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ANTIBACTERIAL ACTIVITY TEST OF PINEAPPLE JUICE (*Ananas comosus* L.) AGAINST THE GROWTH OF *Escherichia coli*

UJI AKTIVITAS ANTIBAKTERI SARI NANAS (*Ananas comosus* L.) TERHADAP PERTUMBUHAN *Escherichia coli*

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Abstract

Pineapple (*Ananas comosus* L.) is known to contain active compounds that have antibacterial potential. This study aims to evaluate the antibacterial activity of pineapple juice and determine the effective concentration required to inhibit the growth of *Escherichia coli*. The antibacterial activity test was conducted using the disc diffusion method at three concentrations (20%, 25%, and 30%), with chloramphenicol as the positive control and distilled water as the negative control. The antibacterial activity test showed that pineapple juice at 20%, 25%, and 30% produced inhibition zones against *Escherichia coli* of 13.83 mm, 16.33 mm, and 18.50 mm, respectively. Based on the study results, pineapple juice shows antibacterial activity against Escherichia coli, with the most effective concentration for inhibiting bacterial growth at 30%.

Keywords: *pineapple juice, Ananas comosus L., antibacterial, Escherichia coli*

Abstrak

Nanas (*Ananas comosus* L.) diketahui mengandung senyawa aktif yang berpotensi sebagai antibakteri. Penelitian ini bertujuan untuk mengevaluasi aktivitas antibakteri sari nanas serta menentukan konsentrasi efektif dalam menghambat pertumbuhan *Escherichia coli*. Uji aktivitas antibakteri dilakukan dengan metode difusi cakram pada tiga konsentrasi (20%, 25%, dan 30%), dengan kloramfenikol sebagai kontrol positif dan aquadest sebagai kontrol negatif. Hasil uji aktivitas antibakteri menunjukkan bahwa sari nanas pada konsentrasi 20%, 25%, dan 30% menghasilkan zona hambat terhadap *Escherichia coli* masing-masing sebesar 13,83 mm; 16,33 mm; dan 18,50 mm. Berdasarkan hasil penelitian, dapat disimpulkan bahwa sari annas memiliki aktivitas antibakteri terhadap *Escherichia coli*, dengan konsentrasi paling efektif dalam menghambat pertumbuhan bakteri yaitu 30%.

Kata Kunci: sari nanas, *Ananas comosus* L., antibakteri, *Escherichia coli*

INTRODUCTION

Infections caused by pathogenic bacteria remain a major health problem worldwide, especially in developing countries. One bacterium that often causes gastrointestinal disorders is Escherichia coli. Although most E. coli strains are commensal and live normally in the human

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intestine, some pathogenic strains can cause diarrhea, food poisoning, and urinary tract infections. These infections are generally treated with synthetic antibiotics, but their overuse has led to bacterial resistance and adverse effects (Rahayu *et al*., 2018). This situation has prompted the search for safer, cheaper, and more readily available natural antibacterial alternatives.

Pineapple (*Ananas comosus* L.) is a tropical fruit that Indonesians widely consume. In addition to being rich in nutrients such as vitamin C, minerals, and fiber, pineapple contains bioactive compounds, including bromelain, flavonoids, tannins, saponins, and phenols (Maulana *et al*., 2021). These compounds are believed to have antibacterial activity through mechanisms that inhibit bacterial growth and damage bacterial cell membranes (Sinaga *et al*., 2025). This potential makes pineapple a candidate for natural antibacterial sources for use in the health sector.

Several previous studies have shown the antibacterial potential of pineapple parts. Research by Sinaga (2025), reported that pineapple peel extracts at concentrations of 75%, 50%, 25%, 12.5%, and 6.25% inhibited the growth of Staphylococcus aureus and Escherichia coli. Juariah and Diana (2020) also reported that pineapple core extract was effective in inhibiting E. coli growth. In addition, research on pineapple leaf ethanol extract also showed antibacterial activity against Propionibacterium acnes (Rishliani, 2022). This confirms that almost all parts of the pineapple plant have antimicrobial potential. Even pineapple peel, which is often considered waste, is rich in secondary metabolites (flavonoids, saponins, tannins, alkaloids, bromelain) that are effective as antimicrobials.

Based on the above description, pineapple has great potential as a natural antibacterial source. However, research specifically testing pineapple juice for its effect on E. coli growth remains limited. Therefore, this research is important for determining the extent to which pineapple juice inhibits E. coli growth and for identifying opportunities to use pineapple as a natural antibacterial agent that is easily accessible to the public.

MATERIAL AND METHOD

Material

The materials used in this study were Pineapple (*Ananas comosus* L.) obtained from Lambaro Market, Aceh Besar, isolates of Escherichia coli, alcohol 70% aquadest, Nutrient Agar (NA) medium, Mueller Hinton Agar (MHA) medium, chloramphenicol, physiological NaCl, 0.5 McFarland, disc paper, and aluminum foil.

Procedures

Plant Determination

Plant determination was conducted at the Biosystematics Laboratory of the Faculty of Mathematics and Natural Sciences, Biology, Syiah Kuala University. The aim was to verify the accuracy of the plant's identity used in the research, thereby preventing errors in material collection.

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Making Pineapple Juice

Peel the pineapple, wash it thoroughly, cut the flesh into small pieces, and blend it without adding water. Then strain it to separate the juice from the pulp.

Antibacterial Activity Test

a. Sterilization
   Glassware and media were sterilized in an autoclave at 121 °C for 15 minutes (Novel et al., 2010).
b. Culture Revival
   One loop of Escherichia coli culture was inoculated into slanted NA medium and incubated at 37 °C for 24 hours (Smith & Aferd, 2022).
c. Preparation of Suspension Test
   A loop of revived bacteria was transferred into a test tube containing 10 mL of sterile 0.9% NaCl solution. It was homogenized using a vortex and compared with the 0.5 McFarland standard solution. The 0.5 McFarland standard corresponds to a cell suspension at 1.5 x 10^8 CFU/mL (Kherid *et al*., 2020).
d. Antibacterial Activity Test
   Antibacterial activity testing was performed using the disk diffusion method (Smith & Alferd, 2022; Koeth & Linda, 2022). A bacterial suspension at 0.5 McFarland was prepared using a sterile cotton swab and spread evenly on the surface of the MHA medium. Discs containing pineapple extract at concentrations of 20%, 25%, 30%, negative control (Aquadest), and positive control (chloramphenicol) per disc were placed on the agar surface. The discs were then incubated at 37°C for 24 hours, and the diameter of the inhibition zone was measured using a caliper.

RESULTS AND DISCUSSION

This study began with plant identification at the Biosystematics Laboratory in the Department of Biology, Faculty of Mathematics and Natural Sciences, Syiah Kuala University. The results of the identification showed that the plant used in this study was indeed from the species *Ananas comosus* L. Testing of the antibacterial activity of pineapple juice (*Ananas comosus* L.) against *Escherichia coli* bacteria was carried out using various concentrations, namely 20%, 25%, and 30%. This test was carried out using the disc diffusion method (Kirby and Bauer disc diffusion). The principle of disc diffusion is to use a disc as a medium to carry the test extract in various concentrations. The test extract on the disc will diffuse into the agar medium inoculated with fungi and will form an inhibition zone after incubation.

Table 1 shows that the higher the pineapple juice concentration, the larger the inhibition zone against E. coli. According to Dewi *et al*. (2025), the larger the inhibition zone formed, the better the antibacterial activity. This aligns with the opinion of Harvey *et al*. (2017), who stated that the concentration of a test material directly affects the size of the inhibition zone formed. The difference in the diameter of the inhibition zone at each concentration reflects the amount of

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active substance contained in pineapple juice. The higher the concentration of pineapple juice, the greater the content of active compounds, so that the inhibitory power against bacteria is stronger. Madigan *et al*. (2021), also emphasize that the concentration of antibacterial compounds is an important factor determining the efficiency and effectiveness of antibacterial activity. Based on the classification by David and Stoud, inhibition zones with a diameter≥20 mm are categorized as very strong, 11–20 mm as strong, 6–10 mm as moderate, and ≤5 mm as weak (Dewi *et al*., 2024). Thus, the variation in inhibition zone diameter provides a clear picture of pineapple juice's antibacterial potential.

Table 1. Results of Antibacterial Activity Test of Pineapple Juice Extract Against *E. coli*

Perlakuan	Average Diameter of the Inhibitory Zone (mm)	Kategori Daya Hambat
Negative control (Aquadest)	00,00	None
20% pineapple juice	13,83	Strong
25% pineapple juice	16,33	Strong
30% pineapple juice	18,50	Strong
Positive control (chloramphenicol)	24,00	Very strong

The results of measuring the inhibition zone diameter against E. coli (Table 1) show variations in antibacterial effectiveness across pineapple juice concentrations. The negative control (aquadest) did not produce an inhibition zone, whereas pineapple juice at 20%, 25%, and 30% produced inhibition zone diameters of 13.83 mm, 16.33 mm, and 18.50 mm, respectively. In comparison, the positive control (chloramphenicol) produced the largest inhibition zone, namely 24 mm. Based on the David & Stout classification, all tested pineapple juice concentrations (20%, 25%, 30%) were classified as strong, while the positive control was categorized as very strong. These findings confirm that increasing the concentration of pineapple juice extract directly increases antibacterial activity, although its effectiveness remains lower than that of chloramphenicol, the clinical standard.

The antibacterial activity of pineapple juice is due to secondary metabolites such as saponins, flavonoids, tannins, and phenols. Each of these active compounds works through specific mechanisms that synergistically weaken bacterial growth and survival. Saponins damage bacterial cell membranes. Flavonoids work by interfering with enzymes and damaging membranes. Tannins work by precipitating proteins and inhibiting metabolic enzymes. Meanwhile, phenols work as antibacterials by damaging bacterial cell membranes, precipitating proteins, and interfering with enzyme function, thereby causing cell death. These mechanisms collectively make pineapple a potential natural source for inhibiting bacterial infections (Anggrahini, 2013; Diyan *et al*., 2015; Madduluri *et al*., 2013).

In the negative control (aqudest), no inhibition zone formed around the paper disc, indicating that the solvent did not affect the test material's antibacterial activity. This proves that the antibacterial activity observed was due to pineapple juice. The inhibition zone in the negative control (distilled water) did not form. This confirms that the observed antibacterial activity is

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solely due to pineapple juice and is not influenced by the solvent. This aligns with Dewi *et al*. (2024), who state that distilled water has no antimicrobial activity; therefore, it does not inhibit microbial growth and is safe to use as a solvent in activity tests. In contrast, in the positive control (chloramphenicol), a larger inhibition zone was observed compared to pineapple. Chloramphenicol is a broad-spectrum antibiotic that inhibits protein synthesis in bacteria. Chloramphenicol inhibits peptidyl transferase, which catalyzes peptide bond formation between amino acids. As a result, polypeptide chain elongation halts, preventing the formation of new proteins and causing bacteria to lose their ability to grow and replicate (Zhang *et al*., 2022).

CONCLUSIONS

The results showed that pineapple juice (*Ananas comosus* L.) had antibacterial activity against *Escherichia coli*, with effectiveness increasing with concentration. A concentration of 30% gave the best results, with the largest inhibition zone of 18.50 mm. These findings reinforce pineapple's potential as a natural source for developing antibacterial agents.

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