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Antibacterial Activity of Clove Flower (*Eugenia aromatica*) Extract Against Escherichia coli Clinical Isolates

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ABSTRACT

Infectious diseases remain a major global health concern, including in Indonesia, with *Escherichia coli* being one of the most common bacterial pathogens responsible for various infections. The overuse and prolonged application of antibiotics have contributed to bacterial resistance, creating a need for alternative treatments based on natural antibacterial agents. This study evaluated the antibacterial activity of ethanol extract of clove flowers (*Eugenia aromatica*) against *E. coli* isolated from clinical samples. Using the well diffusion method, extract concentrations of 10%, 20%, 40%, 60%, and 80% were tested, with amoxicillin as a positive control and sterile water (aqua pro injection) as a negative control. The results showed that all extract concentrations exhibited antibacterial activity, with the highest inhibition zone observed at 80% concentration (18.63 mm), classified as a strong antibacterial effect. These findings indicate that clove flower extract has the potential to be developed as a natural antibacterial agent to support efforts in combating bacterial infections and reducing reliance on synthetic antibiotics.

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ABSTRAK

Penyakit infeksi masih menjadi masalah kesehatan utama di seluruh dunia, termasuk di Indonesia, dengan *Escherichia coli* sebagai salah satu bakteri patogen yang paling umum menyebabkan berbagai jenis infeksi. Penggunaan antibiotik yang berlebihan dan jangka panjang telah berkontribusi pada terjadinya resistensi bakteri, sehingga diperlukan pengembangan alternatif pengobatan berbasis agen antibakteri alami. Penelitian ini mengevaluasi aktivitas antibakteri ekstrak etanol bunga cengkeh (*Eugenia aromatica*) terhadap *E. coli* yang diisolasi dari sampel klinis. Pengujian dilakukan menggunakan metode difusi sumur dengan konsentrasi ekstrak 10%, 20%, 40%, 60%, dan 80%, menggunakan amoksisilin sebagai kontrol positif dan aqua pro injection sebagai kontrol negatif. Hasil penelitian menunjukkan bahwa semua konsentrasi ekstrak memiliki aktivitas antibakteri, dengan zona hambat terbesar pada konsentrasi 80% (18,63 mm) yang tergolong kuat. Temuan ini menunjukkan bahwa ekstrak bunga cengkeh berpotensi dikembangkan sebagai agen antibakteri alami untuk mendukung pengobatan infeksi dan mengurangi ketergantungan terhadap antibiotik sintetis.

Kata Kunci: Ekstrak *Eugenia aromatica*; *Escherichia coli*; Aktivitas Antibakteri; Obat Herbal; Antimikroba Alami

1. Introduction

Infectious diseases remain one of the leading public health concerns worldwide, including in Indonesia. They are responsible for significant morbidity and mortality in both developed and developing countries. These infections can occur in community settings as well as in healthcare facilities, where they are often referred to as nosocomial infections. Several microorganisms have been associated with such infections, including *Staphylococcus aureus*, *Escherichia coli* (*E. coli*), *Candida albicans*, and *Pseudomonas aeruginosa* [1],[2].

Among these, *E. coli* is a prominent Gram-negative bacterium capable of causing various infectious diseases such as diarrhoea, urinary tract infections, and meningitis [3]. The excessive and uncontrolled use of antibiotics in treating *E. coli*-related infections has led to the emergence of antibiotic-resistant strains. This issue has driven the search for safer and more effective alternative treatments, including those derived from natural sources [4].

Clove (*Eugenia aromatica*), a plant native to the Maluku Islands of Indonesia, has been widely used in traditional medicine and as a food preservative. The main active compound in clove is eugenol, which constitutes approximately 72–90% of clove essential oil. Eugenol exhibits a broad spectrum of biological activities, including antibacterial, antifungal, insecticidal, and antioxidant effects [5]. Studies have shown that eugenol can inhibit bacterial growth through mechanisms such as disruption of cell membranes, protein denaturation, and nucleic acid degradation [6].

This study aims to evaluate the antibacterial activity of ethanol extract of clove flowers against *E. coli* isolated from clinical samples obtained from Dr. H. Chasan Boesoirie Regional Hospital, Ternate. This hospital represents the main healthcare referral centre in North Maluku, where infection rates are relatively high. The findings from this study are expected to contribute to the development of natural antimicrobial agents and provide an alternative to synthetic antibiotics in addressing bacterial infections.

2. Research Methods

This study employed a quantitative true experimental design conducted in a laboratory setting. The research was carried out at Dr. H. Chasan Boesoirie Regional Hospital and the Microbiology Laboratory of the Pharmacy Study Program, Faculty of Medicine, Khairun University, Ternate, during the period of February to May 2024. The target population was clove plants (*Eugenia aromatica*), and the accessible population used in this study was clove flowers. The samples tested were *Escherichia coli* bacteria obtained from clinical specimens collected at Dr. H. Chasan Boesoirie Hospital. The antibacterial assay was performed using the well diffusion method with five concentrations of clove flower extract: 10%, 20%, 40%, 60%, and 80%.

Extraction Procedure of Clove Flower (*Eugenia aromatica*)

Dried clove flowers sourced from Ternate City were washed under running water, sun-dried, and re-sorted to ensure uniform dryness. The dried flowers were then ground into powder. A total of 500 grams of clove powder was macerated in 3,000 mL of 96% ethanol for three consecutive 24-hour periods with occasional stirring. The resulting solution was filtered using filter paper, and the combined filtrate was concentrated using a water bath to yield a thick ethanol extract. To confirm the absence of residual ethanol, 1 mL of the thick extract was mixed with 2 drops of sulfuric acid and 2 drops of acetic acid, then heated. The absence of a characteristic ester odour indicated that the extract was ethanol-free [7].

Isolation and Identification of Bacteria

Urine samples (10 mL) were collected in sterile containers and transported in cool boxes to the laboratory. Samples were centrifuged at 2,500–3,000 rpm for 10 minutes to sediment any non-bacterial matter. The sediment was inoculated onto nutrient agar (NA) and incubated at 37 °C for 24 hours. Colonies showing bacterial growth were subcultured onto eosin methylene blue agar (EMBA) and re-incubated under the same conditions. Colonies with a characteristic metallic green sheen were identified as *E. coli* [8].

Preparation of Bacterial Suspensions

Bacterial suspensions were prepared by transferring a single loopful of pure *E. coli* colony into 10 mL of 0.9% NaCl solution. The mixture was vortexed until homogeneous and adjusted to match the turbidity of the 0.5 McFarland standard, equivalent to approximately 1.5×10^8 CFU/mL [8].

Preparation of Extract and Control Solutions

Extract concentrations of 10%, 20%, 40%, 60%, and 80% were prepared using the thick ethanol extract of clove flower. For the positive control, a 500 mg amoxicillin tablet was crushed and dissolved in 20 mL of distilled water. Aqua pro injection was used as the negative control [9].

Antibacterial Activity

The antibacterial activity test was conducted using the well diffusion method. Nutrient agar plates were labeled and divided into sectors. Wells were created using a sterile perforator and filled with 50 µL of the respective extract concentrations or controls

using a micropipette. The plates were incubated at 35–37 °C for 18–24 hours. The diameter of the inhibition zones was measured with a caliper and classified as follows: <5 mm (weak), 5–10 mm (moderate), 10–20 mm (strong), and >20 mm (very strong) [10].

Ethical Approval

This study was approved by the Health Research Ethics Committee of the Faculty of Medicine and Health Sciences, Khairun University, under the approval number 003/UN44/C.9/KEP/2024.

3. Results and Discussion

The antibacterial activity test of ethanol extract of clove flower (*Eugenia aromatica*) against *Escherichia coli* from clinical isolates was carried out using extract concentrations of 10%, 20%, 40%, 60%, and 80%. The results showed the formation of inhibition zones for all extract concentrations, indicating the antibacterial potential of clove flower extract. **Figure 1** shows the visual representation of inhibition zones formed after 24 hours of incubation. The quantitative results of zone diameters are summarized in **Table 1**

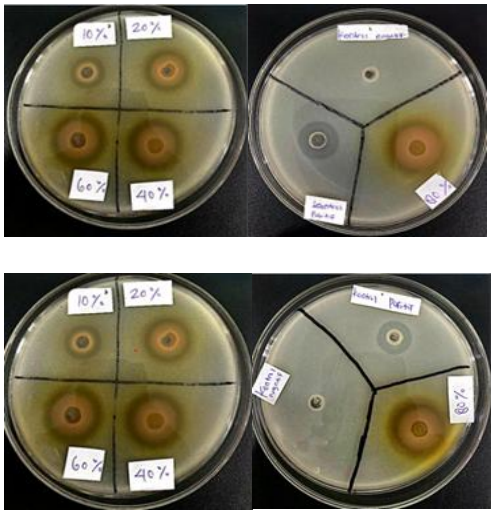

Figure 1. Antibacterial activity test of ethanol extract of clove flower

Based on the test results of five concentrations of clove flower (*Eugenia aromatica*) ethanol extract along with positive and negative controls, it was found that in repetition 1, the greatest inhibition against *E. coli* was observed at the 80% concentration, with a diameter of 18.6 mm. In repetition 2, the highest inhibition occurred at the 60% concentration, measuring 18.5 mm. Meanwhile, in repetition 3, the strongest inhibition was again observed at 80%, with a zone diameter of 19.0 mm. From these findings, the overall average inhibition zone was greatest at the 80% concentration, with a mean diameter of 18.63 mm. This value falls under the "strong" category of antibacterial activity.

Table 1. Inhibition zone measurement data of ethanol extract of flower (*Eugenia Aromatica*)

No	Treatment	Replicate 1 (P1)	Replicate 2 (P2)	Replicate 3 (P3)	Mean	Strength
1	Positive control	14.9 mm	13.5 mm	14.1 mm	14.16 mm	Strong
2	Negative control	-	-	-	-	-
3	10% concentration	14.2 mm	14.1 mm	13.6 mm	13.96 mm	Strong
4	Concentration 20%	16.1 mm	15.9 mm	14.2 mm	15.4 mm	Strong
5	40% concentration	16.9 mm	18.2 mm	17.2 mm	17.43 mm	Strong
6	Concentration 60%	17.4 mm	18.5 mm	15.2 mm	17.03 mm	Strong
7	80% concentration	18.6 mm	18.3 mm	19.0 mm	18.63 mm	Strong

The antibacterial activity testing of *E. coli* using the ethanol extract of clove flower showed that the extract could inhibit bacterial growth at all tested concentrations: 10%, 20%, 40%, 60%, and 80%. The positive control used was 500 mg of amoxicillin, prepared by crushing one tablet and dissolving it in 10 mL of sterile aqua pro injection. The negative control was aqua pro injection alone. The experiment was conducted in three repetitions, and observations were carried out after a 24-hour incubation period.

The following are the inhibition zone diameters for each concentration and repetition:

- 10% concentration: P1 (14.2 mm), P2 (14.1 mm), P3 (13.6 mm) → Average = 13.96 mm
- 20% concentration: P1 (16.1 mm), P2 (15.9 mm), P3 (14.2 mm) → Average = 15.40 mm
- 40% concentration: P1 (16.9 mm), P2 (18.2 mm), P3 (17.2 mm) → Average = 17.43 mm
- 60% concentration: P1 (17.4 mm), P2 (18.5 mm), P3 (15.2 mm) → Average = 17.03 mm
- 80% concentration: P1 (18.6 mm), P2 (18.3 mm), P3 (19.0 mm) → Average = 18.63 mm
- Positive control (amoxicillin): P1 (14.9 mm), P2 (13.5 mm), P3 (14.1 mm) → Average = 14.16 mm
- Negative control: No inhibition observed

These results demonstrate that all tested concentrations of clove flower (*Eugenia aromatica*) ethanol extract were effective in inhibiting the growth of *Escherichia coli*, with higher concentrations producing progressively larger inhibition zones. Notably, even the lowest concentration (10%) showed considerable antibacterial efficacy, suggesting that the extract remains bioactive even at minimal doses. According to the standard classification of antibacterial potency, where inhibition zones of <5 mm are considered weak, 5–10 mm moderate, 10–20 mm strong, and ≥20 mm very strong [10], all extract

concentrations in this study fall within the strong category. These findings underscore the significant antimicrobial potential of clove flower extract against *E. coli*, a pathogen frequently associated with urinary tract infections, diarrheal disease, and nosocomial infections.

The observed pattern of inhibition zone expansion in line with increasing concentration is consistent with previous research. Simanjuntak et al. [11] reported that a mean inhibition diameter of 18.4 mm against *E. coli* indicated strong antibacterial efficacy, supporting the results observed at the 80% extract concentration in this study. Ugha et al. [12] further confirmed that higher extract concentrations led to broader inhibition zones, demonstrating a direct relationship between the concentration of bioactive compounds and antibacterial effectiveness. This correlation is rooted in phytochemical content—lower concentrations of extract naturally contain fewer secondary metabolites, while higher concentrations ensure the delivery of a more potent dose of antibacterial agents such as eugenol, flavonoids, and tannins [13].

Tuntun (2016) [13] emphasized that the higher the extract concentration used, the larger the zone of inhibition observed, due to the cumulative impact of active components. This is consistent with findings from Lianah [14] who noted that plant extracts rich in phenolic compounds exert antibacterial effects by disrupting cell wall synthesis and interfering with bacterial enzyme systems. The concentration-dependent enhancement of activity seen in this study may thus reflect both quantitative and qualitative phytochemical differences at each concentration level.

Furthermore, Utami et al. [15] demonstrated that *Eugenia aromatica* extract shows significant antibacterial properties against Gram-negative and Gram-positive bacteria. Their study suggested that eugenol, the dominant bioactive compound in clove, plays a key role in reducing bacterial viability, with the inhibition zone diameter increasing as the extract concentration rises. This observation aligns with the present study, where the 80% extract concentration consistently produced the largest inhibition zones in all replicates. Kalalo et al. [16] also noted that clove extract's inhibitory action is strongly associated with its chemical constituents, which include eugenol, β-caryophyllene, and acetyl eugenol—all known for their antimicrobial effects.

Eugenol, in particular, has been well documented for its antibacterial mode of action. It exerts its effect by permeabilizing and disrupting bacterial cell membranes, altering the structure and function of membrane proteins, and ultimately causing leakage of vital intracellular contents. Eugenol's high hydrophobicity enables it to integrate into lipid bilayers, thereby compromising the integrity of the bacterial membrane and disturbing metabolic activities [17]. Moreover, it interferes with mitochondrial enzymes and energy production pathways, leading to the collapse of bacterial viability. These mechanisms contribute significantly to the formation of visible inhibition zones around the wells containing clove extract.

This study demonstrated that ethanol extract of clove flower (*Eugenia aromatica*) possesses antibacterial activity against *Escherichia coli*. However, several limitations should be acknowledged. First, the study was conducted solely using an in vitro disc diffusion method, which may not accurately reflect its potential efficacy under in vivo or clinical conditions. As a result, the extract's effectiveness in physiological environments remains uncertain. Second, this study only focused on a single bacterial species, and its broader antibacterial spectrum against other pathogens was not evaluated.

Additionally, the range of extract concentrations tested was relatively narrow, limiting the ability to determine the most effective or minimum inhibitory concentration. The absence of phytochemical profiling also left the key bioactive constituents unidentified. Moreover, no stability or toxicity testing was performed, which are critical for pharmaceutical development. Technical variables in laboratory procedures, such as inconsistencies in inhibition zone measurements, could further influence the reliability of the data. Future studies should address these gaps by including in vivo experiments, comprehensive phytochemical and mechanistic analyses, as well as evaluations of extract stability and safety. Such investigations are necessary to validate clove flower extract's potential as a natural antibacterial agent suitable for clinical application.

4. Conclusion

The findings of this study confirm that ethanol extract of clove flower (*Eugenia aromatica*) has promising antibacterial potential against *Escherichia coli*. Its activity across multiple concentrations supports the value of further developing this extract as a natural alternative to synthetic antibiotics, particularly in addressing bacterial resistance challenges. To move toward clinical relevance, further studies are necessary to investigate the extract's mechanisms of action, safety in in vivo models, and chemical consistency. Exploration of its potential synergy with existing antibiotics, along with standardisation and optimisation of its formulation, will be essential steps to enable its application in pharmaceutical or therapeutic contexts.

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Conflicts of Interest:

The authors declare no conflict of interest regarding the publication of this article.

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