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Research Article

Comparison of Ethanol Solvent Concentration in Jackfruit Leaf (*Artocarpus heterophyllus L.*) Extract on Antibacterial Activity of *Staphylococcus aureus*

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Abstract: Jackfruit (*Artocarpus heterophyllus* L.) is a traditional medicinal plant containing bioactive compounds such as flavonoids, saponins, tannins, and alkaloids with potential antibacterial properties. *Staphylococcus aureus* is a pathogenic bacterium commonly associated with skin infections. This study aimed to compare the antibacterial activity of jackfruit leaf extracts prepared using 70% and 96% ethanol against *S. aureus* at concentrations of 10%, 20%, and 30%. Extraction was conducted by maceration for three days using a 1:5 solvent ratio. The extracts were tested to ensure the absence of residual ethanol, followed by phytochemical screening and thin-layer chromatography (TLC) analysis. Antibacterial activity was evaluated using the well diffusion method on Mannitol Salt Agar (MSA), with clindamycin as the positive control and Dimethyl Sulfoxide (DMSO) as the negative control. Samples were incubated for 24 hours, and all tests were performed in five replicates. The yields of the 70% and 96% ethanol extracts were 20.14% and 16.95%, respectively. Phytochemical screening and TLC confirmed the presence of alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. The 70% ethanol extract produced inhibition zones of 0.736 cm, 0.788 cm, and 0.893 cm, while the 96% ethanol extract produced 0.635 cm, 0.719 cm, and 0.812 cm at 10%, 20%, and 30% concentrations, respectively. Statistical analysis showed $p > 0.05$, indicating no significant difference between the two extracts at each concentration.

Keywords: Antibacterial; Jackfruit Leaves; *Staphylococcus Aureus*

1. Introduction

In both developed and developing countries, bacterial infections remain one of the major public health problems. *Staphylococcus aureus* is a natural pathogenic bacterium in the human body and is one of the most common causes of infection [7]. This bacterium is recognized as a primary agent of pyogenic infections, characterized by local tissue damage in the host. It is most frequently found on mucous membranes, particularly in the nasal cavity, respiratory tract, and gastrointestinal tract. *Staphylococcus aureus* is opportunistic in nature and may cause inflammation, necrosis, abscess formation, and food poisoning due to the enterotoxins it produces, resulting in symptoms such as nausea, vomiting, and diarrhea.

The use of natural products as alternative treatments, including traditional medicinal plants, has gained increasing attention along with growing public awareness of natural-based therapies. Medicinal plants are considered safer alternatives because they are readily available and associated with a lower risk of resistance. One plant known to contain active compounds with bactericidal or bacteriostatic properties is jackfruit leaf (*Artocarpus heterophyllus* L.). This plant is widely distributed throughout Indonesia and belongs to the Moraceae family, which is known for its various health benefits. The active compounds in jackfruit leaves, such as flavonoids, saponins, and tannins, have demonstrated antibacterial potential [3].

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A previous study by Elysa (2018) showed that jackfruit leaf extract was able to inhibit the growth of *Staphylococcus aureus* at a concentration of 50%, producing an inhibition zone diameter of 14.7 mm. In addition to the leaves, jackfruit seeds also contain various vitamins and minerals beneficial to health [4].

In this study, the antibacterial activity of jackfruit leaf extract was evaluated using ethanol solvents at concentrations of 70% and 96%. Differences in solvent polarity may influence the solubility of bioactive compounds and the extraction yield; therefore, the type and concentration of solvent used during the extraction process significantly affect the results obtained. This study aimed to determine the antibacterial properties of 70% and 96% ethanol extracts of jackfruit leaves against the growth of *Staphylococcus aureus*.

2. Method

In this section, you need to describe the proposed method step by step. Explanations accompanied by equations and flow diagrams as illustrations will make it easier for readers to understand your research. The research method explains the activity design, scope or object, main materials and tools, place, data collection techniques, operational definitions of research variables, and analysis techniques. The method used must be reproducible and if taken from other sources be accompanied by citations. The specifications and sources of the materials used are clear.

Research Object

The object of this study was the antibacterial activity of 70% and 96% ethanol extracts of jackfruit (*Artocarpus heterophyllus* L.) leaves against the growth of *Staphylococcus aureus*.

Research Variables

The independent variables were the concentrations of jackfruit leaf extract prepared using 70% and 96% ethanol at levels of 10%, 20%, and 30%. The dependent variable was the diameter of the clear inhibition zone formed, indicating the antibacterial activity of the extract against *Staphylococcus aureus*. Controlled variables included the maceration method, solvent concentrations (70% and 96% ethanol), extraction procedures, preparation of culture media, bacterial strain incubation conditions, and the antibacterial testing method (well diffusion method).

Materials and Instruments

The materials used in this study included jackfruit leaves; 70% and 96% ethanol; silica gel GF 254 plates as the stationary phase for thin-layer chromatography (TLC); mobile phases consisting of acetic acid, ethyl acetate, methanol, n-butanol, chloroform, and n-hexane; and visualization reagents such as Dragendorff reagent, $FeCl_3$, ferric chloride, and anisaldehyde-sulfuric acid.

Microbiological materials included a suspension of *Staphylococcus aureus*, Nutrient Agar (NA), Nutrient Broth (NB), Mannitol Salt Agar (MSA), Dimethyl Sulfoxide (DMSO), and 1/2 McFarland standard solution.

The equipment used included glass jars and porcelain evaporating dishes for extraction; test tubes, water bath, filter paper, and droppers for phytochemical screening; a chromatography chamber, UV lamp (254 nm), capillary tubes, and sprayer for TLC analysis; and inoculating loop, cylinder cup, volumetric flask, autoclave, incubator, micropipette, caliper, and UV spectrophotometer for antibacterial testing

Preparation of Jackfruit Leaf Extract

Two hundred grams of powdered jackfruit leaves were macerated with 70% and 96% ethanol at a ratio of 1:5 for three days with occasional stirring. The mixture was filtered, and the filtrate was evaporated using a rotary evaporator and water bath to obtain a concentrated extract. The extraction yields were 20.14% for 70% ethanol and 16.95% for 96% ethanol.

Ethanol-Free Test

The absence of residual ethanol was evaluated using odor and color tests. In the odor test, the extract was heated with H_2SO_4 and acetic acid; the absence of an ester odor indicated no residual ethanol. In the color test, sulfanilic acid, HCl, $NaNO_2$, and NaOH were added; the absence of a raspberry-red color confirmed ethanol-free extract.

Phytochemical Screening and TLC Analysis

Phytochemical screening was performed by adding specific reagents to the extract samples. Positive results were indicated by characteristic color changes. TLC analysis was conducted using silica gel GF 254 plates.

The extract was spotted and developed to the solvent front limit, observed under UV light at 254 nm, sprayed with visualization reagents, and the Rf values were calculated for compound identification.

Antibacterial Activity Test

All equipment was sterilized prior to use. NA was used for bacterial maintenance, NB for preparing bacterial suspensions, and MSA as a selective medium for *Staphylococcus aureus*. The antibacterial activity was tested using the well diffusion method with five replicates per treatment.

Data Analysis

Data were obtained by measuring the diameter of inhibition zones around the wells. Statistical analysis was performed using SPSS software. Parametric tests were applied if data were normally distributed and homogeneous. Otherwise, non-parametric tests, including Kruskal–Wallis and Mann–Whitney tests, were used.

3. Results and Discussion

The maceration of jackfruit leaf extract using 70% ethanol produced a higher yield of 40.3 grams (20.14%) with a brownish color. In contrast, extraction with 96% ethanol yielded 33.916 grams (16.95%) with a dark green color. The higher yield obtained from 70% ethanol indicates that the chemical compounds present in jackfruit leaves have polarity characteristics similar to 70% ethanol. This solvent contains more water and hydroxyl (–OH) groups compared to 96% ethanol, making it more effective in extracting polar metabolites and resulting in a greater extract yield [1].

The ethanol-free test results showed that both 70% and 96% ethanol extracts of jackfruit leaves were free from residual ethanol, as indicated by the absence of ester odor and the absence of a raspberry-red color. Therefore, it can be concluded that the extracts did not contain residual ethanol.

Phytochemical screening revealed that both 70% and 96% ethanol extracts of jackfruit leaves tested positive for alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. Thin-layer chromatography (TLC) analysis further confirmed the presence of alkaloids, flavonoids, saponins, tannins, and triterpenoids in both extracts. Previous research conducted by Isromarina et al. (2022) also reported that jackfruit leaf extract contains flavonoids, steroids, and triterpenoids.

Table 1. Antibacterial Activity Test of Jackfruit Leaf 70% Ethanolic Extract.

Replication	**Diameter of Inhibition Zone of Jackfruit Leaf Extract Ethanol 70% (Cm)**				
	Concentration				
	10%	20%	30%	Control +	Control -
1	0,746	0,746	0,929	1,568	0,000
2	0,701	0,750	0,970	1,691	0,000
3	0,840	0,856	0,880	1,557	0,000
4	0,734	0,792	0,800	1,682	0,000
5	0,661	0,800	0,890	1,704	0,000
Average	0,736±0,066	0,788±0,044	0,893±0,063	1,640±0,071	0,000±0, 000

Table 2. Antibacterial Activity Test of Jackfruit Leaf 96% Ethanolic Extract.

Replication	Diameter of Inhibition Zone of Jackfruit Leaf Extract Ethanol 96% (Cm)				
	Concentration				
	10%	20%	30%	Control +	Control -
1	0,751	0,781	0,910	1,764	0,000
2	0,693	0,816	0,943	1,879	0,000
3	0,514	0,633	0,709	1,683	0,000
4	0,493	0,601	0,696	1,394	0,000
5	0,725	0,765	0,802	1,825	0,000
Average	0,635 SD±0,122	0,719 SD±0,095	0,812 SD±0,112	1,709 SD±0,190	0,000 SD±0,000

Based on Table 1, the antibacterial activity test showed that the mean inhibition zones of the 70% ethanol extract of jackfruit leaves at concentrations of 10%, 20%, and 30% were 0.736 cm, 0.788 cm, and 0.893 cm, respectively.

Meanwhile, the 96% ethanol extract produced mean inhibition zones of 0.635 cm ± 0.122, 0.719 cm ± 0.095, and 0.812 cm ± 0.112, respectively. These results indicate that all extract concentrations were able to inhibit the growth of *Staphylococcus aureus*, with greater concentrations producing larger inhibition zones.

The positive control (clindamycin solution) for the 70% and 96% ethanol groups produced mean inhibition zones of 1.640 cm and 1.709 cm, respectively. In contrast, the negative control (DMSO) showed no inhibition zone, as DMSO does not possess antibacterial properties [8].

Data analysis using SPSS version 23 showed that the Shapiro–Wilk normality test for the 70% and 96% ethanol extracts yielded significance values of 0.938 and 0.867 ($p > 0.05$), indicating that the data were normally distributed. The homogeneity test also showed a significance value greater than 0.05, confirming that the data were homogeneous. Therefore, a parametric test was conducted, which demonstrated that there was no significant difference between the 70% and 96% ethanol extracts.

4. Conclusions

This study demonstrated that jackfruit leaf extract (*Artocarpus heterophyllus* L.) prepared using 70% and 96% ethanol exhibits antibacterial activity against the growth of *Staphylococcus aureus*. However, statistical analysis indicated that there were no significant differences between the 70% and 96% ethanol extracts at concentrations of 10%, 20%, and 30%.

Based on these findings, it is recommended that future studies explore a wider range of solvents with different polarity levels to determine the most effective solvent for extracting bioactive compounds that provide optimal antibacterial activity.

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Conflicts of Interest : The authors declare no conflict of interest.

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