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BIOACTIVITY TEST AGAINST ANTIBACTERIAL COMPOUND LEAF EXTRACT OF SUGAR APPLE LEAVES (*Annona Squamosa*) AGAINST *Staphylococcus aureus*

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Abstract

Introduction: Infectious diseases are one of the major health problems globally, most often caused by bacteria. Treatment for bacterial infections is done with antibiotics, but their use can cause resistance. Sugar apple (*Annona squamosa*) leaves contain metabolites such as alkaloids, flavonoids, and saponins that have antibacterial potential. This study aims to determine the sensitivity response of *Staphylococcus aureus* bacteria to Sugar apple leaf extract with concentrations of 20%, 40%, 60%, 80%, and 100%. **Methods:** This study used an experimental design with a disk diffusion technique (Kirby Bauer). Sugar apple leaves are collected from the yard of a resident of Harapan Jaya, Bekasi, West Java. Sugar apple leaf extract was obtained by maceration technique using 70% ethanol. Extract concentrations are 20%, 40%, 60%, 80%, and 100%. control (+) chloramphenicol and control (-) distilled water. **Result:** Antibacterial test using disk diffusion method (Kirby Bauer). The average results obtained are concentrations of 20%: 1.5mm, 40%: 2mm, 60%: 2.5mm, 80%: 2.75mm, and 100%: 4.5mm. The higher the concentration of the extract given, the greater the inhibition zone formed. **Conclusion:** The bioactivity of the ethanol extract of Sugar apple (*Annona squamosa*) leaf as an antibacterial can inhibit the growth of *Staphylococcus aureus* bacteria.

***Keywords**: Antibacterial, Kirby Bauer, Maceration, Staphylococcus aureus, Sugar apple (Annona squamosa).*

INTRODUCTION

Infectious diseases are one of the major health problems globally caused by pathogenic microorganisms, which are capable of causing illness and death (Yadav *et al*., 2020). Infectious diseases are often caused by bacteria. One of them is *Staphylococcus aureus* which belongs to the group of gram-positive pathogenic bacteria that can cause various diseases such as abscesses, endocarditis, skin infections, pneumonia, meningitis, and sepsis. The severity of bacterial infections in humans varies, including respiratory tract infections, eye infections, urinary tract infections, and the Central Nervous System (CNS) (Widiastuti *et al*., 2019). Treatment therapy for infectious diseases caused by bacteria is done by giving antibiotics. However, antibiotics that are used inappropriately can trigger antibiotic resistance (Ballo *et al*,. 2021). With this, research is needed in addition to using chemicals, one of which is the development of natural ingredients that contain antibacterial compounds to avoid the occurrence of resistance to antibiotics. A study conducted by Isramilda *et al* (2020) stated that decoction Sugar apple (*Annona squamosa*) leaves with 0.9% NaCl using the good method was able to inhibit *Staphylococcus aureus* with a diameter of 6.35 mm with a concentration of 100%. While research by Dewangga dan Nirwana (2016) stated that the infusion of Sugar apple (*Annona squamosa*) leaves was compared to 70% ethanol extract of Sugar apple (*Annona squamosa*) leaves with concentrations of 50%, 25%, and 12.5%. The inhibition obtained was much better and no maximum concentration was found that compensated for its positive control on the growth of *Staphylococcus aureus*. In the study, Tansil *et al* (2016) stated that the 96% ethanol extract of Sugar apple leaves with concentrations of 50%, 25%, and 12.5% in Escherichia coli was less inhibitory when compared to *Staphylococcus aureus*. Based on previous research, no research has been conducted on the bioactivity test of the antibacterial compound of Sugar apple leaf (Annona squamosa) against *Staphylococcus aureus* at doses of 100%, 80%, 60%, 40%, and 20%. Therefore, researchers are interested in researching bioactivity tests of antibacterial compounds using 70% ethanol extract of Sugar apple leaves against *Staphylococcus aureus* bacteria by disc diffusion method (Kirby Bauer).

METHOD

A. Research design

This research design uses experimental research which aims to determine the emergence of the consequences of the treatment given by the researcher intentionally (Payadnya dan Trisna, 2018). The treatment in this study was Sugar apple (*Annona squamosa*) leaf extract with concentrations of 100%, 80%, 60%, 40%, and 20%.

B. Time and Location of Research

This research was carried out at the Phytochemical and Microbiology Laboratory of STIKes Mitra Keluarga, East Bekasi in February - March 2022.

C. Research Sample

The samples in this study were pure cultures of *Staphylococcus aureus* ATCC25923 and Sugar apple (*Annona squamosa*) leaves.

D. Research Variables

The variables in this study include the independent variable namely the concentration of Sugar apple (*Annona squamosa*) leaf extract of 100%, 80%, 60%, 40%, and 20% (w/v). The dependent variable is diameter zone of inhibition formed from the growth of *Staphylococcus aureus* bacteria and the control variable used chloramphenicol as control (+) and sterile distilled water as control (-)

E. Tools and materials

The tools used are glass and non-glass tools, analytical balance (IKA, Ohaus MB 120), incubator (Memmerth IN-30), autoclave (AGGRAM), Laminar air flow (UK 1200 MM), rotary evaporator (IKA-RC 2), oven (IKA 125), and P100 micropipette (Acura digital 825). The materials used were Sugar apple (*Annona squamosa*) leaves, 70% ethanol, distilled water, NA (*Nutrient agar*) and MHA (*Mueller hinton agar*) medium, *Staphylococcus aureus* bacteria culture ATCC25923.

F. Making Sugar apple Leaf Extract

Wash the Sugar apple leaves and dry them with the wind until they are completely dry. After that, mashed with a blender. Weigh the fine powder as much as 100 grams and give 500 ml of 70% ethanol and stored it in a place protected from light for 3 days while stirring occasionally. Then filtered, the residue was macerated again by adding 250 ml of 70% ethanol and stored for 2 days. Then the concentration was carried out using a rotary evaporator to produce a thick extract of Sugar apple leaves with a concentration of 100%.

G. Qualitative Phytochemical Screening

1. Alkaloid Test

   1 ml of Sugar apple leaf extract was taken into two test tubes, then 5 drops of Mayer and Dragendorff reagents were added to the test tube. Positive results when a precipitate forms on the sample. A white precipitate is formed indicating (+) Mayer, and a yellow-red (+) Dragendorff. the precipitate is formed (Aliwu *et al*., 2020).

2. Flavonoid Test

   As much as 1 ml of Sugar apple leaf extract then add 1 ml of ethanol to a test tube and heat on a bath. Then three drops of concentrated HCl and MgCl2 were added, and the changes were seen. A positive result was obtained with a reddish-brown color change Putri *et al*.,2020).

3. Saponin Test

   2 ml of Sugar apple leaf extract is put into a test tube then add distilled water and shaken for 30 seconds, observe the changes that occur. A positive result is indicated by the formation of a stable foam for 10 minutes with a height of 1 to 3 cm (Rante *et al*, 2020).

4. Terpenoid Test

   Take 5 ml of extracted Sugar apple leaves into a test tube. added with 2 ml of chloroform and 3 ml of concentrated sulfuric acid and 2 ml of chloroform. A reddish-brown color change indicates a positive result (Astuti *et al*., 2021).

H. Sensitivity Test

The inhibition test was carried out using the Kirby Bauer method. Each disc paper was soaked for

± 30 minutes in a solution of Sugar apple leaf extract with various concentrations (20%, 40%, 60%,80%, and 100%), distilled water as a negative control and chloramphenicol as a positive control, then the disc paper was placed on the surface of the MHA media which had been swabbed with bacterial suspension evenly with a sterile cotton swab and incubated at 37ºC for 24 hours. Sugar apple leaf extract is said to be positive if the given treatment forms an inhibition zone around the paper disc (Syarifah *et al*., 2018).

RESULTS

Based on research that has been carried out at the Phytochemistry and Microbiology Laboratory of STIKes Mitra Keluarga, East Bekasi from February 18 – March 30, 2022. The results of the study are as follows:

A. The yield of Sugar apple Leaf Extract

The result of extracting the leaves of Sugar apple (*Annona squamosa*) using the maceration method. Obtained with the % yield value as shown in the following table:

No.	Leaf Weight (grams)	Powder Weight (grams)	Extract Weight (grams)	yield (%)
1.	500	100	11,642	11,642

B. Phytochemical Screening of Sugar apple Leaf Extract

The results of the phytochemical screening test of Sugar apple leaf extract (*Annona squamosa*) obtained include the Alkaloid, Flavonoid, Saponin, and Terpenoid tests as shown in the following table:

No	Identification	Observation result	Information
1.	Alkaloids (Dragondorff's reagent)	The solution is dark brown and there is a yellow-reddish precipitate	+
2.	Alkaloids (Mayer's reagent)	The solution is dark brown and there is a white precipitate	+
3.	Flavonoids	Reddish brown solution	+
4.	Saponins	The solution is brown and stable foam is formed	+
5.	Terpenoids	Brown solution	-

Results :
+ : Positive contains secondary metabolites
- : Negative contains secondary metabolites

C. Sugar apple Leaf Extract Bioactivity Test

The results of the observation of the bioactivity test of Sugar apple leaf extract (*Annona squamosa*) on the growth of *Staphylococcus aureus* bacteria with various concentrations, namely 100%, 80%, 60%, 40%, 20%, control (+) and control (-) which were replicated 3 times. . The results

obtained are shown in the following table:

Treatment	Inhibition Zone Diameter (mm)			Average (mm)	Information
	Replication I	Replication II	Replication III		
20%	1.5	1	2	1.5	resistance
40%	2	1.5	2.5	2	resistance
60%	2.5	2	3	2.5	resistance
80%	2.75	2.5	3	2.75	resistance
100%	5	4.5	4	4.5	resistance
Control (+)	26	28	27	27	sensitive
Control (-)	0	0	0	0	resistance

Results :
Control (+) : Chloramphenicol
Control (-) : Aquadest

DISCUSSION

In this study, the sample used was the leaf part of the Sugar apple (*Annona squamosa*) plant that grows in the gardens of the residents of Harapan Jaya village, Bekasi, West Java. This sample was collected in early February 2022 and determined at the Center for Biological Research – Indonesian Institute of Sciences (LIPI), Cibinong, West Java, to ensure the correctness of the plants to be used, by matching the morphological characteristics of Sugar apple leaves with the literature. After that, the identification of Sugar apple leaf simplicia which includes smell, color, taste, and shape is carried out. In this study, simplicia was dried and extracted by the maceration method and concentrated with a rotary evaporator to obtain a thick extract. Maceration was carried out on Sugar apple leaf powder using 70% ethanol because it was able to attract more compounds in the simplicia. The maceration process was carried out for three days with maceration for two days. Then the % yield value from wet sorting of Sugar apple leaves was 149%. Then on dry sorting of Sugar apple leaves, the % yield value was 43.738%. Meanwhile, in the thick extract of Sugar apple leaves, the % yield value obtained was 11.642%. According to FHI in 2017 the requirement for a good % yield is < 7.2%. So based on the results of the calculation of the % yield value that has been obtained is appropriate because it meets the requirements of a good % yield. Then the % yield value from wet sorting of Sugar apple leaves was 149%. Then on dry sorting of Sugar apple leaves, the % yield value was 43.738%. Meanwhile, in the thick extract of Sugar apple leaves, the % yield value obtained was 11.642%. According to FHI in 2017 the requirement for a good % yield is < 7.2%. So based on the results of the calculation of the % yield value that has been obtained is appropriate because it meets the requirements of a good % yield. Then the % yield value from wet sorting of Sugar apple leaves was 149%. Then on dry sorting of Sugar apple leaves, the % yield value was 43.738%. Meanwhile, in the thick extract of Sugar apple leaves, the % yield value obtained was 11.642%. According to FHI in 2017 the requirement for a good % yield is < 7.2%. So based on the results of the calculation of the % yield value that has been obtained is appropriate because it meets the requirements of a good % yield.

Then in this study phytochemical screening of the extracts that have been obtained to determine the presence or absence of secondary metabolites is observed from the precipitate formed and the color changes that occur. The results of phytochemical screening tests on 70% ethanol extract of Sugar apple (*Annona squamosa*) leaves can be seen in Table 5.2 showed that Sugar apple leaf extract was positive for terpenoids, flavonoids, alkaloids, and saponins. The results terpenoid test obtained negative results because when observed it did not show a reddish-brown color change in the solution. Positive results of Alkaloids and Flavonoids are indicated by the formation of colored precipitates while saponins are

indicated by the presence of stable foam formed above the solution.

In the bioactivity test of Sugar apple leaf extract, concentration variations were made, namely 100%, 80%, 60%, 40%, and 20%. Based on the results of the measurement data obtained in Table 5.3 It is known that the leaf extract of Sugar apple (*Annona squamosa*) has antibacterial activity against the growth of *Staphylococcus aureus* which is indicated by the formation of inhibition of the growth of *Staphylococcus aureus* bacteria in variations in the concentration of the Sugar apple leaf extract. The data obtained from the measurement of the diameter of the inhibition zone of Sugar apple leaf extract with a concentration of 20% obtained an inhibition zone with an average of 1.5 mm, a concentration of 40% obtained an average of 2 mm, a concentration of 60% obtained an average of 2.5 mm, 80% is 2.75mm and 100% concentration is 4.5mm. The data was obtained from the positive control with an average of 27mm while the inhibition zone was not obtained in the negative control. The results of this study are following research conducted by Erlina *et al* (2018) about the antibacterial effect and phytochemical activity of sugar apple leaf extract which states that the high concentration of sugar apple leaf, the greater the antibacterial activity so that bacterial growth can be maximally inhibited.

Activity in inhibiting *Staphylococcus aureus* in the treatment of ethanol extract of Sugar apple leaf (*Annona squamosa*) is due to the presence of active compounds in the extract of Sugar apple leaf. Based on research (Dewangga & Nirwana, 2016). stated that the ethanol extract of Sugar apple leaves contains saponins, alkaloids, flavonoids, and terpenoids that function as antibacterial. In Sugar apple leaf extract, there are active compounds whose respective mechanisms are in inhibiting bacterial growth. According to Jangnga *et al* (2018) Flavonoid compounds work to inhibit bacterial growth by causing damage to the permeability of lysosomes, microsomes, and bacterial cell walls. These flavonoid compounds can inhibit the movement of bacteria actively and spontaneously. Alkaloids are capable of causing changes in the composition of amino acids and cells, resulting in damage and causing bacterial cell death (Gurrapu & Mamidala, 2017). Saponins as antibacterials work through hydrogen bonds by forming complex compounds on the cell membrane so that they can destroy the permeability properties of the bacterial cell wall and cause cell death (Dewangga & Nirwana, 2016). The active compounds in the ethanolic extract of Sugar apple leaves that were previously mentioned above against the growth of *Staphylococcus aureus* bacteria in this study could be inhibited.

The results of the data from these measurements can be seen that the test given the treatment of Sugar apple leaf ethanol extract at various concentrations showed results that were resistant to the growth of Staphylococcus aureus. While the results obtained from the treatment of chloramphenicol antibiotics showed sensitive results. So that the inhibition zone obtained in the positive control using the antibiotic chloramphenicol was greater than the treatment with various concentrations of Sugar apple leaf extract. This is because antibiotics are derived from a substance obtained by chemical synthesis or microorganisms so that they can inhibit and kill microorganisms at low concentrations (Cahyani, 2021). In this study, chloramphenicol was used as a positive control. Chloramphenicol is a broad-spectrum antibiotic and Has activity capable of inhibiting protein synthesis in bacterial cells (Mahdiva *et al.*, 2021). The interpretation of the results of bacterial growth against antibacterial or antibiotic substances in this study was categorized based on the formation of a bacterial inhibition zone around the paper disc which was then compared with the 2018 CLSI table. The standard for assessing the diameter of the antibiotic inhibition zone based on the CLSI (*Clinical Laboratory Standards Institute*) contained a level of resistance. bacteria to antibiotics are grouped into three categories, namely resistant, intermediate, and sensitive.

CONCLUSION

The diameter of the inhibition zone for the growth of Staphylococcus aureus bacteria which was replicated 3 times at concentrations of 100%, 80%, 60%, 40%, and 20% was formed with an average of 4.5mm, 2.75mm, 2.5mm, 2mm and 1, 5mm. So it falls into the category of resistance.

ACKNOWLEDGMENTS

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