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Research Article

Antibacterial Activity of Graded Extract of Sikkam Stem Bark (*Bischofia javanica* Blume) Against *Staphylococcus aureus*

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Abstract

Antibiotic resistance in represents a serious global health challenge, driving the search for alternative antibacterial agents from natural sources. The stem bark of the sikkam tree (*Bischofia javanica* Blume) has bioactive potential, yet systematic evaluation of its antibacterial activity based on solvent polarity remains limited. This study aimed to investigate the antibacterial potential of graded extracts of Sikkam stem bark against *Staphylococcus aureus*. Extraction was performed by graded maceration and antibacterial activity was assessed using the disc diffusion method, with amoxicillin and dimethyl sulfoxide serving as positive and negative controls, respectively. Phytochemical screening revealed the presence of phenolics, flavonoids, alkaloids, tannins, saponins, steroids/terpenoids, and quinones. Among the tested extracts, the ethyl acetate extract showed the highest yield (44.65%) and exhibited the strongest antibacterial activity, producing an inhibition zone of 13.75 ± 0.09 mm at concentration 500 mg/mL The ethanol extract also demonstrated strong and consistent inhibition zone of 12.90 ± 0.13 mm, while the n-hexane extract showed the lowest activity, with an inhibition zone of 10.86 ± 0.20 mm. This study indicates that ethyl acetate and ethanol extracts of sikkam stem bark have potential as natural antibacterial agents against *Staphylococcus aureus*.

Keywords: *Bischofia javanica*; antibacterial; graded extract; *Staphylococcus aureus*

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1 Introduction

Antimicrobial resistance is recognized as a critical worldwide health issue, leading to treatment failure, extended hospitalizations, and significant healthcare costs [1]. A global research study by the Antimicrobial Resistance Collaborators indicates that in 2019, almost 4.95 million deaths were linked to antimicrobial resistance, with 1.27 million directly resulting from bacterial resistance to antibiotics [2]. *Staphylococcus aureus* is a principal pathogen contributing to the significant burden of resistance.

This bacterium is a frequent etiological agent of a wide variety of infections, ranging from skin and soft-tissue infections to potentially lethal invasive infections, such as bacteremia [3][4]. The clinical challenge is further complicated by the emergence of *methicillin-resistant Staphylococcus aureus* (MRSA) strains, which significantly limit antibiotic options and increase the risk of poor clinical outcomes [5]. In Indonesia, the urgency of the MRSA problem is also reflected in national surveillance data. The 2023 Indonesia Network on Antimicrobial Resistance report, which involved 70 hospitals in 15 provinces, revealed that approximately 34% of *S. aureus* isolates were MRSA [6]. This high proportion of MRSA signifies an urgent necessity for supplementary approaches that can reinforce resistance control and infection therapy strategies.

The rise in bacterial resistance to conventional antibiotics has prompted the investigation of natural substances, with a particular focus on medicinal plants as potential sources of antibacterial compounds. A growing body of research has identified the presence of bioactive compounds in plants that possess therapeutic potential [7][8]. These compounds, found in various parts of the plant, including the stem bark of the sikkam (*Bischofia javanica* Blume), have demonstrated efficacy in combating bacterial infections. The bioactive constituents of sikkam's stem bark are particularly noteworthy, as they offer a promising avenue for the development of new therapeutic interventions.

Bischofia javanica Blume, commonly referred to as cingkam, is an edible plant species belonging to the *Euphorbiaceae* family and is recognised for its nutraceutical and therapeutic potential. Various parts of the plant, particularly the leaves and bark, have been used in traditional medicine across Asia to treat inflammatory diseases, tuberculosis, gastrointestinal diseases, and skin infections [9]. Phytochemical investigations have revealed that *B. javanica* contains a wide range of bioactive compounds, including tannins, luteolin, stigmasterol, quercetin, ursolic acid, betulinic acid, and β-sitosterol. Previous studies have reported that extracts obtained using different organic solvents, such as petroleum ether, acetone, dichloromethane, possess inhibitory activity against *Staphylococcus aureus* [10]. Tan et al. (2025) observed that these extracts demonstrate concentration-dependent inhibitory effects on *S. aureus*, with a MIC of 2.60 mg/mL. The antimicrobial effect is facilitated by mechanisms that damage cell membrane integrity, diminish intracellular energy levels, and limit biofilm formation [11]. Conversely, Idramsa et al. (2022) reported that the ethyl acetate extract of sikkam stem bark produced the largest inhibitory zone against *S. aureus*, with a MIC of 6.25 mg/mL [12].

Despite these promising findings, most available studies have relied on single-solvent extraction or have focused on specific fractions, providing limited insight into how solvent polarity influences the recovery of antibacterial constituents from the stem bark of *B. javanica*. Consequently, the relationship between extraction solvent, phytochemical composition, and antibacterial efficacy against *S. aureus* remains insufficiently understood. This knowledge gap hampers the rational selection of extraction strategies for optimizing the antibacterial potential of this medicinal plant. Therefore, the present study aims to systematically evaluate the antibacterial activity of sequential stem bark extracts of *B. javanica* obtained using solvents of increasing polarity (n-hexane, ethyl acetate, and ethanol), with particular emphasis on elucidating how solvent-dependent phytochemical profiles influence inhibitory activity against *S. aureus*.

2 Method

2.1 Materials and Instruments

The instruments utilised in this study included various laboratory glassware (Pyrex), a Bunsen burner, Petri dishes, evaporating dishes, paper discs, forceps, spatulas, an oven (Memmert), autoclave, a

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biological safety cabinet, an incubator, an analytical balance (Shimadzu), muffle furnace, desiccator, vortex mixer, and hot plate. The materials comprised Nutrient Agar (NA) medium, amoxicillin antibiotic discs, dimethyl sulfoxide (DMSO), n-hexane, ethyl acetate, and ethanol extracts of sikkam bark, Mayer's reagent, Dragendorff's reagent, magnesium powder, 6N HCl, and ferric chloride ($FeCl_3$).

2.2 Phytochemical Screening

Phytochemical screening was performed using qualitative methods based on color reactions and precipitation. Tests were conducted to detect flavonoids, alkaloids, tannins, saponins, steroids/terpenoids, and quinones [13].

2.3 Characterization of Simplicia

The obtained simplicia powder was then characterized to assess the quality of the raw material. The characterization of simplicia involves quantifying several constituents, including moisture content, water-soluble extract content, ethanol-soluble extract content, total ash content, and acid-insoluble ash content. Characterisation characteristics are essential for ensuring the quality and consistency of the simplicia before its application in the extraction process. Furthermore, they provide supporting data in the standardization of natural materials [14].

2.4 Extraction of Sikkam Stem Bark Extract

Sikkam bark was extracted using a stepwise maceration technique with solvents of varying polarity. Specifically, n-hexane (nonpolar), ethyl acetate (semipolar), and ethanol (polar) were utilized in this process. The extraction of Sikkam bark powder was conducted with a total of 200 grams of Sikkam bark powder and 2000 milliliters of n-hexane over an experimental period of 3 × 24 hours at ambient temperature. The protocol involved periodic stirring every 6 hours. After maceration, the mixture was filtered to separate the filtrate from the residue. The macerated residue was then dried and re-extracted sequentially using ethyl acetate and ethanol under identical conditions and procedures. The filtrates obtained from each solvent were then evaporated under vacuum in a rotary evaporator, yielding a concentrated extract of Sikkam stem bark [15].

2.5 Sterilization of Tools and Materials

Sterilization of all glass equipment, including beaker glass, measuring cups, and petri dishes, was achieved by placing them in an oven at 170°C for 1 hour. Sterilization of materials, such as Nutrient Agar media, was performed in an autoclave at 121°C for 15 minutes [16].

2.6 Preparation of Test Solution Concentrations

A total of 500 mg of thick extract was weighed and dissolved in 1 mL of DMSO, yielding a stock solution at 500 mg/mL. In the subsequent step, the stock solution is systematically diluted using a dilution equation, with DMSO as the diluent, thereby yielding a range of concentrations of 250, 125, 100, and 50 mg/mL [16].

2.7 Preparations of Test Bacteria Suspension

The bacterial inoculum was prepared by culturing *Staphylococcus aureus* ATCC 25923 on agar at 37°C for 18 to 24 hours. Following this, the bacterial colonies formed were suspended in a sodium chloride solution. The solution was adjusted until a turbidity equivalent to the McFarland 0.5 standard was obtained. This standard corresponds to a bacterial density of 1×10^8 colony-forming units per millilitre (CFU/mL) [17][18].

2.8 Antibacterial Activity Test

A total of 15 mL of liquid Nutrient Agar medium is dispensed into a sterile Petri dish and permitted to solidify. Subsequently, 200 µL of *S. aureus* suspension is pipetted onto the surface of the medium and uniformly disseminated using a sterile spreader. Sterile paper discs were subsequently placed on the surface of the media inoculated with bacteria. Each disc was dripped with 20 µL of extract from each solvent. Amoxicillin served as the positive control, whereas dimethyl sulfoxide functioned as the negative control. The Petri dishes were then incubated at 37 °C for 24 hours. Each treatment was repeated three times [19].

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2.9 Data Analysis

A descriptive and statistical analysis was conducted on the data about the measured diameter of the inhibition zone. Each test was repeated three times. The data are presented as mean ± standard deviation. Data analysis was performed using Microsoft Excel and SPSS version 21 software.

3 Result and Discussion

3.1 Extract Yield

The yield is the ratio of the weight of the extract obtained to the weight of the raw material used in the extraction process, and is generally expressed as a percentage. The extraction yield was determined to assess the efficiency of the extraction process and the ability of each solvent to extract chemical compounds from sikkam bark [20]. The results of the sikkam bark extraction yield are presented in **Table 1.**

Table 1 Extraction yield of Sikkam Stem Bark (*Bischofia javanica* Blume)

Solvent	Simplicia Weight (g)	Extract (g)	Yield (%)
N-Hexane	200	23.59	11.795
Ethyl Acetate		89.29	44.645
Ethanol		45.65	22.825

Based on Table 1, the highest extraction yield was obtained from the ethyl acetate extract at 44.645%, while the lowest yield was observed in the n-hexane extract at 11.795%. Meanwhile, the ethanol extract produced a yield of 22.825%. The variation in yield values indicates differences in the amount of extracted components, which are closely related to the solubility characteristics of secondary metabolites in each solvent. Compounds with specific polarity tend to dissolve more readily in solvents with corresponding polarity properties [15]. Overall, all three extracts met the requirements of the Indonesian Herbal Pharmacopoeia, which stipulates that extraction yields should not be less than 10% [21]. In comparison to the findings of Ati et al. (2021), who reported extraction yields of 5% and 105 for ethyl acetate and ethanol extracts, repectively, the present study demonstrated markedly higher yields for both solvents [22].

3.2 Phytochemical Screening

Phytochemical screening was conducted to ascertain the presence of secondary metabolite compounds contained in the bark extract of Sikkam (*Bischofia javanica* Blume). The objective of this test was to provide an initial overview of the composition of compounds that could contribute to bacterial activity. The results of the phytochemical composition of Sikkam stem bark powder are presented in **Table 2.**

Table 2. Phytochemical composition of Sikkam Stem Bark (*Bischofia javanica* Blume)

Chemical Consituents	Test Results
Phenolics	+
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	+
Steroids/Terpenoids	+
Quinones	+

Note :
+ = indicates the presence of the compound
- = indicates its absence

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Based on **Table 2**, phytochemical screening of sikkam stem bark simplicia powder revealed the presence of several classes of secondary metabolites, including phenolic compounds, flavonoids, alkaloids, tannins, saponins, steroids/terpenoids, and quinones. These findings are consistent with the study reported by Susanto et al. (2022), which documented that sikkam stem bark extracts contain alkaloids, flavonoids, saponins, tannins, and steroids/terpenoids [23].

Simplicia Characterization

Simplicia characterization was conducted to determine the quality of sikkam bark raw materials before use in the extraction stage. The results of Sikkam bark extract simplicia characterization are presented in **Table 3.**

Table 3 Characterization Simplicia of Sikkam Stem Bark (*Bischofia javanica* Blume)

Parameters	Result (%) ± STD
Moisture Content	4.66 ± 0.05
Water soluble essence content	18.34 ± 0.16
Ethanol soluble essemce content	15.30 ± 0.21
Total ash content	4.28 ± 0.12
Acid insoluble ash content	0.54 ± 0.01

Based on Table 3, the moisture content of sikkam bark extract was 4.66%, which meets the Indonesian Herbal Pharmacopoeia requirement that the extract's moisture content should not exceed 10% [21]. This low moisture content indicates that the crude drug is chemically and microbiologically stable, thereby minimizing the potential for enzymatic degradation and microbial growth during storage [24]. The results of determining the water-soluble extract content showed that the water-soluble extract and ethanol-soluble extract contents were 18.34% and 15.30%, respectively, indicating a high content of active compounds that are soluble in both water and organic solvents such as ethanol, thus potentially supporting the effectiveness of the extraction process of bioactive compounds for pharmaceutical and herbal medicine purposes [14]. In addition, the total ash and acid-insoluble ash content were 4.28% and 0.54%, respectively, which provides an overview of the mineral content and indicates low levels of metal contamination and extraneous contamination, such as soil or sand, so that this simplicia is considered suitable for use and does not pose a potential health danger [25].

3.3 Antibacterial Activity of Sikkam Stem Bark (*Bischofia javanica* Blume)

The results of the antibacterial activity assay demonstrated that sikkam bark extracts were capable of inhibiting the growth of *Staphylococcus aureus*, as presented in **Table 4**. The largest inhibition zone diameter was observed for the ethyl acetate extract at a concentration of 500 mg/mL, measuring 13.75 ± 0.09 mm, followed by the ethanol extract at the same concentration, which exhibited an inhibition zone of 12.90 ± 0.13 mm. In contrast, the n-hexane extract at 500 mg/mL produced the smallest inhibition zone, with a diameter of 10.86 ± 0.20 mm.

Table 4. Antibacterial activity of Sikkam Stem Bark against *Staphylococcus aureus*

Solvent	Concentration (mg/mL)	Inhibition Zone Diameter (mm)			Mean ($\bar{X}$ ± STD)	Category
		Rep-1	Rep-2	Rep-3		
N-Hexane	50	7.830	7.860	7.82	7.83 ± 0.02[a]	Moderate
	100	8.495	8.565	8.545	8.53 ± 0.03[a]	Moderate
	125	9.910	9.690	9.680	9.76 ± 0.13[a]	Moderate
	250	10.140	10.130	9.850	10.04 ± 0.16[a]	Strong
	500	11.100	10.720	10.778	10.86 ± 0.20[a]	Strong
Ethyl Acetate	50	10.275	10.245	10.270	10.26 ± 0.01[a]	Strong
	100	10.765	10.655	10.720	10.71 ± 0.05[a]	Strong
	125	11.895	11.780	11.865	11.84 ± 0.05[a]	Strong

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	250	12.95	13.005	12.875	12.94 ± 0,06 [a]	Strong
	500	13.66	13.85	13.75	13.75 ± 0.09 [a]	Strong
	50	10.150	10.090	10.100	10.11 ± 0.03 [a]	Strong
	100	10.510	10.590	10.675	10.59 ± 0.08 [a]	Strong
Ethanol	125	11.220	11.140	11.010	11.12 ± 0.10 [a]	Strong
	250	11.820	12.050	11.970	11.94 ± 0.11 [a]	Strong
	500	12.770	13.035	12.910	12.90 ± 0.13 [a]	Strong
Control Positive		24.150	24.100	23.800	24.01 ± 0.18	Very Strong
Control Negative		0.000	0.000	0.000	0.00 ± 0.00	No Response

Note :
a : indicates significant difference was found between the treatments and the positive control (p = 0.000)
Control Positive : amoxicillin disc antibiotics
Control Negative : dimethylsulfoxide (DMSO)

Based on the data in Table 1, Sikkam bark extract shows potential as an antibacterial agent in inhibiting the growth of *Staphylococcus aureus*. Test results at various concentrations show a tendency for the inhibition zone diameter to increase with increasing extract concentration. This pattern indicates a relationship between concentration and response, whereby the higher the concentration of extract used, the greater the amount of active compounds that diffuse from the disc into the medium, thereby increasing the ability to inhibit bacterial growth, as shown in **Figure 1** [16][26]. A similar phenomenon was also reported by Nasri et al. (2023), who stated that concentration variations affect the inhibition zone area due to differences in the amount of test solution absorbed by the test disc [27].

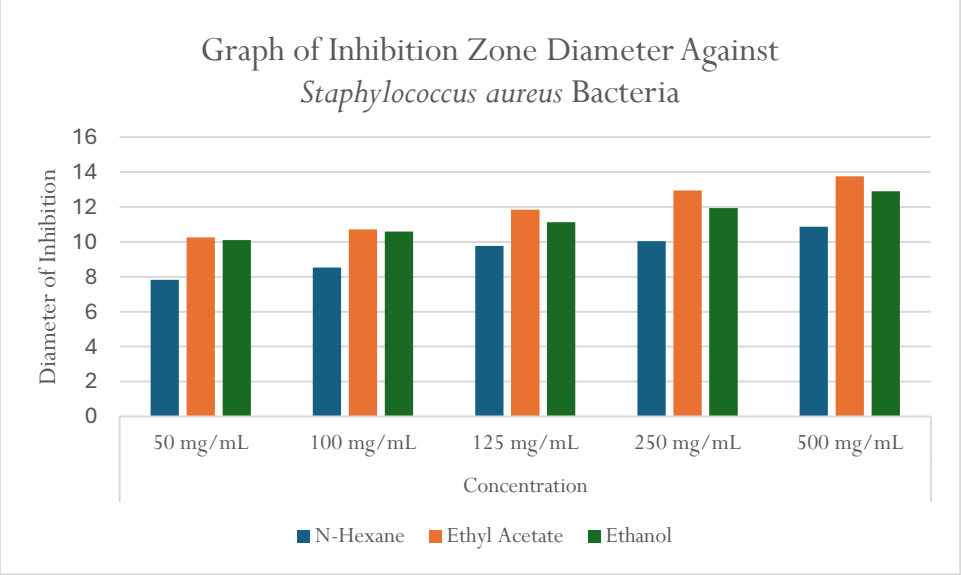

Figure 1 Antibacterial activity of *Bischofia javanica* steam bark extract against *Staphylococcus aureus* based on inhibition zone diameters

The evaluation of antibacterial activity in this study refers to the criteria of Davis and Stout (1971), which classifies antibacterial responses based on the diameter of the inhibition zone, namely inactive if less than 6 mm, moderate activity in the range of 6–10 mm, strong activity in the range of 10–20 mm, and very strong activity if exceeding 20 mm [27]. Based on this classification, all sikkam bark extracts at concentrations of 50–500 mg/mL showed moderate to strong antibacterial activity. Meanwhile, the positive control, amoxicillin, produced an inhibition zone diameter of 24.01 ± 0.18 mm, which is classified as very strong, whereas the negative control did not form an inhibition zone.

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The largest clear zone against *Staphylococcus aureus* was produced by the ethyl acetate extract at 500 mg/mL, with an inhibition zone diameter of 13.75 ± 0.09 mm, which is classified as strong. At concentrations of 250 mg/mL and 100 mg/mL, ethyl acetate extracts produced inhibition zone diameters of 12.94 ± 0.06 mm and 10.71 ± 0.05 mm, respectively, which were still in the strong category, while at a concentration of 50 mg/mL, the diameter of the inhibition zone decreased to 10.26 ± 0.01 mm and was classified as moderate. These results show that the ethyl acetate extract has the highest antibacterial activity among the extracts, especially at high concentrations.

The superiority of ethyl acetate extract in producing the largest clear zone can be explained by the solvent's polarity and the types of secondary metabolites it extracts. According to Harbone, ethyl acetate is a semipolar solvent that effectively extracts compounds with medium polarity, such as flavonoid aglycones, simple phenolic compounds, hydrolyzed tannins, and some alkaloids and terpenoids [28]. These compounds exhibit significant antibacterial activity against Gram-positive bacteria. Flavonoids and phenolic compounds can disrupt the integrity of bacterial cell walls and membranes by increasing membrane permeability, denaturing proteins, and disrupting enzymatic function, thereby causing leakage of intracellular components and inhibiting bacterial growth [29][30]. Higher extract concentrations allow more active compounds to diffuse into the medium, resulting in a larger inhibition zone.

In addition, the presence of alkaloids and flavonoids in ethyl acetate extracts has the potential to produce synergistic antibacterial effects. Alkaloids are known to work by inhibiting DNA synthesis and disrupting the function of essential microbial enzymes. At the same time, flavonoids exhibit antibacterial activity against Gram-positive bacteria by penetrating the peptidoglycan layer, then disrupting the integrity and function of the cell membrane, resulting in inhibited respiration and energy production, accompanied by inhibition of essential intracellular targets such as DNA gyrase and virulence enzymes, as well as modulation of the efflux system, ultimately causing cell dysfunction and bacterial death [31]. The combination of these two groups of compounds in a single extract enables complementary interactions between their mechanisms of action, thereby increasing antibacterial effectiveness compared to the action of each compound alone [32][33]. This synergistic effect has been reported by Liang et al. (2022), showing that complex formulations of secondary metabolites can increase antibacterial efficacy through a combination of complementary mechanisms of action [34].

In addition to the ethyl acetate extract, the ethanol extract also showed consistent antibacterial activity against *Staphylococcus aureus*. At a concentration of 500 mg/mL, ethanol extract produced an inhibition zone diameter of 12.90 ± 0.13 mm, followed by 11.94 ± 0.11 mm at 250 mg/mL, 10.59 ± 0.08 mm at 100 mg/mL, and 10.11 ± 0.03 mm at 50 mg/mL, all of which are still classified as strong. The consistency of this inhibitory power indicates that ethanol extracts contain a mixture of bioactive compounds that are relatively stable in inhibiting bacterial growth. As a polar solvent, ethanol can extract a broader spectrum of secondary metabolites, including flavonoid glycosides, tannins, polar alkaloids, and polar phenolic compounds [35]. Tannins contribute to antibacterial activity by inhibiting important enzymes in bacterial cell metabolism, which occurs due to the formation of complexes between tannins and proteins, including essential enzymes, through hydrogen bonds that trigger protein denaturation. This process disrupts vital enzymatic functions, thereby inhibiting bacterial growth and survival [36][37]. Although the inhibition zone of the ethanol extract at the highest concentration was smaller than that of the ethyl acetate extract, the stability of its activity indicated that its antibacterial potential remained significant.

In contrast, the n-hexane extract showed relatively lower antibacterial activity. At a concentration of 500 mg/mL, this extract produced an inhibition zone diameter of 10.86 ± 0.20 mm, which is still considered strong, but this value decreased to 9.65 ± 0.07 mm at 250 mg/mL, 8.74 ± 0.05 mm at 100 mg/mL, and 7.83 ± 0,02 mm at 50 mg/mL, which is classified as moderate. This more pronounced decrease indicates that the active compounds extracted by n-hexane provide a more limited antibacterial contribution. Phytochemically, n-hexane is a nonpolar solvent that mainly dissolves lipophilic molecules such as hydrocarbons, fatty acids, and nonpolar terpenoids, which often exhibit less antibacterial properties against Gram-positve bacteria compared to phenolic or flavonoid compounds [38].

The differences in antibacterial activity observed among ethyl acetate, ethanol, and n-hexane extracts confirm that solvent polarity significantly affects the nature and quantity of extracted secondary metabolites, thereby directly influencing the potency of antibacterial activity against *Staphylococcus aureus*. Ethyl acetate extract exhibited the largest inhibition zone because it enriched semipolar compounds with high antibacterial potential; ethanol extract showed more stable activity due to its mixture of polar and semipolar compounds; while n-hexane extract showed lower activity because nonpolar compounds dominated it.

4 Conclusion

This study demonstrates that sequential extracts of sikkam stem bark exhibit antibacterial activity against *Staphylococcus aureus*, with effectiveness influenced by solvent type and extract concentration. The ethyl acetate extract showed the strongest activity, particularly at 500 mg/mL (13.75 $\pm$ 0.09 mm), while the ethanol extract displayed consistent inhibition across concentrations and the n-hexane extract showed weaker activity. These findings indicate that solvent polarity affects the extraction of bioactive constituents and their antibacterial efficacy, highlighting the potential of sikkam stem bark, especially the ethyl acetate fraction, as a natural antibacterial source.

5 Declarations

5.1 Acknowledgements

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5.2 Author contributions

Shintjia conceptualized and designed the study, executed the experiments, analyzed the data, and composed the preliminary manuscript. Vera acted as the corresponding author, contributed to data analysis and interpretation, supervised the publication process, and critically revised the text. Astriani contributed to the development of the technique, sample preparation, and data collection. Roy oversaw the research, provided scholarly input, and sanctioned the final manuscript. All authors have reviewed and endorsed the final manuscript.

5.3 Ethics

This research has obtained ethical approval from the Ethics Committee of Universitas Prima Indonesia, under permission number 099/KEPK/UNPRI/V/2025.

5.4 Conflict of Interest

The authors assert that there are no conflicts of interest regarding the publication of this paper.

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