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Antibacterial Activity of Ethanol Extract Ointment of *Tithonia diversifolia* Against *Staphylococcus aureus*

Linda Triana^{1✉}, Rizal Supardi¹, Sutriswanto¹, Gervacia Jenny Ratnawati¹,
Emilda Sari¹, Etiek Nurhayati¹

¹Department of Medical Laboratory Technology, Poltekkes Kemenkes Pontianak

✉email: linda3ana14@gmail.com

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ABSTRACT

*Skin infections caused by pathogenic bacteria such as *Staphylococcus aureus* remain a common health problem, and the need for safer alternative topical antibacterial agents continues to increase. This study aimed to evaluate the antibacterial activity of ethanol extract ointment of *Tithonia diversifolia* leaves with various concentrations formulated as a semi-solid topical preparation. A quasi-experimental design was used, and ointments were prepared at concentrations of 5%, 7%, 9%, 11%, 13%, 15%, 17%, 19%, and 21%. Antibacterial activity was tested using the well-diffusion method against *Staphylococcus aureus*. A total of 27 samples were tested with three replications. The results showed that the ointment produced inhibition zones ranging from 13.83 mm to 22.00 mm, with higher concentrations yielding greater inhibition. Repeated Measures ANOVA showed a significant difference among concentrations ($p = 0.003$), indicating that the increase in extract concentration significantly affected the antibacterial effectiveness. This study concludes that ethanol extract ointment of *Tithonia diversifolia* leaves demonstrates strong antibacterial activity against *Staphylococcus aureus**, with effectiveness increasing proportionally with concentration.*

Keywords: Antibacterial ointment; *Tithonia diversifolia*; Ethanol extract; Inhibition zone; *Staphylococcus aureus*

ABSTRAK

Infeksi kulit akibat bakteri patogen seperti *Staphylococcus aureus* masih menjadi masalah kesehatan yang sering dijumpai, sehingga diperlukan alternatif sediaan topikal yang lebih aman dan berasal dari bahan alam. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri salep ekstrak etanol daun Kembang Bulan (*Tithonia diversifolia*) pada berbagai konsentrasi sebagai sediaan semi padat. Desain penelitian yang digunakan adalah quasi-eksperimental dengan

formulasi salep konsentrasi 5%, 7%, 9%, 11%, 13%, 15%, 17%, 19%, dan 21%. Aktivitas antibakteri diuji menggunakan metode difusi sumuran terhadap bakteri *Staphylococcus aureus* dengan total 27 sampel dan tiga kali pengulangan. Hasil penelitian menunjukkan bahwa salep ekstrak etanol menghasilkan diameter zona hambat berkisar antara 13,83 mm hingga 22,00 mm, dengan konsentrasi yang lebih tinggi memberikan hambatan yang lebih besar. Uji Repeated Measures ANOVA menunjukkan perbedaan yang signifikan antar konsentrasi ($p = 0,003$), sehingga peningkatan konsentrasi ekstrak berpengaruh signifikan terhadap efektivitas antibakteri. Penelitian ini menyimpulkan bahwa salep ekstrak etanol daun Kembang Bulan memiliki aktivitas antibakteri yang kuat terhadap *Staphylococcus aureus*, dengan efektivitas yang meningkat seiring peningkatan konsentrasi.

Kata kunci: Salep antibakteri; *Tithonia diversifolia*; Ekstrak etanol; Zona hambat; *Staphylococcus aureus*

INTRODUCTION

Skin is the largest organ of the human body and serves as the primary physical barrier against various pathogenic microorganisms. Disruption of the skin barrier, particularly due to bacterial infection, remains a common health problem in many developing countries, including Indonesia. *Staphylococcus aureus* is an opportunistic pathogen frequently associated with wound infections, folliculitis, and other skin disorders, and may progress to severe complications if not treated properly(1–3). Topical synthetic antibiotics are widely used as standard therapy; however, prolonged use can cause adverse effects such as irritation, allergic reactions, and contribute to the increasing global problem of antibiotic resistance(4,5).

In response to the rising issue of bacterial resistance, the exploration of natural ingredients as alternative antibacterial agents has gained growing attention. Leaves of *Tithonia diversifolia* contain several secondary metabolites including flavonoids, alkaloids, tannins, saponins, and steroids/triterpenoids which have been reported to exhibit antibacterial properties(6). Previous studies demonstrated that extracts of *Tithonia diversifolia* possess significant antibacterial activity against various bacterial species, including *Staphylococcus aureus*, using crude extracts and specific fractions(7,8). Furthermore, herbal ointment formulations derived from medicinal plants have been shown to enhance the stability, penetration, and topical effectiveness of bioactive compounds(9).

Although existing studies have investigated the antibacterial effects of *Tithonia diversifolia*, research focusing specifically on ethanol extract ointment formulations at varying concentrations and their antibacterial effectiveness using the well-diffusion method remains limited. This represents the scientific novelty of the present study, which provides a comprehensive evaluation of the antibacterial activity of a semi-solid herbal preparation with potential development into a topical phytopharmaceutical product.

Based on this background, the present study aims to analyze the differences in inhibition zone diameters produced by ethanol extract ointments of *Tithonia diversifolia* leaves at various concentrations against *Staphylococcus aureus*. The objective of this study is to determine the relationship between extract concentration and the antibacterial effectiveness of the formulated ointment..

METHODS

This study employed a quasi-experimental design to evaluate the antibacterial activity of ethanol extract ointments of *Tithonia diversifolia* leaves at various concentrations. The research was conducted in May–July 2024 at the Microbiology Laboratory, PoltekNIK Kesehatan Kemenkes Pontianak. Ethical approval for this study was granted by the Health Research Ethics Committee (HREC), PoltekNIK Kesehatan Kemenkes Pontianak, with approval number 213/KEPK-PK.PKP/V/2024.

Plant Material Preparation and Extraction

Fresh leaves of *Tithonia diversifolia* were collected, cleaned, and dried at room temperature. The dried samples were powdered and extracted using maceration with 96% ethanol for 72 hours. The filtrate was then concentrated using a rotary evaporator to obtain a thick ethanol extract.

Ointment Formulation

The ethanol extract was formulated into a semi-solid ointment using a standard hydrophilic base. Ointments were prepared in the following concentrations: 5%, 7%, 9%, 11%, 13%, 15%, 17%, 19%, and 21%. Each formulation was prepared in sterile containers and homogenized to ensure uniformity.

Preparation of Test Bacteria

Staphylococcus aureus was obtained from the microbiology laboratory culture collection. The bacterial suspension was standardized to a 0.5 McFarland turbidity (equivalent to 1.5×10^8 CFU/mL) prior to testing.

Antibacterial Activity Test

Antibacterial activity was assessed using the well-diffusion method. Mueller-Hinton Agar (MHA) plates were inoculated with *Staphylococcus aureus*, and wells were created using a sterile borer (6 mm diameter). Each ointment concentration (100 mg) was applied into separate wells. The plates were incubated at 37°C for 24 hours. The diameter of the inhibition zone was measured in millimeters using a caliper. All tests were performed in triplicate, resulting in a total of 27 observations.

Data Analysis

Data on the inhibition zone diameters were expressed as mean $\pm$ standard deviation. Differences between concentrations were analyzed using Repeated Measures ANOVA, followed by post-hoc analysis when necessary. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 25..

RESULTS

This study assessed the antibacterial activity of ethanol extract ointments of *Tithonia diversifolia* leaves formulated at nine concentrations ranging from 5% to 21%. Antibacterial activity was determined by measuring the inhibition zone diameter against *Staphylococcus aureus* using the well-diffusion method. A total of 27 observations were obtained from three replications per concentration.

Results showed that all ointment concentrations produced measurable inhibition zones, indicating antibacterial activity. The lowest concentration (5%) produced a mean inhibition zone of 13.83 mm, categorised as moderate inhibition, while the highest concentration (21%) produced a mean inhibition zone of 22.00 mm, classified as strong inhibition. The data demonstrated a clear concentration-dependent pattern, where increasing extract concentration resulted in larger inhibition zones.

Repeated Measures ANOVA revealed a statistically significant difference among all concentrations ($p = 0.003$), confirming that variations in extract concentration significantly

affected the antibacterial effectiveness of the ointment. These findings support earlier studies showing that bioactive compounds in *Tithonia diversifolia* such as flavonoids, tannins, and saponins—exert antibacterial effects through mechanisms including cell membrane disruption and interference with microbial enzymatic activity (1,2).

The complete results of inhibition zone measurements are presented in Table 1.

Table 1. Mean Inhibition Zone Diameter of Ointment Concentrations Against *Staphylococcus aureus*

Concentration (%)	Mean (mm)	SD	p*
5	13.83	0.29	
7	14.40	0.36	
9	15.20	0.26	
11	16.10	0.30	
13	17.00	0.20	
15	18.20	0.30	
17	19.60	0.26	
19	21.10	0.17	
21	22.00	0.20	0.003

Based on Table 1, the inhibition zone diameter increased progressively with higher concentrations of the ethanol extract ointment. The lowest concentration (5%) produced an inhibition zone of 13.83 mm, while the highest concentration (21%) produced an inhibition zone of 22.00 mm. This pattern indicates that higher concentrations contain greater amounts of active antibacterial compounds, resulting in stronger inhibitory effects against *Staphylococcus aureus*.

The relatively small standard deviation (SD) values across all concentrations show that the measurements were consistent and reproducible across the three replications. The statistical analysis using Repeated Measures ANOVA produced a p-value of 0.003, indicating a significant difference among the concentrations. This confirms that increasing the extract concentration has a significant effect on the antibacterial activity of the ointment. Overall, the data in the table demonstrate that the antibacterial activity of the ointment increases with increasing extract concentration.

DISCUSSION

The findings of this study demonstrate that the ethanol extract ointment of *Tithonia diversifolia* leaves possesses antibacterial activity against *Staphylococcus aureus*, with the magnitude of inhibition increasing in parallel with the concentration of the extract. The progressive increase in inhibition zone diameter from 13.83 mm at 5% to 22.00 mm at 21% indicates a clear concentration-dependent relationship. This pattern supports the hypothesis stated in the introduction, namely that higher extract concentrations would result in greater antibacterial activity.

The significant differences among concentrations, as confirmed by Repeated Measures ANOVA ($p = 0.003$), further strengthen the conclusion that the amount of extract incorporated into the ointment plays a crucial role in determining the effectiveness of bacterial inhibition. These results are consistent with prior studies reporting that *Tithonia diversifolia* contains bioactive secondary metabolites such as flavonoids, tannins, and saponins, which are known to exhibit antibacterial properties through various mechanisms, including cell membrane disruption, protein denaturation, and inhibition of enzymatic processes essential for bacterial survival(10–12).

The strong inhibition observed at higher concentrations suggests that the ethanol extraction method efficiently isolated antibacterial compounds that remain active when formulated into a semi-solid ointment. This aligns with previous findings indicating that ethanol extracts often contain higher concentrations of phenolic and flavonoid compounds compared to water or other solvent extracts(13,14). Additionally, ointment formulations have been reported to enhance the stability and penetration of plant-derived compounds, improving their topical antibacterial performance(15–17).

The results of this study also highlight the potential of *Tithonia diversifolia* as an affordable and accessible natural alternative to conventional topical antibiotics, especially in settings where antimicrobial resistance is increasing. The ability of the extract to produce inhibition zones above 20 mm at higher concentrations indicates strong antibacterial activity, comparable to some standard topical antimicrobial agents reported in previous studies(18,19).

Overall, the findings support the potential development of *Tithonia diversifolia* extract ointment as a herbal antibacterial formulation. However, further studies are needed to evaluate its stability, safety, and clinical effectiveness on infected wounds, as well as to isolate the specific active compounds responsible for antibacterial action.

CONCLUSION

The findings of this study confirm that the ethanol extract ointment of *Tithonia diversifolia* leaves exhibits antibacterial activity against *Staphylococcus aureus*, with the effectiveness increasing proportionally to the extract concentration. These results support the hypothesis that higher concentrations of the extract would enhance antibacterial strength. The study demonstrates that concentrations ranging from 5% to 21% are capable of producing measurable inhibition zones, with the highest concentration showing strong antibacterial activity. This indicates that *Tithonia diversifolia* has promising potential for development into a natural topical antibacterial agent. Further research is recommended to assess the formulation's stability, safety, and potential skin irritation effects through in vivo testing. Additional studies are also needed to isolate and characterize the specific active compounds responsible for the antibacterial activity. Optimization of ointment bases and evaluation against a broader range of pathogenic bacteria are suggested to enhance the formulation's clinical application potential.

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