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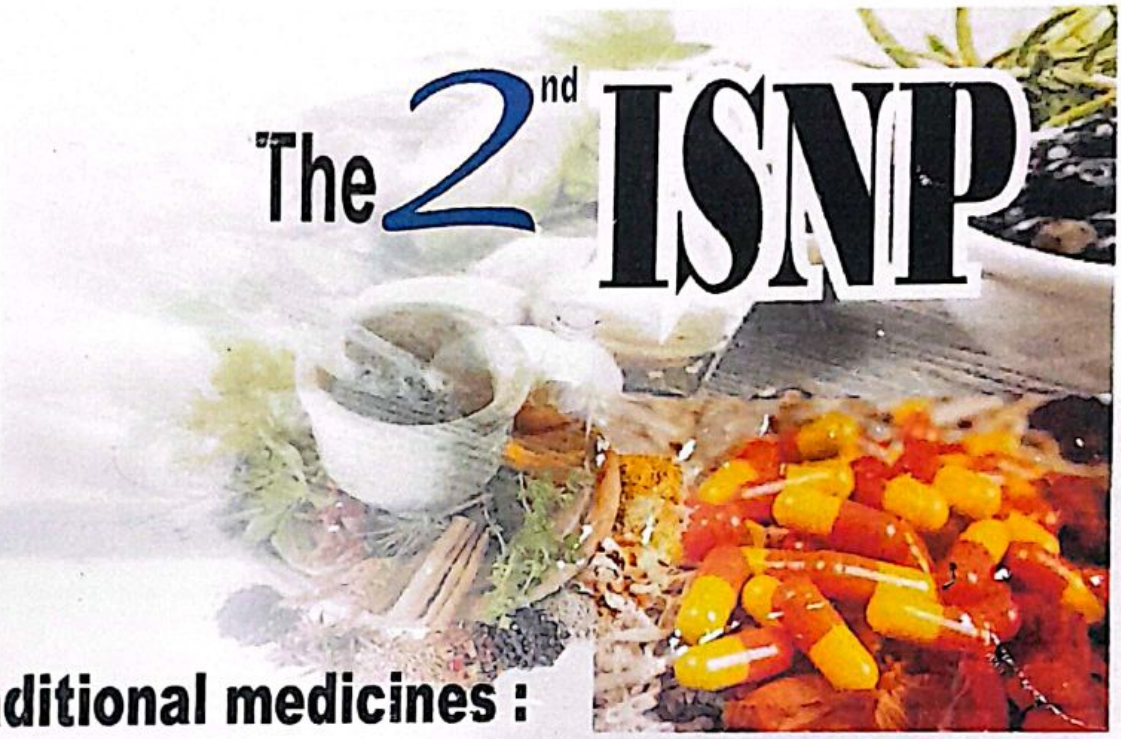

Traditional medicines :

"A Challenge in drug discovery from natural resources"

Grand Clarion Hotel Makassar
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DAFTAR ISI

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EFFECT OF SISIK NAGA LEAF ETHANOLICEXTRACT CONCENTRATION (Drymoglossum pilloscloides(L.) Persl.) ONINHIBITGROWTH OF Staphylococcusaureus

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Abstract

Sisik Naga is an epiphytic plant contains saponins, polyphenols, essential oil, triterpene or sterols, phenols, flavonoids, sugars, and tannins. This plant has many benefits such as its water stew can be used to treat ulcers, arthritis, pulmonary tuberculosis with cough with phlegm, breast cancer, urinary tract infections, pelvic inflammatory and cancer. The aim of this study was to determine the inhibition growth effect and the different concentrations (1%, 2%, and 3%) effect of Sisik Naga leaf ethanolic extract on inhibit growth of Staphylococcus aureus. This is a laboratory experiment with Post Test Only Control Group design. Samples were Sisik Naga leaves collected from Malalayang Manado. Extraction process conducted with maceration method by using ethanol 70% as the solvent. The ethanolic extract made as solution test with concentrations of 1%, 2%, and 3% and then performed tested on Staphylococcus aureus. The diameter of inhibition zone observed every 1 x 24 hours, 2 x 24 hours, 3 x 24 hours. The data were analyzed descriptively and continued with One Way Anova. The results showed that Sisik Naga leaves ethanolic extract have the inhibitory effect and there is an influence of different concentrations of ethanolic extract to inhibit the growth of Staphylococcus aureus.

Keywords: Sisik Naga Leaf Ethanolic Extract, Inhibitory test, Staphylococcus aureus

INTRODUCTION

Indonesia has natural resources such as plants that have benefits in traditional medicine. One of the plants that can be utilized by the community as traditional medicine is sisik nagaleaf (Drymoglossum piloselloides (L.) Presl.). Sisik naga is an epiphytic plant that grows wild in the branches of trees,it can be found in the surrounding environment. Sisik naga containingsaponins, polyphenols, essential oils, triterpenes/sterols, phenols, flavonoids, sugars, andtannins. This plant has many benefits, among others, the stewcan treat ulcers, rheumatism, lung tuberculosis with a productive cough, cancerbreast, urinary tract infections, pelvic inflammation and cancer (Hariana, 2011)

Research conducted by Rizka, et al (Rizka et al, 2012), states that the sisik naga leaves have antibacterial activity against Escherichia coli and Staphylococcus aureus.The study was conducted using maceration method and ethanol 70 % as the solvent at a concentration of 2%. The results showed that, sisik naga leaf ethanol extract has antibacterial activity with inhibition of the bacteria Escherichia coli (6 mm) and Staphylococcus aureus (19 mm).This study using Staphylococcus aureus because the inhibitory effect of the exract on this bacteria according to the last researchis greater than Escherichia coli, and this bacteria can easily found, for example in the wound.

Staphylococcus aureus is a spherical cell with a diameter of 1 µm, arranged in the form of irregular clusters (Brooks et al, 2005). Staphylococcus aureus is a human normal microflora, can be found in the nose,

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skin, eyes, intestines, and liver but if the immune system decreased, these bacteria can cause infection. These bacteria can also be found in the air and the surrounding environment (Syarurachman et al, 1994).

Based on the description above, the problem inthis study are whether the ethanolic extract of sisik naga leaf at concentrations 1%, 2%, 3% have inhibitory effect on the growth of Staphylococcus aureus and whether there is the effect of different concentrations (1%, 2%, 3%) of sisik naga leaf ethanolic extract to inhibit the growth of Staphylococcus aureus? The aim of this study was to determine the inhibition growth effect and the different concentrations (1%, 2%, and 3%) effect of Sisik Naga leaf ethanolic extract on inhibit growth of Staphylococcus aureus.

MATERIAL AND METHODS

Tools: autoclave, analytical balance, blender, flasks, test tubes, measuring cups, glasses Bekker, spiritus lamp, stir bar, tweezers, needle inoculation (ose), petri dish, aluminum foil, jars, rotary evaporator, caliper.

Materials: ethanol 70%, tween 80, aqua pro injection, nutrient agar, pure cultures of Staphylococcus aureus

Research Methods

The type of study is a laboratory experiments with Post Test Only Control Group design with the draft as follows:

Group1 : P1----O1
Group 2 : P2----O2
Group 3 : P3----O3
Group 4 : P4----O4

Specification:

P1 = negative control; P2 = sisik naga leaves ethanolic extract at concentration 1%; P3 = sisik naga leaves ethanolic extract at concentration 2%; P4 = sisik naga leaves ethanolic extract at concentration 3%

O1 = inhibition zone of negative control; O2 = inhibition zone of sisik naga leaves ethanolic extract at concentration 1%; O3 = inhibition zone of sisik naga leaves ethanolic extract at concentration 2%; O4 = inhibition zone of sisik naga leaves ethanolic extract at concentration 3%.

Procedure

- Sterilization of instruments: in the autoclave at the temperature 121^{0}Cfor 15 minutes.
- Preparation of simplisia :sisiknaga leaveswere collected, washed with water, chopped, dried aerated in a place not exposedsunlight, then smoothed by using a blender.
- Preparation ofsisiknaga leaf extract (Depkes RI, 1995; Harborne, 1987): using maceration method by weighing 50 g of sisiknagasimplisia included in the container and soaked in 70% ethanol as much as 350ml. The container is closed and left for 5 days while occasionally protected from light, stirred and then filtered. Dregs rinsed with 70% ethanol to obtain maserat 500 ml. The container is closed and left in a cool, sheltered from sunlight for 2 days and then filtered. Maserat filtered and then put in a round bottom flask, then concentrated in a rotary evaporator and evaporated to obtain a thick extract.
- Preparation of test solution: the test solution at concentration 1% prepared by weighing 0.1 g sisiknaga leaf extract was then added with 0.5% tween 80 and aqua pro injection to 10 ml. The test solution at concentration 2% prepared by weighing 0.2 g sisiknaga leaf extract was then added with 0.5% tween 80 and aqua pro injection to 10 ml. The test solution at concentration 3% prepared by weighing 0.3 g sisiknaga leaf extract was then added with 0.5% tween 80 and aqua pro injection to 10 ml.
- Preparation of nutrient agar medium (Pelczar& Chan, 2007): Nutrient agar weighed to 1 g, then dissolved in 50 ml distilled water, heated until a clear solution of nutrient agar while stirring,

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then sterilized in the autoclave at a temperature of 121°C for 15 minutes. Poured into 3 sterile petri dishes each containing 15 ml. Let out for a moment until it becomes solid.

- Inoculation of bacteriafrompurecultures of Staphylococcus aureus (Pelczar& Chan, 2007): incandescent the ose untill red and then cooled slightly until the red color disappeared. Take the pure cultures of Staphylococcus aureus with sterilized ose, suspended with aqua pro injection in a test tube. Cotton buds dipped into a test tube containing the bacteria Staphylococcus aureus then smeared across the surface of the media that have been solidified evenly.
- Testing: Soaked paper discs at each concentration of 1%, 2%, 3% and a negative control for 5 minutes, then the paper discs were taken with tweezers and placed in petri dishes containing nutrient agar medium inoculated with Staphylococcus aureus. Incubated in the incubator at a temperature of 37°C, observed and measured the diameter of inhibition zone formed around the disc using a caliper every 1 x 24 hours, 2 x 24 hours, 3 x 24 hours.

RESULT AND DISCUSSION

Result

Research on the effect of the concentration of 1%, 2%, and 3% sisik naga leaf extract in inhibiting the growth of Staphylococcus aureus has been done. The extraction of30 gsisik nagaleaves with 300 ml ethanol 70% thick extract obtained as 5.1 g with a yield of 17%. Research results in the form of inhibition zone diameter measurement data can be seen in Table 1 below.

Table 1. Observation DataInhibition Zone Diameter (mm)

est Solution	Petri dish code	Observation DataInhibition Zone Diameter (mm)		
		1 x	2 x	3 x 24
Negative control	I	0	0	0
	II	0	0	0
	III	0	0	0
Mean		0	0	0
Concentration 1 %	I	1	1	1
	II	1	1	1
	III	1	1	1
Mean		1	1	1
Concentration 2 %	I	6	6	6
	II	8	8	8
	III	8	8	8
Mean		7,33	7,33	7,33
Concentration 3 %	I	6	6	6
	II	3	3	3
	III	1	2	2
Mean		3,33	3,67	3,67

To confirm the inhibitory effect of sisik naga leaf ethanolic extract concentration influence the data analysis followed by one way ANOVA test Statistically and obtained the following results.

Table 2. Results of Test One way Anova

ANOVA

Diameter					
	Sum of Squares	Df	Mean Square	F	Sig
Between Groups	94.020	3	31.340	61.957	.000
Within Groups	4.047	8	.506		
Total	98.067	11			

*. The mean diference is significant at the 0,05 level.

To determine differences in concentrations 1%, 2%, and 3% of sisik naga leaves ethanolic extract (Drymoglossum pilloseloides (L.) Persl.) on the inhibition of bacterial growth, Post Hoc test held and tre result can be seen in table 3

Table 3.Results of Post Hoc Test

Multicomparison

Dependent Variable diameter zona bening

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LSD

	(I)Larutan Uji	(J) Larutan Uji	Sig
Tukey LSD	Kontrol Negatif	Konsentrasi 1 %	.123
		Konsentrasi 2 %	.000
		Konsentrasi 3 %	.002
	Konsentrasi 1 %	Kontrol negatif	.123
		Konsentrasi 2 %	.000
		Konsentrasi 3 %	.023
	Konsentrasi 2 %	Kontrol negatif	.000
		Konsentrasi 1 %	.000
		Konsentrasi 3 %	.002
	Konsentrasi 3 %	Kontrol negatif	.023
		Konsentrasi 1 %	.000
		Konsentrasi 2 %	

*. The mean diference is significant at the 0,05 level

Discussion

Research on the effect of sisik naga leaves ethanolic extract (Drymoglossum pilloseloides (L.) Persl.) to inhibit the growth of Staphylococcus aureus has conducted. Sisik naga leaves contain saponins, polyphenols, essential oils, triterpenes / sterols, phenols, flavonoids, sugars, and tannins. This plant has many benefits, among others it can treat ulcers, rheumatism, tuberculosis of the lungs with a productive cough, breast cancer, urinary tract infections, pelvic inflammation and cancer (Hariana, 2011).

Sisik naga leaves are extracted using the maceration method with 70% ethanolas the solvent. This solvent have been choose because it miscible with water in any ratio, and can dissolve flavonoids. Ethanol will extracted potentially polar compounds such as antibacterial flavanoid, tannins, and saponins. Test solution preparation using tween 800.5% to mix the extract homogeneously.

Previous research using only one concentration, ie 2%, whereas in this study made three variations of concentration to see the effect of different concentrations on the inhibitory effect against bacteria and to determine the concentration which has the greatest effect of inhibition.

The observations in Table 1 indicate that the sisik naga leaves ethanolic extract at concentration 1%, 2%, and 3%, have inhibitory effects on the growth of Staphylococcus aureus with the diameter of inhibition zone formedaround the paper disc on the observation for 3 x 24 hours. Negative control showed no inhibition zone diameter. At a concentration of 1% had a mean diameter of 1 mm inhibition zone and no increase in the diameter of inhibition zone for 3 x 24 hours. Concentration of 2% had a mean diameter of 7.3 mm inhibition zone and there is no inhibition zone diameter changes during the observation period. The concentration of 3% gives the average diameter of the inhibition zone varies. In observation of 1 x 24 hour average of 3.33 mm, next at 2 x 24 hour observation and 3 x 24 hours an increase in the mean diameter of the inhibition zone is 3.67 mm.

The results showed that the concentration of 1% has thesmallest effect on inhibitory zone with diameter of 1 mm, while the concentration of 2% had the greatest inhibitory effect. These results indicate that the differences in the concentration of the extract yield different diameter ofinhibition zone. This occurs because of the increased concentration of the extract led to the greater number of antimicrobial compounds that diffuse into the agar so that the zone of inhibition is expected to increase, however, the size of the zone of inhibition depends also on the

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rate of diffusion of antimicrobial compounds used. Extract concentration continuously improved causes diffusion ability of antimicrobial compounds present in the extract will decrease as more viscous extract and as a result the size of the inhibition zone diameter tends to decrease. This can be seen in the inhibition zone resulting from a concentration of 2% greater than the concentration of 3%.

The difference in the average diameter of the inhibition zone between the treatment groupsstatistically analyzed by one way ANOVA test can be seen in Table 2,and it showed that Fh value of 61.957 with significantly by α (0.000 <0.005). With Ftable value of 4.07. Value of F> F table (61.957> 4.07), so H_0 rejected and H_1accepted, showed there is inhibitory effect of sisik naga leaf ethanolic extract at concentration 1%, 2%, 3% to the growth of Staphylococcus aureus.Post Hoc test results in Table 3, show that there is the effect of different concentration of 1%, 2%, and 3% of Sisik naga leaves ethanolic extract in inhibiting the growth of Staphylococcus aureus. However, on the negative control and a concentration of 1% was not significantly different in inhibiting the growth of Staphylococcus aureus.

Antibacterial activity found in sisik naga leaf extract due to the compound contained in the leaves of sisik naga.In general, the inhibition of bacterial growth relatedwith the content of secondary metabolites such as saponins, tannins,and flavonoids.Mechanism of action of saponins will disrupt the cell wall surface tension, the surface tension of the solid when disturbed will antibacterial agent witheasy entry into the cell and will interfere with the metabolism untilthere was inhibition of bacterial growth. Flavanoid compounds have a role asantimicrobial and antiviral. Flavonoids are phenolic compounds that canmendenaturasikan proteins by interfering with cell wall permeabilitybacteria. The mechanism of inhibition of tannin that is the way the walls of bacteria that have been filled due to tannin can easily fit into a bacterial cell and bacterial cell protoplasm mengkoagulasi (Karlina et al, 2013).

CONCLUSION

Based on the research that has been done, it can be concluded that:

1. Sisiknaga leaf ethanolic extract at concentration of 1%, 2%, and 3% have inhibitory effects on the growth of Staphylococcus aureus.
2. There is effect of different concentrations of 1%, 2%, and 3% sisik naga leaf ethanolic extract on the growth of Staphylococcus aureus.

Suggestion

Needed to do more research on the effects of inhibition of sisik nagaleaf on the growth of other bacteria.

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