DOI: https://doi.org/10.21009/JRSKT.112.03

Potential of the Ethyl Acetate Extract of Kesambi (*Schleichera oleosa*) Bark as an Ointment-Based Treatment for Skin Diseases Against *Staphylococcus aureus*

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Received: 11 July 2025
Revised: 27 October 2025
Accepted: 30 October 2025
Online: 15 December 2025
Published: 30 December 2025

Jurnal Riset Sains dan Kimia Terapan
p-ISSN: 2302 - 8467
e-ISSN: 2303 – 0720

Abstract

One plant with potential to treat skin diseases is the Kesambi plant. Based on community experience in Cilegon, a decoction of Kesambi tree bark has been used to treat skin ailments, including itching, insect bites, and scabies. This research aims to reveal the potential of ethyl acetate extract from the tree bark as a standardized ointment preparation. Extraction was performed using the maceration method. Quality testing of the ointment included antibacterial, safety, stability, organoleptic, and bioactive compound content testing. The best formulation was formulation 1, with an antibacterial inhibition zone of 0.6-0.7 mm, classified as moderate. Increasing the amount of extract did not significantly affect antibacterial activity. The bioactive compound content in the ointment consisted of compounds from the groups of triterpenoids, steroids, phenolics, and flavonoids.

Keywords: antibacterial, Kesambi, ointment

Introduction

Skin diseases are among the most frequently reported health problems in tropical countries such as Indonesia, where warm, humid conditions favor the growth of various pathogenic microorganisms (Kirmani et al., 2024). In Indonesia, these conditions are commonly associated with bacterial, fungal, or allergic infections. According to the World Health Organization (WHO), approximately 20% of the global population is affected by cutaneous infections, with dermatophytosis among the most prevalent (Ghosh et al., 2022). National data from the 2019 Indonesia Health Profile further indicate that skin diseases rank third among the ten most common illnesses reported in outpatient hospital visits across the country (Widyawati et al., 2022). Among the bacterial pathogens associated with skin infections, Staphylococcus aureus is one of the most prominent. This aerobic bacterium naturally inhabits the skin, nasal cavity, and throat and can cause a wide range of infections when the skin barrier is compromised (Sari et al., 2019).

One of the medicinal plants with strong potential for treating skin diseases is Schleichera oleosa, commonly known as Kesambi (Pupuk et al., 2018). Empirical evidence from communities in Cilegon City indicates that decoctions of Kesambi bark have long been used traditionally to treat various skin ailments, including itching, insect bites, and scabies (Situmeang et al., 2016). Research conducted by (Sari et al., 2019) demonstrated that the ethyl acetate extract of Kesambi bark exhibits vigorous inhibitory activity against Staphylococcus aureus (Sari, 2020). Similarly, findings by Situmeang et al. (2022) also reported significant antibacterial activity of Kesambi bark extract against S. aureus (Situmeang et al., 2022). The ethyl acetate extract of Kesambi bark has been reported to contain flavonoids and phenolic compounds (Musa et al., 2021). These phytochemicals are widely recognized for their biological activities, including anticancer, antiseptic, antimicrobial, and antiviral properties (Situmeang et al., 2024). Based on these studies, the bark extract of S. oleosa shows strong potential as an ointment for treating skin infections.

Staphylococcus aureus on the skin can cause systemic infections, underscoring the need to develop preventive and therapeutic approaches derived from natural sources (Asefian & Ghavam, 2024). Ointment formulations using a polyethylene glycol (PEG) base are known to release active compounds more effectively compared to oil-based preparations. PEG-based ointments are also suitable for acne-prone skin because they are oil-free. Natural-product-based ointments are considered safer for various skin types and age groups, as they generally have fewer side effects.

Based on the existing literature, no studies have reported the development of a standardized ointment formulated from Kesambi bark extract. Therefore, it is necessary to investigate the potential of an ointment-based preparation formulated with the ethyl acetate extract of *Schleichera oleosa* bark. Furthermore, an in vitro antibacterial assay against *Staphylococcus aureus* will be performed. An ointment dosage form is selected because it serves as an effective carrier for topical medications, a skin lubricant, and a protective agent that is safe for use.

Method

Materials and Instrumentation

The materials used in this research include: *Schleichera oleosa* (Kesambi) bark (fresh and dried/powdered), ethyl acetate (for maceration extraction), methanol (for stability test sample preparation), ointment formulation ingredients (PEG 4000; PEG 400), alpha-tocopherol, propyl paraben, ointment base (PEG-based as described), microbiological media, nutrient broth, sterile cotton swabs, treptomycin (positive control), paper disks (for Kirby–Bauer test), phytochemical screening reagents, Dragendorff reagent (alkaloid test), Liebermann–Burchard reagent (triterpenoid/steroid test), 10% $AlCl_3$ (phenolic test), 5% $FeCl_3$ (flavonoid test). The instrumentation used includes: rotary evaporator (for solvent evaporation), mortar and pestle (sample grinding), water bath/heating apparatus (for ointment fusion method), Incubator at 37°C (for bacterial culture and antibacterial assay), UV–vis spectrophotometer, λ = 510 nm (for stability test), caliper (for inhibition zone measurement), analytical balance (weighing materials), glassware set (beakers, test tubes, etc.), ointment preparation tools (stirring rods, heating container).

Sample Preparation

The bark samples of *Schleichera oleosa* were collected from Kubang Lesung Village, Citangkil District, Cilegon City. A total of 5 kg of fresh bark samples were used in this study. The sample preparation steps included washing, cutting, drying, and pulverizing the bark. The dried bark samples were then ground using a mortar and pestle.

Sample Extraction

The powdered Schleichera oleosa bark samples were subsequently macerated using ethyl acetate for 3 × 24 hours. The resulting ethyl acetate filtrate was then concentrated by rotary evaporation to obtain a thick ethyl acetate extract (Putri et al., 2019). The concentrated extract was then formulated into an ointment preparation.

Preparation of Kesambi Ethyl Acetate Extract Ointment

The ointment was prepared using the fusion method. Polyethylene glycol (PEG) 4000 was heated until melted, and PEG 400 was then added to form a viscous mixture. Propyl paraben was then incorporated and stirred until homogeneous, then cooled. Once the mixture reached homogeneity, alpha-tocopherol was added, and finally, the *Schleichera oleosa* bark extract was incorporated and mixed thoroughly until a uniform ointment was obtained.

Antibacterial Activity test of Ointment using Kirby-Bauer Method

A loopful of *Staphylococcus aureus* from the stock culture was inoculated into a sterile test tube containing 5 mL of nutrient broth and incubated for 24 hours at 37°C. The antibacterial activity of the ointment, along with the negative and positive controls, was evaluated by dipping a sterile cotton swab into the diluted bacterial suspension adjusted to 0.5 McFarland standard. The swab containing the bacterial inoculum was then evenly spread across the surface of solid media (Satari et al., 2019). A volume of 15 µL of the negative control, positive control (streptomycin), and the Kesambi ointment samples (labeled F1, F2, and F3) was dispensed onto paper disks, which were subsequently placed on the surface of the solid medium. The plates were then incubated at 37°C for 24 hours. After incubation, the diameter of the clear inhibition zones surrounding each disk was observed and measured using a caliper (Widyawati et al., 2022).

Analysis of Bioactive Compounds in the Ointment

The analysis of bioactive compounds was performed using phytochemical screening to detect triterpenoids, steroids, flavonoids, alkaloids, and phenolics (Fayyadie et al., 2025). A total of 5 mg of each ointment sample was treated with the corresponding test reagents. Dragendorff's reagent was used for alkaloid detection, while the Liebermann–Burchard reagent was used to identify triterpenoids and steroids. Phenolic compounds were tested using 10% $AlCl_3$, and flavonoid content was examined using 5% $FeCl_3$ (Eisa et al., 2025).

Ointment Safety Assessment

The safety evaluation of the ointment was conducted through irritation and sensitivity tests. A total of 5 mg of the ointment was applied to the skin and left in place for 1 hour. The treated area was then observed for any signs of skin reaction.

Stability Evaluation of the Ointment

The stability test of the ointment was performed by heating 10 mg of the ointment for 1 hour at 30, 35, and 40°C. After heating, the Kesambi ointment samples were dissolved in 3 mL of methanol. The absorbance of the samples was measured at 510 nm using a UV–vis spectrophotometer.

Result and Discussion

The Result of Sample Preparation

The moisture content obtained from the *Schleichera oleosa* bark extract was 39.13%. The net weight of the fresh sample was 2.3 kg, while the net weight of the dried sample was 1.4 kg. From 1 kg of dried

 E-ISSN: 2303-0720

S. oleosa bark (simplicia) subjected to maceration, 96 g of concentrated extract was obtained. The yield of the ethyl acetate extract relative to the dried plant material was 9.6%.

The Result of Kesambi Ointment Preparation

The Kesambi ointment was prepared by formulating the ethyl acetate extract of *Schleichera oleosa* bark with an ointment base consisting of PEG 4000, PEG 400, and alpha-tocopherol in three different formulations. The results of the ointment formulations are presented in **Table 1**.

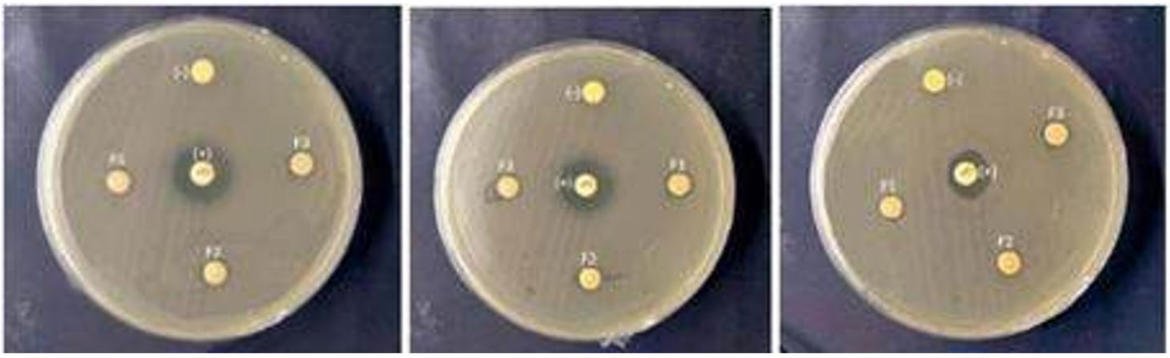
Table 1. The formulation of Kesambi ointment

Material	F1 (g)	F2 (g)	F3 (g)
Kesambi extract	2.5	5	7.5
α-tokoferol	0.5	0.5	0.5
PEG 400	0,5	0.5	0.5
PEG 4000	0.5	0.5	0.5
Bases ointment	46	43.5	41

The addition of an ointment base enhances the absorption of medicinal substances into the skin. In addition, the ointment base provides good hydration to the stratum corneum, ensuring uniform absorption of the active compounds (Anggyadinata et al., 2023). The addition of PEG 4000 and PEG 400 allows the ointment to melt when applied to the skin surface. The inclusion of alpha-tocopherol helps maintain skin moisture and provides nourishment to the skin (Dhyneu Dwi et al., 2022).

Antibacterial Test Results of the Ointment Formulation Samples

The antibacterial activity test of the Kesambi ointment aimed to determine its ability to inhibit the growth of *S. aureus*. The results of the antibacterial activity test of the Kesambi ointment are presented in **Figure 1**.



(a) (b) (c)

Figure 1. Antibacterial test results of ointment formulation samples. Repetition 1 (a), repetition 2 (b), repetition 3 (c)

The inhibition zones were measured using a caliper. The clear zones surrounding the paper disks were observed and measured. The results of the inhibition zone diameter measurements from the antibacterial activity test of the ointment are presented in **Table 2**.

Tabel 2. Inhibiton zone of antibacterial activity test of oitment

Sample	Repetition oh inhibition zone (mm)			Average (mm)
	1	2	3	
Formulation 1	3.13	3.10	3.23	3.15
Formulation 2	2.93	2.93	2.87	2.91
Formulation 3	3.07	3.33	2.87	3.09
Positive control	5.13	5.17	5.13	5.14
Negative control	0	0	0	0

 E-ISSN: 2303-0720

The antibacterial activity test was carried out in three replications (triplicate). Based on **Table 2** above, formulation 1 had the highest antibacterial inhibition zone against Staphylococcus aureus compared to formulations 2 and 3. Widyawati et al. (2022) reported that antibacterial activity with an inhibition zone diameter of 0.5-1.0 mm is categorized as medium, while a diameter below 0.5 mm is considered weak. Based on the antibacterial activity test results, the addition of the ethyl acetate extract did not directly correlate with the size of the bacterial inhibition zone. According to Situmeang et al. (2022), the more concentrated an extract is, the more difficult it becomes for it to penetrate the bacterial cell wall, preventing the bacteria from being killed.

Organoleptic Evaluation Results of the Kesambi Ointment

The organoleptic evaluation of the Kesambi ointment samples included assessments of taste, aroma, color, texture, and form. The results of the organoleptic test of the Kesambi ointment samples are presented in **Table 3**.

Table 3. Organoleptic test results of Kesambi ointment samples

Sample	Flavour	Aroma	Texture	Form	Colour
F1	Slightly warm	Fragrant	Soft	Semi solid	Brown
F2	Cool	Fragrant	Soft	Semi solid	Brown
F3	Slightly cool	Fragrant	Soft	Semi solid	Dark brown

Based on organoleptic testing, the Kesambi ointment had a pleasant fragrance, a soft texture, and a semi-solid consistency. The ointment ranged in color from light brown to dark brown. These characteristics indicate that the Kesambi ointment is well-liked by the community.

Bioactive Compounds of Kesambi Oitment

The results of the bioactive compound analysis of the Kesambi ointment samples for triterpenoid, steroid, flavonoid, alkaloid, and phenolic contents are presented in **Table 4**. Situmeang et al. (2022) reported that phenolic and flavonoid compounds exhibit antibacterial activity against pathogenic bacteria. The results of the bioactive compound analysis of the Kesambi ointment are consistent with the antibacterial activity test findings. Widyawati et al. (2022) also reported that phenolic and flavonoid compounds can penetrate the cell walls of pathogenic bacteria, thereby inhibiting their growth.

Table 4. The result of the bioactive compound test of Kesambi ointment

Sample	Compounds	Reagent	Result
Formulation 1	Triterpenoid/steroid	*Lieberman burchard*	+
	Alaloid	*Dragendroff*	-
	Flavonoid	FeCl3 5%	+
	Phenolic	AlCl3 10%	+
Formulation 2	Triterpenoid/steroid	*Lieberman burchard*	-
	Alkaloid	*Dragendroff*	-
	Flavonoid	FeCl3 5%	+
	Phenolic	AlCl3 10%	+
Formulation 3	Triterpenoid/steroid	*Lieberman burchard*	+
	Alkaloid	*Dragendroff*	-
	Flavonoid	FeCl3 5%	+
	Phenolic	AlCl3 10%	+

Safety Test of the Kesambi Ointment

The safety evaluation of the Kesambi ointment consisted of irritation and sensitivity tests. The purpose of this assessment was to determine whether the ointment caused any skin irritation. The test

 E-ISSN: 2303-0720

was performed by applying the ointment to the skin surface, followed by observations at 15, 30, and 60 minutes. The safety test results for all three formulations indicated that none of them caused skin irritation, allowing the conclusion that the Kesambi ointment is safe for topical application.

Stability Test of Kesambi Ointment

The stability test for temperature parameters was conducted within the range of 30–40°C. Measurements at room temperature (25°C) were used as a negative control. This test aimed to determine the stability of the bioactive compounds in kesambi ointment, particularly at typical storage temperatures (25–40°C). The absorbance measurement results of the Kesambi ointment stability test are presented in **Table 5**.

Table 5. The result of the absorbance measurement of the stability test of the Kesambi ointment

Temperature (ºC)	Absorbance sample		
	F1	F2	F3
25	0.386	0.389	0.387
30	0.389	0.392	0.391
35	0.391	0.397	0.395
40	0.395	0.402	0.401

The insignificant increase in absorbance within the temperature range of 30–40°C was caused by solvent evaporation, which led to sample concentration. It can be concluded that the stability of the bioactive compounds was maintained within this temperature range. This finding is supported by (Alim et al., 2022), who reported that phenolic compounds begin to degrade at temperatures above 50°C.

Conclusion

The ethyl acetate extract of *Schleichera oleosa* (kesambi) bark showed promising potential as an active ingredient in topical ointment formulations to inhibit Staphylococcus aureus growth. Among the three formulations tested, formulation 1 produced the largest inhibition zone and thus exhibited the most potent antibacterial activity, categorized as moderate. The increase in extract concentration in formulations 2 and 3 did not enhance antibacterial performance, indicating that higher extract levels may not improve diffusion or bioavailability within the ointment matrix. Phytochemical analysis confirmed the presence of triterpenoids, steroids, flavonoids, and phenolic compounds in the ointment, aligning with the observed antibacterial properties, as these secondary metabolites are known to contribute to antimicrobial activity. Organoleptic evaluation showed that all formulations possessed acceptable sensory characteristics, while safety testing confirmed the absence of irritation, indicating suitability for topical use. Furthermore, the stability assessment demonstrated that the bioactive compounds remained stable across typical storage temperatures (25–40°C). Overall, the ethyl acetate extract of kesambi bark can be developed into a safe, stable, and functional ointment formulation with antibacterial activity against Staphylococcus aureus. Further research, including optimization of formulation parameters and in vivo effectiveness studies, is recommended to support its potential application as a natural-based topical therapeutic.

Acknowledgement

The authors would like to express their gratitude to Direktorat Pembelajaran dan Kemahasiswaan (BELMAWA), Ministry of Education and Culture of the Republic of Indonesia, for funding the 2023 Student Creativity Program (PKM) in the research category, and to the Sekolah Tinggi Analis Kimia Cilegon for providing both in-kind and in-cash support.

 E-ISSN: 2303-0720

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