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Potential of Alkaloids Isolated from Benzoin Resin (*Styrax benzoin*)

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ABSTRACT

Benzoin resin (Styrax benzoin) is a natural product that contains various bioactive compounds, including alkaloids, which have potential antibacterial properties. This study aimed to evaluate the antibacterial potential of alkaloids obtained from benzoin resin against Staphylococcus aureus. Benzoin resin was extracted using the maceration method with 96% ethanol, followed by alkaloid screening using Mayer, Wagner, and Dragendorff reagents. Alkaloids were isolated through chromatographic separation and tested for antibacterial activity using the disc diffusion method at concentrations of 10%, 20%, and 40%. The extraction process yielded 92.13 g of crude extract with an extraction yield of 76.77%. Alkaloid screening showed positive results for all tested reagents, indicating the presence of alkaloid compounds in the extract. The alkaloid isolate exhibited antibacterial activity against Staphylococcus aureus, with inhibition zone diameters of 8.23 ± 0.38 mm, 8.93 ± 0.32 mm, and 12.43 ± 1.33 mm at concentrations of 10%, 20%, and 40%, respectively. Statistical analysis revealed that variations in alkaloid concentration significantly affected antibacterial activity ($p < 0.05$). These findings suggest that alkaloids derived from benzoin resin possess promising antibacterial potential against Staphylococcus aureus and may serve as a natural source of antibacterial agents.

Keywords: Benzoin Resin; *Styrax benzoin*; Alkaloids; Antibacterial Activity; *Staphylococcus aureus*

1. INTRODUCTION

Bacterial infections remain a significant health concern worldwide. The prolonged and irrational use of antibiotics has contributed to the increasing prevalence of antimicrobial resistance (AMR), thereby reducing the effectiveness of various antibiotics in controlling bacterial infections. The World Health Organization (WHO) reported that antimicrobial resistance causes more than 1.27 million deaths annually and is expected to continue increasing if effective alternative therapies are not developed. This situation highlights the urgent

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need for the development of new antibacterial agents that are effective, safe, and sustainable for combating pathogenic bacterial infections.[1]

One of the promising sources of bioactive compounds for the development of natural antibacterial agents is plant secondary metabolites. Various classes of secondary metabolites, including flavonoids, tannins, terpenoids, saponins, and alkaloids, have been reported to exhibit antibacterial activity against a wide range of pathogenic bacteria. Among these compounds, alkaloids are known for their strong antibacterial properties through several mechanisms, such as disrupting cell membrane permeability, inhibiting protein synthesis, and suppressing the activity of essential enzymes required for bacterial growth.[2] A study conducted by Mabhiza et al. reported that alkaloid isolates from Callistemon citrinus and Vernonia adoensis effectively inhibited the growth of Staphylococcus aureus and Pseudomonas aeruginosa at low minimum inhibitory concentration (MIC) values, demonstrating their considerable potential as natural antibacterial agents.[3]

Indonesia possesses rich biodiversity and abundant natural resources that have considerable potential for the development of traditional medicines and pharmaceutical raw materials. One of the natural products widely utilized by local communities is benzoin resin (Styrax benzoin). Traditionally, benzoin resin has been used as an aromatic material, incense, and traditional medicine. In addition, several studies have revealed that benzoin resin contains various secondary metabolites, including benzoic acid, cinnamic acid, terpenoids, lignans, and alkaloids, which may contribute to a variety of biological activities.[4]

The antibacterial activity of benzoin resin has been reported in several previous studies. Gayatri et al. demonstrated that the ethyl acetate extract of benzoin resin exhibited antibacterial activity against Staphylococcus aureus, with a minimum inhibitory concentration (MIC) of 1 mg/mL and a minimum bactericidal concentration (MBC) of 2 mg/mL.[5] Furthermore, Darmawan et al. reported the presence of antibacterial active bands in benzoin resin using thin-layer chromatography (TLC) bioautography analysis.[6] These findings indicate that benzoin resin has considerable potential as a source of natural antibacterial compounds.

However, studies investigating the antibacterial activity of alkaloids isolated from benzoin resin (Styrax benzoin) remain limited. Most previous studies have focused on crude extracts or resin fractions, while information regarding the antibacterial potential of alkaloids derived from benzoin resin is still scarce. Therefore, this study was conducted to evaluate the antibacterial potential of alkaloids obtained from benzoin resin (Styrax benzoin) against Staphylococcus aureus using the disc diffusion method.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The materials used in this study included benzoin resin (*Styrax benzoin*), 96% ethanol, Mayer reagent, Wagner reagent, Dragendorff reagent, dimethyl sulfoxide (DMSO), Nutrient Agar (NA), Mueller Hinton Agar (MHA), 0.9% NaCl solution, and 30 µg chloramphenicol discs. The equipment used included a blender, 80-mesh sieve, analytical balance, rotary evaporator, autoclave, Laminar Air Flow (LAF) cabinet, incubator, micropipette, vernier caliper, and standard laboratory glassware.

2.2. Research Procedure

2.2.1 Sample Preparation

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Benzoin resin (*Styrax benzoin*) was cleaned to remove adhering impurities and subsequently air-dried at room temperature until a dry sample was obtained. The dried sample was then ground and sieved through an 80-mesh sieve to obtain a uniform particle size prior to the extraction process.[7,8]

2.2.2 Extraction

A total of 120 g of benzoin resin powder was macerated in 600 mL of 96% ethanol for 3 × 24 h at room temperature with occasional stirring. The resulting filtrate was filtered and concentrated using a rotary evaporator to obtain a crude extract. The extraction yield was calculated based on the ratio of the extract mass to the initial mass of the simplicia.[8]

2.2.3 Alkaloid Screening and Isolation

Alkaloid screening was performed using Mayer, Wagner, and Dragendorff reagents. A positive alkaloid result was indicated by the formation of a yellowish-white precipitate with Mayer reagent, a brown precipitate with Wagner reagent, and an orange precipitate with Dragendorff reagent.[8,9] The extract was subsequently separated using chromatographic methods to obtain an alkaloid fraction, which was used for antibacterial activity testing.[7,8]

2.2.4 Antibacterial Activity Assay

The antibacterial activity of the alkaloid fraction was evaluated against *Staphylococcus aureus* using the disc diffusion method. The bacterial suspension was prepared according to the 0.5 McFarland standard (1.5×10^8 CFU/mL).[10] The alkaloid fraction was prepared at concentrations of 10%, 20%, and 40% using dimethyl sulfoxide (DMSO) as the solvent.[11] A volume of 10 µL of each test solution was applied to sterile paper discs (6 mm in diameter) and allowed to dry prior to use.[10,11]

A total of 100 µL of the bacterial suspension was inoculated onto Mueller Hinton Agar (MHA) plates and evenly spread using a sterile cotton swab. Discs containing the alkaloid fraction were then placed on the surface of the inoculated medium. Chloramphenicol (30 µg) was used as the positive control, while DMSO served as the negative control. All plates were incubated at 37°C for 24 h, after which the inhibition zone diameters were measured using a vernier caliper.[10–12]

2.2.5 Statistical Analysis

The inhibition zone diameter data were analyzed using the Shapiro-Wilk normality test and One-Way Analysis of Variance (ANOVA) at a 95% confidence level. When significant differences were observed, the data were further analyzed using the Least Significant Difference (LSD) test. The antibacterial activity was interpreted according to the inhibition zone classification reported by Jungjunan et al.[13]

3. RESULTS AND DISCUSSION

3.1. Extraction Yield

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The extraction of benzoin resin (*Styrax benzoin*) was carried out using the maceration method with 96% ethanol as the extraction solvent. The maceration method was selected because it allows the extraction of secondary metabolites without involving high temperatures, thereby minimizing the degradation of thermolabile bioactive compounds. A total of 120 g of benzoin resin powder was macerated for 3 × 24 h, yielding 92.13 g of crude extract with an extraction yield of 76.77%.

Table 1. Extraction yield of benzoin resin (*Styrax benzoin*)

Parameter	Result
Initial sample mass (g)	120
Extract mass (g)	92.13
Yield (%)	76.77

The high extraction yield indicates that a substantial proportion of the chemical constituents present in benzoin resin were soluble in 96% ethanol. Ethanol is a polar solvent widely used in the extraction of natural products due to its ability to dissolve various secondary metabolites, including alkaloids, phenolic compounds, and other aromatic constituents.[14] Furthermore, the uniform particle size obtained after sieving likely enhanced the contact efficiency between the solvent and sample, thereby facilitating the diffusion of bioactive compounds into the extraction medium.[14,15] These findings suggest that 96% ethanol is an effective solvent for extracting bioactive compounds from benzoin resin.

Figure 1. Crude extract of benzoin resin (*Styrax benzoin*) obtained after evaporation.

3.2 *Alkaloid Screening*

Alkaloid screening was performed using Dragendorff, Wagner, and Mayer reagents to qualitatively identify the presence of alkaloid compounds in the benzoin resin extract. The results showed that the ethanol extract of benzoin resin tested positive for alkaloids with all three reagents.

Table 2. Alkaloid screening results of benzoin resin extract

Reagent	Result
Dragendorff	Positive (+)
Wagner	Positive (+)

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Mayer	Positive (+)

Positive results were indicated by the formation of a brick-red precipitate with Dragendorff reagent, a light brown to yellow precipitate with Wagner reagent, and a white precipitate with Mayer reagent. The formation of precipitates with these reagents suggests interactions between the nitrogen atoms of alkaloid compounds and the metal ions present in the reagents, resulting in the formation of insoluble complexes.[16,17] These findings indicate that the ethanol extract of benzoin resin contains alkaloid compounds that may contribute to various biological activities, including antibacterial activity.

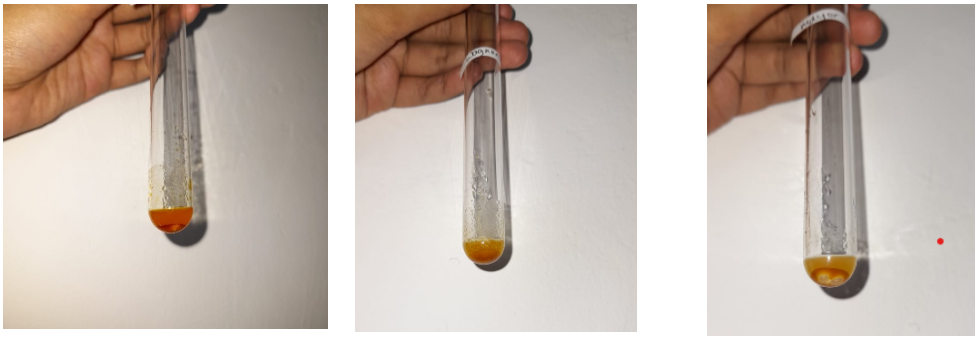

Figure 1. Alkaloid screening results using Dragendorff, Wagner, and Mayer reagents.

3.3 Antibacterial Activity of Alkaloid Isolate Against Staphylococcus aureus

The antibacterial activity of the alkaloid isolate obtained from benzoin resin (*Styrax benzoin*) against *Staphylococcus aureus* was evaluated using the disc diffusion method. Antibacterial activity was indicated by the formation of a clear inhibition zone surrounding the test discs, reflecting bacterial growth inhibition. The inhibition zone diameters are presented in Table 3.

Table 3. Inhibition zone diameters of the benzoin resin alkaloid isolate against *Staphylococcus aureus*

Treatment	Inhibition Zone Diameter (mm)	Category
10%	8.23 ± 0.38	Moderate
20%	8.93 ± 0.32	Moderate
40%	12.43 ± 1.33	Strong
Positive Control	22.43 ± 0.59	Very Strong

The results demonstrated that all tested concentrations of the alkaloid isolate exhibited antibacterial activity against *Staphylococcus aureus*. The inhibition zone diameter increased with increasing alkaloid concentration. The 10% concentration produced an inhibition zone of 8.23 ± 0.38 mm, while the 20% concentration produced an inhibition zone of 8.93 ± 0.32 mm. The highest antibacterial activity was observed at the 40% concentration, which produced an inhibition zone diameter of 12.43 ± 1.33 mm and was categorized as strong. In contrast, the positive control exhibited the largest inhibition zone diameter of 22.43 ± 0.59 mm.

The increase in inhibition zone diameter with increasing concentration suggests that the amount of bioactive compounds diffusing into the medium influences the effectiveness of bacterial growth inhibition. Higher concentrations of the alkaloid isolate provide a greater amount of bioactive compounds capable of interacting with bacterial cells, thereby enhancing antibacterial activity.

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Staphylococcus aureus is a Gram-positive bacterium characterized by a thick peptidoglycan layer and the absence of an outer membrane, allowing antibacterial compounds to diffuse more readily and interact with cell wall components. Consequently, Gram-positive bacteria are generally more susceptible to plant-derived secondary metabolites, including alkaloids. Alkaloids have been reported to exert antibacterial effects through several mechanisms, including disruption of peptidoglycan synthesis, alteration of membrane permeability, and inhibition of enzymes involved in essential bacterial metabolic processes.[18]

The findings of the present study are consistent with those reported by Mabhiza et al., who demonstrated that alkaloid isolates from *Callistemon citrinus* and *Vernonia adoensis* effectively inhibited the growth of *Staphylococcus aureus*. These results further support the potential of alkaloids derived from natural sources as antibacterial agents against Gram-positive bacteria.

To further validate the observed antibacterial activity, statistical analyses were performed on the inhibition zone data obtained against *Staphylococcus aureus*.

Table 4. Statistical analysis of antibacterial activity

Parameter	Value
Shapiro-Wilk (10%)	0.253
Shapiro-Wilk (20%)	0.298
Shapiro-Wilk (40%)	0.583
Shapiro-Wilk (Positive Control)	0.328
Levene's Test	0.081
ANOVA F-value	217.604
ANOVA Significance	0.000

The Shapiro-Wilk normality test indicated that all treatment groups had significance values greater than 0.05, confirming that the data were normally distributed. Additionally, Levene's test yielded a significance value of 0.081 ($p > 0.05$), indicating homogeneity of variance among treatment groups. Therefore, the assumptions required for parametric analysis using One-Way ANOVA were satisfied.

The One-Way ANOVA results revealed an F-value of 217.604 with a significance level of 0.000 ($p < 0.05$), indicating that variations in alkaloid isolate concentration significantly affected the inhibition zone diameter against *Staphylococcus aureus*. In other words, increasing the concentration of the alkaloid isolate significantly enhanced its antibacterial activity.

Subsequently, a Least Significant Difference (LSD) test was performed to determine differences among treatment groups.

Table 5. Least Significant Difference (LSD) test results

Comparison	Significance
10% vs 20%	0.297
10% vs 40%	0.000
10% vs Positive Control	0.000
20% vs 40%	0.001
20% vs Positive Control	0.000
40% vs Positive Control	0.000

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The LSD test showed that the 10% and 20% concentrations were not significantly different ($p = 0.297$), whereas the 40% concentration differed significantly from both the 10% and 20% concentrations ($p < 0.05$). Furthermore, all treatment groups differed significantly from the positive control. These findings indicate that increasing the alkaloid isolate concentration to 40% significantly improved antibacterial activity against *Staphylococcus aureus*. The results further suggest that the observed antibacterial activity is associated with the amount of alkaloid compounds diffusing into the medium, where higher concentrations result in greater bacterial growth inhibition.

4. CONCLUSION

Benzoin resin (*Styrax benzoin*) was successfully extracted using the maceration method with 96% ethanol, yielding an extraction yield of 76.77%. Alkaloid screening using Dragendorff, Wagner, and Mayer reagents confirmed the presence of alkaloid compounds in the extract. The obtained alkaloid isolate exhibited antibacterial activity against *Staphylococcus aureus* at all tested concentrations, with the highest activity observed at the 40% concentration, which produced an inhibition zone diameter of 12.43 ± 1.33 mm and was classified as strong. Statistical analysis demonstrated that variations in alkaloid isolate concentration significantly influenced antibacterial activity ($p < 0.05$). These findings indicate that alkaloids derived from benzoin resin have potential for further development as natural antibacterial agents against *Staphylococcus aureus*.

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