

Original Research Paper

Potential of Soursop Leaf Extract (*Annona Muricata L.*) as an Antimicrobial Against *Staphylococcus epidermidis***Aqil Fauziy¹, Nurelly N. Waspodo^{2*}, Andi Alamanda Irwan³, Yani Sodikah⁴, Dhian karina⁴, Muhammad Wirawan Harahap⁵**¹ Department of Medical Education, Faculty of Medicine, Universitas Muslim Indonesia, RSP Ibnu Sina YW-UMI, Indonesia² Department of Skin and Venerean Health Sciences, Faculty of Medicine, Universitas Muslim Indonesia, RSP Ibnu Sina YW-UMI, Indonesia³ Department of Pharmacology, Faculty of Medicine, Universitas Muslim Indonesia, RSP Ibnu Sina YW-UMI, Indonesia⁴ Department of Microbiology, Faculty of Medicine, Universitas Muslim Indonesia, RSP Ibnu Sina YW-UMI, Indonesia⁵ Department of Anesthesia, Faculty of Medicine, Universitas Muslim Indonesia, RSP Ibnu Sina YW-UMI, Indonesia nurelly.nurelly@umi.ac.id

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Abstract

Staphylococcus epidermidis is an opportunistic bacterium that can cause infections, particularly in individuals with weakened immune systems. The increasing resistance to antibiotics has encouraged the use of medicinal plants such as soursop leaves (*Annona muricata L.*), which contain antibacterial compounds. This study aims to evaluate the antibacterial effectiveness of soursop leaf extract against the growth of *Staphylococcus epidermidis* at concentrations of 50%, 75%, and 100%. The study specifically investigated the inhibition zones formed at each concentration (50%, 75%, and 100%) of *Annona muricata L.* extract against *Staphylococcus epidermidis*, and compared the antibacterial activity across these three concentrations. This research used a laboratory experimental design with the Kirby-Bauer disc diffusion method to assess the antibacterial activity. The extract was prepared using 96% ethanol and tested at the concentrations stated. Gentamicin 10 µg was used as a positive control, while sterile distilled water served as the negative control. The inhibition zones were observed after 24 hours of incubation at 37°C. The results showed that soursop leaf extract at 50% and 75% concentrations produced inhibition zones categorized as resistant. At 100% concentration, the inhibition zone ranges from resistant to intermediate. The comparison across all three concentrations indicates that the 100% concentration demonstrated notable antimicrobial activity against *Staphylococcus epidermidis*.

Keywords: *Annona muricata L.*; activity test; *Staphylococcus epidermidis***1. Introduction**

Disease is a public health problem that is difficult to address completely. Infectious diseases are caused by bacteria. Therefore, efforts are made to kill or suppress bacterial growth. Typically, a formula containing substances is used to inhibit bacterial growth or even kill them. These substances are commonly known as antibacterials and are better known in the medical world as antibiotics. Treating infections with a combination of antibiotics, which was originally believed to be able to eradicate the bacteria that cause infection, has also given rise to a new problem: the emergence of multi-resistant bacteria. Bacterial resistance due to the inappropriate use of chemical drugs is the background to the increased use of herbal medicines (Meitania Utami et al., 2022; Zainab, S., Choesrina, R., & Hazar, 2024).

One such infection is *Staphylococcus epidermidis*, a type of bacteria that has opportunistic properties and tends to attack individuals with compromised or weakened immune systems (Halid & others, 2022). This bacteria is capable of producing various types of staphylococcal enterotoxins A, C,

D, and E toxins as well as mucus that allows it to adhere to various surfaces. This mucus also contributes to the resistance of *Staphylococcus epidermidis* bacteria to the process of phagocytosis and several types of antibiotics, thus giving rise to the use of herbal plants as an alternative. One plant that has long been believed to have quite good antibacterial activity against bacteria is soursop leaves (*Annona muricata* L.). (Meitania Utami et al., 2022; Pomputius et al., 2023; Zainab, S., Choesrina, R., & Hazar, 2024).

According to the WHO (World Health Organization), there are approximately 20,000 species of plants that can be used as medicinal plants. One Indonesian plant that can be used is the soursop leaf (*Annona muricata* L.). (Sagita, 2021). This is because soursop leaves contain several active substances that act as antibacterial agents. These chemical compounds include Flavonoids, Glucosides, Tannins, Formic Acid, Citric Acid, such as annoaceus acetogenin and several minerals (especially calcium and potassium). The soursop plant (*Annona muricata* Linn) is a type of plant that lives in tropical areas, this plant can live for a long time and bear fruit every year, so it is easy to obtain (Made Ionnandha & Andriana, 2023).

According to the Indonesian Minister of Health, medicinal plants have been accepted as alternative medicine and are even officially recommended for use by healthcare practitioners. Pharmaceutical companies, previously chemical manufacturers, are now producing medicines using medicinal plants using traditional recipes. Public awareness of the use of medicinal plants is beginning to be re-accepted as a natural and safe alternative treatment and health maintenance method (Komalasari et al., 2021).

Traditional medicines are still widely used by the public, considered highly beneficial because they have long believed that natural ingredients can treat various diseases and have relatively fewer side effects than synthetic drugs. Soursop leaves are commonly used to prevent and treat abscesses, hypertension, liver disease, headaches, and diabetes. They are also used to treat several bacterial diseases such as pneumonia, diarrhea, urinary tract infections, skin infections, headaches, sleep disorders (insomnia), liver disease, diabetes, hypertension, and as an anti-inflammatory and anti-convulsant drug through various processing techniques. Furthermore, soursop leaves are also used by the public as antibacterial, antiviral, antioxidant, antifungal, antiparasitic, and antihypertensive agents. (Meitania Utami et al., 2022; Nadira et al., 2017).

Previous research conducted by (Rizky et al., 2024) showed that soursop leaf extract was able to inhibit the growth of *Staphylococcus epidermidis* at concentrations of 3%, 6%, 9%, and 12%. Although all concentrations showed inhibitory activity, the increase in antibacterial effectiveness with increasing concentration was relatively small. This indicates that low concentrations have limitations in producing optimal antibacterial effects.

Several other studies on different herbal plants, such as betel leaf and turmeric extracts, have shown that much higher concentrations (above 50%) significantly increase the inhibition zone against various pathogenic bacteria. However, no previous studies have explored the effectiveness of soursop leaf extract at higher concentrations, namely 50%, 75%, and 100%. Therefore, this study is novel in testing the antibacterial efficacy of soursop leaves at higher concentrations, which have the potential to produce more significant activity, while also evaluating whether increasing the concentration can substantially expand the inhibition zone against *Staphylococcus epidermidis* (Komalasari et al., 2021).

The use of high concentrations is also based on the principle that active compounds such as acetogenins, flavonoids, and tannins in soursop leaves can work more effectively in larger quantities, especially against bacteria that have strong defense mechanisms such as biofilm formation. Thus, this study not only tests the antibacterial effectiveness at high concentrations but also addresses the need for empirical data that is not yet available in the context of the use of soursop leaves as a potential antibacterial agent (Rizky et al., 2024).

2. Research Methods

This research is a laboratory experimental study with a true experiment post-test only design, using the Kirby-Bauer disc diffusion method to test the antibacterial effectiveness of soursop leaf extract (*Annona muricata L.*) against *Staphylococcus epidermidis*. The research was conducted in 2024 at the UP3M Laboratory, Faculty of Medicine, Muslim University of Indonesia. Samples were fresh soursop leaves taken directly from the tree, extracted using 96% ethanol through a maceration method for 3x24 hours and evaporated using a rotary evaporator until a thick extract was obtained. This extract was then diluted into three concentrations: 50%, 75%, and 100% using sterile distilled water and 1 mL of DMSO, each with a volume of 5 mL, 7.5 mL, and 10 mL. Inclusion criteria included fresh green soursop leaves and Nutrient Agar (NA) media grown with *Staphylococcus epidermidis*, while exclusion criteria included damaged soursop leaves and contaminated media. The tools used included standard laboratory equipment such as petri dishes, autoclaves, incubators, and measuring calipers. The materials used included soursop leaves, *Staphylococcus epidermidis*, NA, 96% ethanol, distilled water, 10 µg gentamicin as a positive control, and distilled water as a negative control.

Bacteria were prepared by rejuvenating pure cultures in NA and making a suspension in 0.9% NaCl to equivalent to the McFarland turbidity standard of 0.5. A 1 mL suspension was poured into a petri dish, followed by the addition of 12–15 mL of sterile NA and homogenized. Paper discs that had been soaked in the extract for 15 minutes were placed on the media containing the bacteria, then incubated at 37°C for 24 hours. After incubation, the inhibition zone was measured using a measuring caliper in millimeters. Data were analyzed based on the classification of inhibition zones according to the CLSI standard for 10 µg gentamicin, namely resistant (≤ 12 mm), intermediate (13–14 mm), and sensitive (≥ 15 mm), and compared with the results from soursop leaf extract at each concentration to assess its antibacterial effectiveness.

3. Results and Discussion

3.1. Results

Zone results barriers that form in various concentration extract leaf soursop, control positive, and negative controls can seen in Table 1.

Table 1. Inhibition Zones Formed in Various Soursop Leaf Concentration (*Annona muricata L.*)

Concentration	Zone of Inhibition in <i>Staphylococcus epidermidis</i>	Interpretation Response Obstacle Growth	Conclusion
50%	6.3 mm	Resistant	100% Resistant
	6.5 mm	Resistant	
	6.4 mm	Resistant	
75%	9.1 mm	Resistant	100% Resistant
	6.1 mm	Resistant	
	6.4 mm	Resistant	
100%	12.1 mm	Resistant	67% Intermediate
	13.7 mm	Intermediate	
	14.1 mm	Intermediate	
Control (-)	0 mm	Resistant	-
Control (+)	38.17 mm	Sensitive	

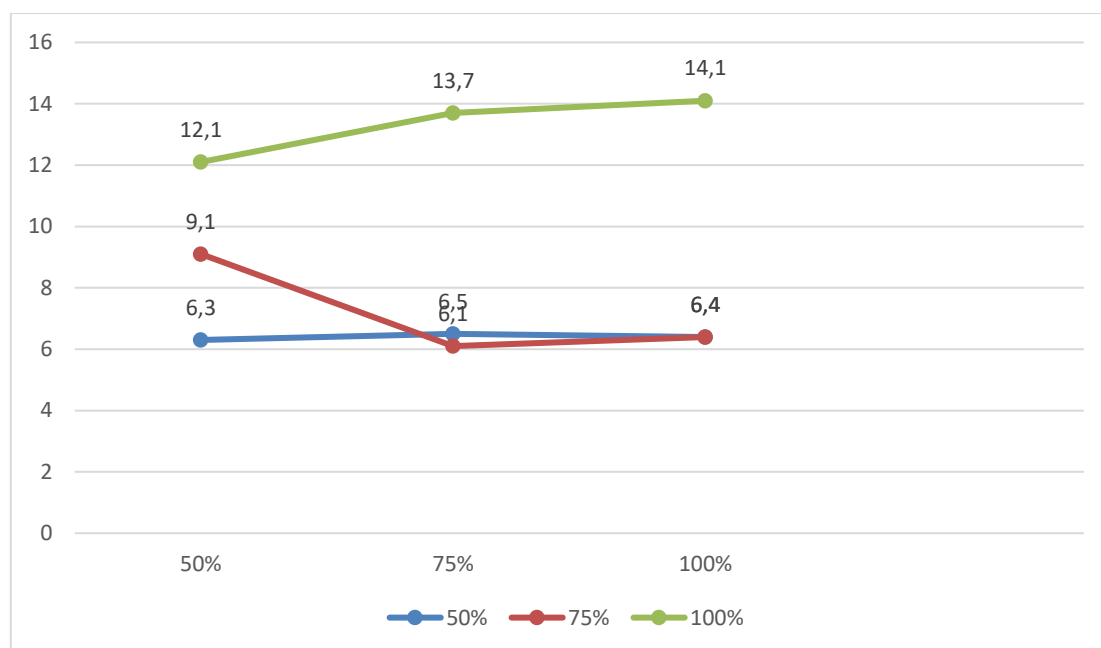


Figure 1. Inhibition Zone Interpretation Chart

Table 1 and Figure 1 show that size concentration something sample can influence the resulting inhibition zone diameter. Extract leaf soursop 50% concentration produces inhibition zones of 6.3 mm; 6.5 mm; 6.4 mm, At 75% it produces inhibition zones of 9.1 mm; 6.1 mm; 6.4 mm, While 100% produces inhibition zones of 12.1 mm; 13.7 mm; 14.1 mm, culture bacteria *Staphylococcus epidermidis*. Control positive with Gentamicin antibiotic produced an inhibition zone of 38.17 mm in culture bacteria *Staphylococcus epidermidis*. Control negative with distilled water No produce inhibition zones Because No have activity as an anti- bacterial (0 mm).

Based on bacterial test results can seen No existence Power resistor soursop leaf extract to growth bacteria *Staphylococcus Epidermidis* in various concentration of 50%, 75% and 100% against the inhibition zone.

3.2. Discussion

Formation of inhibition zone microbes by extract leaf soursop (*Annona Muricata L.*) in research can seen around marked *paper* disk with formation of a clear zone Because existence compound bioactive content in the extract leaf soursop. Based on the results data research obtained start *starting point* from 50% concentration, an inhibition zone was formed which was classified as resistant. Likewise with 75% concentration, inhibition zone formed resistant. At 100%, an inhibition zone was also obtained One resistant and two intermediate. Although the diameter of the inhibition zone formed experience improvement from inhibition zone concentration 50%, At 75% there are two inhibition zone values that are not increase namely 6.1 mm and 6.4 mm while the only one increase that is 9.1 mm will but Still Far from standard optimal dose in control positive (*Gentamicin*) which produces an inhibition zone that is classified as sensitive namely 38.17mm. The size of the inhibition zone formed Keep going increase along with increase concentration leaf soursop (*Annona Muricata L.*). The more tall concentration extract leaf soursop (*Annona Muricata L.*). So the more Lots content substance active in it so that activity antibacterial will the more big and vice versa the more low concentration Soursop leaf extract (*Annona Muricata L.*). the more A little substance active in it so that activity antibacterial will the more reduced. In the inhibition zone method activity antibacterial influenced a number of factor that is leaf itself in the form of leaf age and leaf position, content compound antibacterial, concentration

extract, power diffusion extract, type inhibited bacteria, bacterial sensitivity and incubation time and temperature (Martsiningsih et al., 2023; Rahman et al., 2017).

Based on research by Martsiningsih et al., (2023) comparing the effect of leaf parts (shoots, middle and base) of soursop plants on antibacterial activity on *Staphylococcus aureus* bacteria. and *Pseudomonas aeruginosa*. The results of this study showed that the tips of soursop leaves contain the highest concentration of bioactive compounds. There was no significant difference between leaf position and antibacterial activity against *Staphylococcus aureus*. and *Pseudomonas aeruginosa*, although the leaf tips showed the greatest antibacterial activity. Young leaves also contain antibacterial substances, while older leaves have begun to deteriorate. Soursop leaves have weak inhibitory properties against gram-positive bacteria, so this extract can be considered bacteriostatic. Here, researchers took the location of the middle, base, and slightly tip of the soursop leaves. The age of the soursop leaves was mixed, namely young and old soursop leaves, which can affect the inhibition zone activity test, there were resistant results at concentrations of 50%, 75%, and two intermediates at a concentration of 100% (Martsiningsih et al., 2023; Rahman et al., 2017; Widyananda et al., 2021).

Research conducted by (Rizky et al., 2024) is a laboratory experimental study that tests the effectiveness of an antiseptic hand gel preparation from soursop leaf extract (*Annona muricata* Linn) against *Staphylococcus epidermidis* bacteria. This study used four extract concentration formulations, namely 3% (formulation I), 6% (formulation II), 9% (formulation III), and 12% (formulation IV). The results showed that at concentrations of 3% and 6% produced an inhibition zone diameter of 3 mm (weak inhibition category), while concentrations of 9% and 12% produced inhibition diameters of 5 mm and 7 mm, respectively (strong inhibition category). Although the 12% formulation showed a larger inhibition zone, the results obtained were still relatively small. In this study, a gel preparation was used that may affect the diffusion power of the antibacterial extract to the medium. In contrast to that study, this study used much higher extract concentrations, namely 50%, 75%, and 100%, but the results obtained showed resistance and intermediate categories. This indicates that in addition to concentration, antibacterial effectiveness can also be influenced by other factors, such as the content of active compounds in the leaves used, extraction method, dosage form, and the ability of the active substance to diffuse into the medium.

As for the factors of the type inhibited bacteria and bacterial sensitivity. It is necessary known in microbes bacteria *Staphylococcus epidermidis* have component main wall thick cells Because a single plasma membrane surrounded by peptidoglycan so that including group Gram- positive bacteria. Peptidoglycan compile about 90% of the wall cells and the rest in the form of sour teichoate. Peptidoglycan is strong target antibiotics so that can influence lack of inhibition zone power in hinder growth This could be due to the possible lack of inhibition zone activity in the antibacterial compound from soursop leaf extract. (Lestari & Asri, 2021; Rizky et al., 2024).

The influence of incubation time and temperature can affect the inhibition zone based on researcher a n (Martsiningsih et al., 2023) that variation time incubation affect the inhibition zone each antibiotics. In his research, the time incubation beginning that is 6-18 hours of effect the more wide or big the formation of inhibition zones and at the time incubation furthermore namely 18-48 hours of experience decrease in inhibition zone Antibiotics. The study used a 24-hour incubation period, which could be a factor influencing the inhibition zone.

Need known to be one of the factor important that allows *Staphylococcus e. pidermidis* from species coagulase negative For endure living in a harsh environment is biofilm production. Biofilm formation occurs with adhesion beginning to surface foreign or endothelium, which causes accumulation to in structure multicellular. After formed, biofilm protects to defense host cause manifestation clinical and resistance antibiotics. (Skovdal et al., 2022) Soursop leaves can be an option as a medicinal plant and have been shown to have antibacterial activity. This is because soursop leaves contain tannins,

flavonoids, polyphenols, saponins, and essential oils. Research (Kresnapati & Sofya, 2023) shows that soursop leaf extract contains alkaloids, saponins, flavonoids, steroids, and tannins. The mechanism of inhibition of bacterial growth by antibacterial compounds can be the destruction of the bacterial wall or membrane, changes in the permeability of the cytoplasmic membrane causing the release of food from the cell, changes in protein and nucleic acid molecules, inhibition of enzyme activity and inhibition of enzyme activity and nucleic acids and proteins.

Research conducted (Meitania Utami et al., 2022) A review was conducted on the phytochemical screening test of soursop leaf extract and the results showed that soursop leaf extract contains compounds of the alkaloid, phenol, flavonoid, saponin and tannin groups which have the potential to be antibacterial with a working mechanism that is... different. The bacteria used in this literature study use Gram-positive and Gram-negative bacteria. Soursop leaf extract is able to inhibits the growth of Gram-positive and Gram-negative bacteria because it can suppress the function of the bacterial cell wall so that the compound's biological activity does not develop.

4. Conclusion

This study shows that soursop leaf extract (*Annona muricata* L.) at concentrations of 50% and 75% produced inhibition zones classified as resistant to *Staphylococcus epidermidis*, while a concentration of 100% showed inhibition zones with resistant and intermediate interpretations. This indicates that at high concentrations, soursop leaf extract begins to show antibacterial activity against *Staphylococcus epidermidis*.

Further research is recommended to explore other parts of the soursop plant, such as the fruit or stem, which may potentially contain higher levels of antibacterial compounds, as well as to test other activities, such as antifungal or antiviral. The use of higher extract concentrations should also be considered to obtain results that demonstrate sensitive interpretation. Furthermore, it is recommended to test its effectiveness against other bacteria belonging to the *Staphylococcus epidermidis* -like group.

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