BIOEDUSCIENCE
ISSN: 2614-1558
http://journal.uhamka.ac.id/index.php/bioeduscience

The activity of Jackfruit Leaf Extract (*Artocarpus heterophyllus* L.) Against *Staphylococcus epidermidis*

Sri Wahyu Vita [1*], Putri Vidiasari Darsono [1], Saftia Aryzki [1]

[1] Department of Pharmacy, Health Faculty, Sari Mulia University, Banjarmasin, Kalimantan Selatan 70236, Indonesia
* Correspondence: sriwahyuvita11@gmail.com

Abstract

Background: Acne is an infectious disease that is still a severe problem. The therapy used for acne is the bacterial antibiotic Clindamycin. Still, there is a lot of resistance to these antibiotics, one of which is *Staphylococcus epidermidis*, so it is necessary to find alternative antibacterial drugs to treat acne. Non-pharmacological therapy often used is jackfruit leaf extract (*Artocarpus heterophyllus* L.) which contains secondary metabolites such as flavonoids, saponins, and tannins, which are thought to be effective as antibacterial agents. **Methods:** The type of research used is True Experimental with the Posttest-Only Control Group research design. The research method used healthy diffusion and liquid dilution, and then the data were analyzed using ANOVA. **Results:** It was found that jackfruit leaf extract (*Artocarpus heterophyllus* L.) had antibacterial activity against *Staphylococcus epidermidis* with a diameter of inhibition zone at a concentration of 50 ppm of 11.7 mm, a concentration of 75 ppm of 12.1 mm, a concentration of 100 ppm of 12.3 mm and had a minimum inhibitory ability at a concentration of 50 ppm with a p-value of 0.007 in the Kruskall-Wallis Test and the Mann Whitney Test showed a p-value of 0.025. Still, the extract did not have the minimum killing ability. **Conclusions:** Jackfruit leaf extract can only inhibit the growth of *Staphylococcus epidermidis* bacteria but cannot kill the growth of these bacteria.

Keywords: acne vulgaris; antibacterial; jackfruit leaf; *Staphylococcus epidermidis*

Article history
Received: 08 Dec 2022
Accepted: 24 Dec 2022
Published: 31 Dec 2022

Publisher's Note:
BIEDUSCIENCE stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Citation:
Vita, S.W., Darsono, P.V., Aryzki, S. 2022. The activity of Jackfruit Leaf Extract (*Artocarpus heterophyllus* L.) Against *Staphylococcus epidermidis*. *BIOEDUSCIENCE*, 6(3), 282-287. doi: 10.22236/jbes/6310573

Introduction

Various microorganisms, including bacteria, viruses, fungi, and parasites, cause infectious diseases. Infectious diseases can be transmitted from one person to another. Research conducted by (Radityastuti & Anggraeni, 2017) states that gram-positive Staphylococcus bacteria cause most contagious diseases. One of the causes of wound infection on the skin is the *Staphylococcus epidermidis* bacteria which is associated with acne (Ekawati *et al*., 2018). The highest prevalence of acne sufferers is in women aged 14-17, ranging from 83-85%, and in men aged 16-19 years, ranging from 95-100%. From a survey in Southeast Asia, there are 40-80% of cases of acne vulgaris (Hasanah & Novian, 2020).

Acne treatment can be in the form of topical and oral antibiotics routinely used (Madelina & Sulistiyaningsih, 2018). One of the antibiotics commonly used to treat skin infections is Clindamycin. However, the problem currently faced in medicine is bacterial resistance to antibiotics, so it is necessary to develop research to find alternative antibacterials to inhibit the spread of infection by *Staphylococcus epidermidis*. Jackfruit leaves are one of the plants that have the potential to an antibacterial.

The purpose of this study was to identify the antibacterial activity of jackfruit leaf extract (*Artocarpus heterophyllus* L.) and to identify the Minimum Inhibitory Concentration (MIC) and Minimum Kill Concentration (KBM) of jackfruit leaf extract (*Artocarpus heterophyllus* L.) on the growth of *Staphylococcus epidermidis*.

Methods

Tools and Materials

The tools used in this study were the Biological Safety Cabinet (Thermo Scientific), incubator (ESCO Isotherm), autoclave (GEA YX-280D), measuring flask (Pyrex), measuring cup (Pyrex), beaker glass (Pyrex), Erlenmeyer (Pyrex), hot plate (Thermo Scientific-Cimarec), colony counter, refrigerator, analytical balance (AciS AD-600i), micropipette, stir bar, loop needle, magnetic stirrer, petri dish, cork borer, aluminum foil, label, plastic wrap, glass container, rotary evaporator, calipers, and bunsen.

The materials used in this study were jackfruit leaf Simplicia powder (*Artocarpus heterophyllus* L.), *Staphylococcus epidermidis* bacteria, sterile distilled water, 96% ethanol, clindamycin antibiotic, Nutrient Agar (NA), Nutrient Broth (NB), Dimethyl Sulfoxide (DMSO). 10%, 1% BaCl, 1% H2SO4, and 0.9% NaCl.

Procedure

The initial step is to pour 20 ml of Nutrient Agar (NA) media into a petri dish and wait to solidify. Then the next step is to add 100 µl of the *Staphylococcus epidermidis* bacterial suspension, the turbidity level of which has been adjusted to a standard solution of 0.5 Mc Farland. After that, three holes with a diameter of 6 mm were made using a cork borer in each petri dish. The first petri dish was filled with 50 ppm extract with three replications. The second was filled with 75 ppm extract with three repetitions. The third was filled with 100 ppm extract with three repetitions, the fourth was filled with the positive control (Clindamycin), and the fifth was filled with negative control (DMSO) of 20 µl and incubated for 18-24 hours at 37°C. Furthermore, it can be observed that the inhibition formed by a clear zone can be documented and measured (Mahdiyah *et al*., 2020).

The next step is to put Nutrient Broth (NB) media into each test tube and add jackfruit leaf extract (*Artocarpus heterophyllous* L.) according to the concentrations used 50 ppm, 75 ppm, and 100 ppm. Each tube was added to the bacterial suspension and adjusted to the Mc standard solution. Farland 0.5 and then incubated for 18-24 hours at 37°C. Observe turbidity and compare with positive and negative controls. The lowest concentration without turbidity is the MIC test tube (Noval *et al*., 2019).

Meanwhile, to determine the MBC, 20 µl of each MIC treatment was taken, then poured on solid media and spread using the spread technique using L rods and incubated for 24 hours at 37°C. Observe whether or not bacterial colonies grow in each treatment group by counting the number of colonies using a colony counter. The lowest concentration that does not show colony growth in solid media is the MBC (Mahdiyah *et al*., 2020)

Data analysis

Data analysis on the research results was carried out descriptively and using the One Way ANOVA Test, one of the parametric tests. It was chosen because there were more than two groups of variables in this study. The One Way Anova test requires that the research data is usually distributed and homogeneous. Suppose the significant value of the data is > 0.05 ($p > 0.05$). In that case, the data is usually distributed, but if the significant value of the data is <0.05 ($p < 0.05$), then the data is not normally distributed. Data analysis does not use the One Way ANOVA parametric test if one or all of these conditions are not met. Still, non-parametric data analysis uses the Kruskal-Wallis test.

Result

Table 1. Effects of Antibacterial Activity of Jackfruit Leaf Extract (*Artocarpus heterophyllus* L.) Against *Staphylococcus epidermidis* Using Well Diffusion

No	Treatment	diameter			Average
		I	II	III	
1	Jackfruit Leaf Extract 50 ppm	11,3	12,1	11,7	11,7
2	Jackfruit Leaf Extract 75 ppm	12,5	11,4	12,5	12,1
3	Jackfruit Leaf Extract 100 ppm	12,7	11,8	12,6	12,3
4	Positive Control	37,1	37,5	36,8	37,1
5	Negative Control	0	0	0	0

Based on the data from the screening test of the activity of jackfruit leaf extract (*Artocarpus heterophyllus* L.) on the growth of *Staphylococcus epidermidis* bacteria, it is known that jackfruit leaf extract (*Artocarpus heterophyllus* L.) has antibacterial power which is indicated by the presence of an inhibition zone around the good holes.

Table 2. Research Results of Minimum Inhibitory Concentration (MIC) of Jackfruit Leaf Extract (*Artocarpus heterophyllus* L.) Against *Staphylococcus epidermidis*

No	Concentration Variation	Replication			***P value***
		I	II	III	
1	Concentration 50 ppm	Clear	Clear	Clear	0,007[a] 0,025[b]
2	Concentration 75 ppm	Clear	Clear	Clear	0,007[a] 0,025[b]
3	Concentration 100 ppm	Clear	Clear	Clear	0,007[a] 0,025[b]
4	Positive Control	Clear	Clear	Clear	0,007[a] 0,025[b]
5	Negative Control	Turbid	Turbid	Turbid	0,007[a] 0,025[b]

Table 3. Results of Research on Minimum Kill Concentration (KBM) of Jackfruit Leaf Extract (*Artocarpus heterophyllus* L.) Against *Staphylococcus epidermidis*

No	Concentration Variation	Replication		
		I	II	III
1	Concentration 50 ppm	Growing Colonies	Growing Colonies	Growing Colonies
2	Concentration 75 ppm	Growing Colonies	Growing Colonies	Growing Colonies
3	Concentration 100 ppm	Growing Colonies	Growing Colonies	Growing Colonies
4	Positive Control	Not Growing Colonies	Not Growing Colonies	Not Growing Colonies
5	Negative Control	Growing Colonies	Growing Colonies	Growing Colonies

Based on the results of visual observations in each tube, it is known that jackfruit leaf extract (*Artocarpus heterophyllus* L.) has an inhibitory power or Minimum Inhibitory Concentration (MIC) value for the growth of *Staphylococcus epidermidis* bacteria at a concentration of 50 ppm. Based on the results of statistical tests, it is known that there are differences in the effect of giving jackfruit leaf extract (*Artocarpus heterophyllus* L.) to *Staphylococcus epidermidis* bacteria using the Kruskall-Wallis Test, obtaining a significance value of 0.007 ($p < 0.05$). The Mann-Whitney Test showed significant differences between the concentration variations compared to the negative control with a significance value of 0.025 ($p < 0.05$).

Based on the results of observations using a colony counter, it is known that jackfruit leaf extract (*Artocarpus heterophyllus* L.) has no killing power or Minimum Killing Concentration (KBM) value on the growth of *Staphylococcus epidermidis* bacteria characterized by bacterial colonies growing at all various concentrations.

Discussion

In the results of testing the antibacterial activity of jackfruit leaf extract (*Artocarpus heterophyllus* L.) with the excellent diffusion method, the average diameter of the inhibition zone at a concentration of 50 ppm was 11.7 mm, at a concentration of 75 ppm was 12.1 mm and at a concentration of 100 ppm was 12 .3mm. According to (CLSI, 2020), the diameter of the inhibition zone is included in the resistance category if the diameter of the inhibition zone is ≤14 mm. For Clindamycin positive controls, results were obtained with an inhibition zone diameter of 37.1 mm and included in the Susceptible category where the inhibition zone diameter was ≥21 mm. This is from a study conducted by (Athaillah & Sugesti, 2020), where Clindamycin has an inhibition zone diameter of 35 mm with a powerful category against *Staphylococcus epidermidis*. In the negative control (DMSO), there was no inhibition zone. This is to research conducted by (Indriastuti *et al*., 2018) that DMSO does not have antibacterial abilities, so it does not affect *Staphylococcus epidermidis.*

Based on the antibacterial activity screening, it is known that there is an inhibition zone so that the jackfruit leaf extract (*Artocarpus heterophyllus* L.) used can be continued to the antibacterial activity test to determine the Minimum Inhibitory Concentration (MIC) and Minimum Killing Concentration (MBC). Antibacterial activity testing using the dilution method. Determination of the Minimum Inhibitory Concentration (MIC) value was carried out by determining the lowest concentration of the sample that was able to inhibit the growth of *Staphylococcus epidermidis* as indicated by the clarity in the tube from the sample group of jackfruit leaf extract (*Artocarpus heterophyllus* L.) which was observed visually. Based on table.2, the MIC value of jackfruit leaf extract (*Artocarpus heterophyllus* L.) was obtained against *Staphylococcus epidermidis* bacteria at a concentration of 50 ppm. The test results indicate this on a 50ppm concentration tube that looks clear. The results of this MIC are reinforced by research (Hasnawati, 2017) that the leaves of plants with the genus *Artocarpus* have an inhibitory ability to inhibit *Staphylococcus epidermidis* bacteria.

In the data from the Minimum Inhibitory Concentration (MIC) test results, statistical tests were carried out using non-parametric statistical tests. Based on the results of statistical tests, it is known that there are differences in the effect of jackfruit leaf extract (*Artocarpus heterophyllus* L.) on *Staphylococcus epidermidis* bacteria using the Kruskal-Wallis Test, obtaining a significance value of 0.007 ($p < 0.05$). This means there are differences in all treatment groups (the jackfruit leaf extract treatment group, positive control and negative control). The Mann-Whitney Test was conducted to find the differences in each treatment group. Based on the Mann-Whitney Test, the results showed significant differences between the concentration variation groups and the negative control group with a significance value of 0.025 ($p < 0.05$). In testing between groups of concentration variations with the positive control group, there was no significant difference with a significance value of 1.00 ($p < 0.05$). Therefore, it can be stated that jackfruit leaf extract (*Artocarpus heterophyllus* L.) affects the growth of *Staphylococcus epidermidis* bacteria.

Then testing the antibacterial activity can be continued determining the killing power value or Minimum Killing Concentration (KBM) of jackfruit leaf extract (*Artocarpus heterophyllus* L.) on the growth of *Staphylococcus epidermidis* bacteria. The Minimum Inhibitory Concentration (MBC) value was determined by streaking the Minimum Inhibitory Concentration (MIC) test solution on solid media. The lowest sample concentration with no bacterial growth in the petri dish is the kill power value or Minimum Kill Concentration (KBM). Based on table 3, it was found that jackfruit leaf extract (*Artocarpus heterophyllus* L.) did not have an MBC value against *Staphylococcus epidermidis* bacteria at all concentration variations. This was indicated by the discovery of bacterial

growth on solid media at various extract concentrations. There were no growing bacterial colonies in the Clindamycin positive control because the antibiotics used had a broad-spectrum mechanism of action (Athaillah & Sugesti, 2020). Whereas in the negative control, bacterial colonies were growing because research conducted by (Indriastuti *et al*.,2018) showed that Dimethyl Sulfoxide (DMSO) did not have antibacterial abilities, so it did not affect *Staphylococcus epidermidis*.

The ability of jackfruit leaf extract (*Artocarpus heterophyllus* L.) to inhibit the growth of *Staphylococcus epidermidis* is due to the presence of secondary metabolites such as flavonoids, saponins and tannins, which have antibacterial properties. These secondary metabolites play an essential role in the antibacterial ability of a plant (Darsono & Fajriannor, 2020). This is the same as cat whiskers leaf extract containing secondary metabolites with antibacterial inhibition (MIC), namely flavonoids, alkaloids, and saponins (Madani *et al*., 2021).

Conclusions

Jackfruit leaf extract (*Artocarpus heterophyllus* L.) has been shown to show antibacterial activity as seen from the inhibition zones formed around the suitable holes. The best results were obtained from 100 ppm jackfruit leaf extract with an inhibition zone diameter of 12.3 mm, which was included in the resistant category. Meanwhile, the screening results for antibacterial activity had an inhibitory ability (MIC) at a concentration of 50 ppm with a significance value on the Kruskal-Wallis Test was 0.007 and a significance value on the Mann-Whitney Test was 0.025. Still, jackfruit leaf extract (*Artocarpus heterophyllus* L.) had no antibacterial activity killing power (KBM). Henceforth, it is necessary to carry out further research to increase the concentration of jackfruit leaf extract (*Artocarpus heterophyllus* L.) to obtain the best concentration in killing the growth of *Staphylococcus epidermidis* bacteria.

Acknowledgments

The author would like to thank Sari Mulia University for giving permission and facilitating the completion of this research.

Declaration statement

The authors reported no potential conflict of interest

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