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Research Article

Antibacterial Activity Test of African Leaf Extract (*Gymnanthemum amygdalinum* Del.) against the Growth of *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

The increasing threat of antibiotic-resistant bacterial infections has encouraged the search for safer and more effective natural alternatives. One potential medicinal plant is African leaf, which contains bioactive compounds such as flavonoids and saponins with antibacterial properties. These compounds work by inhibiting bacterial cell wall synthesis and damaging cell membranes. This study aimed to determine the protease enzyme activity of *Staphylococcus aureus* and *Escherichia coli*, evaluate the antibacterial activity of African leaf extract, identify the most effective extract concentration, and compare its inhibitory effects on both bacteria. The study used a qualitative protease test with Skim Milk Agar (SMA) media and an antibacterial assay using the disc diffusion method at concentrations of 20%, 40%, 60%, and 80%. The results showed that *E. coli* exhibited protease activity, indicated by the formation of a clear zone, whereas *S. aureus* did not. African leaf extract inhibited the growth of both bacteria, with stronger activity against *E. coli*. The inhibitory effect increased with higher extract concentrations, reaching optimal activity at 80%.

Keywords: antibacterial, bioactive compounds, *E. coli*, *Gymnanthemum amygdalinum* leaf extract, *S. aureus*

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INTRODUCTION

Bacterial infections pose a serious threat to public health, particularly with the development of antibiotic resistance. Antibiotic resistance occurs due to the irrational use of antibiotics, including inappropriate dosages, overuse, or premature discontinuation of therapy. Infectious diseases occur when interactions with microbes cause damage to the body, resulting in various clinical signs and symptoms. This condition causes many previously effective antibiotics to become ineffective in inhibiting the growth of pathogenic bacteria (Novard *et al*. 2019).

Staphylococcus aureus and *Escherichia coli* are pathogens that frequently cause infections in humans. *S. aureus* is known as a Gram-positive bacterium that can cause skin infections, abscesses, and even systemic infections. Meanwhile, *E. coli* is a Gram-negative bacterium that can cause gastrointestinal and urinary tract infections (Jayanthi *et al*. 2020).

One widely developed approach is the use of medicinal plants as a source of natural antimicrobial compounds. The African plant (*Gymnanthemum amygdalinum* Del.) has long been used in traditional medicine and is known to

possess various pharmacological activities. This plant contains bioactive compounds including flavonoids, saponins, tannins, and alkaloids (Bestari, 2021).

The mechanism of action of these bioactive compounds includes inhibiting bacterial cell wall synthesis and reducing the permeability of microbial cell membranes. Flavonoids work by damaging bacterial cell membranes and proteins. Saponins can increase cell membrane permeability, causing leakage of intracellular components. Tannins play a role in inactivating enzymes and disrupting protein transport within microbial cells (Khameneh *et al*. 2019).

In addition to antibacterial compounds, bacterial physiological characteristics, such as the ability to produce protease enzymes, also influence bacterial pathogenesis and resistance. Protease enzymes play a role in hydrolyzing proteins into peptides and amino acids, which can be used as a nutrient source for bacteria and contribute to the infection process. Based on this background, this study was conducted to determine the activity of protease enzymes and the activity of African leaf extract as an antimicrobial agent against *S. aureus* and *E. coli*.

METHODS

Sample preparation

Fresh African leaf samples were cleaned with running water to remove any dirt. Next, the material was drained and air-dried. 400 g of simplisia was weighed and then extracted with 96% ethanol at a ratio of approximately 1:10 (powder:solvent). The mixture was macerated for 3 days with occasional stirring, then filtered to separate the filtrate from the residue. The filtrate was then evaporated using a rotary evaporator at 40-50°C until a thick extract was obtained (Erwanda *et al*. 2024).

Preparation of test solutions and various concentrations

African leaf extract was formulated into test solutions at concentrations of 20%, 40%, 60%, and 80% using distilled water to a final volume of 10 mL using the volume dilution method (v/v). The positive control used a chloramphenicol antibiotic disc, while the negative control used a disc dipped in distilled water.

Preparation of MHA (Muller Hinton Agar) media

Muller Hinton Agar (MHA) medium was dissolving 22.8 g of the medium in 600 mL of distilled water, heating until homogeneous, and then sterilizing in an autoclave at 121°C for 15 minutes. The medium is poured into approximately 25 mL of petri dishes and allowed to solidify (Nurhayati *et al*. 2020).

Preparation of SMA (Skim Milk Agar) MEDIA

Skim Milk Agar (SMA) media is made from a mixture of agar, yeast extract, and tryptone in distilled water, then sterilized using an autoclave. 1% skim milk solution is sterilized separately using a water bath at 75°C for 15 minutes to prevent protein denaturation, then aseptically mixed into the base medium at 45–50°C. The medium is poured into sterile Petri dishes and allowed to solidify (Vijayaraghavan *et al*. 2019).

Preparation of test bacterial suspension

As much as 0.18 g of 0.9% NaCl media was weighed, dissolved in 20 mL of distilled water, and pipetted into 9 mL of test tubes. Then, it was sterilized in an autoclave at 121°C for 15 minutes (Purniasih *et al*. 2022). The suspension was prepared by taking one loop of test bacteria and inoculating it in a 0.9% NaCl solution with a turbidity that met the 0.5 McFarland standard (Nurhayati *et al*. 2020).

Protease enzyme activity test

S. aureus and *E. coli* colonies were aseptically inoculated into SMA media and then incubated for 24 hours at room temperature (Efendi *et al*. 2017).

Antibacterial activity test (paper disc diffusion method)

Paper discs were soaked in African leaf extract at concentrations of 20%, 40%, 60%, and 80% for 15 minutes. Chloramphenicol was used as a positive control, while sterile distilled water was used as a negative control. The discs were then placed on the surface of the media inoculated with the test bacteria and incubated at 37°C for 24 hours. Antibacterial activity was determined based on the formation of an inhibition zone around the disc (Pratama *et al*. 2025).

RESULTS AND DISCUSSION

Protease enzyme activity test

The results showed that *E. coli* produces protease enzymes, which are indicated by the formation of clear zones in SMA media. In contrast, *S. aureus* did not show any clear zones, indicating the absence or low level of protease activity. Protease test results seen in Figure 1.

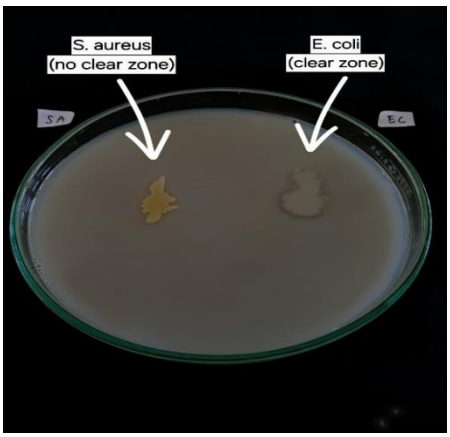

Figure 1. Protease test results

Research results indicate that the formation of a clear zone in SMA media is a qualitative indicator of protease enzyme activity (Winahyu *et al*., 2019). *E. coli* is capable of producing protease, indicated by the formation of a clear zone, while *S. aureus* does not exhibit proteolytic activity. This difference indicates that protease production capacity is specific to each bacterium. Protease activity in *E. coli* is influenced by environmental factors such as temperature, pH, substrate, and incubation time. In this study, an incubation condition of 37°C for 24 hours supported optimal casein hydrolysis (Mbira, 2024). Biologically, proteases play a role in bacterial metabolism, adaptation, and pathogenesis. Therefore, *E. coli* ability to produce proteases indicates higher physiological potential than *S. aureus* (Prihatini and Dewi, 2021).

Antibacterial activity test

African leaf extract demonstrated antibacterial activity against both test bacteria. For *S. aureus*, inhibition zones were only formed at concentrations of 60% and 80%, indicating weak inhibition. This indicates that this Gram-positive bacterium is relatively resistant to the extract. Conversely, for *E. coli*, antibacterial activity was already evident at a concentration of 20% and increased to moderate levels at concentrations of 40–80%. In the positive control, chloramphenicol demonstrated very strong inhibition against both bacteria, while the negative control showed no activity, confirming that the antibacterial effect originated from the extract. The result of inhibition zone diameter in Table 1. Inhibitory diameter of African leaf extract against *S. aureus* and *E. coli* seen in Figure 2 and Figure 3.

Increasing extract concentration was proportional to the increase in inhibition zone diameter, particularly *for E. coli*, indicating that the amount of bioactive compounds diffusing into the medium influences antibacterial effectiveness.

Table 1. The result of inhibition zone diameter

Test Bacteria	Concentration	Repetition			Average Inhibition Zone (mm)	Information
		I	II	III		
S. aureus	K (+)	22,8	21,1	21,7	21,9	Very strong
	K (-)	-	-	-	-	There isn't any
	20 %	-	-	-	-	There isn't any
	40 %	-	-	-	-	There isn't any
	60 %	0,1	0,02	0,02	0,07	Weak
	80 %	4,7	3,1	5,6	4,5	Weak
E. coli	K (+)	26,8	26,2	27,3	26,7	Very strong

K (-)	-	-	-	-	There isn't any
20 %	-	1,2	0,03	0,42	Weak
40 %	6,1	4,2	6,2	5,5	Moderate
60 %	8,9	7,7	7,8	8,1	Moderate
80 %	8,0	7,2	10,2	8,49	Moderate

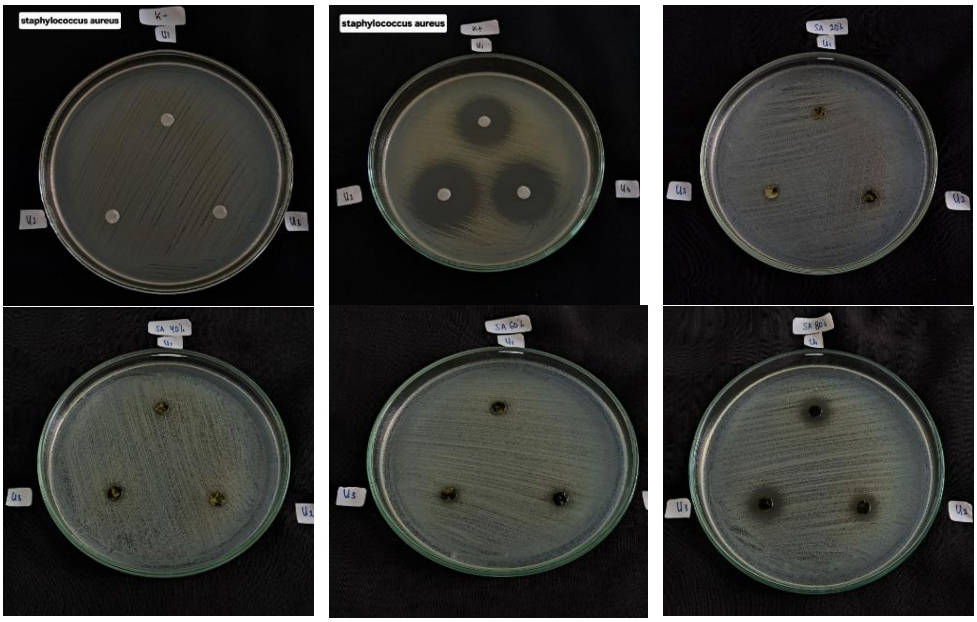

Figure 2. Inhibitory diameter of African leaf extract against *S. aureus* in three repetitions

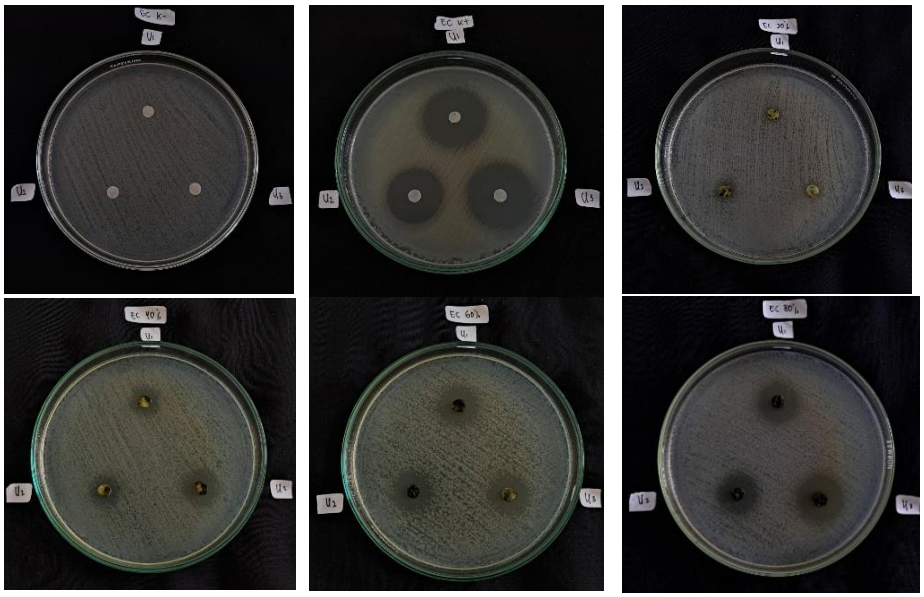

Figure 3. Inhibitory diameter of African leaf extract against *E. coli* in three repetitions

The different responses between the two bacteria suggest the influence of cell wall structure. *S. aureus*, as a Gram-positive bacterium, has a thicker peptidoglycan layer, making it more resistant, while *E. coli* is more susceptible to active compounds (Nurhayati *et al*. 2020).

Limited antibacterial activity at some concentrations can be influenced by factors such as low concentration of active compounds, diffusion capacity in the medium, and extract stability (Andriyana *et al*., 2021). The antibacterial activity of African leaf extract is related to the content of bioactive compounds such as flavonoids, saponins, and

tannins, which work through mechanisms such as cell membrane damage, increased permeability, and inhibition of bacterial enzymes and metabolism (Nasri *et al*. 2025).

CONCLUSION

The results showed that *E. coli* has protease enzyme activity as indicated by the formation of a clear zone in SMA media, while *S. aureus* does not. African leaf extract (*Gymnanthemum amygdalinum*) was able to inhibit the growth of both bacteria, with higher effectiveness against *E. coli*. Increasing the extract concentration increased the inhibitory power, with optimal activity at a concentration of 80%. Antibacterial activity against *E. coli* was moderate, while against *S. aureus* was weak.

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AUTHOR CONTRIBUTIONS

All authors have read and approved the manuscript to be published.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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