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Research Article

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Ethanol Extract of Bajakah Tampala Roots (*Spatholobus littoralis* Hassk) as an Antibacterial Against Escherichia coli O157:H7 : In Vitro Test

Kartika Dwi Wulandari[1], Lina Noviyanti Sutardi[2]*, Safika[3]

[1]Animal Biomedical Science Study Program, Graduate School, IPB University, Indonesia
[2]Division of Pharmacy, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
[3]Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia

*Corresponding author: linans@apps.ipb.ac.id

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ABSTRACT

Background: Gastrointestinal tract infections caused by *Escherichia coli* O157:H7 pose a serious threat, as these bacteria can trigger severe illnesses such as Hemorrhagic Colitis (HC) and Hemolytic Uremic Syndrome (HUS). The increasing cases of antimicrobial resistance in these strains have prompted efforts to find alternative treatments based on natural ingredients. One potential plant is bajakah tampala (*Spatholobus littoralis* Hassk), which has been traditionally used by the people of Kalimantan for generations.

Aims: This study aims to evaluate the antibacterial activity of Bajakah tampala extract as preliminary information for the development of alternative natural antibacterial agents against *E. coli* O157:H7.

Methods: The extract was obtained through maceration using 70% ethanol. Antibacterial activity was tested using the well diffusion method at various concentrations (12.5%, 25.0%, 50.0%, 75.0%, and 100.0%), with enrofloxacin used as a positive control. Bacterial identification was performed using the Polymerase Chain Reaction (PCR) method. Phytochemical screening was conducted to identify bioactive compounds in the extract.

Results: Phytochemical screening revealed that the ethanol extract contains alkaloids, flavonoids, saponins, and tannins, which contribute to antibacterial activity. The antibacterial assay showed that the extract significantly inhibited the growth of *Escherichia coli* O157:H7 ($p < 0.05$). The highest inhibition zone was observed at 100% concentration (16.31 mm). All tested concentrations exhibited strong antibacterial activity.

Conclusion: The ethanol extract of Bajakah tampala roots demonstrates significant antibacterial activity against *E. coli* O157:H7 and shows potential as a natural antibacterial agent for further development.

INTRODUCTION

Gastrointestinal infections represent one of the serious global health issues, particularly in developing countries. One of the common symptoms is diarrhea. Life-threatening diarrhea cases are often associated with various pathogenic microbes, one of which is *Escherichia coli*, a bacterium that is easily spread in the environment and can be transmitted through food. The impact of this infection is significant, especially for vulnerable groups such as infants and the elderly, with more than 1.6 million children losing their lives annually due to diarrhea (Gomes *et al.*, 2016; Halim *et al.*, 2017).

Among various *E. coli* strains, *Escherichia coli* O157:H7 is one of the dangerous strains that frequently causes cases in humans. This strain belongs to the *Enterohemorrhagic Escherichia coli* (EHEC) group, which can cause Hemorrhagic Colitis (HC) and Hemolytic Uremic Syndrome (HUS) (Brooks *et al*., 2013). Cattle are known as the primary reservoir of Verocytotoxin-producing *Escherichia coli* O157:H7 and the main source of transmission of this agent to humans (Suardana *et al*., 2013).

Another issue of concern worldwide, particularly in the field of antibiotic use, is the emergence of Antimicrobial Resistance (AMR) (Suwito *et al.,* 2023). The occurrence of Antimicrobial Resistance (AMR) in *Escherichia coli* O157:H7 needs to be anticipated. Escherichia coli O157:H7 isolated from fresh milk in Sukabumi Regency, West Java, was reported to be resistant to chloramphenicol, sulfamethoxazole, and tetracycline (Suwito & Adji, 2011).

Kebab meat sold around IPB University was reported to be contaminated with *Escherichia coli* O157:H7 at 31.6% and resistant to ampicillin, amoxicillin, clavulanic acid, gentamicin, ciprofloxacin, enrofloxacin, and colistin sulfate (Sari *et al.,* 2021). The emergence of Antimicrobial Resistance (AMR) causes bacteria to become immune to antibiotics, making them difficult to treat and increasing the risk of disease spread.

The use of traditional medicine in Indonesia remains common and has been trusted for generations (BPOM, 2019), including the bajakah tampala plant (*Spatholobus littoralis* Hassk), which is widely known in Kalimantan and utilized by the Dayak community as traditional medicine, among others, for treating cancer (Maulina *et al*., 2019), stomach pain, diarrhea, and dysentery (Saputera & Ayuchecaria, 2018). However, scientific research on its effectiveness is still limited, especially against *Escherichia coli* O157:H7, a pathogen causing severe diarrhea and serious complications.

Therefore, this study focuses on evaluating the extract's ability to inhibit bacterial growth through laboratory testing. The results obtained are expected not only to provide initial evidence of the extract's potential but also to serve as a basis for developing nature-based therapies, while offering alternative solutions to the increasing antibiotic resistance.

MATERIALS AND METHODS

Research Location

The research was conducted at two locations: the Pharmacy Laboratory and the Bacteriology Laboratory of the School of Veterinary Medicine and Biomedical Sciences, IPB University. The Bajakah tampala root (*Spatholobus littoralis* Hassk) used in this study was collected from the Berau Islands, East Kalimantan.

Extraction Process

The method for preparing Bajakah Tampala root extract (*Spatholobus littoralis* Hassk) is carried out using the maceration method. 150 grams of bajakah tampala root powder is placed into a maceration vessel, to which 1,500 milliliters of 70% ethanol solvent are added (1:10 ratio) until the powder is completely submerged. The maceration process was conducted for 2 × 24 hours with occasional stirring. The macerate was then separated from the residue. The maceration result was evaporated and concentrated using a rotary evaporator at a temperature of 50°C (Indonesian Herbal Pharmacopoeia, 2017).

Phytochemical Screening

Phytochemical screening was conducted to identify the presence of secondary metabolite compounds contained in the ethanol extract of bajakah tampala (Spatholobus littoralis Hassk) roots. Phytochemical screening was performed on several groups of compounds, such as alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins, using a color reaction method based on the procedure by Hanifah et al. (2024).

Concentration of Bajakah Tampala Ethanol Extract

The concentrated bajakah tampala extract was then divided into five concentrations: 12.5%, 25.0%, 50.0%, 75.0%, and 100.0%. The process begins by weighing the concentrated bajakah tampala extract according to the concentration, then placing it into a glass beaker using a spatula, followed by adding 3 drops of Tween 80 and 10 milliliters of distilled water, then stirring. Transfer to a labeled vial bottle (Magvirah et al., 2019).

Isolation and Identification of Escherichia coli O157:H7

The isolation process began by re- cultivating archived Escherichia coli O157:H7 isolates in Tryptic Soy Broth (TSB) liquid media. After incubation, the cultures were transferred to Eosin Methylene Blue Agar (EMBA)

media and incubated for 24 hours at 37°C. Growing coliform colonies were characterized by a pink to red color and a mucoid appearance, while metallic green colonies were identified as indicating the presence of E. coli. Next, all coliform colonies, including those suspected of being E. coli, were Gram-stained for confirmation (Bambang et al., 2014).

After the isolation process was complete, the identification stage was carried out using a molecular approach to confirm the presence of E. coli O157:H7. DNA extraction of suspected E. coli colonies was carried out using 100 µl of liquid culture transferred to a 2 ml Eppendorf tube, then centrifuged at 5,000 rpm for 5 minutes. The obtained cell sediment was mixed with TE buffer pH 8. The lysis process was carried out through a heating cycle in boiling water for 15 minutes then vortexed for 5 minutes, which was repeated three times. Identification of the presence of Escherichia coli bacteria in the sample was carried out using the PCR method targeting the uspA (Universal Stress Protein A) gene which produced an amplification product measuring 856 bp. Detection of specific Escherichia coli O157:H7 genes was carried out using the multiplex PCR technique with primers targeting the eaeA (1,118 bp), stx1 (771 bp), fliC (H7) (461 bp), and rfb (339 bp) genes. The PCR mixture consisted of 6 µl PCR mix, 1 µl forward primer, 1 µl reverse primer, 2 µl DNA, and 2 µl NFC in a total volume of 12 µl. The amplification process included one initial cycle (94°C for 5 minutes), followed by 40 main cycles (94°C, 48°C, and 72°C for 30 seconds each), and one final cycle (72°C for 5 minutes). The amplification results were then analyzed by 1% agarose gel electrophoresis using a 100 bp DNA marker as a reference for the size of the DNA fragments, then visualized with a UV illuminator (Rizky et al., 2021).

Antibacterial Activity Test

The antibacterial activity testing procedure was carried out using the agar well diffusion method, and all experimental procedures were performed in triplicate (three repetitions) to ensure the consistency of the results. A suspension of Escherichia coli O157:H7 bacteria adjusted to the McFarland 0.5 standard was taken in an amount of 100 µL using a micropipette, then inoculated evenly on the surface of Mueller Hinton Agar (MHA) media using a sterile stir bar. Wells were made with a diameter of 6 mm on the surface of the petri dish. Extracts prepared at various concentrations (12.5%, 25.0%, 50.0%, 75.0%, and 100.0%) were taken alternately, each containing 50 µl of the extract and placed into the well. Enrofloxacin at a concentration of 5 µg/ml served as a positive control, and distilled water with Tween 80 as a negative control were added to the well. After incubation at 37°C for 24 hours, the clear zone around the wells was observed and counted using a vernier caliper (Yulianti et al., 2018). The diameter of the inhibition zone, or clear zone formed around the well, indicates the bacterial sensitivity to the antibacterial agent used as the test material and is expressed as the diameter of the inhibition zone. The inhibition zone formed around the wells was measured using a vernier caliper. Antibacterial activity is considered weak if the inhibition zone diameter is <5 mm, moderate between 5-10 mm, strong between 10-20 mm, and very strong if >20 mm (Kumowal et al., 2019).

Data Analysis

The data, in the form of inhibition zone diameters from several extract concentrations, were statistically analyzed using One Way ANOVA (Analysis of Variance) using SPSS version 26. ANOVA results showing significant differences between treatments were then further analyzed using Tukey's post hoc test to identify treatment groups with statistically significant differences.

RESULTS AND DISCUSSION

Phytochemical Screening

The identification of secondary metabolite compounds in the ethanol extract of bajakah tampala root aimed to determine the presence of active compounds with potential pharmacological effects, particularly as antibacterial agents. The results of the test are presented in Table 1.

The results of the phytochemical screening of the ethanol extract of bajakah tampala root (Spatholobus littoralis Hassk) revealed the presence of several secondary metabolites that potentially exhibit biological activity, particularly as antibacterial agents. These compounds include alkaloids, flavonoids, saponins, and tannins. The antibacterial activity of the ethanol extract of bajakah tampala root against E. coli O157:H7 is particularly interesting because this bacterium belongs to the Gram- negative group, which is structurally more resistant to natural antibacterial compounds than Gram-positive bacteria. This characteristic is due to the presence of an outer membrane composed of lipopolysaccharides (LPS), which functions as a selective barrier against the entry of foreign substances, including antibacterial compounds (Simpson & Trent, 2019). Therefore, the

ability of the bajakah tampala root extract to produce a strong inhibition zone indicates that the active compounds it contains are likely capable of penetrating or disrupting the outer membrane of Gram-negative bacteria, thereby interfering with essential bacterial cell functions.Alkaloid compounds exhibit potential as antibacterial agents through inhibitory mechanisms that disrupt the structural elements of peptidoglycan in bacterial cells, thereby preventing the complete formation of the cell wall layer and causing cellular death. In addition, alkaloids inhibit protein synthesis, resulting in disruption of bacterial metabolic processes (Anggraini et al., 2019). These alkaloid compounds are effective in inhibiting the proliferation of both Gram-positive and Gram- negative bacteria (Compean & Ynalvez, 2014).

Flavonoids have also been proven effective in inhibiting bacterial proliferation through interference with cell wall synthesis, which is a crucial element for bacterial survival and structural integrity. Flavonoids are known to interact with membrane proteins and phospholipids, thereby increasing the permeability of Gram-negative bacterial cell membranes. The bacterial cell wall, which is primarily composed of peptidoglycan, plays an essential role in maintaining cell morphology and protecting against osmotic pressure. Inhibition of cell wall synthesis results in cellular lysis and ultimately bacterial death. One of the main mechanisms of flavonoids in inhibiting cell wall synthesis is through suppression of the activity of key enzymes involved in peptidoglycan biosynthesis. Flavonoids are capable of targeting enzymes such as peptidoglycan glycosyltransferase, which is essential for the polymerization process and the formation of cross-links within peptidoglycan chains (Liu et al., 2025).

Another identified compound was saponin. Saponins contain active substances located on their surface and function similarly to detergents, with the primary mechanism involving the reduction of surface tension in the bacterial cell wall membrane. This facilitates the penetration of saponin compounds into the cell, which subsequently disrupts bacterial cellular metabolic pathways and leads to cell death (Suryani et al., 2019). This mechanism enables saponins to damage the integrity of the outer membrane of Gram-negative bacteria, thereby increasing bacterial sensitivity to other antibacterial compounds present in the extract. Tannin compounds were also detected in the ethanol extract of Bajakah tampala. The antibacterial mechanism of tannins is achieved through the inhibition of reverse transcriptase and DNA topoisomerase enzymes, which triggers lysis of the bacterial cell wall. As a result, the biosynthesis of the cell wall is inhibited, leading to bacterial cell death. In addition, tannins are known to form complexes with bacterial membrane proteins, thereby disrupting membrane permeability and cellular nutrient transport (Dewi et al., 2014).

Isolation and Identification Results of *Escherichia coli* O157:H7

Confirmation of the presence of Escherichia coli in the samples was carried out using the PCR method

Table 1. Phytochemical Screening Results of Ethanol Extract of Bajakah Tampala Roots (*Spatholobus littoralis* Hassk)

Parameter	Test Results	Information
Alkaloids: Mayer	Negative (-)	No white precipitate formed
Wagner	Positive (+)	A brown solution was formed
Dragendorf	Positive (+)	An orange-colored solution was formed
Flavonoids	Positive (+)	An orange colored solution was formed
Steroids and Terpenoids	Negative (-)	No green color was formed
Saponins	Positive (+)	A stable foam was formed for more than 30 seconds
Tannins	Positive (+)	A dark green color was formed

targeting the uspA gene (856 bp), as shown in Figure 1. Meanwhile, the specific detection of Escherichia coli O157:H7 was performed using multiplex PCR targeting the eaeA (1,118 bp), stx1 (771 bp), fliC (H7) (461 bp), and rfb (339 bp) genes, as presented in Figure 2.

The isolation and identification process of Escherichia coli O157:H7 was carried out using the Polymerase Chain Reaction (PCR) technique to ensure the authenticity and pathogenicity of the strain used in the antibacterial activity test. The PCR results shown in Figure 1 demonstrate that all tested samples produced DNA bands corresponding to the uspA gene (856 bp), indicating that all samples were Escherichia coli. The uspA gene is one of six universal stress protein (usp) genes found in E. coli. This gene plays a crucial role in bacterial survival, particularly under unfavorable environmental conditions or during stress, such as nutrient deprivation, extreme temperatures, or exposure to antimicrobial agents (Kvint et al., 2003). Moreover, the uspA gene is also involved in bacterial growth phases, including adhesion (the ability to attach to host surfaces) and motility (bacterial movement), which are the initial steps in colonization and infection (Nachin et al., 2005).

The amplification analysis for the detection of specific Escherichia coli O157:H7 genes, shown in Figure 2, revealed that three out of five tested samples. Samples number 3, 5, and

6 exhibited the presence of three virulence genes: eaeA, stx1, and fliC. This indicates that these three samples are most likely pathogenic E. coli O157:H7 strains. Meanwhile, samples number 2 and 4 only showed the presence of the fliC (H7) gene, suggesting that these samples were not E. coli O157:H7 since they lacked the eaeA and stx1 virulence genes. Additionally, the rfb gene, which serves as one of the serotype O157

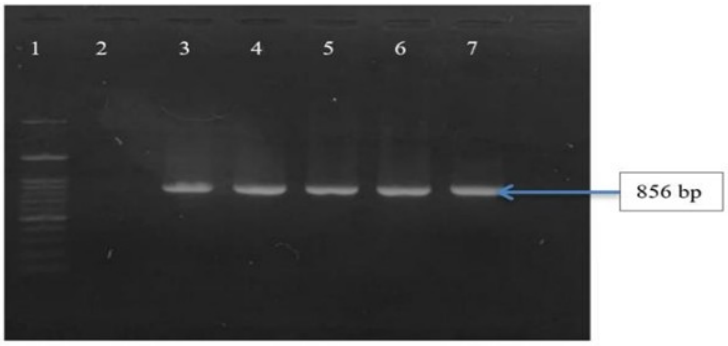

Figure 2. Amplification result of the uspA gene (856 bp) on 1% agarose gel. 1: 100 bp marker; 2: negative control; 3–7: samples.

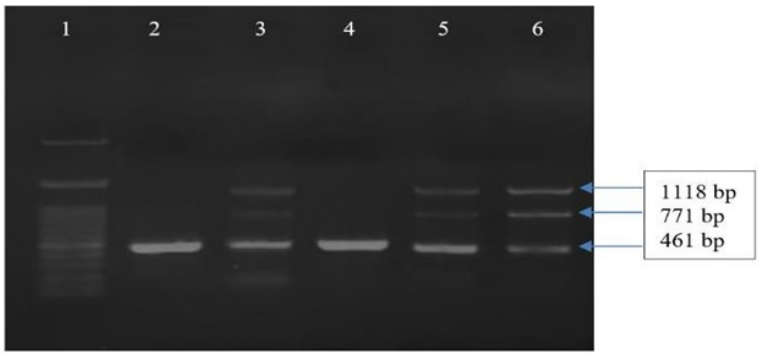

Figure 3. . Detection of *E. coli* O157:H7 genes on 1% agarose gel using primers eaeA (1,118 bp), stx1 (771 bp), fliC (H7) (461 bp), and rfb (339 bp). 1: 100 bp DNA marker; 2–6: E. coli samples.

markers, was not detected in any of the samples. The absence of detection of the rfbE gene may be attributed to genetic variation among strains or technical limitations during the PCR amplification process, such as low concentrations of target DNA or primer–template mismatch. These conditions may prevent primers from properly annealing to the target gene, resulting in unsuccessful amplification and a negative PCR result, eventhough the gene may actually be present (Huang et al., 2024).

This explanation is consistent with the early findings of Feng et al. (1998), who reported the presence of Escherichia coli O157:H7 rough strains that genetically still carried the rfbE gene but did not express the O157 antigen due to alterations or loss of the lipopolysaccharide (O- antigen) structure. This condition indicates that differences in antigen expression may occur in certain strains despite retaining the genetic characteristics of O157:H7. Furthermore, Bertrand et al. (2007) reported that PCR-based detection of E. coli O157 targeting the rfbE gene may have limited sensitivity due to methodological factors and sample conditions, potentially leading to false-negative results. Therefore, detection based on a single gene target alone is not always sufficient to accurately confirm the presence of E. coli O157:H7. Thus, the presence of key virulence genes such as eaeA, stx1, and fliC (H7) can still be used as indicators of the pathogenic potential of E. coli O157:H7, even when rfbE detection yields a negative result, since such a finding does not necessarily indicate the absence of the bacterium or the loss of its virulence properties.

Antibacterial Activity Test of Bajakah Tampala Root Ethanol Extract Against Escherichia coli O157:H7

An antibacterial activity test was conducted using the well diffusion method to determine the effectiveness of Bajakah Tampala (Spatholobus littoralis Hassk) ethanol extract against the growth of Escherichia coli O157:H7. After 24 hours of incubation at 37°C, antibacterial activity was determined based on the formation of a zone of inhibition (clear zone) around the well. The results of the average inhibition zone diameter measurements for each treatment are presented in Figure 3.

Based on the results of the study, it was observed that the higher the extract concentration, the larger the inhibition zone diameter formed. At a concentration of 12.5%, the inhibition zone diameter was 13.56 mm, indicating that the extract was already able to exhibit antibacterial activity classified as strong. Furthermore, at concentrations of 25%, 50%, and 75%, the inhibition zone diameters increased to

14.18 mm, 15.40 mm, and 15.86 mm, respectively, all of which remained within the strong antibacterial category. The 100% concentration produced the largest inhibition zone diameter of 16.31 mm, representing the highest value among all extract concentrations and still classified as having strong antibacterial activity according to the classification proposed by Kumowal et al. (2019).

Although the inhibition zone diameter increased with increasing extract concentration, the differences among concentrations were relatively small. This finding suggests that the active compounds present in the ethanol extract of bajakah tampala root were likely

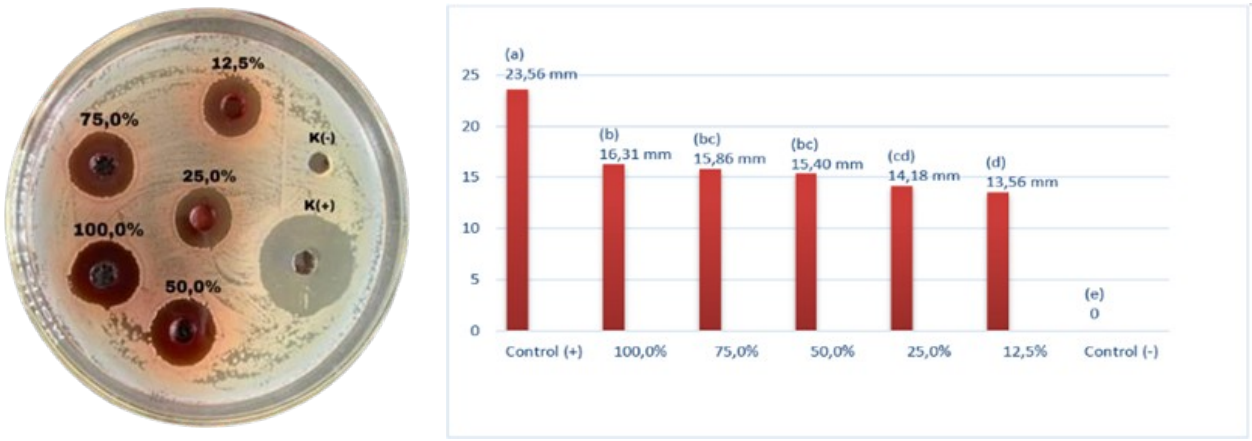

Figure 3. Average inhibition zone diameter of bajakah tampala extract against E. coli O157:H7. Values followed by different letters indicate significant differences according to the Tukey HSD test. Control (+): Enrofloxacin; Control (-): Tween 80 and distilled water.

effective even at low concentrations. In other words, at a concentration of 12.5%, the extract had already achieved a relatively optimal inhibitory effect against the growth of E. coli O157:H7, so that further increases in concentration resulted in only slight additional inhibitory activity. This condition may be associated with the high content of bioactive compounds present in the extract. These results indicate that the ethanol extract of bajakah tampala root possesses significant antibacterial potential against E. coli O157:H7, showing consistently strong antibacterial activity at all treatment concentrations, although the resulting inhibition zones were still smaller than those produced by the positive control, enrofloxacin.

These findings reinforce the evidence that the bioactive compounds present in the ethanol extract of Bajakah tampala, namely alkaloids, flavonoids, saponins, and tannins, contribute significantly to its antibacterial activity. This result aligns with a previous study by Latu et al. (2023), which reported that Spatholobus littoralis Hassk contains flavonoids, tannins, saponins, and phenolic compounds with antibacterial potential. Similarly, research conducted by Hadanu et al. (2023) indicated that bajakah plants originating from Kolaka Regency, Southeast Sulawesi Province, contain various secondary metabolites with potential as natural antibacterial agents, including apocynoside I, quercetin, hexosylphingosine, 3-hydroxy-7- methoxybaicalein, momor-cerebroside I, ambronal, and stigmastan-3,6-dione. These compounds contribute to the inhibition mechanisms against pathogenic microorganisms. Among them, quercetin has been reported to disrupt bacterial cell membrane structure and interfere with cellular metabolic pathways, thereby impairing vital bacterial functions (Nguyen & Bhattacharya, 2022).

CONCLUSION

This study demonstrated that the ethanol extract of Bajakah tampala root contains active compounds such as alkaloids, flavonoids, saponins, and tannins, which play a role in antibacterial activity against Escherichia coli O157:H7. PCR identification successfully confirmed the presence of specific virulence genes, confirming that the isolate used was E. coli O157:H7. In vitro testing demonstrated that all extract concentrations exhibited strong antibacterial activity, with the most effective 100% concentration producing the largest inhibition zone (16.31 mm). These findings indicate the potential of Bajakah tampala extract to be developed as a natural antibacterial.

AUTHORS CONTRIBUTION

K.D.W. conducted the laboratory experiments, collected and analyzed the data, and prepared the initial manuscript draft. L.N.S. conceived and supervised the study, contributed to the research design, data interpretation, and critical revision of the manuscript. S. assisted in microbiological analyses, data validation, and manuscript editing. All authors read and approved the final version of the manuscript.

"The author declares that there is no conflict of interest with the parties involved in this research".

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