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Antibacterial Activity of Ethanol Extract of *Jernang* Resin (*Daemonorops draco*) Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract

Bacterial infections are a growing global health problem. This is primarily due to the emergence of antibiotic-resistant bacteria, necessitating the need for new, effective antibacterial compounds. Jernang resin (*Daemonorops draco*) is known to contain secondary metabolite compounds such as dracorhodin, which are thought to have potential as antibacterial agents. This study aims to determine the percentage yield of ethanol extract of jernang resin, identify its phytochemical content, and evaluate its antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) pathogenic bacteria. The extract was obtained through a maceration method with 96% ethanol solvent. Antibacterial activity testing was carried out using the disc diffusion method at concentrations of 10, 20, 30, and 40 mg/ml. The inhibition zone was measured and compared with the positive control doxycycline (antibiotic) and the negative control (dimethyl sulfoxide). The extraction yielded 48.86% ethanol extract, which tested positive for flavonoid, alkaloid, and triterpenoid compounds. The results showed that the ethanol extract of jernang resin could inhibit the growth of both test bacteria, with the strongest inhibition against *E. coli* at 30 mg/ml (irradical inhibition zone of 22.5 mm, strong) and against *S. aureus* at 20 mg/ml (irradical inhibition zone of 8.6 mm, moderate). In conclusion, the ethanol extract of jernang resin has antibacterial activity, but further research is needed to determine the best concentration in inhibiting bacterial growth.

Keywords: Antibacterial*; Daemonorops draco; Escherichia coli; Jernang; Staphylococcus aureus*.

INTRODUCTION

Infection remains one of the diseases with the highest global prevalence. According to the GBD 2019 Antimicrobial Resistance Collaborators (2022), bacterial infections were associated with approximately 7.7 million deaths in 2019. Infection occurs when microorganisms enter the body and multiply, causing damage to host tissues. *Staphylococcus aureus*, a Gram-positive bacterium that colonizes human skin, causes infections ranging from mild to fatal (Salim et al., 2023) and accounts for 25-65% of skin infections, with a national average of 38% (Nurjannah et al., 2022). *Escherichia coli*, a Gram-negative bacterium, can also cause digestive tract infections by producing toxins that lead to diarrhea (Fatonah et al., 2022) and, if left untreated, potentially fatal dehydration (Ardila et al., 2024). These two pathogens are among the most frequently isolated bacteria in clinical and environmental samples, making them common indicator organisms for evaluating antibacterial efficacy.

Infections caused by both *S. aureus* and *E. coli* are generally treated with antibiotics. However, antibiotic resistance is rising due to uncontrolled drug use, the slow pace of new antibiotic development, and the emergence of super-resistant bacteria, threatening the success of treatment (Fachdiana, 2024). This growing challenge has renewed interest in plant-derived antibacterial alternatives, such as *jernang* fruit (*Daemonorops draco*), a rattan species that produces a blood-red resin known as dragon's blood. This resin has long been used by local communities, including the Anak Dalam tribe in Jambi Province, to treat wounds, stomach aches, coughing up blood, postpartum hemorrhage, and other ailments (Andriani et al., 2021). Its antibacterial potential is attributed to dracorhodin, a natural anthocyanin belonging to the flavonoid group of secondary metabolites, which damages bacterial cell membranes by interacting with membrane proteins and lipids, increasing permeability until the cells can no longer function and eventually die.

Several previous studies have demonstrated the antibacterial activity of *jernang*. Waluyo and Pasaribu (2013) tested n-hexane, ethyl acetate, and methanol extracts of *jernang* rambai and kalamuai resins using the disc method against *Bacillus subtilis* and *S. aureus*, obtaining inhibition zones ranging from inactive (0 mm) to 15 mm depending on the solvent and resin type, with

the n-hexane extract showing the broadest activity against both bacteria. Ringo et al. (2020) used the well diffusion method to test ethanol and chloroform extracts of *jernang* resin against *S. aureus*, *E. coli*, and *V. cholerae*, reporting the strongest inhibition zones of 9-11 mm at concentrations between 15 and 25 mg/ml. Wahyuni et al. (2018) further tested n-hexane, ethyl acetate, and methanol extracts of *D. draco* resin against both *S. aureus* and *E. coli* using the agar diffusion method, and determined the minimum inhibitory concentration (MIC) of the methanol extract to be 0.5 mg/mL. However, none of these studies evaluated an ethanol extract of *jernang* resin using the disc diffusion method, nor examined a concentration range as wide as 10-40 mg/ml, leaving the antibacterial potential of *jernang* resin against *S. aureus* and *E. coli* under these specific conditions largely unexplored.

This study therefore evaluated the ethanol extract of *jernang* resin using the disc diffusion method at concentrations of 10, 20, 30, and 40 mg/ml, with doxycycline as the positive control and dimethyl sulfoxide (DMSO) as the negative control. Building on previous findings that *jernang* resin can inhibit bacterial growth through its flavonoid-derived secondary metabolites, this study aims to determine the percentage yield and phytochemical content of the ethanol extract of *jernang* resin, and to evaluate its antibacterial effectiveness against *S. aureus* and *E. coli* across this concentration range in order to identify the concentration with the strongest inhibitory activity.

MATERIALS AND METHODS

Study area

The *jernang* fruit used in this study was obtained from Merangin Regency, Jambi Province, Indonesia.

Procedures

Sample Preparation

One kilogram of *jernang* fruit was collected and then fed into a milling machine for the crushing process (Saifuddin et al., 2019). The crushed fruit was filtered using a 3 mm sieve to obtain a uniformly sized *jernang* resin powder. The resulting resin powder was then stored in a clean, tightly closed sample container until ready for extraction.

Maceration

A total of 5 g of *jernang* resin was extracted using 96% ethanol solvent with a ratio of 1:40 or as much as 200 ml and stored in a closed container for 3 days (Waluyo and Pasaribu, 2013). During the soaking process, the solution was stirred periodically so that the sediment could be reabsorbed. After the maceration process was complete, the extract was filtered using filter paper to separate the solution from the coarse sediment. The obtained filtrate was then concentrated using a water bath until all the solvent evaporated. The resulting concentrated extract was then weighed to determine the percentage yield of *jernang* resin using the formula:

$$\text{Jernang Resin Yield (\%)} = \frac{\text{A}}{\text{B}}\ x\ 100\%$$

Description:

A= Weight of *jernang* resin extract (g)

B= Weight of *jernang* resin before extraction (g)

Phytochemical Screening

Phytochemical screening was carried out to determine the content of secondary metabolite compounds in the ethanol extract of *jernang* resin.

a. *Flavonoid test*
   Place 1 ml of sample extract into a test tube. Next, add a few drops of concentrated HCl and Mg powder. A positive sample is indicated by a yellow or orange color (Ulfah et al., 2024).
b. *Alkaloid test*
   A 1 ml sample extract was placed in a test tube and several drops of Dragendorff, Wagner, and Mayer reagents were added. A positive result with Dragendorff's reagent was indicated by the formation of a red to orange sediment. A positive result with Wagner's reagent was indicated by the formation of a brown sediment. A positive result with Mayer's reagent was indicated by the formation of a white sediment (Ulfah et al., 2024).
c. *Saponin test*
   Place 1 ml of sample extract into a test tube and add water. Next, add a few drops of HCl and shake until foam forms. A positive result is indicated by the presence of foam that persists for 10 minutes (Ulfah et al., 2024).
d. *Tannin test*
   Place 1 ml of sample extract into a test tube. Next, add a few drops of 10% NaCl and $FeCl_3$. A positive result is indicated by a blue or green color (Ulfah et al., 2024).
e. *Steroid and triterpenoid test*
   The sample extract is placed in a test tube. Next, add a few drops of Liebermann-Burchard reagent. A positive result for steroids is indicated by a green or blue color, while a positive result for triterpenoids is indicated by a purple or orange color (Ulfah et al., 2024).

Antibacterial Activity Test

a. *Sterilization of tools*
   The equipment used is first washed and dried. Next, the equipment is sterilized using an autoclave for 15 minutes at 121°C (Kosasi et al., 2019). This method is effective for sterilizing heat- and steam-resistant instruments.
b. *Preparation of Positive and Negative Control Solutions*
   The control solutions used in this study were dimethyl sulfoxide (DMSO) as a negative control and

doxycycline as a positive control. DMSO is a polar aprotic solvent. DMSO can dissolve polar chemical compounds in samples without causing inhibition and is non-toxic. Meanwhile, doxycycline is a semi-synthetic bacteriostatic antibiotic derived from tetracycline. This antibiotic is widely used in the treatment of infections (Kamilah, 2024). The negative control solution was prepared by taking 1 ml of DMSO solution and putting it into a vial. Next, the positive control solution was prepared by dissolving 0.1 mg of doxycycline in 1 ml of distilled water in a vial (Fauziyah, 2021). Each vial was labeled accordingly.

c. *Preparation of Concentrations 10, 20, 30 and 40 mg/ml*

The concentration variations used in this study were 10, 20, 30, and 40 mg/ml. A total of 10, 20, 30, and 40 mg of extract were each placed in separate vials and dissolved in 1 ml of DMSO with a few drops of Tween 80 solution to obtain concentrations of 10, 20, 30, and 40 mg/ml, respectively (Fadli et al., 2021). The addition of Tween 80 solution at various concentrations functions as an emulsifier that can reduce surface tension (Sholikhah and Cahyaningrum, 2020).

d. *Preparation of TSA and NB Media*

This study used Tryptic Soy Agar (TSA) media to grow bacteria. TSA media provides essential nutrients for bacterial growth and development (Muhammad and Widayati, 2024). TSA was prepared by dissolving 5 g of TSA in 120 ml of distilled water in an Erlenmeyer flask. The media was then sterilized using an autoclave at 121°C for 15 minutes. After that, the media was poured into sterilized petri dishes. Nutrient Broth (NB) was prepared by dissolving 0.4 g of media in 50 ml of distilled water in an Erlenmeyer flask. It was then tightly covered with aluminum foil and sterilized using an autoclave at 121°C for 15 minutes (Kolopita et al., 2022).

e. *Bacterial rejuvenation*

Bacterial rejuvenation is performed to reactivate the metabolism of bacteria stored in culture. This process produces new, young bacterial cultures that are ready for use. Bacterial rejuvenation is performed by taking a loop of pure bacterial inoculum from a Petri dish using a sterilized loop needle. The bacteria are then inoculated into 50 ml of NB media by dipping the bacteria into an Erlenmeyer flask and incubated for 24 hours at 37°C (Rizki et al., 2025).

f. *Antibacterial Activity Testing*

Antibacterial activity testing was performed using the disc method. The principle of disc diffusion is to inoculate the agar with bacterial culture and allow the antibacterial substance to diffuse into the agar medium (Fauziyah, 2021). Antibacterial activity testing began by taking a rejuvenated bacterial colony using a cotton swab. Next, the bacteria were inoculated onto TSA media by scratching the surface to form a zigzag pattern. The paper discs were then immersed in the negative and positive control solutions. The discs were then immersed in *jernang* resin extract at varying concentrations of 10, 20, 30, and 40 mg/ml until the extract is absorbed through the paper discs. After that, the soaked disc paper was placed on the surface of the solidified media at a distance of approximately 3 cm apart, with a distance of 2 cm from the edge of the media. It was then incubated for 24 hours at 37 °C. Observation of the inhibition zone can be seen from the clear area which indicates a reaction between the bacteria and the *jernang* resin extract. The inhibition zone can be calculated using the following formula.

$$\text{Inhibition Zone Diameter} = \frac{(\text{Dv} - \text{Dc}) + (\text{Dh} - \text{Dc})}{2}$$

Description:
Dv= Vertical Diameter
Dh= Horizontal Diameter
Dc= Disc Diameter

The inhibition zone is measured by observing the shape of the results on the agar medium. If the inhibition zone formed is a perfect circle, then the measurement is carried out by measuring the diameter directly by observing the position of the vernier caliper which is straight parallel to the center point of the paper disc in the inhibition zone. However, if the inhibition zone is not a perfect circle, then the measurement is carried out by measuring the longest and shortest diameters or taking measurements several times at points that have different lengths. According to Elgayyar et al. (2000), the response to bacterial growth inhibition based on its diameter can be divided into several categories, namely strong (inhibition zone >11 mm), moderate (inhibition zone >6-<11 mm), and inactive if the zone of inhibition for the tested bacteria is <6 mm.

Data analysis

Data obtained from the antibacterial activity test were analyzed descriptively. The diameter of the inhibition zone for each sample was measured and classified into categories of antibacterial response—strong (>11 mm), moderate (>6-<11 mm), or inactive (<6 mm)—based on the classification criteria of Elgayyar et al. (2000).

RESULTS AND DISCUSSION

Sample Preparation

The *jernang* fruit used in this study was obtained from Merangin Regency, Jambi Province. One kilogram of *jernang* fruit was crushed using a milling machine.



(a) (b)

Figure 1. (a) Process of grinding *jernang* fruit, (b) *Jernang* resin

Figure 1 shows the *jernang* resin preparation process, where (a) shows the milling of *jernang* fruit using a milling machine and (b) shows the *jernang* resin filtered through a 3 mm sieve.

Maceration

A total of 5 g of *jernang* resin was macerated using 96% ethanol solvent (200 ml) for 3 days. The macerate was filtered using filter paper to separate the filtrate from the residue, and the filtrate was then concentrated using a water bath to produce a thick (concentrated) extract. The extract obtained was 2.443 g, resulting in an extract yield of 48.86%.

Phytochemical Screening

Phytochemical screening was conducted to identify the secondary metabolite compounds contained in the ethanol extract of *jernang* resin. The results of the phytochemical screening are shown in Table 1.

Table 1. Phytochemical screening of ethanol extract of *jernang* resin.

No	Phytochemical Test		Results	Picture	Information
1.	Flavonoid		+		Yellow or orange in color
2.	Alkaloid	Dragendorff	+		Red to orange in color
		Wagner	+		Brown color
		Mayer	-		White

No	Phytochemical Test	Results	Picture	Information
3.	Saponin	-		Foam that can last for 10 minutes
4.	Tannin	-		Blue or green in color
5.	Steroid	-		Green or blue in color
	Triterpenoid	+		Purple or orange in color

Description:
(+) indicates the presence of secondary metabolite compounds
(-) indicates the absence of secondary metabolite compounds

Table 1 shows the results of phytochemical screening of the ethanol extract of *jernang* resin, which showed positive secondary metabolites from the flavonoid, alkaloid, and triterpenoid groups.

Antibacterial Activity Test

An antibacterial activity test was conducted to determine the ability of the ethanol extract of *jernang* resin to inhibit bacterial growth using the disc diffusion method. Antibacterial activity is indicated by the formation of an inhibition zone around the paper disc. This test was conducted on Gram-positive (*S. aureus*) and Gram-negative (*E. coli*) bacteria at several extract concentrations, namely 10, 20, 30, and 40 mg/ml.

Figure 2 shows the antibacterial activity of ethanol extract of *jernang* resin against *E. coli* bacteria. E+ is *E. coli* bacteria with a positive control, while E- is *E. coli* bacteria with a negative control. E1, E2, E3 and E4 are variations in concentrations of 10, 20, 30 and 40 mg/ml used against *E. coli* bacteria. Figure 3 shows the antibacterial activity of ethanol extract of *jernang* resin against *S. aureus* bacteria. S+ is *S. aureus* bacteria with a positive control, while S- is *S. aureus* bacteria with a negative control. S1, S2, S3 and S4 are variations in concentrations of 10, 20, 30 and 40 mg/ml used against *S. aureus* bacteria.

The test results showed a radical inhibition zone in the positive control and an irradical zone at various concentrations for each test bacterium. The radical zone is characterized by the formation of a clear zone around the paper disc that is completely devoid of test bacterium growth, whereas the irradical zone is where bacterial growth is inhibited by the antibacterial agent but not killed, seen as less vigorous growth compared to areas outside the antibacterial influence. The results of the antibacterial activity test are shown in Table 2.

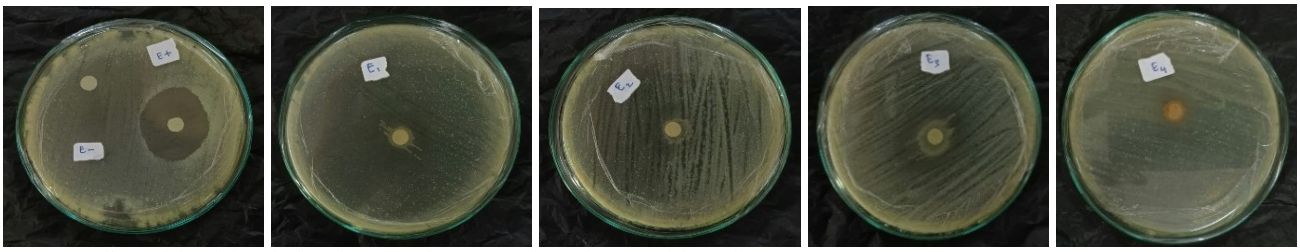

Figure 2. Antibacterial activity of ethanol extract of *jernang* resin against *E. coli* bacteria.

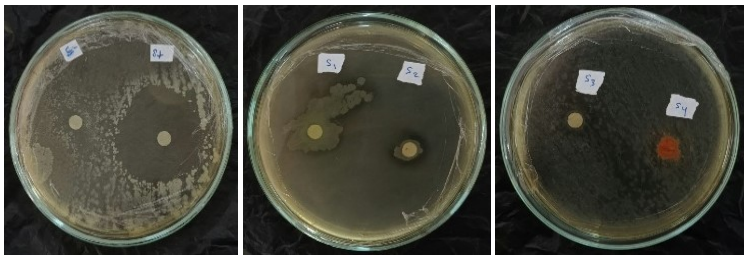

Figure 3. Antibacterial activity of ethanol extract of *jernang* resin against *S. aureus* bacteria.

Table 2. Antibacterial activity of ethanol extract of *jernang* resin against *E. coli* and *S. aureus.*

Sample	Bacteria	Inhibition Zone Diameter	Category
Positive control	*E. coli*	21.4 mm	Strong
	S. aureus	30.75 mm	Strong
Negative control	*E. coli*	0 mm	-
	S. aureus	0 mm	-
10 mg/ml	*E. coli*	17.5 mm	Strong (irradical)
	S. aureus	0 mm	-
20 mg/ml	*E. coli*	0 mm	-
	S. aureus	8.6 mm	Moderate (irradical)
30 mg/ml	*E. coli*	22.5 mm	Strong (irradical)
	S. aureus	7.4 mm	Moderate (irradical)
40 mg/ml	*E. coli*	0 mm	-
	S. aureus	0 mm	-

Table 2 shows that the best inhibition of *E. coli* growth occurred at a concentration of 30 mg/ml, with an inhibition zone diameter of 22.5 mm, categorized as strong. Meanwhile, the best inhibition of *S. aureus* growth occurred at a concentration of 20 mg/ml, with an inhibition zone diameter of 8.6 mm, categorized as moderate. The negative control (DMSO) did not inhibit the test bacteria, while doxycycline, as the positive control, produced a strong inhibition zone against both test bacteria. The diameter of the inhibition zone in the test results increased and decreased irregularly with increasing concentration.

Discussion

Grinding the *jernang* fruit prior to extraction aims to obtain a fine powder, thereby increasing the sample's surface area so that the solvent can interact more optimally with the sample, resulting in more efficient extraction of bioactive compounds (Arief and Meilisari, 2025). The maceration method was chosen because the equipment required is simple and does not damage the bioactive compounds contained in the sample. This method does not involve heating, thus preventing changes in the compounds contained within the extract. Stirring during maceration prevents the powder from solidifying, which would otherwise make it difficult for the solvent to penetrate and extract the active compounds. Ethanol was used as the solvent because it has a polarity similar to that of the target compounds, allowing for more effective extraction.

The positive phytochemical screening results are consistent with the research of Muhaimin et al. (2021), which similarly used an ethanol (polar) solvent. The red pigment in *jernang* resin is attributed to the presence of anthocyanins (flavonoid group) and carotenoids (tetraterpenoid group), which give it a yellowish-red color (Rizki et al., 2025). In the flavonoid test, the addition of concentrated HCl and Mg metal reacts with the benzopyrone nucleus in the flavonoid structure to form a yellow to orange flavylium salt, indicating a positive result. These secondary metabolites are believed to act together to inhibit bacterial growth: flavonoids can disrupt the peptidoglycan component of bacterial cells, preventing the cell wall from forming properly; alkaloids

can inhibit the topoisomerase enzyme involved in DNA replication, causing bacterial lysis; and triterpenoids damage bacterial cell membranes by increasing cell permeability and causing cell leakage, followed by the release of intracellular material.

The irradical inhibition zone observed at various concentrations indicates that the antibacterial activity of the ethanol extract of *jernang* resin is bacteriostatic, meaning it can inhibit bacterial population growth but does not kill the bacteria (Ningsih et al., 2013). The difference in sensitivity between Gram-positive and Gram-negative bacteria observed in this study is likely influenced by cell wall structure. The cell wall of Gram-negative bacteria such as *E. coli* consists of a thin peptidoglycan layer with an outer membrane and does not contain teichoic acid, which may explain why *E. coli* was more susceptible than *S. aureus* to the extract in this study (Mustary et al., 2021).

The negative control (DMSO) did not inhibit the test bacteria, consistent with its properties as a polar aprotic solvent with a high boiling point that allows it to evaporate slowly at normal air pressure without antibacterial effect. Doxycycline, used as the positive control, produced a radical (complete) inhibition zone against both bacteria, which is likely related to its known Minimal Inhibitory Concentration (MIC) as a standardized antibiotic. The MIC of the ethanol extract of *jernang* resin itself was not determined in this study, as the disc diffusion method used is not designed to quantify the amount of antibacterial compound diffusing into the agar and is therefore not considered appropriate for establishing MIC values (Eloff, 2019); this would instead require a complementary broth microdilution assay, such as that used by Wahyuni et al. (2018), who reported an MIC of 0.5 mg/ml for the methanol extract of *D. draco* resin. Nonetheless, numerous studies have shown that *jernang* resin extract has antibacterial activity against various types of bacteria.

The irregular increase and decrease in inhibition zone diameter with increasing concentration observed in this study was likely due to differences in the diffusion rate of antibacterial compounds through the agar medium (Hayon et al., 2023). This is a recognized limitation of agar-based diffusion assays: many antibacterial compounds in plant extracts are relatively non-polar and diffuse poorly and inconsistently through the aqueous agar matrix, so that factors other than compound concentration alone can strongly influence the resulting zone diameter (Eloff, 2019). This limitation should be taken into account when interpreting the concentration-response pattern observed in this study, and supports the use of a complementary dilution-based assay in future work to obtain a more quantitative measure of antibacterial potency.

CONCLUSION

Based on the research results, it can be concluded that the ethanol extract of *jernang* resin produces a yield of 48.86%. Phytochemical screening of the ethanol extract of *jernang* resin showed positive results in the flavonoid, alkaloid (Dragendorff and Wagner reagents) and triterpenoid tests. Testing the antibacterial activity of the ethanol extract of *jernang* resin against *S. aureus* and *E. coli* bacteria showed antibacterial activity characterized by the irradical zone.

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