Phytochemical Screening And Antibacterial Activity Test Of Ethanol Extract Of Jengkol Leaves (*Archidendron Pauciflorum* Benth.) I.C. Nielsen Against *Staphylococcus Epidermidis* And *Propionibacterium Acnes*

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***Abstract**.*
Skin disease was a disease that attacked the surface of the body, and was caused by various agents, one was bacteria. One of the plants that had antibacterial activity was jengkol leaf (Archidendron pauciflorum Benth.) I.C. Nielsen because it contained compounds that had antibacterial properties that had previously been studied by other researchers against Staphylococcus aureus and Escherichia coli bacteria. So that researcher was interested in researching on the antibacterial activity of jengkol leaves (Archidendron pauciflorum Benth.) I.C. Nielsen against Staphylococcus epidermidis and Propionibacterium acnes. Fresh Jengkol leaves were processed into simplicia and extracted using 96% ethanol. Phytochemical screening was carried out on simplicia powder and extracts of jengkol leaves. Ethanol extract jengkol leaves was made in several concentrations, namely 20%, 25%, and 30%, positive control using Tetracycline HCl, and negative control using 1% DMSO. There were several tests carried out on jengkol leaves in addition to phytochemical screening, namely examination of simplicia characteristic including macroscopic examination, microscopic examination, examination of water content, examination of water-soluble extract levels, examination of ethanol-soluble extracts, examination of total ash content, and also an examination of acid-insoluble ash levels and antibacterial activity test of jengkol leaves. The results of phytochemical screening showed that the compound of jengkol leaves (Archidendron pauciflorum Benth.) I.C. Nielsen contained a class of secondary metabolites of alkaloids, flavonoids, tannins, saponins, steroids/triterpenoids, and glycosides. And for the results of the antibacterial activity research also showed that jengkol leaves could be used as antibacterial because it has a strong inhibitory power at a concentration of 20% and the strongest at a concentration of 30% against Staphylococcus epidermidis, namely 15.06 mm and 17.83 mm, while in diameter of growth inhibition zone Propionibacterium acnes was 15.86 mm and 18.1 mm.

***Keywords**: Jengkol leaves (Archidendron pauciflorum Benth.) I.C. Nielsen, jengkol leaves extract, antibacterial activity, Staphylococcus epidermidis, Propionibacterium acnes*

I. INTRODUCTION

Acne or *Acne vulgaris* is one of the most common skin diseases found globally in adolescents and young adults. Acne appears caused by 4 factors, namely overactive oil glands, clogged pores, skin bacterial activity, and inflammation (Fauzi dkk, 2012). The bacteria that cause acne are *Propionibacterium acnes*, *Staphylococcus aureus, Staphylococcus epidermidis*. Among these bacteria, *Propionibacterium acnes* has the most important role in the development of acne. *Propionibacterium acnes* is a gram-positive, air-tolerant, anaerobic bacteria that is a normal flora of the skin. *Propionibacterium acnes* produces various biological molecules and enzymes that act as inflammatory agents in acne (Pothitirat dkk., 2010). Plants are a source of various types of chemical compounds that have efficacy.

To determine the content of chemical compounds in plants, a phytochemical analysis is carried out. Phytochemical screening is a qualitative examination of chemical content to determine the class of compounds contained in a plant. The examination was carried out on secondary metabolites that have health benefits such as alkaloids, glycosides, flavonoids, terpenoids, tannins, and saponins (Harborne, 2006). Jengkol plant (Archidendron pauciflorum Benth) I.C. Nielsen is one of the plants used by the Indonesian people as traditional medicine. Jengkol leaves are efficacious as medicine for eczema, scabies, wounds, and ulcers, the skin of the fruit is used as medicine for ulcers. Seeds, jengkol leaf cortex contain saponins, flavonoids, and tannins (Whitmore, 1987). The chemical content of jengkol leaves has been tested and proven to contain flavonoids, tannins, and saponins. The content of flavonoids can be used as an antibacterial (Yunitasari dkk,2016). Flavonoids are derivatives of phenolic compounds that can disrupt the integrity of bacterial cell walls and membranes which can be seen from changes in the size and morphology of bacterial cells (Zahrah.dkk, 2018).

In general, a lot of jengkol leaves are wasted and become garbage because they cannot be used, only jengkol fruit is used or processed by the community to be used as food ingredients. And traditionally jengkol leaves have been used to treat infectious diseases, it is suspected that the jengkol plant contains antibacterial compounds. Previously, a study by Salni et al. (2011) was successful to prove the antibacterial activity of jengkol (Archidendron pauciflorum Benth.) I.C. Nielsen leaves against Staphylococcus aureus and Escherichia coli which can cause disease in humans. Antibacterial activity was measured in vitro to determine the ability of an antibacterial substance. Antibacterial activity is determined by the spectrum of action (broad spectrum or narrow spectrum), mode of action (bactericidal or bacteriostatic), and the Minimum Inhibitory Concentration (MIC). An antibacterial is to have high activity if MIC occurs at low levels but has great killing or inhibiting power (Brook et al, 2008). This study used ethanol extract of jengkol leaves with concentrations of 20%, 25%, and 30%. The results of the study analyzed using SPSS ANOVA showed that the ethanol extract of jengkol leaves with a concentration of 30% had the strongest inhibitory power in inhibiting the growth of antibacterial activity.

II. METHOD

2.1 Simplified Characterization Examination

Examination of simplicia characterization includes macroscopic, microscopic examination, determination of water content by the azeotropic method, determination of water-soluble extract content, determination of ethanol-soluble extract content, determination of total ash content, and determination of acid-insoluble ash content (Depkes RI, 1995).

2.2 Phytochemical Screening Test

2.2.1 Alkaloid Examination

Ethanol extract of Jengkol Leaves was weighed 0.5 g added 1 ml HCl 2 N added 9 ml distilled water, then heated on a water bath for 2 minutes, cooled and filtered the filtrate was used for the examination of alkaloids:

1. 3 drops of filtrate are added with 2 drops of Mayer reagent, a white or yellow lumpy residue will be formed.
2. 3 drops of filtrate are added with 2 drops of Bouchardat reagent, a brown to black residue will be formed.
3. 3 drops of filtrate are added with 2 drops of Dragendroff reagent to form brown or orange.

If there is a residue or turbidity in at least 2 test tubes in the above experiment, the alkaloid is positive (Ditjen POM, 1979).

2.2.2 Flavonoid Examination

As much as 10 g of ethanol extract of jengkol leaves was weighed and then added 100 ml of hot distilled water, boiled for 5 minutes, and filtered in a hot state, into 5 ml of the filtrate added magnesium powder and 1 ml of concentrated HCl and 2 ml of amyl alcohol, shaken vigorously and allowed to separate. The presence of flavonoids is indicated by the presence of a red, yellow, or orange color on the amyl alcohol layer (Ditjen POM, 1979).

2.2.3 Saponin Examination

As much as 0.5 g of ethanol extract of jengkol leaves was put into a test tube, then added 10 ml of hot distilled water and cooled, then shaken vigorously for 10 minutes. If the foam is formed with a height of 1-10 cm which is stable for not less than 10 minutes and does not disappear with the addition of 1 drop of 2 N HCl, it indicates the presence of saponins (Ditjen POM, 1979).

2.2.4 Tannin Examination

As much as 1 g of ethanol extract of jengkol leaves with 10 ml of distilled water and then filtered the filtrate was diluted with distilled water until it was colorless. 2 ml of the solution was taken and 1-2 drops of 1% iron (III) chloride reagent were added. If a blue-black or green-black color occurs, it indicates the presence of tannins (Ditjen POM, 1979).

2.2.5 Glycoside Examination

A total of 1 gram of simplicia powder and jengkol leaf extract was extracted with 30 ml of a mixture of 96% ethanol - distilled water (7:3), then added 10 ml of 2N HCl, refluxed for 10 minutes, then cooled and

filtered. 20 ml of the filtrate was taken plus 25 ml of distilled water and 25 ml of 0.4 M lead (II) acetate, shaken, allowed to stand for 5 minutes, and then filtered. The filtrate was extracted 3 times, each time with 20 ml of the chloroform-isopropanol mixture (3:2). In the collection of juices added anhydrous sodium to taste. Filtered and evaporated at a temperature of not more than 50˚C. The remainder was dissolved with 2 ml of ethanol, then 0.1 ml of the experimental solution was taken into a test tube, evaporated over a water bath. 2 ml of water and 5 drops of Molisch reagent were added to the residue, 2 ml of concentrated sulfuric acid was added carefully, a purple ring was formed at the boundary of the two liquids indicating the presence of glycosides (Depkes RI, 1995).

2.2.6 Steroid/Triterpenoid Examination

As much as 1 g of jengkol leaves. Simplicia powder and ethanol extract were each macerated with 20 mL of n-hexane for 2 hours and then filtered. The filtrate is evaporated in a vaporizer cup. To the remainder, a few drops of Liebermann-Buchard reagent were added. The appearance of blue or green-blue indicates the presence of steroids, red, pink, or purple colors indicate the presence of triterpenoids (Depkes RI, 1995).

2.3 Antibacterial Activity Test

2.3.1 Equipment Sterilization

The tools used in this antibacterial activity test were sterilized before being used. Glass utensils are sterilized in the oven at 170˚C for 1-2 hours. Bacterial growth media were sterilized in an autoclave at 121ºC for 15 minutes. While the ose needles are sterilized by burning them in a spirit lamp until they glow.

2.3.2 Making Tilt Media

The prepared and sterile NA media was poured into a 5 mL test tube under warm conditions of 40-45˚C. The test tube containing the media is then tilted at an incline of about 30-45˚. The mouth of the test tube was gagged with cotton wrapped in sterile gauze, then the solid media was stored in a refrigerator at 5˚C so that the media was tilted (Depkes RI, 1995).

2.3.3 NaCl solution 0,9%

Weighed as much as 0.9 grams of sodium chloride then dissolved in sterile distilled water little by little in a 100 mL volumetric flask until completely dissolved. Sterile distilled water was added up to the marked line, put in a sterile Erlenmeyer with a lid, and then sterilized in an autoclave at 121˚C pressure at 1 atm for 15 minutes.

2.3.4 Suspension Standard McFarland

McFarland standard suspension showed that the concentration of turbidity of the bacterial suspension was equal to 108 CFU/mL. The two solutions, namely 1% sulfuric acid solution 9.95 mL and 1.1% barium chloride solution 0.05 mL were mixed in a sterile 100 mL volumetric flask, shaken until homogeneous and closed. If the turbidity of the bacterial suspension is the same as the turbidity of the standard suspension McFarland, the bacterial concentration was 108 CFU/mL. The purpose of making suspension solution McFarland's reference was to adjust the turbidity of the bacterial suspension so that the number of bacteria was within a given range for standardizing microbial testing. If the suspension used is too concentrated or too dilute, incorrect results (false resistance or false susceptibility) for any given antimicrobial agent can occur (Fitri, 2015).

2.3.5 Bacteria Identification

To ensure the test bacteria used, bacterial identification was carried out, namely by Gram staining and cultivating on selective media for each bacterium, the method was: cleaned the glass slide, then the needle was ignited, waited for it to cool, then the bacteria were taken from the media and leveled on the object. The glass is then incandescent to dry. Furthermore, it was dripped with a solution of Crystal violet, allowed to stand for 1 minute, then the glass object was given distilled water and dried. Then it was dripped with Lugol's solution and allowed to stand for 1 minute, washed with acid alcohol, and rinsed with distilled water, then dripped with safranin, left for 1 minute, washed with distilled water and dried over a Bunsen fire and observed under a microscope.

2.3.6 Preparation of Bacterial Suspension

The bacterial suspension was made using 0.9% physiological NaCl. The suspension was made by adding several cycles of the test bacterial culture into 0.9% physiological NaCl and then vortexing until homogeneous. The results are compared with McFarland standards. If the bacterial suspension is still too clear when compared to the standard solution, then a few more bacteria cultures are added. Meanwhile, if the bacterial suspension is too turbid, 0.9% NaCl can be added to obtain a bacterial suspension solution that is as turbid as the standard solution.

2.3.7 Antibacterial Activity Testing

The antibacterial test was carried out by the diffusion method, consisting of 5 treatment groups, namely 3 concentrations of extract, and (20%, 25%, and 30%), 1 negative control group (DMSO 1%), and 1 positive control group (Tetracycline HCl) with 3 repetition times. The suspension of *Staphylococcus epidermidis* and *Propionibacterium acnes* were adjusted to the standard turbidity McFarland took 0.5 ml of the suspension solution and placed it into a petri dish then the sterilized Mueller Hinton agar media was put into a petri dish as much as ± 20 ml and leveled and allowed to solidify. Empty discs were dipped and soaked in each concentration of ethanol extract of jengkol leaves and then placed on the surface of the media. Then all the petri dishes that had been treated were incubated into the incubator. Incubation was carried out at 37ºC for 18-24 hours. Then the diameter of the clear zone was measured using a caliper (Oxoid, 1982).

III. RESULT AND DISCUSSION

Tabel 1. Results of Simplicia Characterization Examination

No	Inspection	Earning Rate %
1	Determination of water content	8%
2	Determination of water-soluble extract content	25%
3	Determination of ethanol-soluble extract content	17%
4	Determination of total ash content	4.975%
5	Determination of acid-insoluble ash content	0.085%

Description : ≥ : No more than
≤ : Not less than

No.	Group of Chemical Compounds	Identification
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannins	+
4.	Saponins	+
5.	Glycoside	+
6.	Steroids/Triterpenoids	+

Table 2. Results of Phytochemical Screening of Powder and Ethanol Extract of Jengkol Leaves
Description:
(+) = contains the substance being examined
(-) = does not contain the substance examined

Table 3. The results of testing the antibacterial activity of ethanol extract of jengkol leaves against *Staphylococcus epidermidis*.

Test Sample	Concentration (%)	inhibition zone (mm)			Average inhibition zone (mm)
		Replikasi			
		1	2	3	
EEJL (Ethanol Extract of Jengkol Leaves)	20%	15.4 mm	14.7 mm	15.1 mm	15.06 mm
	25%	16.1 mm	15.3 mm	16.7 mm	16.03 mm
	30%	18.4 mm	17.3 mm	17.8 mm	17.83 mm

Positive Control Tetracycline HCl		21.5 mm	18.9 mm	15.6 mm	18.66 mm
Negative Control DMSO 1%		-	-	-	-

Table 4. The results of testing the antibacterial activity of ethanol extract of jengkol leaves against the bacteria *Propionibacterium acnes*.

Test Sample	Concentration (%)	inhibition zone (mm)			Average inhibition zone (mm)
		Replikasi			
		1	2	3	
EEJL (Ethanol Extract of Jengkol Leaves)	20% 25% 30%	16.3 mm 17.5 mm 18.3 mm	15.4 mm 18.3 mm 17.1 mm	15.9 mm 18.7 mm 18.9 mm	15.86 mm 18.16 mm 18.1 mm
Positive Control Tetracycline HCl		17.1 mm	17.4 mm	17.8 mm	17.43 mm
Negative Control DMSO 1%		-	-	-	-

Discussion

Based on Tables 3 and 4, it can be seen that the inhibition of the growth of *Staphylococcus epidermidis* and *Propionibacterium acnes* bacteria is different in each concentration. However, for a concentration that has a large inhibitory power, it is at a concentration of 30%. Where in this 30% concentration has more ethanol extract of jengkol leaves than the solvent. According to Davis and Stout (1971), the criteria for antibacterial power are as follows: an inhibition zone diameter of 5 mm or less is categorized as weak, an inhibition zone of 5-10 mm is categorized as moderate, an inhibition zone of 10-20 mm is categorized as strong and an inhibition zone of 20 mm or more is categorized as very strong. Thus, it is known that the extract concentrations of 20%, 25% and 30% are effective concentrations to inhibit the growth of *Staphylococcus epidermidis* and *Propionibacterium acnes* bacteria.

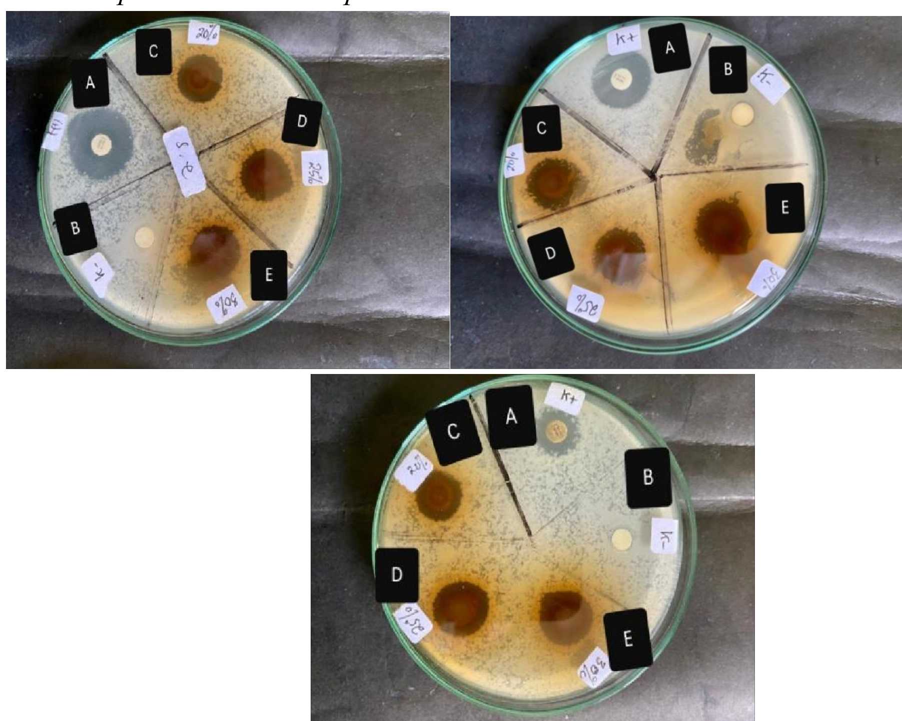

Fig 1. Antibacterial Activity Test Results of Ethanol Extract of Jengkol Leaves (Archidendron pauciflorum Benth.) Against *Staphylococcus epidermidis* Bacteria.

Description: A. Positive Control
B. Negative Control

C. Concentration of 20%
D. Concentration of 25%
E. Concentration of 30%

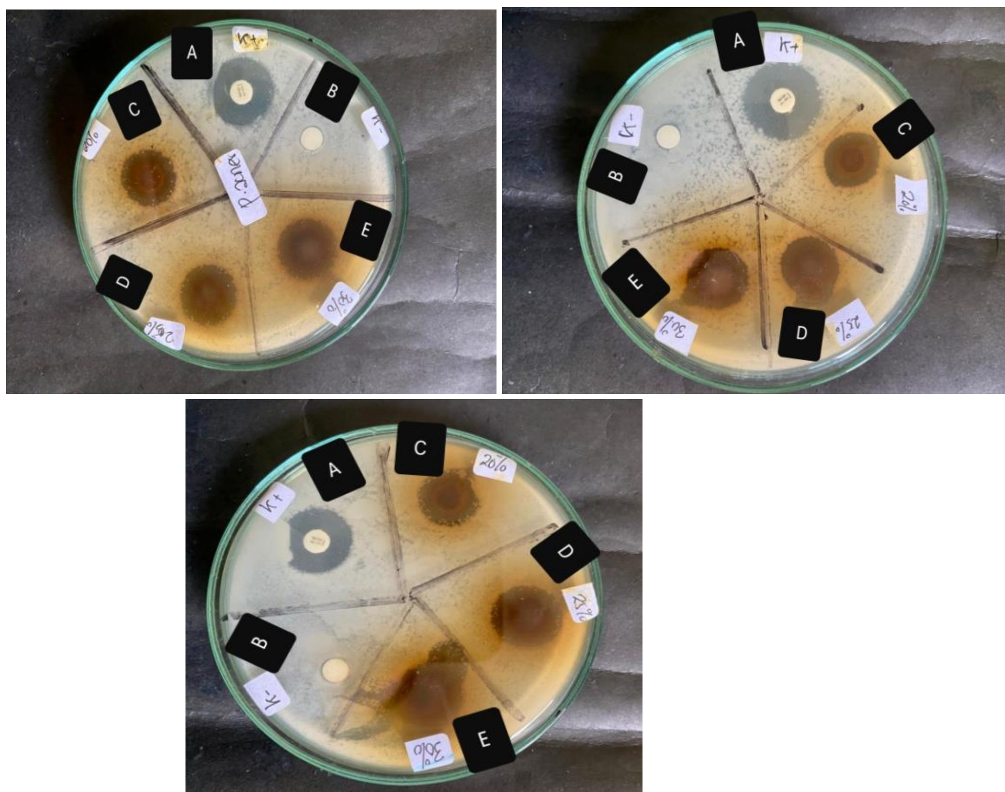

Fig 2. Antibacterial Activity Test Results of Ethanol Extract of Jengkol Leaves (Archidendron pauciflorum Benth.) against *Propionibacterium acnes* bacteria.

Description: A. Positive Control
B. Negative Control
C. Concentration of 20%
D. Concentration of 25%
E. Concentration of 30%

IV. CONCLUSION

Ethanol extract of jengkol leaves (Archidendron pauciflorum Benth.) can be used as an antibacterial because it has a strong inhibitory power at a concentration of 20%, and the strongest at a concentration of 30% against *Staphylococcus epidermidis*, namely 15.06 mm and 17.83 mm, and *Propionibacterium acnes* is 15.8 mm. and 18.1 mm. So it can be concluded that the ethanol extract of jengkol leaves has a strong inhibitory power against Staphylococcus epidermidis and Propionibacterium acnes bacteria based on the concentrations tested in this study.

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