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Formulation And Antibacterial Effectiveness Test Of Ethyl Acetate And Methanol Extract Gel From Patchouli Leaf (Pogostemon Cablin Benth) Against Acne-Causing Bacteria

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ABSTRACT

Background: Acne is an infection characterized by inflammation of the polysebocyte layer caused by microorganisms such as Staphylococcus aureus, Staphylococcus epidermidis, and Propionibacterium acnes. Antibiotic use can lead to drug resistance, making natural ingredients a viable alternative. Patchouli leaves (Pogostemon cablin Benth) contain flavonoids, saponins, and tannins with antibacterial properties.
Methods: This was a laboratory experimental study involving maceration extraction of patchouli leaf using ethyl acetate and methanol solvents, followed by gel formulation and antibacterial effectiveness testing (well diffusion method) against S. aureus, S. epidermidis, and P. acnes at 3%, 5%, and 7% extract concentrations.
Results: Physical stability evaluation showed all gel preparations met semisolid criteria (organoleptic, homogeneity, pH 4.5–8.0, adhesiveness >1 s, spreadability 5–7 cm, viscosity 3000–50000 cPs, thixotropic flow). At 7% concentration, ethyl acetate and methanol extracts showed the greatest inhibitory zones against all tested bacteria.
Conclusion: Gel preparations of ethyl acetate and methanol extracts of patchouli leaf (Pogostemon cablin Benth) showed good physical stability and effective antibacterial activity at all concentrations, with 7% showing the highest inhibitory effect.

ABSTRAK

Latar Belakang: Jerawat merupakan infeksi yang ditandai dengan inflamasi pada lapisan polisebosit yang disebabkan oleh mikroorganisme seperti Staphylococcus aureus, Staphylococcus epidermidis, dan Propionibacterium acnes. Penggunaan antibiotik dapat menyebabkan resistensi obat sehingga pemanfaatan bahan alam menjadi alternatif pengobatan. Daun nilam (Pogostemon cablin Benth) mengandung flavonoid, saponin, dan tanin yang berfungsi sebagai antibakteri.
Metode: Penelitian eksperimental laboratorium dengan ekstraksi maserasi bertingkat menggunakan pelarut etil asetat dan metanol, formulasi sediaan gel, dan pengujian efektivitas antibakteri dengan metode sumuran terhadap S. aureus, S. epidermidis, dan P. acnes pada konsentrasi ekstrak 3%, 5%, dan 7%.
Hasil: Evaluasi stabilitas fisik menunjukkan semua sediaan gel memenuhi persyaratan sediaan semisolid yang baik (organoleptis, homogenitas, pH 4,5–8,0, daya lekat >1 detik, daya sebar 5–7 cm, viskositas 3000–50000 cPs, aliran tiksotropik). Pada konsentrasi 7%, ekstrak etil asetat dan metanol memberikan zona hambat terbesar terhadap semua bakteri uji..
Kesimpulan: Sediaan gel ekstrak etil asetat dan metanol daun nilam (Pogostemon cablin Benth) menunjukkan stabilitas fisik yang baik dan efektivitas antibakteri pada semua konsentrasi, dengan konsentrasi 7% memberikan efek hambat tertinggi.

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INTRODUCTION

Infection is a serious disease that can arise due to the entry of harmful microorganisms into the body. Staphylococcus aureus, Staphylococcus epidermidis, and Propionibacterium acnes are pathogenic bacteria that can cause infections on the skin, including acne or acne vulgaris. These bacteria are gram-positive and play a role in decomposing lipid-free fatty acids in the skin, which triggers inflammation and acne (Mangkey et al., 2023).

Acne (acne vulgaris) is an infection characterized by inflammation of the polyscebocyte layer, followed by keratin buildup, which generally occurs in adolescence. The incidence rate of acne globally is quite high and is the most common skin health problem. Acne treatment generally uses antibiotics such as erythromycin, clindamycin, and tetracycline. However, the continuous use of antibiotics can cause drug resistance and kidney damage in the long term (Sifatullah, 2021).

Alternatively, the use of herbal plants is becoming an increasingly popular option. The patchouli plant (Pogostemon cablin Benth) is one of the plants known to have flavonoids, saponins, and tannins that function as antibacterial. Previous research by Karim et al. (2022) proved that patchouli leaves are able to inhibit the growth of S. aureus, S. epidermidis, and P. acnes with a strong category at a concentration of 5%. Active compounds such as delta-guaiene, patchoulol, and beta-patchoulene play a role in such antibacterial mechanisms (Adhayani et al., 2021).

Dalam upaya memformulasikan bahan aktif daun nilam ke dalam bentuk sediaan yang praktis, gel dipilih sebagai bentuk sediaan topikal yang lebih efektif dibandingkan krim, karena tidak mengandung minyak yang dapat memperburuk kondisi jerawat dan lebih mudah diaplikasikan pada kulit (Yasir et al., 2021). Penelitian ini bertujuan mengetahui stabilitas fisik sediaan gel ekstrak etil asetat dan metanol daun nilam serta efektivitas antibakterinya terhadap bakteri penyebab jerawat.

METHOD

This study is a laboratory experimental study to see the effectiveness of methanol and ethyl acetate extract gels of patchouli leaf (Pogostemon cablin Benth) against acne-causing bacteria. The research will be carried out in June 2025 at the Pharmaceutical Laboratory, Bachelor of Pharmacy Study Program, Faculty of Medicine and Health Sciences, Alauddin State Islamic University Makassar.

Sample Preparation and Extraction

A sample of 5 kg of patchouli leaves (Pogostemon cablin Benth) was taken from Amowe Village, North Pakue District, North Kolaka Regency. The samples are cleaned, sliced, dried by aerating protected from direct sunlight, then blended and sifted using mesh 40 to obtain simplicia.

Extraction is carried out by the multi-stage maceration method. Simplisia 500 g is macerated with 5 L of ethyl acetate for 3 x 24 hours. The obtained pulp was then re-macerated with 5 L of methanol for 3 x 24 hours. The maserate of each solvent is evaporated using a rotary evaporator to obtain a viscous extract. Before formulation, solvent-free tests and phytochemical tests were carried out on both extracts.

Formulation of Gel Preparations

The gel preparation is made in three formulas with extracts concentrations of 3%, 5%, and 7%, as well as one negative control formula (without extract). The gel formula uses carbopol 940 as a base, triethanolamine as a pH regulator, propylene glycol as a humectant, phenoxyethanol as a preservative, and aquades as a solvent.

Table 1. Formula of Ethyl Acetate and Methanol Extract Gels of Patchouli Leaves (*Pogestemon cablin* Benth)

Material Name	Uses	F1	F2	F3	F4	Range
Ethyl Acetate/Methanol Extract Of Patchouli Leaf	Active substances	3	5	7	-	-
Carbopol 940	Basis	0,5	0,5	0,5	0,5	0,5%-2%
Trietanolamin	pH regulator	0,4	0,4	0,4	0,4	-
Phenoxyethanol	Preservatives	0,5	0,5	0,5	0,5	0,5%-1%
Propilenglikol	Humectant	15	15	15	15	±15%

Material Name	Uses	F1	F2	F3	F4	Range
Aquades ad	Solvents	100	100	100	100	-

Description: F1=3%; F2=5%; F3=7% ethyl acetate/methanol extract; F4=negative control (no extract). All values are in % (b/v) for 100 mL.

The gel is made by developing carbopol 940 in hot aquades (70°C), then adding TEA, propylene glycol, phenoxyethanol, and patchouli leaf extract which has been gradually dissolved while homogenizing.

Evaluation of Gel Preparations

Physical stability evaluation included organoleptic test, homogeneity, pH (SNI 16-4399-1996: 4.5-8.0), adhesion (>1 second), dispersion (5-7 cm), viscosity (3000-50000 cPs, SNI 1995), and flow type, carried out before and after cycling tests (6 cycles, alternating temperatures of 4°C and 40°C). Statistical analysis used the Wilcoxon test (abnormal data) and the Paired T-test (normal data).

Antibacterial Activity Test

Antibacterial effectiveness testing was carried out by the sewage diffusion method against S. aureus, S. epidermidis, and P. acnes. Mueller Hinton Agar (MHA) media is poured into a petri dish, then bacteria are inoculated on the surface of the media. The well hole is made with a 6 mm diameter reserve, then filled with a gel preparation (50 µL) at various concentrations, negative controls, and positive controls (Medi-Klin® 1.2%). Incubation is carried out at 37°C for 24 hours. The parameter measured is the diameter of the barrier zone using the caliper. Data analysis using one-way ANOVA and Tukey test.

RESEARCH RESULTS

Phytochemical Extraction and Test Results

Table 2. Results of Ethyl Acetate and Methanol Extract of Patchouli Leaves (*Pogestemon cablin* Benth)

Sample	Sample Weight(g)	Thick Extract Weight (g)	Rendamen (%)
Daun nilam – etil asetat (*Pogestemon cablin* Benth)	500	39,65	0,793
Daun nilam – metanol (*Pogestemon cablin* Benth)	500	68,14	13,628

From 500 g of simplicia, 39.65 g of ethyl acetate condensed extract (0.793% immersion) and methanol extract of 68.14 g (13.628% immersion) were obtained. Solvent-free tests showed no ethyl acetate or methanol odor in both extracts.

Phytochemical tests showed ethyl acetate extract contained alkaloids, tannins, and saponins. While methanol extract contains alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids. This difference in content is related to the difference in polarity of the two solvents; Methanol is more polar so it can attract more types of polar compounds.

Evaluation of the Physical Stability of Gel Preparations

The results of the organoleptic test showed that all formulas were green, smelled like extracts, had a semi-solid consistency, and remained stable after the cycling test. The homogeneity test shows all homogeneous formulas before and after the cycling test.

The pH value before the cycling test ranged from 6.28–8.07 and after the cycling test ranged from 6.25–8.02, still within the range of the quality requirements of the skin preparation (4.5–8.0). The Wilcoxon test showed a P>0.05, meaning there was no significant difference between before and after the cycling test.

The adhesion of all formulas before the cycling test >1 second (range 4.87–17.73 seconds) and after the cycling test still met the requirements despite the decrease. The dispersion before the cycling test ranged from 5.42–6.72 cm and after the cycling test was 5.67–6.91 cm, still in the range of 5–7 cm. The viscosity of all formulas before and after the cycling test is in the range of 3000–50000 cPs. The

flow type test showed that all formulas had the properties of a positive cyclotropic (non-Newtonian plastic) flow.

Antibacterial Effectiveness Test Results

Tabel 3 Average Diameter of Inhibition Zone (mm) of Ethyl Acetate (EA) and Methanol (M) Patchouli Leaf Extract Gel Preparations against Acne-Causing Bacteria

Bacteria	F1 (3%EA)	F2 (5%EA)	F3 (7%EA)	F4 (3% M)	F5 (5% M)	F6 (7% M)	F7 (-)	K (+)
S. aureus	3,31	4,19	5,52	3,37	5,09	5,88	0,00	30,15
S. epidermidis	3,65	4,28	5,19	3,64	4,43	9,14	0,00	22,48
P. acnes	3,16	3,66	6,14	4,97	6,30	7,31	0,00	37,91

Description: EA = Ethyl Acetate Extract; M = methanol extract; F7(-) = Negative control; K(+) = Medi-Klin® 1.2%

All gel formulas of ethyl acetate and methanol extract of patchouli leaves show antibacterial activity against S. aureus, S. epidermidis, and P. acnes which are characterized by the formation of inhibition zones around the well. Negative controls (F7) do not indicate an inhibition zone, while positive controls (Medi-Klin® 1.2%) provide a very large inhibition zone (>20 mm, very strong category).

DISCUSSION

The low soaking of ethyl acetate extract (0.793%) compared to methanol (13.628%) was related to the difference in solvent polarity. Methanol is more polar so it can dissolve almost all compounds, while ethyl acetate, which is semipolar, is more selective in extracting certain compounds (Domithesa et al., 2021). The maceration method was chosen because the process is simple, does not damage the active ingredients that are not heat-resistant, and is able to extract large quantities (Nugroho, 2017).

The difference in the phytochemical profile of the two extracts affects the antibacterial activity. Methanol extracts containing more active compounds (alkaloids, flavonoids, tannins, saponins, steroids, triterpenoids) consistently produce greater inhibitory zones than ethyl acetate extracts at the same concentration. Alkaloid compounds work to inhibit enzymes on bacterial protein synthesis, while saponins damage cell membranes through their amphiphilic properties that resemble detergents (Karim et al., 2022). Tannins play a role in binding to bacterial cell wall proteins so as to inhibit cell growth.

On the physical stability evaluation, all the parameters of the preparation meet the quality requirements of the gel for the skin preparation. Maintained pH stability in the range of 4.5–8.0 is important to prevent skin irritation. The decrease in adhesion after the cycling test is directly proportional to the decrease in viscosity, according to the principle that the higher the viscosity, the greater the adhesion. Viscosity changes are caused by temperature fluctuations (4°C and 40°C) during cycling tests that affect the structure of the carbomer polymer (Arifin et al., 2022). The ticsotropic flow properties that all formulas have are advantageous because the viscosity is reduced when applied (swiped) and increases again after rest.

Based on the classification of the inhibition zone (Alifiar et al., 2024), the antibacterial activity of gel formulas against S. aureus and S. epidermidis in F1–F6 is mostly in the weak category (≤5 mm). However, F6 (7% methanol) against S. epidermidis reaches the medium category (9.14 mm). For P. acnes, F3 (7% ethyl acetate), F5 (5% methanol), and F6 (7% methanol) are in the medium category (6–10 mm). Increased inhibition zones as the extract concentration increases shows a consistent dose-response relationship. The ANOVA test showed a significant difference ($P<0.05$) between treatment groups for all test bacteria.

P. acnes showed a higher sensitivity to both extracts than S. aureus and S. epidermidis. This can be attributed to the nature of P. acnes as an anaerobic bacterium that is more susceptible to phenolic compounds from plants. Although the resulting inhibition zone was smaller than the positive control (Medi-Klin®), the use of patchouli leaf extract gel still has potential as an acne adjuvant therapy based on natural ingredients.

CONCLUSIONS AND SUGGESTIONS

Based on the results of the study, it can be concluded: (1) Gel-prepared extract of ethyl acetate and methanol of patchouli leaf (Pogostemon cablin Benth) showed good physical stability before and after the cycling test, including organoleptis, homogeneity, pH, adhesion, dispersibility, viscosity, and flow type; (2) All gel formulas are effective in inhibiting the growth of S. aureus, S. epidermidis, and P. acnes which are characterized by the formation of inhibition zones around the radiation; (3) The 7% concentration of the extract (both ethyl acetate and methanol) provides the greatest inhibition zone, with methanol extracts consistently producing a greater inhibition zone than ethyl acetate extracts at the same concentration.

It is recommended that before adding the extract to the gel preparation, the gel base pH measurement is first carried out to ensure that the initial pH is within the required range. Further research is needed with clinical trials and increased concentration of extracts to obtain more optimal antibacterial activity.

Author's Contribution Statement:
Muhammad Taufiq Duppa: Conceptualization, Methodology, Investigation, Project Administration, Resources, Formal Analysis, Writing – Original Draft, Writing – Review & Editing, Funding Acquisition, Supervision. **Anshari Masri:** Conceptualization, Methodology, Investigation, Data Curation, Validation, Writing – Review & Editing, Resources. **Syafruddin:** Methodology, Investigation, Data Curation, Formal Analysis, Writing – Review & Editing, Validation. Genevieva **Ermawati:** Conceptualization, Methodology, Formal Analysis, Writing – Original Draft, Writing – Review & Editing, Visualization, Resources.

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