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EXPLORING THE NATURAL ANTIBACTERIAL POTENTIAL: EFFECTIVENESS OF LEUCAENA LEUCOCEPHALA SEED EXTRACT AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

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ABSTRACT
The global rise of antibiotic resistance has encouraged researchers to investigate natural plant-based alternatives. Leucaena leucocephala (lamtoro) seeds contain bioactive compounds such as alkaloids, flavonoids, saponins, and tannins, which are suspected to possess antibacterial activity. This study aimed to assess the inhibitory effect of ethanol extract of Leucaena leucocephala seeds on the growth of Staphylococcus aureus and Escherichia coli. Method: Antibacterial activity was determined using the disc diffusion method on Mueller Hinton Agar. Extract concentrations of 5%, 10%, and 15% were tested. Aquadest served as the negative control, vancomycin (30 µg) as the positive control for S. aureus, and chloramphenicol (30 µg) as the positive control for E. coli. Each treatment was conducted in triplicate, and the inhibition zones were measured using a vernier caliper. The extract demonstrated measurable antibacterial effects on both tested bacteria. The average inhibition zones against S. aureus were 6.17 mm (5%), 6.20 mm (10%), and 6.42 mm (15%), while the positive control vancomycin showed 15.49 mm. For E. coli, inhibition zones were 6.24 mm (5%), 6.26 mm (10%), and 6.44 mm (15%), compared with 21.66 mm for chloramphenicol. The activity of the extract was classified as moderate, and higher concentrations yielded slightly larger zones. Ethanol extract of Leucaena leucocephala seeds exhibits moderate antibacterial potential against both Gram-positive and Gram-negative bacteria.

Keywords: antibacterial activity; escherichia coli; ethanol extract; leucaena leucocephala; natural compounds; staphylococcus aureus

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INTRODUCTION

The world's largest user of medicinal herbs is Indonesia, a nation abundant in these plants. With the importance and effectiveness of plants in promoting health, Indonesia's plant diversity is a natural resource that should be preserved. Because of their therapeutic qualities and chemical makeup, a variety of wild and conventionally grown plants can be utilized as medicines (simple mixtures). Men have used plants as a source of food, housing, clothing, medicine, cosmetics, and as a means of seeking solace from the hardships of life in an ongoing effort to better their quality of life. On Earth, there are thought to be between 250,000 and 500,000 plant species, some of which have been extensively used in medicine. Research on medicinal plants has received a lot of interest recently. Different elements of plant chemicals antibacterial, antifungal, antiviral, and antiprotozoal activities have been studied. Traditional herbal medicine practitioners have documented many native plants' therapeutic effectiveness for various ailments. Because plants contain a variety of components necessary for life, scientists have been compelled to study them to identify any potential medical benefits (Banu et al., 2024a).

Given their lengthy history of usage in traditional medicine, medicinal plants present an interesting research topic. According to a thorough assessment by (Clark, 2020), medicinal plants are essential in the fight against diseases that are resistant to antibiotics. There are 459 chemicals that are known to strongly suppress antibacterial activity and are generated from plant sources, it can be used for a variety of purposes, including as curing tropical ulcers, promoting sleep, treating sexual illnesses, reducing chest congestion, and even treating malaria traditionally.

Native to southern Mexico and northern Central America, Leucaena leucocephala (Lam.) de Wit (family Fabaceae) is a tiny, versatile, fast-growing tropical mimosoid tree (Elbanoby et al., 2024a). Since Leucaena seeds have around 5.5% fat (MAKMUR et al., 2019), they are utilized as vegetables in cooking. The primary fatty acids—palmititic, behenic, stearic, oleic, lignoceric, and linoleic acids—are used as coffee alternatives (Dijkman, 1950). The tree's legumes make high-protein bovine feed (De Angelis et al., 2021), but the seeds contain mimosine, a non-protein amino acid and anti-nutritional component that is known to be harmful to ruminants. The anti-proliferative and cancer chemopreventive properties of L. leucocephala seed gum were induced by polysaccharides (Elbanoby et al., 2024a). Strong antibacterial and antifungal properties of the plant extracts have been shown. It has been demonstrated to be highly effective in inhibiting the growth of Agrobacterium tumefaciens and Dickeya solani. Certain plant pathogenic fungi have been shown to have their mycelial development and spore germination inhibited by botanical preparations. Wood samples from Melia azedarach treated with pomegranate peel extracts demonstrated promising antifungal activity against Rhizoctonia solani. The HPLC analysis revealed the presence of phenolic acid compounds, including syringic, p-coumaric, benzoic, cafeic, gallic, ferulic, salicylic, cinnamic, and ellagic compounds, along with catechol and pyrogallol (Mosa et al., 2022).

Staphylococcus aureus is a Gram-positive, facultative anaerobic bacterium that functions as both a commensal and a significant pathogen affecting humans and animals (Pal et al., 2023). It naturally colonizes the nasal cavity and skin, appearing as grape-like clusters under the microscope (Tigabu & Getaneh, 2021). S. aureus causes a wide range of infections, from skin conditions such as furuncles and impetigo to systemic diseases including osteomyelitis, sepsis, and toxic shock syndrome. It is the third most common cause of foodborne illness worldwide and has a significant impact on agricultural environments through livestock infections (Tigabu & Getaneh, 2021). This pathogen exhibits remarkable adaptability, with methicillin-resistant S. aureus (MRSA) strains exhibiting resistance to multiple classes of antibiotics and reaching a prevalence of 60% in US intensive care units. This antibiotic resistance, combined with the bacterium's ability to transfer between humans and livestock, poses a significant public health challenge (Park & Ronholm, 2021). Staphylococcus aureus is a leading cause of human infections. Nearly everyone has experienced a Staphylococcus aureus infection at some point in their life, ranging from food poisoning and minor skin or wound infections to severe, potentially fatal infections. The use of antibiotics to treat infections increases with the rise in bacterial infections. However, continued antibiotic use can increase the likelihood of bacterial resistance to antibiotics. Therefore, researchers are constantly seeking alternative sources of antibacterial compounds.

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped bacterium belonging to the Enterobacteriaceae family that naturally inhabits the lower intestinal tract of warm-blooded animals. E. coli is a versatile bacterium that functions as both a commensal organism and a highly dangerous pathogen, causing a variety of diseases in humans. At least eleven pathotypes of this species have been identified, with the most common strains divided into intestinal pathogenic E. coli (InPEC) and extraintestinal pathogenic E. coli (ExPEC). However, horizontal gene transfer produces hybrid strains that are difficult to classify (Geurtsen et al., 2022). One of the first bacteria to colonize the newborn's gastrointestinal tract is E. coli, which has remarkable metabolic capabilities and can survive in a variety of environments (Sina et al., 2022). Multidrug-resistant serotypes of the bacterium cause gastrointestinal and urinary tract infections, making this bacterium even more

problematic (Mayer et al., 2023). In recent years, E. coli has become the most common cause of water and food contamination in Brazil, representing a significant public health problem (SILVA & MENDES, 2022). This bacterium controls virulence and pathogenesis factors through a sophisticated quorum sensing system (Mayer et al., 2023).

Research demonstrates significant antibacterial potential of Leucaena leucocephala seed extracts against Staphylococcus aureus and Escherichia coli. (Mora-Villa et al., 2021) found that methanolic extracts of L. leucocephala seeds showed minimum inhibitory concentrations (MIC) of 2000 µg/mL against E. coli and 4000 µg/mL against S. aureus, with minimum bactericidal concentrations of 6000 µg/mL proving effective within 10 hours of treatment. The extracts contained bioactive compounds including alkaloids, triterpenes, steroids, saponins, and phenolic compounds responsible for antimicrobial activity.

Elbanoby et al., (2024) confirmed L. leucocephala's antimicrobial properties, identifying key phytochemicals in seed extracts including benzoic acid (1520.44 mg/kg), myricetin (848.73 mg/kg), and rosmarinic acid (792.46 mg/kg) through HPLC analysis. These findings align with broader research by (Banu et al., 2024b) and (Imran et al., 2021) demonstrating that natural plant extracts offer promising alternatives to chemical preservatives for controlling bacterial pathogens, supporting the traditional medicinal use of L. leucocephala. Additionally, (Hazim et al., 2022a) showed that leaf extract-synthesized silver nanoparticles displayed enhanced antibacterial activity against both Gram-positive and Gram-negative bacteria, suggesting potential for nanotechnology applications. The aim of this study is to evaluate the antibacterial effectiveness of Leucaena leucocephala (Lam.) de Wit seed extract against the growth of Staphylococcus aureus and Escherichia coli, as well as to measure its inhibition zones at concentrations of 5%, 10%, and 15%.

METHOD

Standardization Testing of Simplisia

Simplisia standardization is carried out to maintain stability and safety, as well as to maintain the consistency of the active compound content in the simplisia.

Determination of Water Content

This method for determining water content uses the gravimetric method, which relies on the evaporation of water contained in the sample at a temperature of 105°C. Heat a porcelain crucible for 30 minutes, then cool it in a desiccator for 15 minutes. Next, weigh 2 g of the sample and place it in the porcelain crucible. Dry it for 4 hours at 105°C, then reweigh it. The drying process is continued, and reweighing is performed at 1-hour intervals until the difference between two consecutive weighings is no more than 0.25%.

Determination of Total Ash Content

Weigh 2 g of the powdered medicinal herb into a preheated porcelain crucible and weigh it. Gently heat the crucible until the carbon is completely gone. Heat the crucible at 600°C for 3 hours, then cool and weigh until a constant weight is achieved.

Determination of Water-Soluble Extract Content

To determine the water-soluble extract content, accurately weigh approximately 5 grams of air-dried powdered medicinal herb. Transfer it to a stoppered flask, add 100 mL of chloroform-saturated water, shake frequently for the first 6 hours, and let it stand for 18 hours. Filter, evaporate 20 mL of the filtrate to dryness in a shallow, flat-bottomed dish preheated to 105°C and tared. Heat the remainder at 105°C until a constant weight is achieved. Calculate the % water-soluble extract content. According to Materia Medika Indonesia, the water-soluble extract content should be at least 5%.

Determination of Water-Soluble Extract Content in Ethanol
5 g of powdered medicinal plants were macerated with 100 mL of 70% ethanol for 24 hours using a stoppered flask, occasionally shaking for the first 6 hours, and then allowed to stand for 18 hours. Quickly filtered to prevent ethanol evaporation, 20 mL of the filtrate was evaporated in a tared, flat-bottomed dish over a water bath to dryness, and the remainder was heated at 105°C until a constant weight was achieved. The percentage concentration was calculated for the air-dried material.

Determination of Acid-Insoluble Ash Content
The ash obtained from the total ash content test was boiled with 25 mL of 0.1 N sulfuric acid for 5 minutes. Collect the acid-insoluble portion, filter it using ash-free filter paper, wash it with hot water, and then ignite it until a constant weight is achieved. The acid-insoluble ash content was calculated for the air-dried material.

Phytochemical Screening
Alkaloid Identification
A 0.5 mg sample was weighed and placed in a test tube, then H2SO4 was added and shaken until thoroughly mixed. The mixture was filtered and then Meyer's, Wagner's, and Dragendorff's reagents were added. Samples were confirmed positive for alkaloids by observing the presence of white, brown, and orange precipitates in each reagent.

Flavonoid Identification
A 0.5 gram sample was weighed, placed in a test tube, and 20 mL of ethanol was added. The sample was heated for 5 minutes. After heating, 10 drops of concentrated HCl were added. 0.2 grams of magnesium (Mg) powder was then added. The presence of flavonoids was indicated by the appearance of a red color.

Saponin Identification
The saponin test was performed by adding 20 ml of distilled water to 0.5 ml of lamtoro seed extract, shaking vigorously, and adding 1-3 drops of HCl. If foaming occurs, this indicates a positive result for saponins.

Tannin Identification
A 0.5 mg sample was weighed, placed in distilled water, and then added 1-2 drops of 1% FeCl3 solution. The presence of tannins is indicated by the appearance of a dark green or bluish-green color.

Antibacterial Testing
Antibacterial effectiveness testing was conducted using a diffusion method using paper discs. Paper discs with a diameter of 0.5 cm were aseptically taken using sterilized tweezers. The discs were dipped for 1 hour in varying concentrations of lamtoro seed ethanol extract, then placed on a medium containing the test bacteria. Each treatment used 5%, 10%, and 15% ethanol extract concentrations. The positive control (K(+) for S. aureus bacteria was vancomycin 30 mg, while the positive control (K(+) for E. coli bacteria was chloramphenicol 30 mg). Each test was repeated three times. The effectiveness of the lamtoro seed extract was determined by the inhibition zone obtained. The inhibition zone appeared clearer than the surrounding area and was free of bacteria. The inhibition zones were measured in millimeters using a digital caliper. Each treatment was carried out in triplicate, and the data were expressed as mean ± standard deviation (SD). The results were analyzed statistically using one-way analysis of variance (ANOVA), followed by post-hoc testing (Tukey’s HSD) to determine significant differences between extract concentrations and controls. Antibacterial activity was further categorized based on inhibition zone diameter following the criteria of Davis and Stout, where <5 mm was considered weak, 5–10 mm moderate, and >10 mm strong.

RESULT

Determination and Standardization of Simplisia

The initial research results, namely plant determination, showed that the plant sample used in this study was indeed *Leucaena leucocephala*, belonging to the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Fabales, Family Fabaceae, Genus Leucaene, and Species Leucaena leucocephala.

Figure 1. Lamtoro Seed Drying Process

Characterization parameters conducted on lamtoro seed simplisia included analysis of water content, total ash content, water-soluble extract content, ethanol-soluble extract content, and acid-insoluble extract content. The results of the simplisia standardization are in Table 1.

Table 1.
Standardization Results of *Leucaena leucocephala* Seeds

Determination	Result (%)	Requirement According to MMI (%)	Remark
Moisture content	0.076%	< 10%	Requirement
Total ash content	0.806%	< 8%	Requirement
Acid-insoluble ash content	6.968%	< 11%	Requirement
Water-soluble extractive	36%	> 5%	Requirement
Ethanol-soluble extractive	66%	> 5%	Requirement

Phytochemical Screening Results

Qualitative examination of chemical compounds, commonly known as phytochemical screening, aims to determine the types of compounds contained in a plant.

Table 2.
Phytochemical Screening Results

Secondary Metabolite Content	Reagent	Test Result	Observation
Alkaloid	Meyer	+	Formation of white/yellowish precipitate
	Wagner	+	Formation of brownish-black precipitate
	Dragendorff	+	Formation of orange precipitate
Flavonoid	HCl and Mg powder	+	Produces orange-yellow color
Saponin	Frothing test	+	Produces stable foam
Tannin	$FeCl_3$	+	Produces dark purple coloration

Note: (+): detected, (–): not detected

Antibacterial Test Results

This test aimed to observe the antibacterial effectiveness of ethanol extract of *Leucaena leucocephala* (Lam.) de Wit seeds against the growth of *S. aureus* and *E. coli*.

Table 3.
Dilution Results

No.	Aquadest (mL)	Extract (mL)	Dilution Result
1	3.4 mL	1.6 mL	5%
2	1.7 mL	3.3 mL	10%
3	1 mL	5 mL	15%

The test results were observed by measuring the diameter of the inhibition zone around the paper disc. The criteria for antibacterial activity strength were determined according to Davis and Stout (1971), namely: very strong if the diameter is ≥ 20 mm, strong if 10–20 mm, moderate if 5–10 mm, and weak if < 5 mm (Sarmira et al., 2021).

Table 4.
Test Results of Concentration Against the Growth of *S. aureus* and *E. coli*

Test Sample	Concentration	Bacterial Growth
Extract	5%	(+)
Ethanol	10%	(+)
Leucaena Seeds	15%	(+)

Table 5.
Results of Antibacterial Effectiveness Tests on *S. aureus* Using Lamtoro Seed Extract

No.	Concentration (%)	U1 (mm)	U2 (mm)	U3 (mm)	Average (mm)
1	5%	6.15	6.18	6.18	6.17
2	10%	6.20	6.22	6.20	6.20
3	15%	6.34	6.58	6.36	6.42
4	Vancomycin 30 µg (K+)	15.80	15.38	15.30	15.49
5	Aquadest (K–)	0	0	0	0

Table 6.
Results of Antibacterial Effectiveness Tests on *E.coli* Using Lamtoro Seed Extract

No.	Concentration (%)	U1 (mm)	U2 (mm)	U3 (mm)	Average (mm)
1	5%	6.18	6.28	6.26	6.24
2	10%	6.18	6.30	6.32	6.26
3	15%	6.38	6.46	6.50	6.44
4	Chloramphenicol 30 µg (K+)	21.70	21.66	21.62	21.66
5	Aquadest (K–)	0	0	0	0

Disc Diffusion Against *S. aureus* and *E. coli*

The antibacterial effectiveness of lamtoro seed extract against *S. aureus* and *E. coli* was tested using a 6 mm disc diffusion method using agar media. The results of the lamtoro (*Leucaena leucocephala*) seed extract measurement against the test bacteria are shown in Figure 2 below.

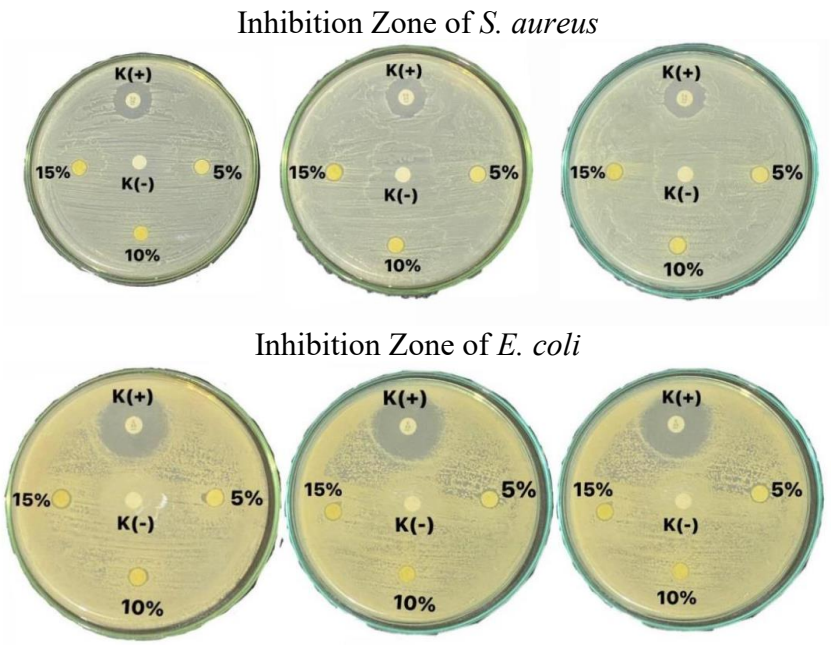

Figure 2. Results of the Antibacterial Effectiveness Inhibition Zone of Ethanol Extract of Lamtoro Seeds Against *S. aureus* and *E. coli* Bacteria Using the Disc Diffusion Method

DISCUSSION

Lamtoro (Leucaena leucocephala), belongs to the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida, Order Fabales, Family Fabaceae, Genus Leucaene, Species Leucaena leucocephala. The characterization parameters carried out on the lamtoro seed simplisia include analysis of water content, total ash content, water-soluble extract content, ethanol-soluble extract content, and acid-insoluble extract content. The results of the standardization of the simplisia are in Table 1. The purpose of determining the water content is to determine how much water is present in the simplisia. The quality and safety of the simplisia will be affected by the high amount of water, which will facilitate the growth of microorganisms. This water content test is to determine the water residue after the drying process. The water content obtained from the lamtoro seed simplisia is 0.076%, which is in accordance with the requirements of MMI (Materia Medika Indonesia), which is not more than 10%. Too high a water content allows microbes to grow the material, which can reduce the stability and pharmacological activity of the simplisia. The total ash content of lamtoro seed simplisia is 0.806%, which meets the quality requirements of <8%. The purpose of testing the total ash content is to determine the internal and external mineral content from the beginning to the end of the process.

The physical properties of simplisia can be affected by the levels of inorganic compounds or minerals contained therein. The low total ash content produced in simplisia indicates that the simplisia does not contain many minerals. The purpose of testing the acid-insoluble ash content is to determine the level of product cleanliness during the processing process. Mineral or metal contamination can cause acid-insoluble ash. According to research that has been conducted, the acid-insoluble ash content of lamtoro seed simplisia is 6.968%, which meets the quality requirements of less than 11%. The acid-insoluble ash content indicates that the lamtoro seed simplisia contains silicates derived from soil or sand (Andini & Putri, 2021). The results show that the water-soluble essence content in lamtoro seed simplisia is 36%, meeting the requirements according to MMI (Materia Medika Indonesia) which is more than 5%. The determination of water-soluble extract content is used to identify polar compounds with the same polarity as water. The determination of an ethanol-soluble extract content of 66% indicates that the ethanol-soluble extract content in the lamtoro seed simplisia meets the MMI (Materia Medika Indonesia) requirement of more than 5% (Warnis et al., 2021).

Table 2 shows that the ethanol extract of lamtoro seeds is known to contain secondary metabolites in the form of alkaloids, flavonoids, saponins, and tannins. A test is considered positive if a color change occurs after the extract reacts with a specific reagent. Testing for alkaloids in the ethanol extract of lamtoro seeds yields a positive result. This is indicated by the appearance of a white precipitate in Mayer's reagent, a blackish-brown precipitate in Wagner's reagent, and an orange-yellow precipitate in Dragendorff's reagent. A light brown to orange color is a positive indicator of alkaloids, and increasing color intensity indicates a higher alkaloid content. This precipitation mechanism occurs because the nitrogen atom in the alkaloid structure has a lone electron pair that can replace ions in the test solution (Isda & Nilasari, 2024). Alkaloids themselves function as antibacterials by inhibiting the formation of peptidoglycan in bacterial cell walls, causing the cells to lose integrity and eventually die (Febrianti et al., 2022).

Flavonoid tests also showed positive results, as evidenced by the formation of an orange to reddish-orange solution. The flavonoid content in the ethanol extract of lamtoro seeds is relatively high. Flavonoid compounds act as antibacterials by disrupting cytoplasmic membrane function through the release of transduction energy and reducing bacterial motility. The hydroxyl groups in flavonoids cause changes in organic components and inhibit nutrient transport, ultimately being toxic to bacteria (Isda & Nilasari, 2024). A positive saponin result is indicated by the formation of foam after the extract is shaken for approximately 10 seconds, which persists even after the addition of HCl. Saponins have antibacterial activity by denaturing proteins. Furthermore, these compounds also act as antioxidants because they can neutralize superoxide through the formation of hyperoxide intermediates, thereby protecting biomolecules from free radical damage (Hasan et al., 2022). The tannin test on the ethanol extract of lamtoro seeds also yielded positive results. The reaction with FeCl3 produces a purple-black to dark black solution, indicating the presence of condensed tannins (Isda & Nilasari, 2024). Tannins, which are polyphenols with a polyhydroxy phenol core, can bind to proteins, precipitate them, and inhibit protein synthesis. The antibacterial activity of tannins is supported by the ability of their phenolic compounds to precipitate proteins, thus disrupting bacterial survival.

This test aims to determine the antibacterial effectiveness of ethanol extract of Leucaena leucocephala (Lam.) de Wit seeds against the growth of S. aureus and E. coli. The antibacterial activity of Leucaena leucocephala seed extract was tested using the disc diffusion method against Staphylococcus aureus and Escherichia coli. Prior to testing, the turbidity of the bacterial suspension was adjusted to a McFarland 0.5 standard. The treatment consisted of three extract concentration variations: 5%, 10%, and 15%. Vancomycin was used as a positive control for S. aureus and chloramphenicol for E. coli, while distilled water served as a negative control. The extract was diluted to obtain the desired test concentration. This dilution process reduces the solute content in the stock solution. From the ethanol extract of Leucaena seeds, test solutions were prepared at concentrations of 5%, 10%, and 15%, depending on the antibacterial testing requirements (Nadhm & AL-Waheeb, 2025).

According to Table 5, the average diameter of the inhibition zone formed in the 5% lamtoro seed ethanol extract was 6.17 mm, 6.20 mm at 10%, and 6.42 mm at 15%. The positive control using 30 µg vancomycin produced an average inhibition zone of 15.49 mm, while the negative control, distilled water, showed no inhibition zone (0 mm). These results demonstrate that the lamtoro seed ethanol extract is capable of inhibiting the growth of S. aureus at all tested concentrations, although its activity was much lower than that of the positive control. This is understandable, considering that vancomycin is a bacteriostatic compound with a specific mechanism of action, while plant extracts contain naturally occurring active compounds with weaker effectiveness. Furthermore, there was a tendency for the inhibition zone diameter to increase with increasing extract concentration. The highest concentration (15%) produced the largest inhibition zone, indicating that

the amount of bioactive compounds (Hazim et al., 2022b) in the extract directly influences the level of bacterial inhibition. A diagram of the inhibition zone measurement results is presented.

According to Table 6, the average inhibition zone diameter of the lamtoro seed ethanol extract against E. coli at a concentration of 5% was 6.24 mm, at a concentration of 10% it was 6.26 mm, and at a concentration of 15% it reached 6.44 mm. The positive control using 30 µg chloramphenicol showed an average inhibition zone of 21.66 mm, while the negative control, distilled water, did not produce an inhibition zone. These results indicate that the lamtoro seed ethanol extract has antibacterial activity against E. coli at all tested concentrations, although the resulting activity was still much lower than that of the positive control antibiotic. This is understandable because chloramphenicol is an antibiotic with a specific mechanism of action, while lamtoro extract contains natural bioactive compounds with relatively weaker effectiveness. Furthermore, the research results also showed a pattern of increasing inhibition zones with increasing extract concentration. The highest concentration (15%) produced the largest inhibition zone, indicating that the active compounds in the extract contribute concentration-dependently to bacterial inhibition.

According to the Indonesian Pharmacopoeia, IV edition (1995), an inhibition zone is categorized as effective if the diameter formed is in the range of 14–16 mm. Based on this reference, the research results showed that ethanol extract of lamtoro seeds at concentrations of 5%, 10%, and 15% was classified as moderate against S. aureus and E. coli. As a positive control, the use of 30 µg of vancomycin against S. aureus produced strong inhibition, while 30 µg of chloramphenicol against E. coli demonstrated very strong antibacterial activity. Conversely, the negative control, distilled water, showed no inhibition zones for either S. aureus or E. coli, confirming its lack of antibacterial activity.

This proves that lamtoro seed extract has antibacterial potential, although its effectiveness is lower than standard antibiotics. This antibacterial activity is thought to originate from the content of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and mimosine. These compounds work through various mechanisms, including damaging cell membranes, disrupting DNA and protein synthesis, inhibiting energy metabolism, and binding ions essential for bacterial growth. Because S. aureus is a Gram-positive bacterium with a relatively simpler peptidoglycan cell wall structure, the active compounds in lamtoro seeds are able to penetrate and suppress bacterial growth in a concentration-dependent manner (Odekanyin et al., 2024).

The ethanol extract of lamtoro seeds against E. coli was tested three times. Alkaloids work by damaging the peptidoglycan component so that the bacterial cell wall is not formed properly, flavonoids form complexes with extracellular proteins that disrupt membrane integrity, tannins can damage cell permeability by shrinking the cell wall, while saponins are generally more effective against Gram-positive bacteria. Because E. coli is a Gram-negative bacterium with a cell wall composed of lipoprotein, lipopolysaccharide, phospholipid, and thin peptidoglycan, its resistance to active compounds is higher than that of S. aureus. In comparison, the positive control for E. coli (chloramphenicol) produced a larger inhibition zone (21.66 mm) than the positive control for S. aureus (vancomycin, 15.49 mm). This indicates that antibacterial effectiveness is greatly influenced by the type of bacteria tested, the nature of the active compound contained in the extract, and the concentration used. The higher the extract concentration, the larger the diameter of the inhibition zone formed, because the greater amount of active substance is working.

CONCLUSION

The ethanol extract of Leucaena leucocephala seeds was found to contain secondary metabolites such as alkaloids, flavonoids, saponins, and tannins, which contribute to its antibacterial activity. Standardization results showed that the simplicia met the quality requirements of Materia Medika

Indonesia. Antibacterial testing against Staphylococcus aureus and Escherichia coli revealed inhibition zones ranging from 6.17–6.44 mm at concentrations of 5–15%, indicating moderate activity with increasing inhibition along with higher concentrations. Although the activity was weaker compared to standard antibiotics (vancomycin 15.49 mm and chloramphenicol 21.66 mm), the findings confirm the potential of Leucaena leucocephala seed extract as a natural antibacterial agent that warrants further purification and optimization.

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