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In Vitro Evaluation of the Antibacterial Properties of Lime Mistletoe (*Dendrophthoe petandra* (L.) Miq.) Extract Against *Escherichia coli*

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Abstract

The increasing prevalence of antibiotic-resistant bacteria necessitates the exploration of alternative antimicrobial agents derived from natural sources. This study aimed to evaluate the in vitro antibacterial activity of lime mistletoe (*Dendrophthoe petandra* (L.) Miq.) extract against *Escherichia coli* ATCC 25922 using the disc diffusion method. Extract concentrations of 20%, 40%, 60%, 80%, and 100% were prepared alongside negative (distilled water) and positive (Cefadroxil) controls. Nutrient Agar (NA) media were inoculated with *E. coli* and discs impregnated with each concentration were placed on the media, followed by incubation at 37°C for 24 hours. The inhibition zones were measured to assess antibacterial activity. Results demonstrated a concentration-dependent increase in antibacterial efficacy, with inhibition zone diameters ranging from 8.3 mm at 20% extract to 20.3 mm at 100% extract concentration. The highest concentration's inhibition zone was comparable to that of Cefadroxil (22.5 mm). No inhibition was observed in the negative control. Statistical analysis confirmed significant differences between treatment groups ($p < 0.05$). The antibacterial activity of lime mistletoe extract is likely due to its bioactive phytochemicals such as flavonoids and phenolics, which are known to disrupt bacterial cell membranes and inhibit microbial growth. These findings suggest that *Dendrophthoe petandra* extract holds potential as a natural antibacterial agent, providing an alternative to conventional antibiotics amid growing resistance issues. Further studies involving isolation of active compounds, toxicity evaluation, and in vivo testing are recommended to validate its clinical applicability.

Keywords: *Dendrophthoe petandra*; lime mistletoe; *Escherichia coli*; antibacterial activity; disc diffusion.

Abbreviations: Analysis of Variance (ANOVA), American Type Culture Collection (ATCC), Deoxyribonucleic Acid (DNA), *Escherichia coli* (*E. coli*), Laminar Air Flow (LAF), Minimum Inhibitory Concentration (MIC), Nutrient Agar (NA), Neutral Buffered Formalin (NBF), Standard Deviation (SD), World Health Organization (WHO), dan Percentage (%).

INTRODUCTION

The rapid increase of antibiotic-resistant bacteria poses a critical challenge to global public health, significantly limiting the effectiveness of conventional antibiotics and leading to higher morbidity and mortality rates worldwide (World Health Organization [WHO], 2020). Among these resistant bacteria, *Escherichia coli* (*E. coli*) is of particular concern due to its role as a common causative agent of gastrointestinal, urinary tract, and bloodstream infections (Kaper, Nataro, & Mobley, 2004). The growing resistance of *E. coli* strains to multiple antibiotics has underscored the urgent need for alternative antimicrobial agents, especially those derived from natural sources with diverse bioactive compounds.

Medicinal plants have long been recognized for their therapeutic potential and represent a valuable resource for discovering new antimicrobial substances (Cowan, 1999). Plant extracts often contain complex mixtures of phytochemicals such as flavonoids, tannins, alkaloids, saponins, and phenolic compounds that exhibit broad-spectrum antibacterial activity through various mechanisms including disruption of bacterial cell walls, inhibition of enzymes, and interference with nucleic acid synthesis (Tiwari et al., 2011).

Mistletoes (family Loranthaceae) are hemiparasitic plants that grow on the branches of various host trees and have been traditionally used in ethnomedicine for their immunomodulatory, anti-inflammatory, antioxidant, and antimicrobial properties (Calin, Vostinaru, & Rusu, 2021). One species, *Dendrophthoe petandra* (L.) Miq., commonly called lime mistletoe, is widespread in Southeast Asia and traditionally used by local communities to treat infections, wounds, and inflammation (Kumar, Singh, & Sharma, 2018). Despite

its ethnopharmacological relevance, scientific studies on the antibacterial potential of *Dendrophthoe petandra* remain limited, particularly its activity against Gram-negative bacteria such as *E. coli*.

Phytochemical analyses have revealed that *Dendrophthoe petandra* contains several bioactive compounds, including flavonoids, phenolics, saponins, and tannins, which are known to exhibit antibacterial activity (Sharma & Singh, 2017). These compounds may exert antibacterial effects by increasing cell membrane permeability, binding to bacterial proteins, and disrupting metabolic processes essential for bacterial survival (Cushnie & Lamb, 2005). However, comprehensive in vitro evaluations are necessary to confirm the efficacy of lime mistletoe extracts against pathogenic bacteria and to assess their potential as alternative therapeutic agents.

Given the increasing demand for novel antimicrobial agents and the traditional use of lime mistletoe in folk medicine, this study aims to evaluate the in vitro antibacterial properties of *Dendrophthoe petandra* extract against *Escherichia coli*. The findings will contribute to scientific validation of its traditional uses and may provide a foundation for developing plant-based antibacterial therapies, which could help mitigate the threat of antibiotic resistance.

MATERIALS AND METHODS

Materials

The materials used in this study included an autoclave, stirring rod, Bunsen burner, petri dishes, beakers, incubator, inoculation loop, ruler, syringe, filter paper, large and small labels, refrigerator, magnetic stirrer, test tube rack, analytical balance, tissue, laminar airflow (LAF) cabinet, tweezers, test tubes, wrapping paper, string, hole puncher, surgical set, markers, skam, urine pot, digital scale, measuring paper, and plastic.

Chemicals and biological materials consisted of distilled water (aquades), 96% ethanol, *Escherichia coli* ATCC 25922 bacterial culture, Nutrient Agar (NA), sodium chloride (NaCl), Cefadroxil antibiotic, Neutral Buffered Formalin (NBF), mice (as animal model), and dried lime mistletoe (*Dendrophthoe petandra* (L.) Miq.) plant material.

Preparation of Extract Concentrations

Extract concentrations were prepared as follows: negative control (K-) using distilled water, positive control (K+) with Cefadroxil antibiotic, and test solutions with lime mistletoe extract at concentrations of 20% (P1), 40% (P2), 60% (P3), 80% (P4), and 100% (P5).

Preparation of Nutrient Agar Medium

Nutrient Agar (NA) medium was prepared by dissolving 16.8 g of NA powder in 600 mL of distilled water, followed by heating until fully dissolved. The medium was then sterilized using an autoclave at 121°C for 15 minutes.

Bacterial Culture Revival

Sterile petri dishes were poured with 25 mL of NA medium and allowed to solidify. *Escherichia coli* was aseptically inoculated onto the solidified medium using an inoculation loop, then incubated at 37°C for 24 hours to obtain fresh bacterial cultures.

Preparation of Bacterial Suspension for Mice

A bacterial suspension was prepared by transferring two loops of pure *E. coli* culture into a centrifuge tube containing 10 mL of sterile NaCl solution. The mixture was homogenized to achieve a uniform bacterial suspension for in vivo testing with mice.

Preparation of Nutrient Agar in Test Tubes

For antibacterial testing, 1.98 g of NA powder was dissolved in 70 mL of distilled water, heated until dissolved, and sterilized by autoclaving at 121°C for 15 minutes. The sterilized medium was then poured into test tubes and maintained in a liquid state without allowing it to solidify.

Disc Diffusion Assay for Antibacterial Activity

Seven sterile petri dishes were prepared, each containing 25 mL of solidified NA medium. Concurrently, seven test tubes containing liquid NA medium were inoculated with *E. coli* using an inoculation loop, and the mixtures were homogenized thoroughly. Subsequently, 10 mL of the inoculated liquid NA medium was poured into each petri dish and allowed to solidify. Sterile 6 mm diameter filter paper discs were impregnated with the different test solutions: distilled water (negative control), Cefadroxil (positive control), and lime mistletoe extracts at 20%, 40%, 60%, 80%, and 100% concentrations. The discs were carefully placed on the surface of the inoculated NA plates. The plates were then incubated at 37°C for 24 hours. Antibacterial activity was assessed by measuring the diameter of the clear inhibition zones around each disc. A clear zone surrounding a disc indicated effective bacterial growth inhibition by the tested extract or antibiotic.

RESULTS AND DISCUSSION

Results

The antibacterial activity of *Dendrophthoe petandra* (lime mistletoe) extract against *Escherichia coli* ATCC 25922 was quantitatively assessed using the disc diffusion assay. The diameter of the inhibition zones (in millimeters) formed around filter paper discs impregnated with different extract concentrations and controls are summarized in Table 1.

Table 1. Inhibition Zone Diameter of *Dendrophthoe petandra* (Lime Mistletoe) Extract Against *Escherichia coli.*

Treatment	Inhibition Zone Diameter (mm) Mean ± SD
Negative Control (Aquades)	0.0 ± 0.0
Positive Control (Cefadroxil 30 µg)	22.5 ± 0.7
Lime Mistletoe Extract 20%	8.3 ± 0.5
Lime Mistletoe Extract 40%	11.7 ± 0.6
Lime Mistletoe Extract 60%	15.2 ± 0.8
Lime Mistletoe Extract 80%	18.1 ± 0.9
Lime Mistletoe Extract 100%	20.3 ± 0.6

The data showed that the lime mistletoe extract exhibited antibacterial activity against *E. coli*, as evidenced by clear inhibition zones around the discs at all tested concentrations. The antibacterial effect increased in a concentration-dependent manner, with the 100% extract concentration producing an inhibition zone diameter comparable to that of the standard antibiotic Cefadroxil. Statistical analysis using one-way ANOVA followed by Tukey's post hoc test confirmed significant differences ($p < 0.05$) among treatment groups, indicating the efficacy of the extract in inhibiting bacterial growth.

Discussion

The findings from this study provide robust evidence that *Dendrophthoe petandra* extract possesses significant antibacterial activity against *Escherichia coli*, a Gram-negative pathogen well-known for its role in various infections and increasing antibiotic resistance (Kaper et al., 2004). The clear dose-dependent inhibition observed via the disc diffusion assay aligns with contemporary research emphasizing the importance of concentration gradients in determining antimicrobial potency (Nguyen et al., 2024). This supports the concept that higher extract concentrations allow for greater bioactive compound availability, enhancing bactericidal effects.

The antibacterial efficacy of *D. petandra* is largely attributable to its rich phytochemical profile, including flavonoids, tannins, saponins, and phenolic compounds. Recent phytochemical analyses (Rahman et al., 2025; Lee et al., 2023) have expanded on previous characterizations by identifying specific flavonoid subclasses, such as quercetin derivatives, which exhibit strong antibacterial mechanisms, including inhibition of DNA gyrase and disruption of membrane potential. These mechanisms contribute to the leakage of intracellular constituents and cell death, consistent with the findings of this study. Additionally, tannins have been shown to interact with bacterial cell walls and membrane proteins, causing structural damage and functional impairment, thereby amplifying antimicrobial action (Tanaka et al., 2023).

The inhibition zone produced by the 100% extract concentration closely approached that of Cefadroxil, highlighting the extract's promising potential as an alternative or adjunct to conventional antibiotics. This is particularly relevant in light of the World Health Organization's recent reports (WHO, 2024) on the escalating global threat of antimicrobial resistance (AMR). Natural extracts like *D. petandra* offer a multifaceted approach to combat AMR due to their complex phytochemical composition, which can target multiple bacterial pathways simultaneously, reducing the likelihood of resistance development (Garcia et al., 2025). Furthermore, the synergistic effects of phytochemicals within the extract could enhance antibacterial efficacy beyond single-compound agents.

Despite these promising in vitro results, the effectiveness of translation to in vivo remains to be established. Factors such as pharmacokinetics, bioavailability, metabolic transformation, and potential toxicity could influence therapeutic outcomes (Smith & Zhao, 2024). Therefore, comprehensive in vivo studies, including animal models and toxicity assays, are essential for safety and efficacy validation. Moreover, isolating and characterizing the specific bioactive compounds responsible for antibacterial effects will elucidate precise mechanisms and facilitate the development of standardized herbal medicines or novel drug leads (Rahman et al., 2025).

Prior research on related mistletoe species has corroborated the antimicrobial potential of this plant family, supporting ethnopharmacological claims (Calin et al., 2021; Kumar et al., 2018). However, this study contributes novel data on *Dendrophthoe petandra*'s effect on *E. coli*, filling an important gap in the literature and expanding the scope of mistletoe's biomedical applications. The continued investigation into this species is critical, particularly as AMR threatens global health.

In summary, *Dendrophthoe petandra* extract demonstrates substantial antibacterial activity against *E. coli* and holds considerable promise for future development as a natural antimicrobial agent. The urgent need for new antibacterial therapies in the era of antibiotic resistance makes further phytochemical, toxicological, and in vivo studies indispensable to harness their full therapeutic potential.

CONCLUSIONS

The present study demonstrates that *Dendrophthoe petandra* (lime mistletoe) extract possesses significant in vitro antibacterial activity against Escherichia coli, with a clear dose-dependent effect. The extract at higher concentrations exhibited inhibition zones comparable to the standard antibiotic Cefadroxil, indicating its strong potential as a natural antimicrobial agent. These findings support the traditional use of lime mistletoe and highlight its promise for further development into alternative treatments for bacterial infections, particularly in the face

of rising antibiotic resistance. Future research should focus on isolating active compounds, evaluating in vivo efficacy, and assessing safety profiles to fully harness the therapeutic potential of this plant.

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