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Antibacterial Activity Test of Srikaya Leaf Ethanol Extract (Annona Squamosa L) Against Staphylococcus Epidermidis with Typical Layer Chromatography Profile

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Abstract

Srikaya leaves have long been used in traditional medicine for the treatment of cough, fever, rheumatism, diarrhea, and dysentery, as well as an antibacterial and dysentery. The aim of this study is to determine the phytochemical and antibacterial compounds of Srikaya leaf ethanol extract, as well as its antibacterial against Staphylococcus epidermidis bacteria. They also determined the concentration of Srikaya leaf ethanol extract that was most effective in stopping the growth of Staphylococcus epidermidis bacteria. After the Srikaya leaf extract is extracted through the maceration process, phytochemical screening is carried out before the dilution of the extract, which produces 25%, 50% and 75% ethanol concentrations. Disc diffusion method is used to enhance the antibacterial properties of Srikaya leaves on the media Mueller Hinton Agar (MHA). There are alkaloid compounds, flavonoids, tannins, and saponins, according to the findings of this study. With an optimal barrier zone at a concentration of 75% of 11.26 mm, Srikaya leaf extract shows antibacterial capabilities of Staphylococcus epidermidis. It suggests that Srikaya leaf extract contains alkaloids, flavonoids, tannins, and saponins. Further research is needed to find out the antibacterial properties of Srikaya leaves against bacteria such as Bacillus anthraces, Pseudomonas aeruginosa.

Keywords: Annona Squamosa L., Antibacterial, Staphylococcus Epidermidis

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Introduction

Srikaya is one of the plants that has long been used in traditional medicine. From the time of Dutch colonization Srikaya has been known in Indonesia (Isramilda, Sahreni & Saputra, 2020). Srikaya leaves are widely consumed to boost the body's immunity. Srikaya contains secondary metabolite compounds alkaloids, saponins, flavonoids, tannins, phenolic compounds. Srikaya leaves can be used for antibacterial, antioxidant, antidiabetic, hepatoprotective and antitumor activity (Aryantini, Sari & Wijayanti, 2020).

One of the bacteria that commonly infects acne is the bacterium Staphylococcus Epidermidis, which is not a dangerous disease, but can affect anxiety and can cause psychological effects such as depression and lack of self-confidence (Fitriyanti, Pratama & Astuti, 2019). Acne is a chronic skin disease caused by abnormal sebum production according to sebaceous glands that arise in the oil glands in the active skin. The acne is

visible with comedowns and nodules (Apriana, Rahmawanty & Fitriana, 2017).

According to a previous study of the concentration of the barrier strength of Srikaya leaf decoction against the growth of Staphylococcus aureus gave the result that the decoctions of the leaves of Srikaya are able to inhibit the growth of the bacteria Staphyllococcus Aureus. The leaves contain antimicrobial chemicals, including flavonoids, terpenoids and alkaloids (Isramilda, Sahreni & Saputra, 2020).

Research Purposes

1. To find out the phytochemical compounds of Srikaya leaf ethanol extract that can inhibit Staphylococcus Epidermidis bacteria.
2. To find out the Srikaya leaf ethanol extract has antibacterial activity and at what concentration is optimal inhibiting the growth of bacteria Staphylococcus Epidermidis.

Research Method

The type of research used is True Experimental research, using the research design Post Test Only Control Group Design (Notoatmodjo, 2018). The research was conducted at the Pharmaceutical Biology Laboratory and the Bacteriological Laboratory of Bhakti Wiyata Kediri Institute of Health Sciences. The study was conducted in March-June 2022. The tools used in the research include: analytical scales, ovens, blenders, measuring glasses, aluminum foil, Erlenmeyer, cups, water baths, KLT plates, chambers, UV rays 254 nm and 366 nm, reaction tubes, plating, autoclaves, incubators, tubing shelves, oxygen, sterile swabs, lengths. Substances used in the study include: Srikaya leaves, ethanol 70% as a solvent, distilled water, Mc. Farland solution, clindamycin, DMSO 1%, blank disk, Mayer reaction, HCl, FeCl3, BaCl2, Mg powder, ammonia vapour, Nutrient Agar (NA), Mueller Hinton Agar (MHA), Staphylococcus epidermidis bacteria.

Plant Determination

The Srikaya leaf plant used in this study was determined first. The purpose of determination is to obtain the validity of plants as objects of research. Determination is done at MMB Malang.

Simplicia Making

Srikaya leaves taken from the village of Pojok district of Mojoroto City Kediri. The Srikaya leaves are distorted wetly to separate from the nuts and parts of the plant that are not used in the research, then the Srikaya sheets are washed clean under flowing water. Then cut to dry the leaves quickly. The drying process is performed by winding at room

temperature without direct sunlight. Then Srikaya leaves are smoothed with a blender and soaked. The result is inserted in a closed container (Legi, Edy & Abdullah, 2021).

Srikaya Leaf Ethanol Extract Manufacture

Srikaya leaf extract is made by maceration. 350 grams of simplicial powder is inserted into a brown-coloured bottle, then soaked with a 70% ethanol solution as much as 1750 ml. The drug is sealed with aluminium foil and inhaled for 3 days with occasional mixing. After 3 days, the samples are filtered with flannel cloth to produce filter I and dregs I. The existing dregs are then added with a solution of 875 ml of 70% ethanol solution, covered with aluminium foliage and inhabited for 2 days with occasionally mixed (Rumanti et al., 2020).

Ethanol Free Test

Test free ethanol extract of Srikaya leaf, extract inserted into a cup added 1 ml of acetate acid and 1 ml concentrated sulphate acid, then homogenized and heated. If you don't smell the ester, then it's ethanol-free (Sandy, Wardani & Septiarini, 2021).

Phytochemical Screening

1. Alkaloids

In the amount of 0.5 g of Srikaya leaf extract added 1 ml of 2 N chloric acid and 9 ml of water, heated over the water dryer for 2 minutes, cooled and filtered. The first reaction tube drops Mayer's reaction solution, the second reaction tube drops Wagner, and the third reaction pipe drops Dragendrof 2 drops, respectively. If there are alkaloids, then Mayer will form white or yellow clumps. Wagner's reaction will form brown to black clumps. Dragendorf clumps will form yellow dwarf clusters. The extract is said to contain alkaloids if two of the three above reactions give a positive result (Jannah, Husni & Nursanty, 2017).

2. Flavonoids

Extract thick Srikaya leaves of 0.5 g added Mg powder 2 mg, then added 3 drops of concentrated HCL. Positive flavonoid results will indicate the color of red or yellow dwarf (Jannah, Husni & Nursanty, 2017).

3. Tannin

A thick extract of Srikaya leaves of 0.5 g is inserted into the reaction tube, added distilled water 10 ml for 15 minutes and filtered. Then the filter is added 2 drops of a concentrated FeCL3 solution, observing the color formed. When formed green or blue indicates the presence of tannins (Jannah, Husni & Nursanty, 2017).

4. Saponins

An extract of a thick leaf of Srikaya of 0.5 g is inserted into the reaction tube. Add 10 ml of hot water, cool and mix hard until a foam is formed for at least 10 minutes. At the

addition of 1 drop of 2 N chloric acid, the foam does not disappear (Jannah, Husni & Nursanty, 2017).

5. Typical Layer Chromatography Analysis

Typical Layer Chromatography (KLT) separation method uses silica gel F254 silica plates that are already activated with heating in the oven at a temperature of 100oC for 10 minutes, with a length of 10 cm and a width of 2 cm. While the motion phase uses n-butanol: acetic acid: water (4:1:5) which is the best eluent for separating flavonoid compounds. The sample is bottled on an activated F254 gel silica plate by heating the oven for 5-10 minutes at a temperature of 100oC, after which the plate is inserted into a chamber containing the saturated developer solution. Elution is carried out until the Rf sample is separated, after completion of the wind-wishing plate and given ammonia vapour, the positive result of the ammonium reaction is marked by the presence of yellow colour on the stained sample with the colour becoming clearer then detected at UV rays 366 and 254 nm, sample Rf (Yuda, Cahyaningsih & Winariyanthi, 2017; Erlina, Hesturini & Nurvita, 2018).

6. Antibacterial Activity Test

The bacterial activity was measured using a disc diffusion method by scratching Staphylococcus Epidermidis on a plate containing MHA media. Tests were carried out on 3 cups of petri. The blank disk with a diameter of 6 mm is soaked with ethanol extract of Srikaya leaves with a concentration of 25%, 50% and 75%, the blank disk is also soaked with a solution of positive control of clindamycin and negative control of DMSO 1 %, after which the disc is placed on a medium that has been saturated and given bacteria. The plates were incubated in incubators for 24 hours at a temperature of 37°C and then observed and measured the diameter of the lymphatic zone formed (Fitri & Widiyawati, 2017).

7. Data Analysis Techniques

The data analysis used in this study is a quantitative descriptive that shows the size of the barrier zone of growth of Staphylococcus epidermidis bacteria around the disc. The data obtained will be analysed first with the normality test using the Wilk Shapiro test with a significant value › 0.05, the homogeneity test with the Levene test with an important value › 0.05, if the data are not distributed normally and homogeneous then proceed with the Wallis Kruskal test, if the normal distribution data proceeds with the ANOVA one-way test with error level ‹0.0.5, if there are differences then proceeds with the Tukey test.

Results and Discussion

Plant Determination

Srikaya determination was carried out at Materia Medica Batu, Malang, East Java. The results of the determination of the Srikaya plant showed that the plant used was truly Srikaya.

Srikaya Leaf Extraction Results

Results of extraction of Srikaya leaf simplicial powder. 350 grams of Srikaya leaf simplicial was macerated using 1750 mL of 70% ethanol for 3 days. The extract weight was 46,497 grams with a yield percentage of 13,289%.

Ethanol Free Test Results

The ethanol content in the extract can affect the antibacterial test results because ethanol can give false positive results as an antibacterial, therefore the extract must be free of ethanol content (Sandy, Wardani & Septiarini, 2021). The ethanol-free test showed that the concentrated Srikaya leaf extract did not contain ethanol. This result is indicated by the absence of an ester odor during the test.

Results of Phytochemical Screening of Srikaya Leaf Extract

Identification of the chemical content of Srikaya leaf extract was carried out qualitatively. Identification was carried out on alkaloids, flavonoids, tannins and saponins. The results of identifying the chemical content of Srikaya leaf extract can be seen in table 1.

Table 1. Results of Phytochemical Screening for Active Compounds in Srikaya Leaf Extract

Chemical Content	Reactor	Results (+) Literature	Extract Test Results	Information
Alkaloids	HCl + Reactor Wagner, Mayer, Dragendorf	Wagner (brown-black precipitate), Mayer (yellow or white precipitate), Dragendorf (orange yellow precipitate)	Wagner = brown-black precipitate, Mayer = white precipitate, Dragendorf = orange yellow precipitate	Positive (+)
Flavonoids	Mg powder + concentrated HCl	Orangeyellow	Saffron	Positive (+)
Tannin	$FeCl_3$	Blackish green	Blackish green	Positive (+)
Saponins	Aquadest	If the foam is stable for 10 minutes, it is positive for saponin	Bubbly	Positive (+)

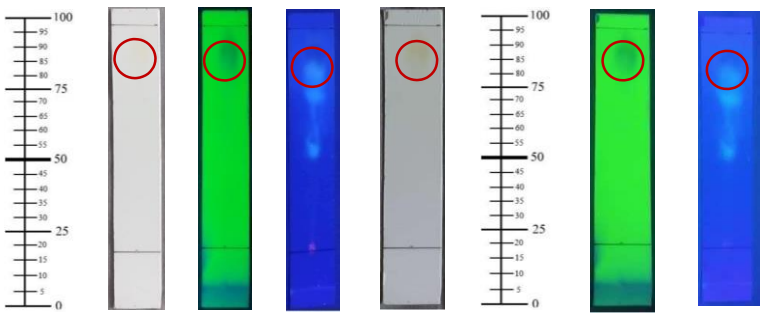

Figure 1. TLC results with mobile phase comparison of n- butanol: acetic acid: water (4:1:5)

(a) Observations in visible light, (b) Observations in UV light 254 nm, (c) Observations in UV light 366 nm, (d) Observations in visible light after being sprayed with Ammonia vapor, (e) Observations in UV light 254 nm after Ammonia vapor, (f) Observation under 366 nm UV light after Ammonia vapor.

The TLC test was carried out to determine the content of flavonoid compounds in Srikaya leaf extract. The TLC test was carried out by eluting the F254 silica gel plate extract using the mobile phase n-butanol: acetic acid: water (4:1:5) then observing the stain under a 366 nm and 254 nm UV lamp. The TLC test results show that the Rf value of Srikaya leaf extract is 0.8. The stains produced in this study acquired a clearer yellow color after being evaporated with ammonia. In line with this research, according to (Erlina, Hesturini and Nurvita, 2018), the TLC test on Srikaya leaves showed a yellow color on the sample spots with the color becoming clearer after being steamed with ammonia. In Figure 1. Results of the Antibacterial Activity Test. The results of the antibacterial activity test of the ethanol extract of Srikaya leaves against Staphylococcus Epidermidis using the disc diffusion method in various concentrations of the ethanol extract of Srikaya leaves contained inhibitory power (clear zone) which can be seen in table 2.

Table 2. Antibacterial Activity Test Results of Srikaya Leaf Ethanol Extract Against Staphylococcus Epidermidis Bacteria

Concentration	Obstacle zone (mm)			Average ± SD (mm)	Categories (Datta *et al.*, 2019)
	R1	R2	R3		
25%	6.07	5.53	5.18	5.59 ± 0.44	Medium
50%	8.53	8.42	7.33	8.09 ± 0.66	Medium
75%	13.04	11.4	9.34	11.26 ± 1.85	Strong
Control (+)	24.05	23.64	23.66	23.78 ± 0.23	Strongest
Control (-)	0	0	0	0 ± 0	-

According to (Datta et al., 2019), antimicrobial inhibition zone activity is grouped into four categories, namely: Weak activity: (<5 mm), moderate activity: (5-10 mm), strong activity: (10-20 mm), very strong activity: (20- 30 mm).

The antibacterial activity test of the ethanol extract of Srikaya leaves against Staphylococcus Epidermidis bacteria was carried out 3 times with the aim of obtaining more accurate data (Devi & Mulyani, 2017). Optimum test results were obtained at a concentration of Staphylococcus epidermidis bacteria of 75% (11.26). Compared with other concentrations because the resulting inhibition zone is closest to the positive control. Concentrations of 25% and 50% are included in the medium category (5-10 mm) with a value

of 5.59 and 8.09 and in the strong category (10-19 mm) at a concentration of 75% with a value of 11.26. The normality test was carried out using the Shapiro-Wilk technique, showing that the data were normally distributed (p>0.05) with significance values, namely for control (+) (0.083), 25% concentration (0.766), 50% concentration (0.159), and concentration 75% (0.875). The results of the homogeneity of variance test, carried out using the Levene Statistics test, show that the data variance is homogeneous with a significance value of 0.053 (p>0.05). The results of the One Way ANOVA test show that each concentration has an average difference with a significance value of 0.000 (p<0.05). The results of the Post Hoc Tukey HSD test showed that there was a significant difference between the extract and the control (+) clindamycin. Based on the results of statistical analysis tests, it was found that the control (+) clindamycin was significantly different from the ethanol extract of Srikaya leaves at concentrations of 25%, 50% and 75% with a significance value of <0.05. There is a significant difference between clindamycin, extract concentrations of 25%, 50%, and 75% because clindamycin is known to be effective against so it is in accordance with its indications for treating acne, whereas extract concentrations of 25%, 50%, and 75% due to differences in concentration in the dilution of the extract for the antibacterial test.

Conclusion

1. There are phytochemical compounds contained in the ethanol extract of Srikaya leaves which can inhibit Staphylococcus Epidermidis bacteria, namely in the form of Alkaloid, Flavonoid, Tannin and Saponin compounds.
2. The ethanol extract of Srikaya leaves has antibacterial activity and at a concentration of 75% Srikaya leaves are optimal as an antibacterial against Staphylococcus Epidermidis bacteria.

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