ACTIVITY OF INHIBITING THE GROWTH OF STAPHYLOCOCCUS AUREUS ATCC 25923 LEAVES OF DURI SPINACH (AMARANTHUS SPINOSUS LINN.) USING THE MACERATION METHOD

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ABSTRACT

Antibacterial is a substance that can inhibit or kill the growth of bacteria. One of the plants that can be used for antibacterial is spinach thorns. Spinach (Amaranthus spinosus Linn.) is a plant originating from the tropical lowlands of America, widely distributed in the tropics and subtropics of Africa, Southeast Asia and in India and even in Indonesia. The purpose of this study was to determine the activity of spinach leaves (Amaranthus spinosus Linn.) on inhibiting the growth of Staphylococcus aureus bacteria by maceration method. This study uses an experimental method. Spinach leaves (Amaranthus spinosus Linn.) were macerated using 70% ethanol solvent. The concentrations used for each method were concentrations of 30%, 40%, 50%, ciprofloxacin positive control and 1% DMSO negative control. Each of these concentrations was tested for antibacterial activity against Staphylococcus aureus using the diffusion and dilution method. The results of the antibacterial activity test by diffusion in the maceration method obtained an average inhibition zone result at a concentration of 30% of 8.73mm, a concentration of 40% of 9.93mm, a concentration of 50% of 11.53mm and a positive control of 12.73mm.

KEYWORDS Antibacterial, Thorn Spinach Leaves, Staphylococcus aureus, Maceration.

INTRODUCTION

Staphylococcus aureus is a Gram-positive facultative anaerobic bacterium, 0.5-1.0 µm in diameter, spherical in shape, with grape-like colonies, and does not have spores (Putri, 2021). These bacteria are normal human microflora normally found in the upper respiratory tract and skin. Nearly everyone has some type of Staphylococcus aureus

infection, with symptoms ranging from food poisoning or mild skin infections to serious infections. the inappropriate use of antibiotics to treat bacterial infections can cause several problems after years of use, which can cause bacteria to become resistant to these antibiotics (Putri, 2021).

The development of new antibacterial drugs as an alternative treatment can use an ethnopharmaceutical approach to select plants with high potential as drugs through empirical knowledge that is believed by people in certain areas (Ningsih, 2015).

One of the plants that is widely used in traditional medicine is spinach thorn (Amaranthus spinosus Linn.). Spinach (Amaranthus spinosus Linn.) is a plant originating from the tropical lowlands of America, widely distributed in the tropics and subtropics of Africa, Southeast Asia and in India and even in Indonesia, which is known as a wild plant that grows in empty fields (Arraissa, 2017) . Spinach is used as medicine because it contains several chemical substances that have pharmacological effects, such as tannins and flavonoids which can be used as antibacterial and antiviral (Djindadi et al., 2020).

Antibacterials are substances that can kill or inhibit the growth of bacteria so they can be used to prevent or treat bacterial infections. This substance is the result of secondary metabolites from several microbes (antibiotics) isolated from plants or animals and synthetic). The frequent use of synthetic antibacterials (modern medicine) can reduce host resistance, thereby making the host more susceptible to infection (Sulistyaningsih & Tjitraresmi, 2016).

Research related to antibacterial effectiveness using the maceration method on soursop leaves has antibacterial effectiveness with an inhibition zone of 12.3 mm. (Pratama and Astuti, 2012)

With the background of previous research, a study was conducted on Comparison of Growth Inhibition Activity of Staphylococcus aureus ATCC 25923 Spinach Spinach Leaves (Amaranthus spinosus Linn.) Using the Maceration Method.

RESEARCH METHOD

RESEARCH VARIABLE

The main variable in this study was the inhibition of bacterial growth from spinach leaves (Amaranthus spinosus Linn.) and 70% ethanol extract of spinach leaves (Amaranthus spinosus Linn.) against Staphylococcus aureus bacteria.

RESEARCH STAGE

Determination of Thorn Spinach Plant (Amaranthus spinosus Linn.),

The determination was carried out at the Center for Research and Development of Medicinal Plants and Traditional Medicines (B2P2TOOT) Tawangmangu.

Intake of Materials

Spinach leaves (Amaranthus spinosus Linn.) were obtained from Wonosari, Gondangrejo, Karanganyar, Central Java in a fresh condition.

Standardization of Simplicia

Determination of drying shrinkage, determination of water content, determination of ash content

Preparation of Spinach Duri (Amaranthus spinosus Linn.)

Leaf Ethanol Extract As much as 300 grams of simplicia powder was carried out by a maceration process using 70% ethanol, the filtrate of spinach leaf extract was concentrated with a rotary evaporator to obtain a viscous extract that was free of solvents (Djindadi et al., 2020).

Standardization of Spinach Duri (Amaranthus spinosus Linn.)

Leaf Ethanol Extract, Determination of Drying Shrinkage, determination of water content, ethanol-free test,

Phytochemical Screening of Spinach Spinach (Amaranthus spinosus Linn.)

Leaf Ethanol Extract. Alkaloid test, tannin test, terpenoid test, flavonoid test, saponin test

Antibacterial Activity Testing

Sterilization, preparation of nutrient agar (NA) media, rejuvenation of Staphylococcus aureus bacteria, Identification of Staphylococcus aureus by Gram Staining, Preparation of Muller Hinton Agar (MHA) Media, Preparation of Nutrient Broth (NB) Media, Preparation of Staphylococcus aureus Bacterial Suspensions, Testing Antibacterial Activity by Diffusion Method , Dilution Method Antibacterial Activity Testing

Data Analysis

Data Analysis Using SPSS (Statistical for Service Solutions), Research data using SPSS. Then a normality test is carried out to measure whether the data has a normal distribution so parametric statistics are used and if the data is not normally distributed, non-parametric statistics are used. The normality test was carried out with Shapiro-Wilk (Sujarweni, 2012). The data are normally distributed, then proceed with the homogeneity test, the homogeneity test is carried out using Levene statistics. After the data is normally distributed and homogeneity is analyzed using One Way Anova to see whether there is a significant difference, if there is, it is necessary to carry out a follow-up test, namely the Post Hoc Test. Post Hoc test was carried out using the Tukey method.

RESULT AND DISCUSSION

SKRINING FITOKIMIA

Proses ekstraksi daun bayam *(Amaranthus spinosus* Linn.*)* dilakukan dengan menggunakan metode maserasi. Uji skrining fitokimia yang dilakukan dengan metode tabung. Hasil skrining fitokimia ekstrak daun bayam duri *(Amaranthus spinosus* Linn.*)*

Table 1. Results of Phytochemical Screening of Spinach Duri Leaf Extract (Amaranthus spinosus Linn.)

Reagent Phytochemical	Reactants	Result	Description
Alkaloid	*Mayer*	Formed a yellowish color	(+)
	Dragendroff	There is a brown precipitate	(+)
Tannin	$FeCl_3$ 5%	Blackish green color formed	(+)
Terpenoid	*Lieberman-Burchard*	Dark green color formed	(+)
Flavonoid	Serbuk Mg + HCl pekat	Formed orange or yellowish color	(+)
Saponin	Aquadest	Stable foam formed for 10 minutes	(+)

Identify Staphylococcus aureus

The results of gram staining Staphylococcus aureus are purple in color, round in shape, clustered in arrangement, and are gram-positive bacteria (Anggraini et al., 2021).

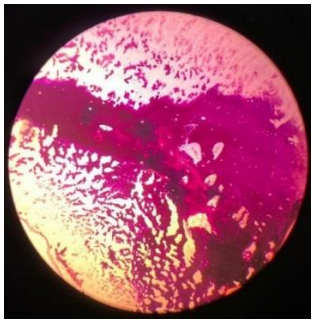


Figure 1. Microscopy of Staphylococcus aureus ATCC 25923
(personal documentation)

Staphylococcus aureus is a gram-positive bacteria so that in gram staining it will turn purple because gram-positive bacteria are able to retain gram A (crystal violet), gram-positive bacteria will bind the substance. (Anggraini et al., 2021).

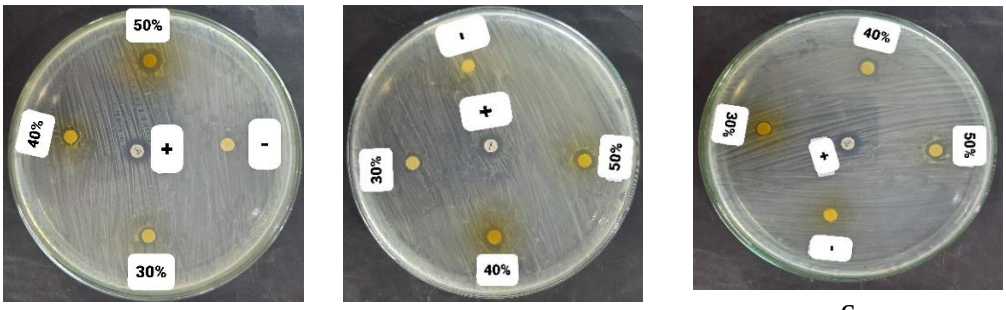
Antibacterial activity testing

Antibacterial activity testing using the diffusion method. The results of the inhibition zones for the maceration and infusion methods can be seen in the table (Anggraini et al., 2021).

Table 2. Results of the Inhibition Zone Diameter of the Maceration Method Antibacterial Activity Test

Concentration	Repetition I	II	III	Mean Zone Inhibition	Growth Restraint Response
50%	10,9mm	11,6mm	12,1mm	11,53mm	Strong
40%	9,4mm	9,1mm	11,3mm	9,93mm	Medium
30%	8,4mm	8,2mm	9,6mm	8,73mm	Medium
K +	12,2mm	12,7mm	13,3mm	12,73mm	Strong
K -	0mm	0mm	0mm	0mm	-

The results showed that the maceration method for spinach leaves (Amaranthus spinosus Linn.) was able to inhibit the growth of Staphylococcus aureus bacteria. Administration of ethanol extract with varying concentrations of 30%, 40%, 50% indicated an inhibition zone with an average inhibition zone on maceration of 11.53mm, 9.93mm, 8.73mm with a strong, moderate inhibition response. The higher the concentration of the ethanol extract of spinach leaves (Amranthus spinosus Linn.), the greater the content of the active compound, thus the greater the inhibition zone formed. The inhibition zone formed can be seen in Figure 2.



a b c

Figure 2. Hasil zona hambat aktivitas antibakteri metode difusi (Dokumentasi pribadi, 2023)

The dilution test was carried out from the results of the diffusion test of the ethanol extract of spinach leaves (Amaranthus spinosus Linn.) which had been tested for antibacterial activity showing that the Minimum Inhibitory Concentration (MIC) in the maceration method was at a concentration of 12.5%. It was indicated by the clarity of the tube which had been filled with ethanol extract and added bacterial suspension and NB media. The concentrations used were 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%. The results of testing the antibacterial activity using the maceration method can be seen in table 3.

Table 3. Dilution Antibacterial Activity of the Maceration Method

Concentration	**Replikasi**		
	I	**II**	**III**
K (+)Suspensi bakteri dan *ciprofloxacin*	-	-	-
50%	-	-	-
25%	-	-	-
12,5%	-	-	-
6,25%	+	+	+
3,12%	+	+	+
1,56%	+	+	+
K (-) Ekstrak etanol	+	+	+

Keterangan : (-) no bacterial growth
(+) there is bacterial growth

The test was continued by inoculating from the test tube into MHA solid media in a petri dish and incubating it for 24 hours at 37°C in an incubator to see the Minimum Kill Concentration (KBM) produced by observing whether there was growth of bacterial colonies (Monica et al, 2021). The results of the KBM can be seen in Figure 3.

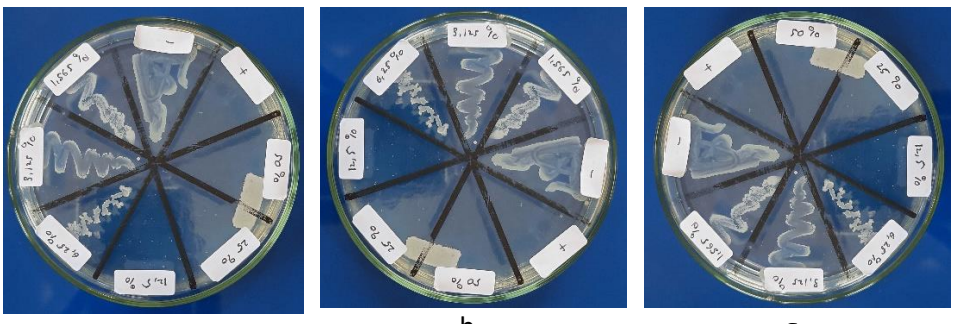


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Figure 3. Hasil KBM metode maserasi (Dokumentasi pribadi, 2023)

From the results of inoculation of bacteria found bacterial growth in all three plates, in the maceration method plates found bacterial growth at concentrations of 1.56% to 6.25% and at concentrations of 12.5% to 50% no bacterial growth was found. So it can be concluded that the Minimum Kill Concentration (KBM) in the maceration method is 12.5%

Data analysis in this study used SPSS (Statistical for Service Solutions). From the results of the Shapiro-Wilk test on the maceration method, a sig of 0.900 was obtained where the value was > 0.05, which means that the data obtained was stated to be normally distributed (Chen, 1968).

Next, the homogeneity test was carried out using the Levene test. From the homogeneity test results obtained in the maceration method, sig 0.263 was obtained, where

the value was > 0.05, which means that the data group came from populations that have the same (homogeneous) variance (Sudjana, 2005).

After carrying out the normality test and homogeneity test the results obtained are normally distributed and have the same variation, then a one way ANOVA test is carried out. From the results of the one way ANOVA test obtained in the maceration method obtained sig 0.002, where the value is <0.05, which means that there is a significant difference in each method and it is necessary to carry out a further test, namely the post hock test (Singgih, 2013).

The test that was then carried out was the Post hock Tukey Test. From the results of the tukey post hock test obtained in the maceration method there was a difference in the concentration of 50% with a concentration of 30% obtained 2.8000 with a sig of 0.013, a difference in concentration of 40% with a positive control obtained -2.8000 with a sig of 0.013, a difference in concentration of 30% with 50% concentration obtained -2.8000 with sig 0.013, 30% concentration difference with positive control obtained -4.0000 with sig 0.001, positive control difference with 40% concentration obtained 2.8000 with sig 0.013, positive control difference with 30% concentration obtained 4.0000 with a sig of 0.001. The results obtained are significant because sig < 0.05 (Singgih, 2013).

CONCLUSION

Spinach ethanol extract (Amaranthus spinosus Linn.) has antibacterial activity against Staphylococcus aureus ATCC 25923 with an average inhibition zone diameter of 10.06mm.

Minimum Inhibitory Concentration (MIC) of spinach leaves (Amaranthus spinosus Linn.) on the activity of inhibiting the growth of Staphylococcus aureus ATCC 25923 in the maceration method at a concentration of 12.5%.

Minimum Kill Concentration (KBM) of spinach leaves (Amaranthus spinosus Linn.) on the activity of inhibiting the growth of Staphylococcus aureus ATCC 25923 in the maceration method, namely at a concentration of 12.5%

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