the spreadability of the gel formula preparation was 5.69, 6.01, and 6.137 cm, respectively. According to Setyaningsih et al. (2022), the spreadability of the preparation was influenced by its viscosity. The greater the viscosity value, the lower the spreadability. The spreadability test was conducted to determine whether the preparation could be easily applied to the skin by assessing its ability to spread.

Cycling test

The cycling test was one of the stability testing methods in the presence of temperature changes (cold and hot). According to Rohmani and Kuncoro (2019), the cycling test was conducted with six cycles, and then observed whether there was a change in the phase, color, odor, and consistency (shape) of the formula preparation. The results of the accelerated stability test (cycling test) are presented in Table 7.

Table 5. Results of the homogeneity test of acne spot emulgel.

Formulation	Parameter	Observation
F0	The presence or absence of particles of an ingredient that have not been mixed evenly or clumped (homogeneous/inhomogeneous)	Homogeneous
F+		Homogeneous
F1		Homogeneous
F2		Homogeneous
F3		Homogeneous

F0: Base emulgel; F+: Emulgel with the addition of *M. leucadendron* essential oil; F1: Emulgel with 5% *M. leucadendra* dichloromethane extract; F2: Emulgel with 10% *M. leucadendra* dichloromethane extract; F3: Emulgel with 15% *M. leucadendra* dichloromethane extract.

Table 6. Spreadability test results of acne spot emulgel.

Formulation	Spreadability (cm)	
	Before Cycling test	After Cycling test
F0	4.8	5.3
F+	5.6	5.9
F1	5.1	5.4
F2	5	5.1
F3	4.4	4.9

F0: Base emulgel; F+: Emulgel with the addition of *M. leucadendron* essential oil; F1: Emulgel with 5% *M. leucadendra* dichloromethane extract; F2: Emulgel with 10% *M. leucadendra* dichloromethane extract; F3: Emulgel with 15% *M. leucadendra* dichloromethane extract.

Table 7. Organoleptic results of the cycling test for six cycles.

Cycling test	Formulation	Test result		
		Color	Odor	Shape
Cycle 0	F0	White	Typical odor of the base	Semi solid
	F+	White	Characteristic smell of eucalyptus extract	
	F1	Faint green		
	F2	Green		
	F3	Intense green		
Cycle 1	F0	White	Typical odor of the base	Semi solid
	F+	White	Characteristic smell of eucalyptus extract	
	F1	Faint green		
	F2	Green		
	F3	Intense green		
Cycle 2	F0	White	Typical odor of the base	Semi solid
	F+	White	Characteristic smell of eucalyptus extract	
	F1	Faint green		
	F2	Green		
	F3	Faint green		
Cycle 3	F0	Green	Typical odor of the base	Semi solid
	F+	Intense green	Characteristic smell of eucalyptus extract	
	F1	Faint green		
	F2	Green		
	F3	Intense green		
Cycle 4	F0	White	Typical odor of the base	Semi solid
	F+	White	Characteristic smell of eucalyptus extract	
	F1	Faint green		
	F2	Green		
	F3	Intense green		
Cycle 5	F0	White	Typical odor of the base	Semi solid
	F+	White	Characteristic smell of eucalyptus extract	
	F1	Faint green		
	F2	Green		
	F3	Intense green		
Cycle 6	F0	White	Typical odor of the base	Semi solid
	F+	White	Characteristic smell of eucalyptus extract	Semi solid
	F1	Faint green		
	F2	Green		
	F3	Intense green		

The results of the cycling test, as shown in Table 7, indicate that the organoleptic properties of the emulgel formula preparation remained unchanged, with no change in color, odor, or phase separation

(shape) during the 6-cycle test. The results of the cycling test showed that the components of the five emulgel formula preparations were evenly dispersed and stable. The stability of the formula preparation

was attributed to the presence of emulgators such as tween and span.

Irritation test

The irritation test was conducted to determine the irritating effect caused by the use of the preparation after it was applied to the skin, allowing the safety level of the preparation to be determined before use. This test aimed to minimize the occurrence of skin side effects. The Irritation test was performed by applying the preparation to the skin under the arm for 60 minutes, and had previously been approved by the USK nursing ethics committee under Research Number 113013100624. The irritation test results showed no signs of irritation, including heat, erythema, edema, itching, or burning. Therefore, of the 10 volunteers who used the emulgel preparation, only one volunteer experienced edema. Based on the calculation of the primary irritation index for the emulgel formula preparation, it was concluded that the acne spot emulgel did not cause skin irritation. The emulgel formula did not cause irritation because its pH was consistent with the natural pH of the skin.

Antibacterial test

Antibacterial activity testing of extracts and emulgel formulations is presented in Table 8.

The antibacterial activity of *S. epidermidis* on acne spot emulgel formulations F1, F2, and F3 showed significant differences, as evidenced by the inhibition zones produced in Table 8. However, formulas F0 and F+ were unable to inhibit the two bacteria that cause acne. It was observed that the F3 acne spot formula inhibited *S. epidermidis* and *P. acnes* bacteria more strongly than other formulas. According to Cockerill

et al. (2012), if the inhibition zone was ≤ 14 mm, the bacteria were classified as resistant to the test material; if the inhibition zone ranged from 15-19 mm, the bacteria were considered intermediate, and if the inhibition zone was ≥ 20 mm, the bacteria were deemed sensitive to the test material.

According to previous research by Gunana et al. (2025), the dichloromethane extract of *M. leucadendra* leaves contains antibacterial compounds, including eucalyptol, fenchol-exo, endoborneol, α -terpineol, and terpinenol. Also, the n-hexane extract showed antibacterial activity against *S. epidermidis* and *P. acnes* bacteria at a 20% concentration, with inhibition zones of 11.56 mm and 10.36 mm, respectively. The dichloromethane extract demonstrated antibacterial activity against *S. epidermidis* bacteria at a 20% concentration, with an inhibition zone of 17.41 mm, and against *P. acnes* at a 10% concentration, with an inhibition zone of 12.29 mm. The methanol extract showed antibacterial activity against *P. acnes* at a 20% concentration, with an inhibition zone of 10.47 mm, but no activity against *S. epidermidis* bacteria. Zouine et al. (2024) reported that terpenoids and other secondary metabolite compounds inhibited bacterial growth through several mechanisms. These included the disruption of cell wall synthesis, interference with membrane-bound enzymes, and the induction of oxidative stress via the production of reactive oxygen species (ROS). Through these multiple modes of action, these compounds demonstrated a significant antibacterial effect.

Alipanah et al. (2022) reported that nanogels containing *Eucalyptus globus* essential oil are stronger against *S. aureus* and *P. aeruginosa* bacteria with an IC_{50} of 187 and greater than 5000 $\mu\text{g/mL}$.

Table 8. Antibacterial test result of acne spot formula.

Sample	Formulation	Mean inhibition zone $\pm$ SD, n = 3	
		<i>S. epidermidis</i> (mm)	<i>P. acnes</i> (mm)
FEML	K(+)	27.57 $\pm$ 0.34	31.33 $\pm$ 0.39
	F0	0	0
	F+	0	0
	F1	11.63 $\pm$ 0.31	11.23 $\pm$ 0.11
	F2	16.59 $\pm$ 0.28	12.48 $\pm$ 0.08
	F3	19.49 $\pm$ 0.10	14.27 $\pm$ 0.17
	Emulgel commercial	12.23 $\pm$ 0.18	15.14 $\pm$ 0.10

K(+): Chloramphenicol 30 $\mu\text{g/mL}$ for *S. epidermidis* bacteria and vancomycin 30 $\mu\text{g/mL}$ for *P. acnes*. F0: Base emulgel; F+: Emulgel with the addition of *M. leucadendron* essential oil; F1: Emulgel with 5% *M. leucadendra* dichloromethane extract; F2: Emulgel with 10% *M. leucadendra* dichloromethane extract; F3: Emulgel with 15% *M. leucadendra* dichloromethane extract.

Table 9. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of acne spot formula against *Staphylococcus epidermidis* and *Propionibacterium acnes* causing acne.

Sample	<i>S. epidermidis</i>		<i>P. acnes</i>	
	MIC	MBC	MIC	MBC
K(+)	16 µg/mL	16 µg/mL	2.5 µg/mL	2.5 µg/mL
F0	-		-	
F+	-		-	
F1	1000 mg/mL	1000 mg/mL	500 mg/mL	500 mg/mL
F2	500 mg/mL	500 mg/mL	250 mg/mL	250 mg/mL
F3	250 mg/mL	250 mg/mL	125 mg/mL	125 mg/mL

K(+): Chloramphenicol for *S. epidermidis* bacteria and vancomycin for *P. acnes*. (-): no inhibition of bacterial growth occurred; F0: Base emulgel; F+: Emulgel with the addition of *M. leucadendron* essential oil; F1: Emulgel with 5% *M. leucadendra* dichloromethane extract; F2: Emulgel with 10% *M. leucadendra* dichloromethane extract; F3: Emulgel with 15% *M. leucadendra* dichloromethane extract.

Based on Table 9 showed that the MIC and MBC values in the acne spot treatment formulations F1 to F3 have the same inhibitory and lethal concentrations for both bacteria. We conducted the MIC test using the dilution method and measured it using an ELISA reader. Furthermore, the MBC test used Mueller Hinton Agar medium to confirm the presence or absence of bacterial growth at the lowest concentration. In this study, we varied the concentration of the preparation from 1000 mg/mL to 31,250 mg/mL. The controls used in this study were negative control (media and methanol solvent), normal control (media without bacteria), and growth control (media and bacteria).

Based on the results of the diffusion inhibition zone test, it was found that the formula with the strongest antibacterial activity was F3 with a concentration of 1000 mg/mL with inhibition zones in *S. epidermidis* and *P. acnes* bacteria of 19.49 mm and 14.27 mm, respectively (Table 8). Furthermore, to determine the minimum concentration in inhibiting *S. epidermidis* and *P. acnes* bacteria, formula F3 had the lowest concentration, namely 250 mg/mL against *S. epidermidis* bacteria and 125 mg/mL against *P. acnes* bacteria. From this data that the minimum concentration and bactericidal concentration of the acne spot treatment emulgel preparation had the same results.

Based on previous research by Bennabi et al. (2025), it has been reported that clove essential oil-based emulgel preparations have antibacterial activity against *Escherichia coli* bacteria with an MIC of 12.6 mg/mL and an MBC of 50 mg/mL.

Antityrosinase test

Antityrosinase activity was assessed using the enzymatic method with L-tyrosine as the substrate. Antityrosinase activity testing of acne spot formulas began with variations in the concentration of each formula. Then, measurements were made using an ELISA Reader with a wavelength of 510 nm. The re-

sults of the antityrosinase activity test are presented in Table 10.

Table 10 shows the percentage inhibition and IC₅₀ values of the acne spot emulgel preparation. Based on the results obtained, formula F3 was the most effective emulgel formulation for inhibiting the tyrosinase enzyme compared to F0, F+, F1, and F2; however, it was still categorized as weak.

Tyrosinase is an enzyme that catalyzes the formation of melanin. In humans, tyrosinase can cause spots, age spots, and hyperpigmentation (Rachkeeree et al., 2020). According to Moon et al. (2010) antityrosinase activity is measured by how small the IC₅₀ value. The smaller the IC₅₀ value, the stronger the antityrosinase activity inhibited by a sample (Garcia-Molina et al., 2022). The criteria for IC₅₀ stated that a sample was considered to have strong inhibition activity if its IC₅₀ was less than 50 µg/mL. If a sample showed an inhibition value of less than 60% at a concentration of 500 µg/mL, it was classified as having low inhibition and was categorized as very low if the percent inhibition was less than 3% at the same concentration. Previous research by Sholikha and Wulandari (2022) reported that the methanol extract, fractionated using semipolar solvents such as butanol, had an IC₅₀ value of 517.94 µg/mL.

CONCLUSION

The results showed that the acne spot emulgel F3 demonstrated the most favorable physical characteristics and the highest antibacterial activity among the three formulations evaluated. A direct correlation was observed between extract concentration and antibacterial efficacy. However, the anti-tyrosinase activity was classified as weak. These results indicate that *M. leucadendra* extract emulgel is a promising topical agent for acne spots. Further investigations, including cytotoxicity and *in vivo* studies, are recommended to confirm its therapeutic potential and safety profile.

Table 10. Antityrosinase test result of acne spot formula.

Sample	Concentration (µg/mL)	Inhibition (%)	IC ₅₀ value (µg/mL)
Kojic acid	2.397	97.474	1.035
	2.096	90.193	
	1.795	87.667	
	1.498	68.202	
	1.197	56.909	
	0.896	40.546	
F0	2000	56.994	1498
	1000	45.158	
	500	33.068	
	250	24.445	
F+	2000	76.704	1079
	1000	59.564	
	500	29.564	
	250	19.901	
F1	2000	92.800	804
	1000	65.863	
	500	48.070	
	250	24.473	
F2	2000	93.703	395
	1000	74.048	
	500	59.567	
	250	46.810	
F3	2000	96.431	271
	1000	78.169	
	500	61.519	
	250	46.767	

F0: Base emulgel; F+: Emulgel with the addition of *M. leucadendron* essential oil; F1: Emulgel with 5% *M. leucadendra* dichloromethane extract; F2: Emulgel with 10% *M. leucadendra* dichloromethane extract; F3: Emulgel with 15% *M. leucadendra* dichloromethane extract.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHOR CONTRIBUTION:

Contribution	Ginting B	Isniani N	Khairan K	Saidi N	Murniana M	Bahi M	Yahya M	Gunana TZT	Saleha S	Riski CD
Concepts or ideas	x	x	x	x	x	x	x	x	x	
Design	x	x	x					x		x
Definition of intellectual content	x	x	x	x	x	x	x			
Literature search	x	x	x				x	x		x
Clinical trial			x		x	x	x			
Experimental studies	x			x				x		
Data acquisition	x	x	x		x					
Data analysis	x						x	x		
Statistical analysis	x	x	x	x	x			x	x	
Manuscript preparation	x						x	x		x
Manuscript editing	x	x	x					x	x	x
Manuscript review	x	x	x	x	x	x	x	x	x	

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