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Original Article

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Antibacterial Activity Test of Karamunting Leaf Extract (*Melastoma malabathricum* L.) Against Eggshell Bacteria

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Abstract. This study aimed to evaluate the antibacterial activity of karamunting leaf extract (*Melastoma malabathricum* L.) against bacteria isolated from eggshells. Eggshells are known as one of the primary contamination sources for foodborne bacteria, which can pose potential health risks to consumers. The ethanolic extract of karamunting leaves was tested using the disk diffusion method with amoxicillin as the positive control and 96% ethanol as the negative control. The inhibition zones were measured after 24 hours of incubation at 37°C. The results showed that karamunting leaf extract produced an inhibition zone of 3.35 mm, categorized as weak antibacterial activity. In contrast, the positive control, amoxicillin, generated a much larger inhibition zone of 22.89 mm, while the negative control showed no inhibition. The antibacterial effect of the extract is believed to result from the presence of bioactive secondary metabolites, including flavonoids, tannins, saponins, steroids, and phenolic compounds, which function by disrupting bacterial cell walls, altering membrane permeability, denaturing proteins, and inhibiting enzyme activity. Although the antibacterial potential of karamunting leaf extract is lower than that of synthetic antibiotics, it still demonstrates promising potential as a natural antibacterial agent that is safer and more environmentally friendly. Further studies are recommended to assess the activity at different extract concentrations and solvent fractions to achieve more optimal antibacterial effects.

Keywords: Antibacterial Activity; Eggshell Bacteria; Karamunting Leaf Extract; *Melastoma malabathricum;* Natural Bioactive Compounds

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INTRODUCTION

Karamunting (*Melastoma malabathricum* L.), a tropical plant that grows abundantly in Indonesia, has long been recognized and utilized in traditional medicine systems (Isnaini et al., 2019). This empirical use is supported by findings that extracts from various parts of the plant, particularly its leaves, are rich in secondary bioactive compounds (Gani et al., 2020). The main identified phytochemical constituents flavonoids, tannins, glycosides, alkaloids, and steroids are groups of metabolites known for their strong antibacterial activity (Dongoran et al., 2023). Studies have demonstrated that the pharmacological potential of Karamunting leaves is significant, making it a promising candidate for exploring natural resources as health solutions (Susanti et al., 2025).

On a global scale, bacterial resistance to synthetic antibiotics has created a health crisis that necessitates the development of new antimicrobial agents (Rajapaksha et al., 2024). The phenomenon of multidrug resistance (MDR) in pathogens such as *Staphylococcus aureus* and *Escherichia coli* has heightened the urgency to discover alternative therapeutic options (Susanti et al., 2025). This issue intersects with food safety concerns, where contamination by foodborne pathogens poses a major threat (Nathanael et al., 2024). Animal-based food products, particularly eggs, often serve as a transmission source of diseases to consumers (Sa'adah et al., 2024).

Bacterial contamination on eggshells serves as a critical transmission pathway for pathogens into the egg contents and to consumers. *Staphylococcus aureus*, one of the major foodborne pathogens, has been identified in processed egg products in Indonesia (Thaha et al., 2024). Furthermore, research has reported the presence of enteropathogenic bacteria such as *E. coli* on the shells of non-broiler chicken eggs sold in markets, highlighting the contamination risk from the surrounding environment (Arumugam et al., 2015). Similar contamination has also been detected on duck eggs, where populations of *E. coli* exceeded safe limits, reinforcing the role of eggshells as protective habitats for pathogenic microorganisms (Ginting & Azzahirah, 2024). This threat is further aggravated by bacterial capabilities, such as *E. coli*'s ability to form biofilms, which enhance bacterial resistance to conventional sanitizing agents (Sabrina et al., 2025)

To address these urgent food safety challenges, the antibacterial potential of Karamunting leaves has been extensively tested against various pathogens. Ethanolic extracts from the leaves and flowers of *Melatosma malabathricum* have been shown to effectively inhibit the growth of foodborne pathogens such as *S. typhi, E. coli*, and *S. aureus* (Isnaini et al., 2019). Furthermore, this antibacterial activity extends to clinical pathogens, with leaf extracts exhibiting bactericidal effects against eight mastitis causing pathogens in dairy cows, including *S. aureus* and *E. coli* (Tan et al., 2024). Specifically, the ethyl acetate fraction of Karamunting leaf extract has demonstrated antimicrobial activity against *S. aureus* and *E. coli*, both relevant to bacteria found on eggshells (Dongoran et al., 2023). Additionally, the n-hexane fraction of Karamunting leaves has been successfully formulated into an antibacterial soap effective against *S. aureus* (Leny et al., 2022)

Based on consistent scientific evidence regarding the antibacterial efficacy of Karamunting against food related pathogens and contamination threats on eggshells, a more detailed investigation is required. Therefore, this study aims to evaluate the antibacterial activity of Karamunting leaf (*Melastoma malabathricum L.*) extract specifically against bacteria contaminating eggshells, to validate the potential use of this extract as a natural, safe, and effective preventive agent against microbial contamination.

MATERIALS AND METHODS

Research Instruments

The equipment used in this study included an analytical balance (Ohaus) for weighing the simplicia and extracts, an oven dryer (Memmert) for drying the leaves, and a blender to pulverize the dried samples into fine powder. Glassware such as beakers, measuring cylinders, volumetric pipettes, and Erlenmeyer flasks (250–1000 mL) were utilized during the extraction and filtration processes. Filtration was performed using glass funnels and Whatman No. 1 filter paper. The concentrated extract was obtained using a rotary evaporator (Buchi) and stored in a desiccator before testing. Sterilization of media and glassware was carried out using an autoclave (All American), while aseptic procedures were conducted inside a laminar air flow cabinet. Other essential instruments included an incubator maintained at 37°C for bacterial incubation, micropipettes with sterile tips, Petri dishes, test tubes, tube racks, and a vortex mixer for homogenizing bacterial suspensions. Metal instruments such as tweezers and inoculating loops were sterilized with a Bunsen burner. A colony counter was used to quantify bacterial colonies, and a digital caliper was employed to measure the diameter of inhibition zones. All experimental observations were documented using a digital camera.

Research Materials

The materials used in this study included karamunting (*Melastoma malabathricum* L.) leaves collected from Pasi Village, Berampu District, Dairi Regency, North Sumatra, which served as the primary sample for extract preparation. Ethanol 96% (technical grade) was used as the solvent for maceration, while sterile distilled water (aquadest) was used for dilution and cleaning purposes. For antibacterial testing, Nutrient Agar (NA) and Mueller Hinton Agar (MHA) were employed as bacterial growth and testing media. The antibacterial test materials consisted of ethanolic extract of *M. malabathricum* leaves, amoxicillin antibiotic as the positive control, and 96% ethanol as the negative control. Sterile paper disks with a diameter of 6 mm (Whatman No. 1) were used as carriers for the extract and control solutions. The bacterial suspension used in the test was obtained from bacterial isolates previously collected from eggshell surface swabs and identified before use. Additional materials included 0.9% physiological NaCl solution for bacterial suspension preparation, cotton and 70% alcohol for work area sterilization, and aluminum foil to cover glassware during sterilization. All chemical reagents used in this research were of analytical grade and were prepared under sterile conditions prior to use.

Plant Determination

Plant determination was conducted to confirm the taxonomic identity of the karamunting plant (*Melastoma malabathricum* L.) used in this study. Fresh plant samples, including leaves, stems, and flowers, were collected from Pasi Village, Berampu District, Dairi Regency, North Sumatra, Indonesia. The samples were identified and authenticated at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan (UNIMED). The identification process was carried out based on morphological characteristics and comparison with taxonomic reference materials. The results confirmed that the plant used in this study was *Melastoma malabathricum* L., belonging to the family Melastomataceae.

Preparation of Simplicia

The fresh leaves of karamunting (*Melastoma malabathricum* L.) were washed thoroughly with clean running water to remove dirt and impurities. The leaves were then air-dried at room temperature for several days until they reached constant weight, avoiding direct exposure to sunlight to prevent the degradation of bioactive compounds. After drying, the leaves were ground into a fine powder using an electric blender. The powdered leaves (simplicia) were then stored in a tightly closed container protected from moisture and light until further use for extraction.

Extraction of Karamunting Leaves

The powdered simplicia of karamunting leaves (*Melastoma malabathricum* L.) was extracted using the maceration method. A total of 150 grams of powdered leaves were soaked in 96% ethanol with a solvent-to-sample ratio of 1:5 (w/v) in a closed container. The mixture was left to stand for 3 × 24 hours at room temperature and stirred periodically every 12 hours to facilitate the extraction process. After this period, the filtrate was separated from the residue using Whatman filter paper, and the residue was remacerated for an additional 3 × 24 hours using the same procedure to ensure maximum extraction of bioactive compounds. All filtrates were combined and concentrated using a rotary evaporator at 40–50°C until a thick, dark-green extract was obtained. The concentrated extract was then stored in a clean, tightly sealed container and kept in a refrigerator at 4°C until further antibacterial testing.

Sterilization of Equipment and Materials

All equipment and materials used in the antibacterial testing were sterilized to prevent contamination. Glassware such as petri dishes, test tubes, and pipettes were sterilized by autoclaving at 121°C for 15–20 minutes. Metal tools, including forceps and spatulas, were sterilized using a hot air oven at 160°C for 2 hours or by flaming over a Bunsen burner when needed. All culture media and solutions were also sterilized by autoclaving at 121°C for 15 minutes. Sterile working conditions were maintained throughout the experiment to ensure the accuracy and reliability of the results.

Isolation of Bacteria from Eggshells

Bacteria were isolated from the surface of eggshells to obtain test organisms for antibacterial activity. The eggshells were first cleaned to remove visible dirt, then swabbed using sterile cotton swabs moistened with sterile saline solution. The swabs were streaked onto Nutrient Agar (NA) plates and incubated at 37°C for 24–48 hours. Distinct bacterial colonies that appeared were picked and subcultured repeatedly on fresh NA plates

to obtain pure isolates. Pure bacterial cultures were maintained on NA slants at 4°C until used for further experiments.

Preparation of Pure Bacterial Cultures

To obtain pure bacterial cultures, distinct colonies obtained from the primary isolation were picked using a sterile inoculating loop and streaked onto fresh Nutrient Agar (NA) plates using the streak plate method. The plates were incubated at 37℃ for 24–48 hours. This process was repeated as necessary until colonies of a single bacterial type were obtained, ensuring purity. The pure cultures were then maintained on NA slants at ℃ for short-term storage until they were used to prepare bacterial inoculum for antibacterial testing.

Bacterial Identification by Gram Staining

The pure bacterial cultures were identified using Gram staining to determine their cell wall characteristics and morphology. A small amount of bacterial culture was spread on a clean glass slide, air-dried, and heat-fixed. The slide was then stained sequentially with crystal violet, iodine solution, decolorized with alcohol, and counterstained with safranin. The stained slides were examined under a light microscope at 1000× magnification using oil immersion. Bacteria were classified as Gram-positive (purple) or Gram-negative (pink/red) and their shape (cocci, bacilli, or others) was recorded for further characterization.

Antibacterial Activity Testing

Preparation of Mueller-Hinton Agar (MHA)

Mueller-Hinton Agar (MHA) was prepared according to the manufacturer's instructions by dissolving the appropriate amount of MHA powder in distilled water and heating while stirring until completely dissolved. The medium was sterilized by autoclaving at 121℃ for 15 minutes. After cooling to 45–50℃, it was poured aseptically into sterile Petri dishes and allowed to solidify at room temperature. The solidified plates were stored at 4℃ until use.

Bacterial Suspension Preparation (0.5 McFarland Standard)

Bacterial isolates obtained from eggshell surfaces were grown in nutrient broth at 37℃ for 18–24 hours to achieve active growth. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard, corresponding to approximately 1–2 × 10^8 CFU/mL, to ensure uniform bacterial density.

Antibacterial Testing Using the Disc Diffusion Method

Sterile MHA plates were evenly inoculated with the standardized bacterial suspension. Sterile paper discs (6 mm in diameter) were impregnated with specific concentrations of the karamunting leaf extract or its fractions and placed gently on the surface of the inoculated plates. Amoxicillin discs were used as positive control, and etanol were used as negative control. The plates were incubated at 37℃ for 24 hours.

Measurement and Data Analysis of Inhibition Zones

After incubation, the clear zones around each disc where bacterial growth was inhibited (zones of inhibition) were measured in millimeters using a ruler or caliper. The sizes of the inhibition zones were recorded and analyzed to determine the antibacterial activity of the extracts and fractions, with larger zones indicating stronger antibacterial effects. Data were expressed as mean ± standard deviation from triplicate measurements.

RESULTS AND DISCUSSION

Antibacterial Activity Test of Karamunting Leaf Extract

The antibacterial activity of karamunting leaf extract (*Melastoma malabathricum* L.) against bacteria isolated from eggshells was identified based on the diameter of the inhibition zones formed in each control treatment can be seen in Table 1

Table 1. Measurement results of antibacterial inhibition zone diameters of karamunting (*Melastoma malabathricum* L.) leaf extract, positive control (amoxicillin), and negative control (ethanol 96%)

Treatment	Dv (mm)	Dh (mm)	verage zone	D cakra m	Clear inhibition zone	Description
Test exstract	9,34	9,35	9,35	6	3,35	Inhibition zone present
Control (+)	28,89	28,89	28,89	6	22,89	Strong inhibition zone
Control (-)	0	0	0	6	0	No inhibition zone

Based on Table 1, the ethanol extract of karamunting leaves (*Melastoma malabathricum* L.) produced an inhibition zone of 3.35 mm, which falls into the category of weak antibacterial activity. In contrast, the positive control (amoxicillin) showed an inhibition zone of 22.89 mm, categorized as very strong antibacterial activity, while the negative control (96% ethanol) showed no inhibition zone at all. These results indicate that, although the inhibitory effect of the extract is lower compared to synthetic antibiotics, it still contains bioactive compounds capable of inhibiting the growth of bacteria from eggshells can be seen in Figure 1.

The results of the antibacterial activity test after 24 hours of incubation at 37°C can be seen in Figure 1.

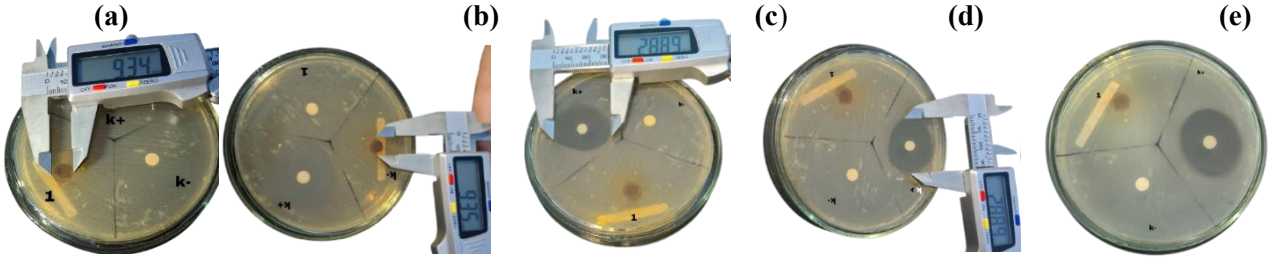

Figure 1. Antibacterial activity inhibition zone. (a) Inhibition zone of the tested extract in the vertical position, (b) inhibition zone of the tested extract in the horizontal position, (c) positive control in the vertical position, (d) positive control in the horizontal position, and (e) overall view of the test plate. The diameter of the inhibition zones was measured using a digital caliper.

The results showed that the karamunting leaf extract (*Melastoma malabathricum* L.) produced an inhibition zone of 3.35 mm, indicating weak antibacterial activity against the bacteria isolated from the eggshell surface. The inhibition zone is classified as weak when it has a diameter of ≤ 5 mm, moderate when ranging from 6–10 mm, strong when 11–20 mm, and very strong when ≥ 21 mm (Dhani et al., 2023). As a comparison, the positive control (amoxicillin) produced a much larger inhibition zone of 22.89 mm (very strong) because amoxicillin is an antibiotic commonly used in various treatments and is effective against both Gram-positive and Gram-negative bacteria (Maida & Lestari, 2019). The antibacterial mechanism occurs by inhibiting protein synthesis in bacteria. The active compounds in the extract inhibit the enzyme peptidyl transferase, which acts as a catalyst in the formation of peptide bonds between amino acids during protein synthesis. This inhibition disrupts the bacterial protein synthesis process, thereby preventing bacterial growth and reproduction (Sukadiasa et al., 2023). Meanwhile, the negative control (96% ethanol) showed no inhibition zone at all. This is because ethanol acts as a neutral compound that cannot inhibit bacterial growth (Sukadiasa et al., 2023). According to Sarmira et al., (2021), the larger the inhibition zone, the greater the antibacterial capability of a substance. This indicates that both the natural antimicrobial compounds in the karamunting leaf extract and the positive control (amoxicillin) possess inhibitory power against bacteria. Hence, the inhibitory effect observed in the karamunting leaf extract (*Melastoma malabathricum* L.) is purely due to its bioactive compounds, not the ethanol solvent.

The antibacterial activity of karamunting leaf extract is closely related to the presence of secondary metabolites such as steroids, triterpenoids, saponins, tannins, flavonoids, and phenols (Sugiantina & Leliqia, 2022). These compounds have been widely reported to inhibit bacterial growth through various mechanisms, including inhibition of cell wall synthesis, disruption of membrane permeability, protein denaturation, and inhibition of essential enzyme activity. Flavonoids exhibit bacteriolytic effects, which interfere with the synthesis of proteins, DNA, and RNA, and form complexes with extracellular and soluble proteins, thereby damaging the bacterial membrane and causing the leakage of intracellular compounds (Sujana et al., 2024).

Tannins function by precipitating membrane proteins, leading to the loss of osmotic stability in the cell wall and ultimately causing cell lysis (Lovianie et al., 2018).

The results of Gram staining on the bacterial isolates showed that the dominant bacteria were Gram-negative rod-shaped bacteria, morphologically resembling *Escherichia coli* can be seen in Figure 2.

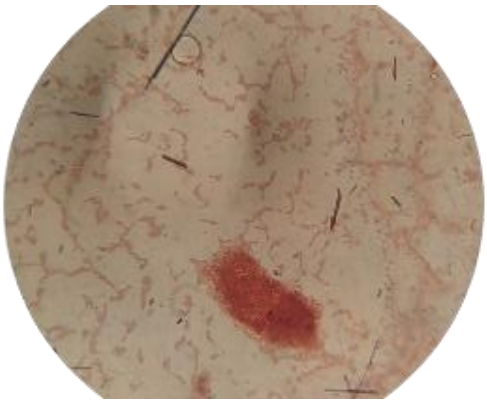

Figure 2. Gram staining results of bacterial isolates obtained from eggshell surfaces showing pink to red, rod-shaped (bacillus) Gram-negative bacteria, morphologically resembling *Escherichia coli*.

The bacterial cells appear pink to red in color with a rod-shaped (bacillus) form, indicating that the isolate is a Gram-negative bacterium morphologically resembling *Escherichia coli*. He red coloration in the bacteria appears because Gram-negative bacteria are unable to retain the crystal violet dye due to their thin peptidoglycan layer (Cahyaningtyas et al., 2024). This finding is consistent with the reports of (Ulfah et al., 2017) which state that E. coli is the main contaminant frequently found on eggshells in traditional markets.

The extract of *Melastoma malabathricum* leaves has the ability to inhibit the growth of both Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) through mechanisms involving cell wall disruption and enzyme inactivation (Mayasari et al., 2021). The smaller inhibition zone compared to the positive control indicates that the antibacterial compounds present in the karamunting leaf extract (*Melastoma malabathricum* L.) possess lower effectiveness than synthetic antibiotics. However, this still demonstrates a significant potential as a safer and more environmentally friendly natural antibacterial agent. The relatively weak inhibitory activity may be attributed to the low concentration of active compounds, the polarity of the ethanol solvent, and the suboptimal separation of synergistic interactions among secondary metabolites (Kairupan et al., 2024). The extract's activity against eggshell bacteria also indicates that karamunting leaves have the potential to inhibit contaminant microorganisms commonly found in food products such as eggs, particularly Gram-negative bacteria like *E. coli* and Gram-positive bacteria like *S. aureus*.

Analysis of Effectiveness and Inhibition Mechanism

The results of this study demonstrate that karamunting leaf extract possesses significant antibacterial activity, although it is not as strong as that of standard antibiotics. This activity is presumed to result from the combined effects of several active compounds, such as flavonoids, tannins, and saponins, which act through multiple mechanisms, including protein and nucleic acid denaturation, disruption of cell membrane permeability, and inhibition of key bacterial metabolic enzymes. These findings strengthen the potential of karamunting leaves as a promising natural antibacterial agent. Further research is recommended to evaluate its effectiveness at various extract concentrations and using different solvent fractions to achieve more optimal antibacterial inhibition results.

CONCLUSION

The karamunting leaf extract (*Melastoma malabathricum* L.) was proven to exhibit antibacterial activity against bacteria isolated from eggshells, with an average inhibition zone of 3.35 mm, classified as weak. Although its activity is lower than that of the antibiotic amoxicillin, these results indicate that karamunting leaves contain bioactive compounds with potential as a natural antibacterial agent for controlling microbial contamination in food products.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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