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Test Of Inhibitory Power Of Cotton Leaf Extract (Gossypium Hirsutum L.) As Anti Bacterial Staphylococcus Aureus

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ABSTRACT

Utilization of natural ingredients as medicine tends to increase in recent times to replace modern medicines such as cotton leaves. Many plant species have antibacterial properties, including the cotton plant (Gossypium hirsutum L.). Cotton leaf plant (Gossypium hirsutum L.). It is known that natural compounds that have the potential as antibacterials generally contain saponins, alkaloids, polyphenols, and flavonoids are cotton plants (Gossypium hirsutum L.). Therefore, it is necessary to conduct research on the inhibition of Cotton leaf extract (Gossypium hirsutum L.) as an anti-bacterial agent for Staphylococcus aureus. The general objective of this study was to determine the effectiveness of cotton leaves (Gossypium hirsutum L.) as an antibacterial for Staphylococcus aureus. The results of this study are expected to provide basic information about the use of cotton leaves (Gossypium hirsutum L.) as an alternative treatment for infectious diseases, especially those caused by Staphylococcus aureus bacteria so that they can become one of the efforts to develop treatments derived from natural ingredients. This type of research is a laboratory experimental design with a one shoot case study design, namely a research design with treatment of the independent variables followed by observations or measurements of the independent variables. Based on the data from this study it can be concluded that cotton leaf extract (Gossypium hirsutum L.) is able to inhibit the growth of Staphylococcus aureus, with a sensitive category where the greatest antibacterial activity is produced at an extract concentration of 100% which is indicated by the formation of an inhibition zone of 18.10 mm.

INTRODUCTION

The use of natural ingredients as medicine tends to increase with the awareness to return to nature to achieve optimal health. Sugianti (2005) wrote that the advantages of using plants as traditional medicine include being relatively safer, easy to obtain, not causing resistance, and relatively harmless to the surrounding environment. Traditional medicines have side effects that are far less dangerous than modern medicines, so the human body is relatively more receptive to them. Plants used as traditional medicine can be in the form of fruit, vegetables, herbs, ornamental plants and plants that live in inland and sea waters to even wild plants that grow in any place.

Microbes that cause infection in order to be killed usually use drugs containing synthetic antibiotics. Infection therapy with synthetic antibiotics can cause bacterial resistance to the anti-bacterial drugs themselves. Natural compounds that have potential as antibacterials generally contain steroids, tannins, polyphenols, flavonoids (Rahman et.al., 2011), alkaloids, saponins (Ahmad et.al., 2008).

Based on the experience of the people of Lamlhom village, G. hirsutum leaves are often used as cough medicine, as a medicine for diabetes, asthma, menstrual pain, and skin diseases in Unani and Ayurveda (Arshiya et al. 2012). In Indonesia, G. hirsutum is also used as a medicine for coughing up blood, diabetes, menstruation, skin diseases, and others (Soedibyo, 1998). G. hirsutum has anticancer, antimicrobial, antiviral, antiparasitic, insecticidal, and antifertility properties, cotton leaf (Gossypium hirsutum L.) is antimicrobial because it contains saponins, flavonoids, polyphenols, alkaloids (Jagt et al. 2000). Therefore, it is necessary to conduct research on the Inhibitory Power of Cotton Leaf Extract (Gossypium hirsutum L.) as an Anti-Bacterial Staphylococcus aureus.

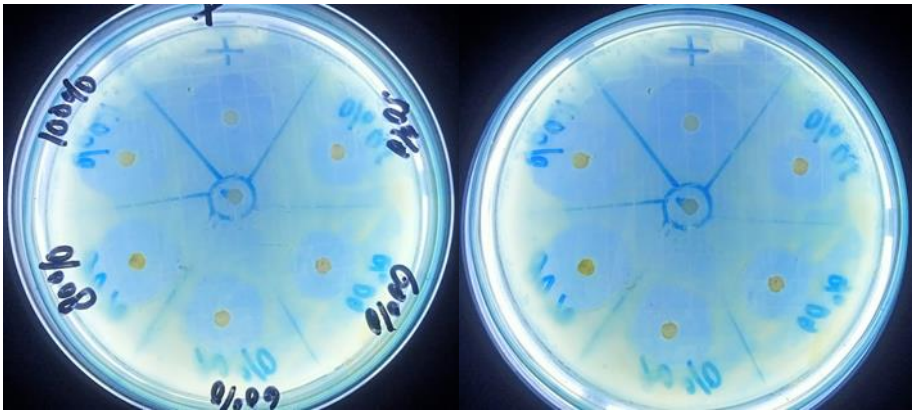
RESEARCH METHODS

This type of research is a laboratory experimental design with a one shoot case study design, namely a research design with treatment of the independent variables followed by observations or measurements of the independent variables. This research was conducted in the laboratory of the Faculty of Medicine, Halu Oleo University in June 2022. The subject of this study was cotton leaf extract (Gossypium hirsutum L.) which was tested against Staphylococcus aureus bacteria. Material collection was carried out by taking a sample of 2000 g of cotton leaves (Gossypium hirsutum L.) taken in Konawe, Southeast Sulawesi. Sampling was carried out by taking cotton leaves (Gossypium hirsutum L.) from their habitat. The selected cotton leaves are whole leaves, green in color and almost the same size of the leaves, washed thoroughly with water, then dried and then extracted. Cotton leaf

extract (Gossypium hirsutum L.) is made by grinding the cotton leaves. Then extracted by soaking for 3 x 24 hours with 95% ethanol. Furthermore, concentration is carried out with an evaporator until a thick extract is obtained. Antibacterial activity testing was carried out by pouring the test bacteria which had been diluted with a concentration of 106/ml by 1 ml into warm media and homogenized. Then paper discs containing cotton leaf extract (Gossypium hirsutum L.) with various concentrations, positive control (ampicillin) concentration of 30 µg were attached to the surface of the agar media in a petri dish (disc paper diffusion method). The petri dishes were incubated in an incubator for 24 hours at 37oC. Furthermore, measurements were taken (in mm units) on the clear area formed around the paper disc containing cotton leaf extract (Gossypium hirsutum L.) and ampicillin. The test was carried out 3 times. Data analysis in this study was carried out in a descriptive way based on the criteria for antibacterial potential, namely the inhibition area of 18 mm or more is classified as sensitive, the inhibition area of 13-17 mm is in the intermediate category, the inhibition area is below 12 mm in the resistant category (CLSI, 2014).

RESULTS

Figure 1 Inhibition zone of Cotton Leaf Extract (Gossypium hirsutum L.) on Staphylococcus aureus Bacteria



Based on the picture above, the results of measuring the diameter of the inhibition zone show that cotton leaf extract (Gossypium hirsutum L.) has an inhibitory effect on the growth of Staphylococcus aureus bacteria from the resistant to the sensitive category.

Table 1. Results of Measurement of the Inhibitory Zone of Cotton Leaf Extract (Gossypium hirsutum L.) on the Growth of Staphylococcus aureus Bacteria

Konsentrasi	Pengulangan (mm)			Rata-rata (mm)	Interpretasi Hasil
	1	2	3		
50%	11,65	11,80	11,90	11,78	Resisten
60%	13,17	13,29	13,22	13,23	Intermediet
70%	14,14	14,04	13,96	14,05	Intermediet
80%	15,41	15,43	15,40	15,41	Intermediet
100%	17,99	18,08	18,22	18,10	Sensitive
Kontrol (+)	23,87	24,35	24,19	24,14	Sensitive
Kontrol (-)	0	0	0	0	Resisten

In this study, concentrations of 50%, 60%, 70%, 80% and 100% cotton leaf extract were tested by evaporation. Antibacterial activity testing was carried out by pouring the Staphylococcus aureus test bacteria. Then paper discs containing cotton leaf extract (Gossypium hirsutum L.) with various concentrations, positive control (ampicillin) concentration of 30 µg were attached to the surface of the agar media in a petri dish (disc paper diffusion method). The petri dishes were incubated in an

incubator for 24 hours at 37oC. Furthermore, measurements were taken (in mm units) on the clear area formed around the paper disc containing cotton leaf extract (Gossypium hirsutum L.) and ampicillin. The clear area indicates an obstacle to the growth of Staphylococcus aureus bacteria on the surface of the agar medium. The test was carried out 3 times.

DISCUSSION

In the study of the inhibition test of cotton leaf extract (Gossypium hirsutum L.) which was tested on Staphylococcus aureus bacteria using the extraction method made in 5 variations of concentration, namely concentrations of 50%, 60%, 70%, 80% and 100% which were carried out in the laboratory Halu Oleo University Faculty of Medicine.

Testing the inhibition of cotton leaf extract (Gossypium hirsutum L.) on the growth of Staphylococcus aureus bacteria was carried out in several stages, starting from the selection of leaves to testing the inhibition of bacteria. The leaf selection stage is carried out by selecting leaves that are still in good condition and taken manually and then carried out until the stage of making concentrations for testing inhibition.

Testing the inhibition of the growth of Staphylococcus aureus bacteria was incubated for 1 x 24 hours in an incubator with an inhibition zone marked by the formation of a clear area around the paper disc. The test was carried out with 3 repetitions using ampicillin as a positive control and DMSO as a negative control.

The inhibition power of cotton leaf extract (Gossypium hirsutum L.) at a concentration of 50% of the inhibition zone formed in the first repetition was 11.65 mm, in the second repetition it was 11.80 mm and in the third repetition it was 11.90 mm with an average of 11.78 mm. The concentration of 60% inhibition zone formed in the first repetition was 13.17 mm, in the second repetition it was 13.29 mm and in the third repetition it was 12.22 mm with an average of 13.23 mm. The concentration of 70% inhibition zone formed in the first repetition was 14.14 mm, in the second repetition it was 14.04 mm and in the third repetition it was 13.96 mm with an average of 14.05 mm. The concentration of 80% inhibition zone formed in the first repetition was 15.41 mm, in the second repetition it was 15.43 mm and in the third repetition it was 15.40 mm with an average of 15.41 mm. The concentration of 100% inhibition zone formed in the first repetition was 17.99 mm, in the second repetition it was 18.08 mm and in the third repetition it was 18.22 mm with an average of 18.10 mm. So that from the 5 concentrations a clear area is formed around the paper disc which is referred to as the inhibition zone. The inhibition zone formed at a concentration of 50% is still categorized as resistant (weak) because the size of the inhibition zone formed is less than 12 mm, the inhibition zone formed at a concentration of 60%, 70% and 80% is categorized as intermediate because of the large inhibition zone formed 13- 17mm. At a concentration of 100%, the inhibition zone that formed was categorized as sensitive because the size of the inhibition zone that formed was more than 18 mm. The negative control using DMSO did not form an inhibition zone and the positive control used the antibiotic ampicillin which was included in the sensitive category and the highest inhibition zone in this test was 24.14 mm, this is because the antibiotic ampicillin is a penicillin derivative which has a broad spectrum that can inhibit the growth of gram-positive bacteria and gram-negative, so the diameter of the inhibition zone produced by the antibiotic is larger than the inhibition zone produced by cotton leaf extract (Gossypium hirsutum L.).

The results of measuring the diameter of the inhibition zone showed that cotton leaf extract (Gossypium hirsutum L.) had an inhibitory ability from the resistant to the sensitive category. In the first, second and third repetitions, both at a concentration of 50% to a concentration of 100%, there was a difference in the size of the inhibition zone, because in general the diameter of the inhibition zone tends to increase in proportion to the increasing concentration of the extract, the greater the concentration, the stronger the effect of the extract being tested (Hamidy et al., 2006).

Cotton leaf extract (Gossypium hirsutum L.) contains saponins, flavonoids, polyphenols, alkaloids (Jagt et al. 2000) which are antibacterial, so this cotton leaf extract has antibacterial activity.

One of the chemical compounds that have antibacterial activity in cotton leaf extract is alkaloids. Alkaloids have an inhibitory mechanism by binding to DNA (Cowan, 2009). This is presumably because the alkaloids have a base group that contains nitrogen. This base group will react with acidic compounds present in bacteria such as DNA which is the main constituent of the cell nucleus. With the disruption of DNA, the synthesis of proteins and nucleic acids in cells will be disrupted. This results in disrupted cell metabolism so that bacterial growth is inhibited or experiences death.

Saponins are also active compounds that are antibacterial in the extract of the leaves of the control. Saponins have soap-like properties. Saponins are active compounds that cause foam when

shaken in water. Saponins work by increasing the permeability of cell membranes so that the membrane becomes unstable and results in cell hemolysis (Robinson, 1995).

Another class of compounds that act as antibacterials are flavonoids. The activity of flavonoids against bacteria is thought to be due to their ability to interfere with the activity of peptidoglycan transpeptidase so that the formation of the cell wall is disrupted. As a result, the cell cannot withstand an internal osmotic pressure which can reach 5 to 20 atmospheres. This pressure is sufficient to break the cell if the cell wall is damaged (Cowan, 2009). Damage to the membrane or cell wall causes various important components to come out of the bacterial cell, namely proteins, nucleic acids, nucleotides, etc., which originate from the cytoplasm and the bacterial cell undergoes lysis.

Polyphenols have a distinctive feature that is having many phenol groups in their molecules. Polyphenolic compounds are widely distributed compounds as natural dyes that cause color in flowers, wood and fruit. The mechanism of polyphenols as antibacterial agents acts as a toxin in the protoplasm, damaging and penetrating the cell wall and precipitating bacterial cell proteins. Large molecular phenolic compounds are capable of inactivating essential enzymes in the nature of bacterial cells even at very low concentrations. Polyphenols can cause damage to bacterial cells, denature proteins, inactivate enzymes, and cause cell leakage (Heyne, 1987).

This study proves that cotton leaf extract (Gossypium hirsutum L.) containing saponins, flavonoids, polyphenols, alkaloids effectively inhibits the growth of Staphylococcus aureus, where the greatest antibacterial activity is produced at an extract concentration of 100% which is characterized by the formation of an inhibition zone 18,10 mm with a sensitive category according to CLSI.

This research is in line with research conducted by Nugrahani, et al (2020) that the ethanol extract of cotton leaves at a concentration of 50% has an inhibitory effect on other bacteria, namely Staphylococcus epidermidis bacteria of 12.28 ± 0.63 mm and a concentration of 70% has an inhibitory effect the largest is 11.40 ± 0.32 mm against Propionibacterium acnes. The active compounds that are thought to inhibit the growth of the two bacteria are compounds belonging to the flavonoid group.

CONCLUSIONS AND RECOMMENDATIONS

Based on the data from this study it can be concluded that cotton leaf extract (Gossypium hirsutum L.) is able to inhibit the growth of Staphylococcus aureus, with a sensitive category where the greatest antibacterial activity is produced at an extract concentration of 100% which is indicated by the formation of an inhibition zone of 18.10 mm.For further researchers, it can be used as further research on inhibition tests, especially in the field of microbiology by using different concentrations to obtain more effective inhibition results.

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