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Analysis of Antibacterial Potential of Tingkem Extract (*Bischofia javanica* Blume) Leaves Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Bischofia javanica Blume (*Tingkem*) is a traditional medicinal plant known for its diverse biological activities, including antidiabetic, anti-inflammatory, antioxidant, and antibacterial properties. This study was conducted to evaluate the antibacterial potential of the methanolic leaf extract of Tingkem against *Staphylococcus aureus* and *Escherichia coli* using the disc diffusion method. The extract was tested at concentrations of 5%, 10%, 20%, and 40%, while its chemical constituents were characterized using liquid chromatography-mass spectrometry (LC-MS). The antibacterial assay demonstrated that all tested concentrations were capable of inhibiting the growth of both bacterial species, producing inhibition zones within the strong activity category (10–20 mm). LC-MS analysis revealed the presence of 53 compounds in the methanolic leaf extract of Tingkem. Among these, 15 compounds showed high similarity scores ranging from 80.6% to 99.5%, including seven compounds reported to possess antibacterial activity: Diprophylline, Furaltadone, Acefylline, Drazidox, Emodin 8-glucoside, Algestone, and Androstane-3,6,17-triol. These findings indicate that the methanolic leaf extract of *B. javanica* has promising antibacterial potential and may serve as a valuable source for the development of natural antibacterial agents.

Keywords: Antibacterial; *B. javanica* Blume; *E. coli*; LC-MS; *S. aureus*.

INTRODUCTION

Indonesia is a country with rich biodiversity, encompassing numerous plant species with potential applications as sources of natural medicinal compounds, one of which is *Tingkem* (*Bischofia javanica* Blume) (Panjaitan, 2023). The species was identified by the Herbarium Medanense, with its morphological characteristics and taxonomic classification presented in Figure 1 and Table 1.



Figure 1. Morphology of Tingkem Plant (*B. javanica* Blume).

Table 1. Classification of Tingkem Plants Based on Medanense Herbarium Identification.

Taxonomy	Classification
Kingdom	Plantae
Division	Spermatophyta
Class	Dicotyledoneae
Order	Malpighiales
Family	Phyllanthaceae
Genus	*Bischofia*
Species	*B. javanica* Blume
Local Name	Tingkem/Gadog

Medicinal plants remain important sources of novel therapeutic agents, particularly in addressing infectious diseases and antibacterial resistance. Among the medicinal plants traditionally used in Indonesia, *B. javanica* Blume has gained attention due to its diverse pharmacological properties and long-standing use in traditional medicine (Ihwan et al., 2018).

Phytochemical studies have revealed that *B. javanica* Blume leaves contain various secondary metabolites, including flavonoids, alkaloids, tannins, steroids, saponins, phenolic compounds, and triterpenoids (Butar Butar, 2023). These bioactive compounds exhibit multiple biological activities, such as anti-inflammatory,

antileukemic, antiparasitic, antinociceptive, and antibacterial effects, highlighting the potential of *B. javanica* Blume as a natural source of antibacterial agents (Sarmah, 2023).

Several studies have demonstrated the antibacterial activity of *B. javanica* Blume extracts obtained using various solvents, including methanol, n-hexane, ethanol, and ethyl acetate. Extracts from both the bark and leaves have shown inhibitory effects against pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli*, which are attributed to the plant's secondary metabolites (Ati et al., 2021; Idramsa et al., 2022; Sarmah et al., 2020). Methanol is commonly used as an extraction solvent because of its effectiveness in extracting a wide range of polar and semi-polar bioactive compounds (Indarto et al., 2019).

The present study focuses on *S. aureus* and *E. coli*, two clinically significant bacterial species that represent Gram-positive and Gram-negative groups, respectively. Their distinct cell wall structures influence their susceptibility to antibacterial compounds, making them suitable models for evaluating the spectrum of antibacterial activity. Moreover, both bacteria are commonly associated with human infections, ranging from skin and soft tissue infections to gastrointestinal diseases (Islamiyati and Husen, 2025; Salim et al., 2023; Sumampouw, 2018).

Given the continuing burden of infectious diseases worldwide, the search for effective antibacterial agents from natural resources remains an important area of research (Pratiwi, 2017; Utomo et al., 2018). Antibacterial activity is commonly evaluated using the disc diffusion method because it is simple, reliable, sensitive, and easy to interpret (Nurhayati et al., 2020; Rezaldi et al., 2023). In addition, the characterization of bioactive compounds can be performed using liquid chromatography–mass spectrometry (LC-MS), a powerful analytical technique capable of identifying chemical constituents based on retention time, molecular mass, and fragmentation patterns (Sumartini et al., 2020).

Although the antibacterial activity of various parts of *B. javanica* Blume, including its leaves, has been reported, information on the antibacterial effectiveness of its methanolic leaf extract remains limited, and the bioactive compounds potentially responsible for this activity have not been comprehensively characterized by LC-MS. Therefore, the present study aimed to evaluate the antibacterial activity of the methanolic leaf extract of *B. javanica* Blume against *S. aureus* and *E. coli* using the disc diffusion method, and to identify, through LC-MS analysis, the bioactive compounds that may contribute to the observed antibacterial effects.

MATERIALS AND METHODS

Plant material

Leaves of *B. javanica* Blume (*Tingkem*) were collected in 28 August 2021 from Ketambe Village, Southeast Aceh, Indonesia, at the foothills of Mount Leuser. The plant material was identified and authenticated by the Herbarium Medanense, University of North Sumatra, with identification certificate number 6562/MEDA/2021. A voucher specimen (HM-003-21-UNSAM) was deposited at the herbarium for future reference.

The equipment used was LC-MS, rotary evaporator, analytical balance, Laminar Airflow (LAF), incubator, autoclave, refrigerator, petri dish, inoculating loop, Erlenmeyer flask, measuring cylinder, glass funnel, rack and test tube, micropipette, spirit burner, spray bottle, disc paper, caliper, stirring rod, spatula, tweezers, cotton swab, aluminum foil, paper, gauze, syringe, and cotton.

The chemicals used were methanol, 0.1% formic acid in water, 0.1% formic acid in acetonitrile, distilled water, 70% ethanol, spirit, Mueller Hinton Agar (MHA), Nutrient Agar (NA), Nutrient Broth (NB), chloramphenicol, McFarland standard solution, 0.9% NaCl, *S. aureus* culture, and *E. coli* culture.

Procedures

Sample Extract Preparation

The collected leaves of *B. javanica* Blume were cleaned, air-dried, and ground into powder. Approximately 500 g of the dried powder was extracted by maceration with methanol at room temperature for 3 × 24 h, followed by filtration. The extraction procedure was repeated until a clear filtrate was obtained. The resulting filtrates were pooled and concentrated under reduced pressure using a rotary evaporator to obtain the crude methanolic leaf extract (Halimatussakdiah et al., 2025).

Tool Sterilization

All equipment is cleaned and rinsed using distilled water. The sterilization method is adjusted to the type of material, for heat-resistant equipment it is autoclaved at 121°C and 2 atm for 15 min, metal equipment is heated directly with a spirit lamp until it glows and equipment that is not heat-resistant is sterilized with 70% ethanol (Yanti and Mitika, 2017).

Making Nutrient Agar (NA) Media

The NA medium was prepared by dissolving 1.4 g of NA in 50 mL of distilled water. The solution, which had been placed in an Erlenmeyer flask, was sterilized by autoclaving at 121°C and 2 atm for 15 min (Yanti and Mitika, 2017).

Making Nutrient Broth (NB) Media

NB media serves as a material for preparing bacterial inoculum solutions which is done by dissolving 1.3 g of NB in 100 mL of distilled water, then stirred until completely dissolved. The dissolved NB media is divided

into two Erlenmeyer flasks with 50 mL each and then closed using cotton. One flask was used for each bacterium. The media is then sterilized using an autoclave at 121°C and 2 atm for 15 min (Yanti and Mitika, 2017).

Bacterial Rejuvenation

Twenty-five milliliters of NA medium was poured into sterile Petri dishes and allowed to solidify. Each bacterial strain was then aseptically streaked onto the NA surface and incubated at 37°C for 24 h. After incubation, one colony of each bacterium was aseptically transferred from the solid NA using a sterile inoculating loop into NB medium and incubated at 37°C for 24 h (Mahmudah and Atun, 2017).

Making Bacterial Suspensions

A loopful of each pure bacterial culture from the NB medium was suspended in a test tube containing 3 mL of sterile 0.9% NaCl solution and mixed until homogeneous. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) (Misna and Diana, 2016).

Preparation of Mueller Hinton Agar (MHA) Media

A total of 3.8 g MHA was weighed and dissolved in 100 mL of distilled water for each type of bacteria tested. The media was then sterilized using an autoclave at 121°C for 15 min at 121°C (Mahmudah and Atun, 2017).

Antibacterial Activity Test of the Methanolic Leaf Extract of B. javanica Blume

Antibacterial activity testing was conducted against *S. aureus* and *E. coli* using the disc diffusion method. In this study, the extract was tested at four concentrations: 5%, 10%, 20%, and 40%. The positive control used was chloramphenicol, and the negative control was distilled water.

Bacteria cultured in NB medium were spread onto MHA plates using a sterile cotton swab. Paper discs impregnated with different concentrations of the extract, positive control, and negative control were then placed on the agar surface. Each treatment was performed in triplicate for both *S. aureus* and *E. coli*. The plates were incubated at 37°C for 24 h. After incubation, the inhibition zones formed around the discs were measured using a vernier caliper (Mahmudah and Atun, 2017; Suherman et al., 2018). Antibacterial activity was categorized based on inhibition zone diameter according to Davis and Stout (1971), as cited in Suherman et al. (2018) (Table 2).

Table 2. Categories of antibacterial activity based on inhibition zone diameter (Davis and Stout, 1971), as cited in Suherman et al., 2018.

Inhibition Zone Diameter (mm)	Growth Inhibition Category
< 5	Weak
5 – 10	Moderate
11 – 20	Strong
> 20	Very Strong

LC-MS Analysis

Approximately 1.5 mg of the methanolic leaf extract of Tingkem (*B. javanica* Blume) was dissolved in 1.5 mL of methanol, sonicated for 15–30 min, and filtered. A 50 µL aliquot of the filtrate was diluted with methanol to a final volume of 1000 µL and vortexed to obtain a 50 ppm solution. Subsequently, 20 µL of this solution was diluted with 0.1% formic acid in water to 1000 µL and vortexed again, giving a final concentration of approximately 1 ppm (1 µg/mL) for injection. A 10 µL aliquot of the prepared sample was injected into an LC-MS system equipped with a quadrupole time-of-flight (QTOF) analyzer. Separation was performed on a ZORBAX Eclipse Plus C18 RRHD column (2.1 mm × 50 mm, 1.8 µm) at 30°C with a flow rate of 0.300 mL/min. The mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Ions were detected over a range of 50–1200 *m/z* in positive electrospray ionization (ESI) mode.

RESULTS AND DISCUSSION

Extraction of *B. javanica* Blume

Maceration of *B. javanica* Blume leaves with methanol yielded a crude methanolic leaf extract (Figure 2).



Figure 2. Methanolic Leaf Extract of *B. javanica* Blume.

Antibacterial Activity of *B. javanica* Blume against *S. aureus*

In the present study, the methanolic leaf extract of *B. javanica* Blume demonstrated the ability to inhibit the growth of *S. aureus*, as evidenced by the inhibition zones observed and presented in Table 3 and Figure 3.

Table 3. Inhibition Zone Diameter in *S. aureus*.

Sample	Sample Concentration (%)	Replicates (mm)			Average (mm)	Inhibition Zone Category
		1	2	3		
Methanolic leaf extract	5	10.1	11.9	12.9	11.6	Strong
	10	13.3	14.8	15.1	14.4	Strong
	20	15.2	16.5	17.3	16.3	Strong
	40	16.9	17.1	18.0	17.3	Strong
Positive Control		36.1	36.3	37.3	36.6	Very Strong
Negative Control		0	0	0	0	No Inhibition

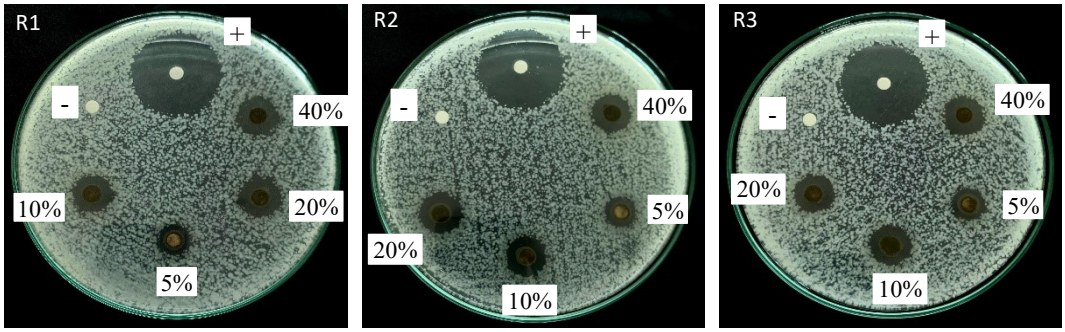

Figure 3. Inhibition zones of the methanolic leaf extract of *B. javanica* Blume against *S. aureus*. R1–R3 indicate the three replicates; 5%, 10%, 20%, and 40% are extract concentrations (w/v); (+) is the positive control (chloramphenicol); and (−) is the negative control (distilled water).

According to the data shown in Table 3 and Figure 3, the inhibition zones produced by the methanolic leaf extract at concentrations of 5%, 10%, 20%, and 40% were categorized as exhibiting strong antibacterial activity based on the classification proposed by Davis and Stout (1971), as cited in Suherman et al. (2018). Furthermore, the antibacterial assay against *S. aureus* was performed in triplicate and showed a high degree of consistency with no significant variation. This consistency indicates that the experimental data were reliable and valid. Similar approaches have been reported by Syari and Aprilla (2022), who emphasized that conducting experiments in triplicate contributes to improving the accuracy and reproducibility of research findings.

Antibacterial Activity of *B. javanica* Blume against *E. coli*

The methanolic leaf extract of *B. javanica* Blume also exhibited inhibitory activity against *E. coli*, as indicated by the formation of inhibition zones at all tested concentrations, which were classified as showing strong antibacterial activity according to the criteria of Davis and Stout (1971), as cited in Suherman et al. (2018). Consistent results across triplicate assays further confirmed the reliability of the experimental data, as shown in Table 4 and Figure 4.

Table 4. Inhibition Zone Diameter in *E. coli.*

Sample	Sample Concentration (%)	Replicates (mm)			Average (mm)	Inhibition Zone Category
		1	2	3		
Methanolic leaf extract	5	13.2	11.4	11.6	12.1	Strong
	10	15.6	16.3	16.0	16.0	Strong
	20	16.3	17.3	17.1	16.9	Strong
	40	18.1	18.3	18.1	18.2	Strong
Positive Control		36.0	34.9	35.5	35.5	Very Strong
Negative Control		0	0	0	0	No Inhibition

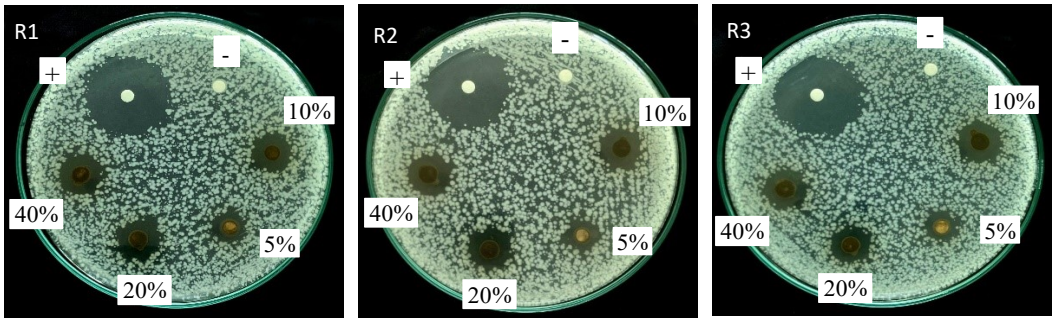

Figure 4. Inhibition zones of the methanolic leaf extract of *B. javanica* Blume against *E. coli*. R1–R3 indicate the three replicates; 5%, 10%, 20%, and 40% are extract concentrations (w/v); (+) is the positive control (chloramphenicol); and (−) is the negative control (distilled water).

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

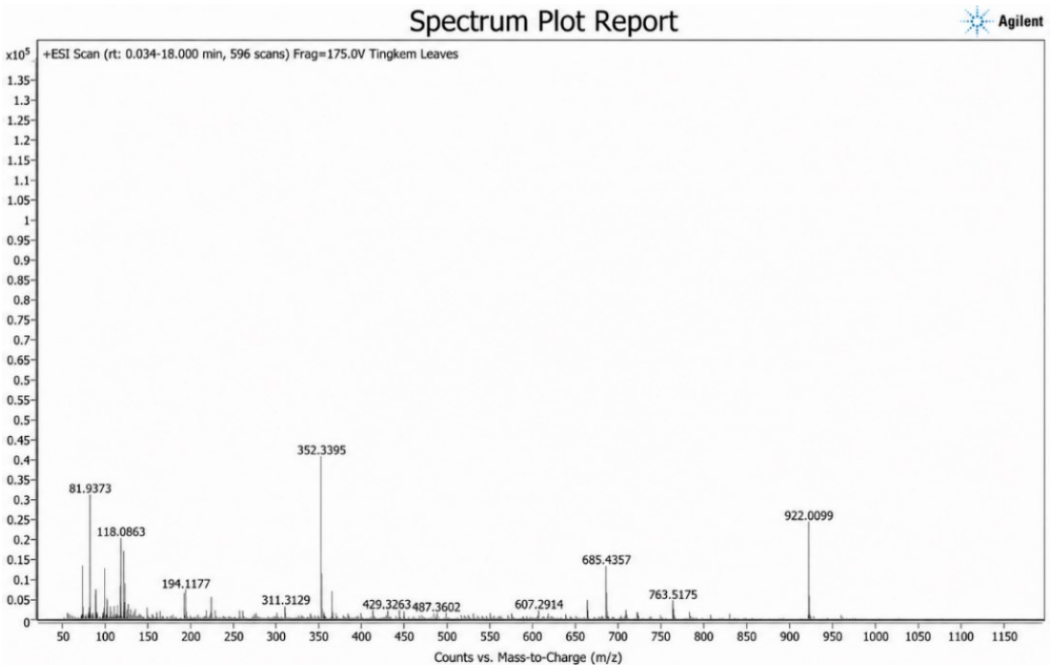

Figure 5. Mass Spectrum of the Methanolic Leaf Extract of *B. javanica* Blume.

The principle of LC-MS is based on the separation of compounds in a mixture according to their polarity, which are then analyzed by a mass spectrometer to obtain information on the structure, molecular mass, and identity of the compounds. This system consists of a chromatography column (stationary phase) and a solvent (mobile phase) that carries the sample through the column. Each compound has a different retention time depending on its interaction with the stationary phase. Once separated, the compounds are detected as a mass spectrum consisting of peaks representing each compound. LC-MS analysis aims to identify organic, inorganic, and biological compounds in a complex sample, generally originating from the natural environment (Lestari, 2022). LC-MS analysis of the extract produced a mass spectrum (Figure 5), which shows the abundance of ions detected as a function of their mass-to-charge ratio (*m/z*). Each peak corresponds to an ion of a specific *m/z* value, from which the molecular mass and elemental formula of the compounds can be inferred (Bolani et al., 2024).

LC-MS analysis identified 53 compounds in the methanolic leaf extract of *B. javanica* Blume. Among these, 15 compounds with similarity scores of 80.6–99.5% are listed in Table 5.

Table 5. Results of LC-MS Analysis of the Methanolic Leaf Extract of *B. javanica* Blume.

Compound	Formula	RT (min)	Mass (Da)	Abund (counts)	Score (%)
MADU / 2-Deoxy-5-(methylamino) uridine	$C_{10}H_{15}N_3O_5$	0.509	257.1023	36444.40	91.39
Trehalose	$C_{12}H_{22}O_{11}$	0.517	342.1164	35941.26	96.73
Diprophylline	$C_{10}H_{14}N_4O_4$	0.612	254.1005	123509.54	93.90
Furaltadone	$C_{13}H_{16}N_4O_6$	0.647	324.1064	54533.68	80.62
Triacetin	$C_9H_{14}O_6$	0.821	218.0794	21005.61	99.38
Acefylline (Theophyllineacetic acid)	$C_9H_{10}N_4O_4$	1.024	238.0691	91834.12	90.29
Cymoxanil (Curzate)	$C_7H_{10}N_4O_3$	1.032	198.0764	52877.93	91.98
Acid Yellow 11	$C_{16}H_{14}N_4O_4S$	1.932	358.0719	67864.87	86.61
trans-trans Muconic acid	$C_6H_6O_4$	3.661	142.0266	80737.50	87.35
Drazidox	$C_{10}H_{10}N_4O_3$	3.723	234.0741	132772.13	91.11
Emodin 8-glucoside	$C_{21}H_{20}O_{10}$	4.277	432.1056	51565.77	99.50
N-Lauryldiethanolamine	$C_{16}H_{35}NO_2$	7.058	273.2671	67199.11	96.44
Algestone	$C_{21}H_{30}O_4$	10.611	346.2121	68914.70	86.21
DIDP / Diisodecyl phthalate	$C_{28}H_{46}O_4$	11.578	446.3399	105172.98	98.45
Androstane-3,6,17-triol	$C_{19}H_{32}O_3$	12.286	308.2353	36756.75	99.40

Based on Table 5, there are seven secondary metabolite compounds that show significant biological activity against bacterial growth, both Gram-positive and Gram-negative, namely Diprophylline and Acefylline which are alkaloid derivatives (Ghosh et al., 2024; Kitanov et al., 2015). Furaltadone belongs to the nitrofuran class (Najah et al., 2024), Drazidox is a quinoxaline derivative (Pereira et al., 2015), Emodin 8-glucoside is an anthraquinone derivative (Okon et al., 2023), Algestone and Androstane-3,6,17-triol are steroid derivatives (Kumar et al., 2019; Sharma et al., 2022).

Discussion

Extraction is a separation process used to isolate bioactive compounds from a material matrix, either in solid or liquid form, through the use of an appropriate solvent (Sholihah et al., 2017). In the present study, Tingkem leaves were extracted using the maceration method. This technique involves immersing the plant material in a suitable solvent to facilitate the dissolution of bioactive constituents without the application of intensive heat. Several factors may affect the efficiency of the extraction process, including the type of solvent, extraction duration, temperature, solvent-to-material ratio, and particle size of the sample. One of the main advantages of maceration is its ability to preserve the stability of thermolabile compounds by minimizing exposure to high temperatures. During the soaking process, differences in osmotic pressure between the intracellular and extracellular environments cause disruption of the cell walls and membranes, allowing secondary metabolites contained within the cytoplasm to be released and dissolved into the organic solvent (Pratiwi et al., 2021).

In this study, the antibacterial efficacy of the extract was assessed against two representative bacterial species, namely the Gram-positive bacterium *S. aureus* and the Gram-negative bacterium *E. coli* (Goetie et al., 2022). *S. aureus* is a commensal bacterium that naturally inhabits the skin, glandular tissues, and the anterior nasal cavity (anterior nares). While it is generally considered part of the normal human microbiota, this bacterium can become an opportunistic pathogen and cause infections when host immune defenses are compromised (Nadhilla, 2014). *E. coli* is a bacterium that naturally inhabits the gastrointestinal tract of humans and other warm-blooded animals. Although it is generally considered a normal component of the intestinal microbiota, *E. coli* can become pathogenic when its population increases excessively within the intestine or when it spreads to sites outside the digestive tract, potentially leading to conditions such as diarrhea (Hutasoit, 2020).

This assay evaluated the antibacterial activity of the methanolic leaf extract of *B. javanica* Blume against *S. aureus* and *E. coli*. As shown in Tables 3 and 4, the extract exhibited strong antibacterial activity against both species, as indicated by the inhibition zones formed. The size of the inhibition zones is influenced by several factors, including the presence and concentration of bioactive compounds in the extract, the type and density of the test microorganisms, and the concentration of the extract applied. In general, higher concentrations of antibacterial compounds and greater extract concentrations tend to produce larger inhibition zones, reflecting a stronger inhibitory effect on bacterial growth (Lestari, 2022).

The inhibition zone diameters observed against *E. coli* and *S. aureus* were relatively similar, however *E. coli* generally exhibited slightly larger inhibition zones. This difference may be attributed to variations in the cell wall structures of Gram-negative and Gram-positive bacteria. Although Gram-negative bacteria such as *E. coli* possess a more complex cell envelope, consisting of an outer membrane containing lipopolysaccharides and a thin peptidoglycan layer, this structure did not completely prevent the penetration of antibacterial compounds present in the extract. In contrast, the cell wall characteristics of *S. aureus*, a Gram-positive bacterium, may have reduced its interaction with or absorption of the active compounds, resulting in

comparatively smaller inhibition zone diameters (Maharani et al., 2016).

Chloramphenicol was used as the positive control because of its broad-spectrum antibacterial activity, which enables it to inhibit the growth of a wide range of Gram-positive and Gram-negative bacteria. Distilled water was employed as the negative control due to its absence of antibacterial properties, ensuring that the solvent itself did not influence the observed bacterial growth or inhibition patterns (Pattipeilohy et al., 2022). The results also demonstrated that increasing the extract concentration was associated with a corresponding increase in the size of the inhibition zone, indicating enhanced antibacterial activity. This relationship is likely due to the higher concentration of active antibacterial compounds present in the extract at increased concentrations. Greater amounts of these bioactive compounds are believed to improve their ability to penetrate bacterial cells, disrupt essential metabolic processes, and ultimately induce bacterial cell death, even in the deeper regions of the microbial cell structure (Fransisca et al., 2020).

The antibacterial assay further revealed that the methanolic leaf extract of *B. javanica* Blume effectively inhibited the growth of pathogenic bacteria within 24 h of incubation. These findings are consistent with previous studies that have reported the antibacterial potential of *B. javanica* Blume and its ability to suppress the growth of pathogenic microorganisms (Ati et al., 2021; Idramsa et al., 2022; Sarmah et al., 2020). Therefore, the antibacterial activity observed in this study can be attributed to the presence of active compounds in the methanolic leaf extract. The results of the biological assays support the hypothesis that the antibacterial effects of *B. javanica* Blume are closely associated with its secondary metabolites and other bioactive constituents, which contribute significantly to its inhibitory activity against pathogenic bacteria.

Diprophylline and Acefylline have antibacterial potential as alkaloid derivatives, the most common secondary metabolites in plants, which generally contain nitrogen atoms in heterocyclic rings. These compounds have biological activity, primarily as antibacterials, by disrupting peptidoglycan formation, preventing bacterial cell walls from forming properly and causing cell death (Ghosh et al., 2024; Kitanov et al., 2015). Furaltadone belongs to the nitrofuran class of antibiotics, which exhibit broad-spectrum antibacterial activity (Najah et al., 2024).

Furthermore, Drazidox is a quinoxaline derivative. Quinoxaline is a heterocyclic compound in which two nitrogen atoms replace two carbon atoms (at positions 1 and 4) in the naphthalene ring, forming a benzene ring fused to a pyrazine ring. Quinoxaline derivatives have been applied in corrosion inhibition, porphyrin synthesis, and electroluminescent materials, and exhibit a broad range of biological activities, including effects on the nervous system as well as antifungal, antiviral, antimalarial, anticancer, and antibacterial effects (Pereira et al., 2015). Anthraquinone derivatives such as Emodin 8-glucoside are also known to have various pharmacological activities, such as antiviral, anti-inflammatory, anticancer, and antibacterial (Okon et al., 2023). Meanwhile, Algestone and Androstane-3,6,17-triol have antibacterial activity through their derivatives, namely steroids, by interacting with the phospholipid membrane of bacterial cells, disrupting its integrity, thus causing morphological changes, leakage, and ultimately cell death (Kumar et al., 2019; Sharma et al., 2022). Therefore, the methanolic leaf extract of *B. javanica* Blume has potential as an antibacterial agent.

CONCLUSIONS

The methanolic leaf extract of *B. javanica* Blume demonstrated antibacterial activity against both *S. aureus* (Gram-positive) and *E. coli* (Gram-negative). All tested concentrations (5%, 10%, 20%, and 40%) produced inhibition zones within the strong activity category (10–20 mm) against both *S. aureus* and *E. coli*. These findings indicate that the methanolic leaf extract of *B. javanica* Blume has promising potential for development as a natural antibacterial agent. LC-MS analysis of the methanolic leaf extract of *B. javanica* Blume revealed a diverse chemical profile. A total of 53 compounds were detected, of which 15 showed high similarity scores ranging from 80.6% to 99.5%. Among these compounds, Diprophylline, Furaltadone, Acefylline, Drazidox, Emodin 8-glucoside, Algestone, and Androstane-3,6,17-triol have been reported to possess antibacterial activity, suggesting that these compounds may contribute to the observed antibacterial effects of the extract.

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Authors' Contributions: Halimatussakdiah designed the study. Alfi Syahra carried out the laboratory work and wrote the manuscript. Yulida Amri and Ulil Amna conducted data analysis. Misdi assisted with plant sample collection. All authors read and approved the final version of the manuscript.

Competing Interests: The authors declare that there is no conflict of interest.

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