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Antibacterial Activity of *Abelmoschus manihot* L. Leaf Extract Ointment Against *Pseudomonas aeruginosa*

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Abstract. *Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from insulin resistance or deficiency. One of its severe complications is diabetic foot ulcers, which are prone to infection, especially by Pseudomonas aeruginosa—a Gram-negative opportunistic bacterium capable of forming biofilms and highly resistant to common antibiotics. This resistance complicates wound healing and increases the risk of amputation, emphasizing the need for alternative treatments. Abelmoschus manihot L. (Gedi leaves) contains bioactive compounds such as flavonoids, tannins, and phenolics, which are believed to possess antibacterial properties. This study aims to evaluate the potential of ethanolic Gedi leaf extract as an active ingredient in antibacterial ointment formulations. Ethanolic extracts were prepared and formulated into ointments at concentrations of 2%, 4%, 6%, and 8%. The formulations were tested for physical properties (pH, spreadability, organoleptic characteristics) and antibacterial activity against P. aeruginosa using the disk diffusion method. Results showed that the ointments had a uniform semi-solid texture, a characteristic herbal aroma, green color, and pH values ranging from 6.3 to 6.7—suitable for topical application. Spreadability ranged between 4.1 cm and 5.3 cm. The inhibition zones for the 2%, 4%, 6%, and 8% formulations were 6.67 mm, 8.00 mm, and 10.67 mm, respectively. No inhibition was observed with the negative control (DMSO), while the positive control (Bioplacenton) showed a 15.00 mm zone. In conclusion, Gedi leaf extract ointment demonstrated effective antibacterial activity against P. aeruginosa, with consistent physical properties across all concentrations. These findings suggest its potential as a natural topical therapy for treating bacterial infections in diabetic foot ulcers.*

Keywords: *Abelmoschus Manihot L., Ointment, Antibacterial, Diabetic Foot Ulcer, Pseudomonas Aeruginosa*

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Abstrak. Diabetes mellitus adalah gangguan metabolik kronis yang ditandai dengan hiperglikemia akibat resistensi insulin atau kekurangan insulin. Salah satu komplikasi seriusnya adalah luka kaki diabetes, yang rentan terinfeksi, terutama oleh *Pseudomonas aeruginosa*—bakteri Gram-negatif oportunistik yang mampu membentuk biofilm dan sangat resisten terhadap antibiotik umum. Resistensi ini mempersulit penyembuhan luka dan meningkatkan risiko amputasi, sehingga menekankan perlunya pengobatan alternatif. *Abelmoschus manihot* L. (daun Gedi) mengandung senyawa bioaktif seperti flavonoid, tanin, dan fenolik, yang diyakini memiliki sifat antibakteri. Studi ini bertujuan untuk mengevaluasi potensi ekstrak etanol daun Gedi sebagai bahan aktif dalam formulasi salep antibakteri. Ekstrak etanol disiapkan dan diformulasikan menjadi salep dengan konsentrasi 2%, 4%, 6%, dan 8%. Formulasi diuji untuk sifat fisik (pH, kelembutan, karakteristik organoleptik) dan aktivitas antibakteri terhadap *P. aeruginosa* menggunakan metode difusi cakram. Hasil menunjukkan bahwa salep memiliki tekstur semi-padat yang seragam, aroma herbal khas, warna hijau, dan nilai pH berkisar antara 6,3 hingga 6,7—cocok untuk aplikasi topikal. Kelembutan berkisar antara 4,1 cm hingga 5,3 cm. Zona penghambatan untuk formulasi 2%, 4%, 6%, dan 8% masing-masing adalah 6,67 mm, 8,00 mm, dan 10,67 mm. Tidak ada zona penghambatan yang diamati pada kontrol negatif (DMSO), sementara kontrol positif (Bioplacenton) menunjukkan zona 15,00 mm. Kesimpulannya, salep ekstrak daun Gedi menunjukkan aktivitas antibakteri yang efektif terhadap *P. aeruginosa*, dengan sifat fisik yang konsisten pada semua konsentrasi. Temuan ini menyarankan potensinya sebagai terapi topikal alami untuk mengobati infeksi bakteri pada luka kaki diabetes.

Kata kunci: *Abelmoschus Manihot* L. Salep, Antibakteri, Luka Kaki Diabetes, *Pseudomonas Aeruginosa*

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by impaired metabolism of carbohydrates, fats, and proteins due to insulin deficiency or decreased insulin sensitivity (Saeedi et al., 2020). Globally, diabetes has become a significant public health concern, with its prevalence increasing steadily each year. The Indonesian Society of Endocrinology (2021) estimates that the number of people living with diabetes worldwide will reach approximately 21.3 million by 2030. This condition not only affects individuals but also exerts a substantial burden on public healthcare systems. Persistent hyperglycemia—resulting from inadequate insulin production or ineffective insulin utilization—can lead to a range of severe complications that negatively impact patients' quality of life (Papachristoforou et al., 2020).

Beyond its direct effects, diabetes contributes to decreased productivity, elevated long-term healthcare costs, and increased risk of complications such as cardiovascular diseases, nephropathy, neuropathy, and chronic wounds. Therefore, optimal diabetes

management is essential, integrating both pharmacological and non-pharmacological approaches. While pharmacological therapy involves oral antidiabetic agents or insulin injections, non-pharmacological strategies include dietary management, regular physical activity, stress control, and intensive foot and wound care. One such intervention is foot exercise, which is recommended for diabetic patients to enhance lower extremity blood circulation, reduce neuropathic symptoms, and maintain foot mobility. Studies have shown that foot exercises improve nerve sensitivity and blood flow, which can help prevent diabetic foot ulcers and potential amputations (Nur Azizah & Samodra, 2022).

Diabetic foot ulcers (DFUs) remain one of the most severe and prevalent complications of diabetes. According to Haskas and Ikhsan (2021), the incidence of DFUs increases by approximately 40 to 60 million cases annually. Diabetic foot infections (DFIs), commonly caused by microbial invasion of the tissue—particularly around the malleolar region—are among the leading reasons for hospitalization in diabetic patients and greatly affect clinical outcomes (Sofyanti et al., 2022). These infections can be exacerbated by several factors such as peripheral neuropathy, vascular insufficiency, immune system dysfunction, and biomechanical abnormalities, all of which hinder the healing process (Dinata & Yasa, 2021).

Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen, is frequently isolated in DFIs and among ICU patients. It demonstrates high adaptability to various environmental conditions and exhibits strong antimicrobial resistance, posing a major threat especially in immunocompromised individuals, including those with diabetes (Ningrum & Jafar, 2024). Its antibiotic resistance mechanisms—such as β -lactamase production, altered membrane permeability, and biofilm formation—make it particularly difficult to treat and contribute to increased morbidity and mortality rates in hospital settings .

In response to these challenges, natural antibacterial agents are being explored as alternatives to synthetic antibiotics. One such candidate is *Abelmoschus manihot* L. (commonly known as Gedi), a tropical plant of the Malvaceae family widely used in traditional medicine. It has been reported to possess various pharmacological properties, including antihypertensive, antidiabetic, anti-inflammatory, antioxidant, and antidepressant effects. Its leaves contain bioactive compounds such as phenolics, flavonoids, tannins, and steroids—many of which are known to exhibit antibacterial

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activity through mechanisms like membrane disruption, inhibition of microbial enzymes, and prevention of biofilm formation (Hendrawati et al., 2020). However, despite its traditional uses, the scientific exploration of Gedi as an antibacterial agent remains underdeveloped, primarily due to limited public awareness and lack of clinical validation (Suliasih & Mun'im, 2022).

Topical preparations such as ointments are among the most effective pharmaceutical forms for treating skin infections. These semi-solid formulations offer prolonged contact with the skin, enabling sustained release and deeper penetration of active compounds. Currently, many commercial antibacterial ointments contain synthetic antibiotics, which can lead to resistance if used excessively. Hence, developing herbal-based ointments using plant extracts such as *A. manihot* L. represents a promising strategy for safer and more sustainable antimicrobial therapy (Bawotong et al., 2020). The formulation of such ointments could contribute to the advancement of affordable, accessible, and standardized phytopharmaceutical products.

Given the growing concern over antibiotic resistance and the need for effective alternative therapies, this study aims to evaluate the antibacterial potential of an ointment formulated with ethanolic extract of *Abelmoschus manihot* L. leaves against *Pseudomonas aeruginosa*. The specific objectives include developing a stable and effective herbal ointment, assessing its antibacterial activity through in vitro testing, and evaluating its physical characteristics—such as pH, spreadability, and consistency—to ensure its suitability as a topical preparation.

The findings of this study are expected to contribute both theoretically and practically. Theoretically, the research adds scientific evidence to the antibacterial efficacy of *A. manihot* L. and supports further pharmacological investigation of its bioactive constituents. Practically, it offers a foundation for developing natural-based topical treatments that are safe, effective, and potentially valuable in managing bacterial skin infections, especially those caused by *P. aeruginosa*.

RESEARCH METHODS

This study was an in vitro laboratory-based experimental research employing the disk diffusion method, with a posttest-only control group design. The aim was to

evaluate the antibacterial activity of ethanolic extract from *Abelmoschus manihot* L. leaves against *Pseudomonas aeruginosa*.

Experimental Procedure

1. Sample Collection and Identification

Leaf samples of *A. manihot* L. were collected randomly from the vicinity of Universitas Prima Indonesia. Botanical identification was conducted at the Herbarium Medanese, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera, to confirm species authenticity.

2. Preparation of Equipment and Materials

- a. Sterilization of Equipment Glassware and culture media were sterilized using an autoclave at 121°C for 15 minutes. Inoculating loops and tweezers were sterilized by direct flaming (Gerung et al., 2021).
- b. Sample Preparation Fresh leaves were washed with running water to remove impurities, air-dried at room temperature for seven days until crisp, and then ground into powder using a blender. The powdered leaves were stored in airtight containers, away from direct sunlight.
- c. Ethanolic Extraction via Maceration A total of 100 g of powdered leaves was macerated in 1000 mL of 96% ethanol (1:10 b/v) in a closed vessel for 24–48 hours at room temperature with occasional stirring. The extract was filtered and concentrated using a rotary evaporator at 40–50°C. The concentrated extract was further dried as needed and stored in sealed containers at room temperature (Saerang et al., 2023).

Phytochemical Screening

Preliminary phytochemical screening of the *Abelmoschus manihot* L. ethanolic leaf extract was conducted to identify the presence of major secondary metabolites, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds. This screening was carried out using standard qualitative methods as described by Harborne (1987), which involve colorimetric reactions based on specific reagent interactions. These phytochemical constituents are known to contribute to antimicrobial activity, particularly flavonoids and tannins, which have been widely reported for their ability to inhibit bacterial growth.

Ointment Formulation

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Table 1. Formulation of Gedi Leaf Extract Ointment

Ingredient	F1	F2	F3	F4
Gedi Leaf Extract (%)	2	4	6	8
Adeps Lanae (%)	2	2	2	2
Cetyl Alcohol (%)	5	5	5	5
Propylene Glycol (%)	15	15	15	15
Methylparaben (%)	0.1	0.1	0.1	0.1
White Soft Paraffin	q.s. to 30 g			

Ointment Preparation

All ingredients were weighed and prepared according to formulation. Adeps lanae and white soft paraffin were triturated until uniform. Cetyl alcohol was melted and mixed with methylparaben. Propylene glycol was added to the melted phase. The ethanolic extract was incorporated into the base according to each concentration. A negative control was prepared without extract. The ointments were stored in sealed containers (Putri et al., 2023).

Evaluation of Physical Characteristics

The physical properties of the ointment formulation were evaluated through several tests. Organoleptic examination assessed the consistency, color, and odor of the preparation. Homogeneity was verified by checking samples taken from the upper, middle, and lower sections of the container for uniform distribution. The pH was measured by dispersing 0.5 g of ointment in 5 mL of distilled water and determining the pH using a calibrated pH meter, with an ideal pH range between 4.5 and 6.5. Spreadability was assessed by placing 0.5 g of the ointment between two glass plates, with the diameter of the spread measured before and after applying a 150 g weight. Adhesion was evaluated by placing the ointment between two glass slides, pressing them with a 1 kg load for 5 minutes, and then measuring the time required for detachment under an 80 g load, with acceptable adhesion defined as detachment within ≤ 4 seconds. Stability testing involved alternating storage of the ointment at $5 \pm 2^\circ\text{C}$ and 40°C for 24-hour intervals over six cycles (12 days total) to assess the formulation's resilience under temperature fluctuations (Sugiyono et al., 2020).

Antibacterial Assay

The antibacterial activity of the ethanolic extract of *Abelmoschus manihot* L. leaves against *Pseudomonas aeruginosa* was evaluated using the disk diffusion method. Nutrient Agar (NA) was prepared by dissolving 3 g of NA powder in 100 mL of distilled water, followed by heating at 180°C for 30 minutes with continuous stirring. The medium was then sterilized in an autoclave at 121°C for 15 minutes and poured into sterile Petri dishes under aseptic conditions (Dajoh et al., 2020).

A bacterial suspension was prepared by transferring several colonies of *P. aeruginosa* into 0.9% NaCl solution and adjusting its turbidity to match the 0.5 McFarland standard (Widyaningrum & Nurjanah, 2020). Subsequently, 0.1 mL of the standardized suspension was spread evenly on the surface of the solidified NA medium.

Sterile paper disks were impregnated with different concentrations of the extract (2%, 4%, 6%, and 8%) and placed on the surface of the inoculated agar. Bioplacenton® was used as a positive control, while dimethyl sulfoxide (DMSO) served as a negative control. The plates were incubated at room temperature for 36–48 hours. Antibacterial activity was determined by measuring the diameter of the inhibition zones formed around each disk (Nurhayati et al., 2020).

The inhibition zone diameters were statistically analyzed using one-way Analysis of Variance (ANOVA) with a significance level of $\alpha = 0.05$. The analysis was conducted using SPSS version 23. Duncan's multiple range test was employed as a post-hoc analysis to identify significant differences among treatment groups (Ernawati et al., 2022).

RESULTS AND DISCUSSION

Extraction Results

The ethanolic extract of *Abelmoschus manihot* L. leaves was obtained using the maceration method with 96% ethanol as the solvent. The yield of the extraction process is presented in the following table:

Table 2. Extraction Yield of *Abelmoschus manihot* L. Leaves

Sample	Weight of Simplicia (g)	Extract Weight (g)	Yield (%)
<i>Abelmoschus manihot</i> L.	1,515.48	158.67	10.46%

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Sample	Weight of Simplicia (g)	Extract Weight (g)	Yield (%)
leaves			

A total of 1,515.48 grams of fine powdered *Abelmoschus manihot* L. leaves was extracted using 15 liters of 96% ethanol. The extraction process was carried out by maceration, where the powdered leaves were soaked in solvent for three consecutive 24-hour cycles in a sealed container, protected from sunlight and stirred occasionally for uniform mixing. After each soaking cycle, the mixture was filtered to separate the filtrate from the residue. This maceration process was repeated three times. The combined filtrates were then evaporated using a water bath followed by a rotary evaporator to obtain a concentrated extract. The final yield was 158.67 grams of thick extract, corresponding to a yield of 10.46%.

Phytochemical Screening Results

The phytochemical screening of *Abelmoschus manihot* L. leaf extract revealed the presence of various secondary metabolites, as summarized in Table 4.2. Alkaloids were identified by the formation of white to cream-colored precipitates with Mayer's reagent and orange to reddish-orange precipitates with Dragendorff's reagent. Flavonoids were confirmed by a yellowish-red to reddish-orange coloration upon the addition of magnesium and hydrochloric acid. Tannins produced dark precipitates—either black or dark green—when reacted with FeCl_3 , while phenolic compounds gave a dark bluish-black precipitate with the same reagent. The presence of saponins was evidenced by the formation of stable foam approximately 2 cm in height, which persisted even after the addition of 2N HCl. Additionally, steroids and triterpenoids were indicated by the appearance of a brown ring in the Liebermann-Burchard reaction. These findings collectively demonstrate that the ethanolic extract of *A. manihot* leaves contains multiple bioactive constituents—namely alkaloids, flavonoids, tannins, saponins, phenols, steroids, and triterpenoids—which support its potential for application in pharmaceutical and therapeutic fields.

Physical Properties of the Ointment Formulation

The physical properties of the *Abelmoschus manihot* L. leaf extract ointment were evaluated through organoleptic, pH, homogeneity, spreadability, and stability tests.

35 Organoleptic observations conducted at room temperature revealed that all formulations had a semi-solid consistency, with Formula 0 (without extract) appearing white, smooth, and homogenous. As the extract concentration increased from 2% to 8% (Formulas 1–4), the ointments exhibited progressively darker shades (from dark green to greenish-brown) and thicker textures, with a distinct odor attributable to the *A. manihot* extract. pH measurements showed values ranging from 5.92 to 6.50, which fall within the acceptable range for topical application (4.5–6.5), indicating suitability for skin use without causing irritation. All formulations were homogenous, as evidenced by the even distribution of active and base ingredients. Spreadability tests demonstrated that while higher extract concentrations slightly reduced the spread due to increased viscosity, the ointments still maintained good application characteristics, with spreadability ranging from 29.82 mm² to 36.86 mm². Stability testing over two weeks showed consistent organoleptic properties, homogeneity, and only slight reductions in pH (5.37–4.56) and spreadability (~52 to ~50 mm²), confirming that the ointments remained stable and suitable for continued topical application.

Inhibition Zone Measurement Against *Pseudomonas aeruginosa*

Table 3. Inhibition Zone Diameter (mm)

Concentration	P1	P2	P3	Mean
Positive Control (K+)	21.89	22.3	22.0	22.06
Negative Control (K–)	0	0	0	0
2%	9.2	9.8	10.14	9.71
4%	11.4	11.0	10.08	10.82
6%	12.68	13.21	12.98	12.95
8%	13.3	13.8	14.0	13.7

8 The disc diffusion method on Mueller-Hinton Agar was used to assess antibacterial activity against *Pseudomonas aeruginosa*. Each concentration was tested in triplicate. The positive control (Bioplacenton) showed the largest inhibition zone, while the negative control (10% DMSO) exhibited no activity. The antibacterial effect increased with higher extract concentrations.

Statistical Analysis

6 The statistical analysis began with the Shapiro-Wilk normality test, which showed that all extract concentration groups had p-values greater than 0.05, indicating that the

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data were normally distributed and suitable for parametric analysis. This was further supported by Levene's test for homogeneity of variance, which yielded a p-value of 0.068 (>0.05), confirming that the variance among groups was homogeneous. These results validated the use of a one-way ANOVA to assess differences in antibacterial activity. The ANOVA test revealed a highly significant difference ($p < 0.001$) in inhibition zone diameters among the various extract concentrations, suggesting that increasing the concentration of *Abelmoschus manihot* L. leaf extract was associated with a greater antibacterial effect against *Pseudomonas aeruginosa*.

CONCLUSION AND SUGGESTIONS

The ethanol extract of *Abelmoschus manihot* L. leaves, obtained via maceration with 96% ethanol, showed a good yield (10.46%) and contained active secondary metabolites such as alkaloids, flavonoids, tannins, saponins, phenols, steroids, and triterpenoids. The formulated ointment met the required physical properties for topical use, with increasing antibacterial activity against *Pseudomonas aeruginosa* in line with higher extract concentrations. The 8% formulation exhibited the highest inhibition zone (13.70 mm). Statistical analysis confirmed significant differences between groups ($p < 0.001$), supporting the potential of this extract as a natural antibacterial agent for topical applications, particularly in managing skin infections like diabetic foot ulcers.

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