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Anti Bacterial Activity Test of Ethanol Extract of Papaya Leaves (*Carica papaya* L) on the Growth of *Staphylococcus epedermidis*

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ABSTRACT

Papaya is a tropical plant from the Caricaceae family, botanically defined as *Carica papaya* L. Traditional use of papaya leaves is to prevent bleeding in wounds and to treat wounds, lower high blood pressure, treat dengue fever, eliminate acne and smooth the skin and digestive disorders. *Staphylococcus epidermidis* is a Gram-positive bacterium, immobile, non-sporing, in solid culture media in the form of cocci in irregular groups, the arrangement is similar to grapes, prominent, shiny, does not produce pigment, white in color. *Staphylococcus epidermidis* bacteria can cause swelling (abscess) diseases such as acne, skin infections, urinary tract infections and swelling of the kidneys. This study was conducted with the aim of knowing the antibacterial activity of the ethanol extract of papaya leaves (*Carica papaya* L) with concentrations of 5%, 10%, 15%, and 20% against the growth of *Staphylococcus epidermidis* bacteria. This research method used an experimental method, namely to determine the antibacterial activity of the ethanol extract of papaya leaves (*Carica papaya* L) against the growth of *Staphylococus epidermidis* bacteria. This research used 96% ethanol as solvent. The test was carried out through several stages including material collection, preparation of simplicia, manufacture of ethanol extract from papaya leaves by maceration and testing of antibacterial activity against Staphylococus epidermidis bacteria. Based on the results of the phytochemical screening test of the thick extract, papaya leaves contain a class of alkaloid compounds, flavonoids, saponins, steroids/triterpenoids and tannins. The results of the antibacterial activity test of thick papaya leaf extract against Staphylococus epidermidis bacteria had an average inhibitory activity at concentrations of 5% (7.33 mm), 10% (8.44 mm), 15% (9.44 mm) and 20 % (10.3mm). As a comparison, Cloramfenocol 30 g was used to produce an inhibitory power of 32.1 mm and the best results obtained were at a concentration of 20% which had an inhibitory power of 10.3 mm.

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1. INTRODUCTION

North Sumatra is a fertile and rich area with various plants. Some of these plants have been used traditionally by the community as medicinal ingredients [1]. One of them is Papaya Leaf (*Carica papaya* L). Papaya leaves have a leaf shape that is fingered and has a long stalk that is hollow in the middle. The shape of the fruit of this papaya plant is oval, with a pointed tip. The color of the fruit when it is still young is dark green, and after the fruit is ripe it is light green to yellowish. Papaya

plants are plants that bear fruit every year so the fruit of this papaya plant will be easy to find at any time [2].

Antibacterial is one of the potentials that is good enough to be developed in the health sector to treat various diseases caused by bacteria because bacteria often occur in the environment around us, one of which is acne which is generally found in adolescence. *Staphylococcus epidermidis* bacteria can cause swelling (abscess) diseases such as acne, skin infections, urinary tract infections and swelling of the kidneys. *Staphylococcus epidermidis* is a Gram-positive bacterium, immobile, not spore-forming, in solid culture media in the form of irregular clusters of cocci, the arrangement is similar to grapes, prominent, shiny, does not produce pigment, is porcelain white so that *Staphylococcus epidermidis* is called *Staphylococcus albus*. These bacteria grow optimally at a temperature of 30-37°C and grow well at 1-7% NaCl. 28 Colonies 1-2 mm in diameter, are facultative anaerobes that can be reproduced by aerobic respiration or by fermentation.

Based on the above, it is necessary to do research by testing the effect of ethanol extract from papaya leaves (*Carica papaya* L) on antibacterial activity by using agar diffusion method using paper backing.

2. METHOD

This research was conducted at the Laboratory of Sari Mutiara Indonesia University, Jalan Kapten Muslim Medan. This research was conducted with an experimental method. The research methods include sample collection, manufacture of simplicia, phytochemical screening, examination of Simplicia characterization and antibacterial test of ethanol extract of papaya leaves against pure culture pathogenic bacteria from the Microbiology Laboratory of Sari Mutiara Indonesia University with agar diffusion method and paper backing, the bacteria used is *Staphylacoccus epidermidis* bacteria. The sample used was papaya leaves (*Carica papaya* L) as much as 5 kg taken from Aek Loba Village, Aek Kuasan District, Asahan Regency. The tools used in this study include laboratory glassware, particle measuring devices, aluminum foil, blender (Philip), chamber, filter paper, drying cabinet, rough balance, porcelain crucible, rubber ball, desiccator, electric balance (Mettler). Toledo), electric oven (Memmert), rotary evaporator, Laminar Air Flow Cabinet, refrigerator, water bath, paper backer, petri dish, tweezers, micro pipette, set of moisture determination apparatus and kiln.

Making Ethanol Extract of Papaya Leaves (*Carica papaya* L)

A total of 500 g of simplicia powder was put in a closed container, added 3.75 liters of 96% ethanol (75 parts) then the container was closed and left for 5 days protected from light while stirring frequently. Then filtered (maserat I) and added 1.25 liters of 96% ethanol (25 parts) then the container was closed and left for 3 days protected from light while stirring frequently. Then filtered (maserat II). The maserates (maserates I, and II) were accommodated in dark bottles, then poured, then the extract was concentrated using a rotary evaporator. The concentrated extract obtained was used for testing [3].

Antibacterial Testing

The antibacterial activity test of the ethanol extract was carried out by the agar diffusion method using a paper backer, the bacteria used was *Staphylococcus epidermidis*.

Preparation of Papaya Leaf Ethanol Extract Test Solution

The ethanol extract was weighed 1g, dissolved with dimethylsulfoxide (DMSO) to 10 ml, so that the extract concentration was 100 mg/ml (100%), then the next dilution was made to obtain an extract with a concentration of 20 mg/ml (20%), 15 mg/ml (15%), 10 mg/ml(10%) and 5 mg/ml (5%).

Making To Tilt

Into a sterile test tube 3 ml of sterile NA media was inserted, allowed to stand at room temperature until the preparation solidified at an angle of approximately 450. Then stored in a refrigerator at 5ºC [4].

Bacterial Rejuvenation

One bacterial colony was taken using a sterile ose needle, then implanted on the media so that it was tilted by scraping. Then incubated in an incubator at a temperature of 36-37ºC for 18-24 hours [4].

Inoculum Making

Bacterial colonies were taken from the culture stock with a sterile ose needle and then suspended in a test tube containing 10 ml of nutrient broth solution. Then the turbidity was measured using Mc. Farland (concentration 1 x 10 6 cfu/ml) [4].

Antibacterial Testing

Antibacterial testing was carried out on the ethanolic extract of papaya leaves by the agar diffusion method using a paper backer. A total of 0.1 ml of bacterial inoculum was mixed homogeneously with 15 ml of MHA in a sterile petri dish, then left until the media solidified. In the media that has been solid, paper backing is implanted, then in each backing it is inserted into the ethanol extract of papaya leaves with various concentrations and blank solutions, for the ethanol extract using dimethylsulfoxide as solvent. Then incubate at a temperature of 36 -37ºC for 18-24 hours. Furthermore, the ethanol extract of papaya leaves was measured the diameter of the clear area around the paper backer using a caliper. Each test is carried out 3 times [4].

3. RESULTS AND DISCUSSION

Plant identification was carried out at the Herbarium Medanense (MEDA) University of North Sumatra with the results of the identification of the sample being Papaya (*Carica papaya* L).

Characteristic Test

Macroscopic Examination

The results of macroscopic examination of papaya leaves (*Carica papaya* L) of California type are single leaves, the leaf shape resembles the palm of the hand with 7-9 parts. Papaya leaves have spines, the upper part of the leaf is dark green and the lower surface is light green, tastes very bitter. Leaf blade diameter 25-75 cm.

Microscopic Examination

The results of microscopic examination of papaya leaf simplicia powder showed the presence of prismatic calcium oxalate crystals, long folded glandular trichomes, anomocytic type stomata, upper epidermis and lower epidermis. Microscopic examination using a microscope with a magnification of 10 x 40.

Simplified Characterization Examination

From the examination of the characterization of papaya leaf simplicia powder (*Carica papaya* L) can be seen in the table below.

Table 1. Characterization Examination of Papaya Leaf Simplicia Powder

No	Characteristics of Simplicia Powder	Simplicia	
		Rate (%)	Req. MMI Edition V
1	Drying shrink	88,8 %	-
2	Total ash content	11,78 %	Not less than 12%
3	Acid insoluble ash content	0,83 %	No more than 1%
4	Water soluble essence	32,19 %	Not less than 30%
5	The content of the juice is soluble in ethanol	16,65 %	Not less than 15%
6	Water content	33,99 %	No More Than 10%

The results of the determination of drying shrinkage on papaya leaf simplicia is 88.8%, the determination of drying shrinkage cannot be determined depending on the sample being tested because the higher the % value of drying shrinkage the sample is, the better for the simplicia to be tested. Drying shrinkage is the percentage of compounds lost during the heating process (not only describing the water lost, but also the volatile compounds lost). In special cases (if the material does not contain volatile/essential oils and residual organic solvents) it is identical to the water content, namely the water content because it is in the atmosphere/open air environment [5].

Phytochemical Screening Test Results

Based on the results of the phytochemical screening test, the ethanol extract of papaya leaves (*Carica papaya* L) contains alkaloids, flavonoids, glycosides, saponins and tannins.

Table 2. Phytochemical Screening Test Results of Papaya Leaf Ethanol Extract

No	Secondary Metabolic Compound	Reactor	Results
1	Alkaloids	Dragendroff	+
		Bouchardat	+
		Meyer	+
2	Flavonoids	Powder Mg+ Amyl Alcohol + HCl (P)	+
3	Saponins	Hot water/shake	+
4	Tannins	$FeCl_3$	+
5	Triterpenoids/Steroids	Lieberman-Bourchat	+

Desc : (+) Positive = Contains a class of compounds

The results of phytochemical screening of papaya leaf ethanol extract (*Carica papaya* L) gave positive results for flavonoid compounds, alkaloids, saponins, tannins and steroids/triterpenoids.

Result of Antibacterial Activity of Papaya Leaf Ethanol Extract (*Carica papaya* L).

The results of the antibacterial effectiveness test of Ethanol Extract on *Staphylococcus epidermidis* ATCC 12228 bacteria, where at concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL and 20 mg/mL had inhibitory power against the growth of the test bacteria.

Table 3. Results of antibacterial activity of papaya leaf ethanol extract.

Concentration (%)	Inhibition Zone Diameter (mm)					Average Inhibition Zone (mm)	Resistance Response
	P1	P2	P3	P4	P5		
5%	7,2	7,6	7	7,4	7,4	7,32	Weak
10%	8,1	8,5	8,8	8,8	8	8,44	Weak
15%	9,5	9,8	9,5	9,1	9,2	9,44	Weak
20%	10,8	10	10,5	10,4	9,8	10,3	Currently
Control (+) Cloramfenocol						32,1	Very strong
Control (-) DMSO 10%						-	-

In this study, the method used was agar diffusion using paper backers at extract concentrations of 5%, 10%, 15%, and 20% to obtain an inhibitory zone at each concentration. In the first treatment with a concentration of 5% the inhibition zone obtained was 7.2 mm, the concentration of 10% was 8.1 mm, the concentration of 15% was 9.5 mm and the concentration of 20% was 10.8 mm. For the second treatment with a concentration of 5% the inhibition zone was 7.6 mm, the concentration of 10% was 8.5 mm, the concentration of 15% was 9.8 mm and the concentration of 20% was 10 mm.

For the third treatment with a concentration of 5%, the inhibition zone was 7 mm, the concentration of 10% was 8.8 mm, the concentration of 15% was 9.5 mm and the concentration of 20% was 10.5 mm. For the fourth treatment with a concentration of 5% the inhibition zone was 7.4 mm, the concentration of 10% was 8.8 mm, the concentration of 15% was 9.1 mm and the concentration of 20% was 10.4 mm. For the fifth treatment with a concentration of 5%, the inhibition zone was 7.4 mm, the concentration of 10% was 8 mm, the concentration of 15% was 9.2 mm and the concentration of 20% was 9.8 mm. Thus, the average inhibition zone was obtained. at a concentration of 5% by 7.32 mm, at a concentration of 10% by 8.44 mm, at a concentration of 15% by 9.44 mm and at a concentration of 20% by 10.3 mm This is because the higher the concentration of ethanol extract from papaya leaves (*Carica papaya* L), the diameter of the inhibitory power obtained is greater due to the higher levels of active compounds (secondary metabolites) that can inhibit the antibacterial activity contained in the ethanol extract of papaya leaves (*Carica papaya* L).

Based on the results of the research conducted, it can be concluded that papaya leaf (*Carica papaya* L) at concentrations of 5%, 10%, and 15% has a weak category of inhibition, it can be seen from the average inhibition zone obtained ranging from 6 - 9 mm, while at a concentration of 20% it was said to be in the moderate category, it could be seen from the average inhibition zone obtained ranging from 10-15 mm and the ethanol extract of papaya leaves (*Carica papaya* L) had a smaller inhibition zone than chloramphenicol which was used as a positive control while DMSO 10% which was used as a negative control had no inhibition as an anti-bacterial.

From the results of this study, the active substance was able to inhibit the growth of *Staphylococus epidermidis* bacteria. contained in papaya leaves (*Carica papaya* L) based on phytochemical screening tests, namely Flavonoids, Saponins, Tannin Alkaloids, Sterids/triterpenoids and Tannins. The mechanism of action of flavonoids is that they can inhibit the function of cell membranes by forming complex compounds with extracellular and dissolved proteins so that they can damage the bacterial cell membrane and followed by the release of intracellular compounds. Another study stated that the mechanism of flavonoids can inhibit the function of cell membranes by disrupting the permeability of cell membranes and inhibiting the binding of enzymes such as ATPase and phospholipase. The mechanism of action of Tannins is that they have anti-bacterial inhibition by precipating the antibacterial effect of tannins proteins through reactions with cell membranes, inactivation of enzymes and inactivation of genetic material functions. The mechanism of action of tannins as antibacterial is to inhibit the reverse transcriptase and DNA topoisomarase enzymes so that bacterial cells cannot be formed. The mechanism of action of saponins as antibacterial is to cause leakage of proteins and enzymes from within the cells. surface tension of bacterial cell walls and impair membrane permeability. Damage to the cell membrane is very disturbing the survival of bacteria. Saponins diffuse through the outer membrane and vulnerable cell walls, then bind to the cytoplasmic membrane so that it disrupts and reduces the stability of the cell membrane. This causes the cytoplasm to leak in and out of the cell resulting in cell death.

4. CONCLUSION

Papaya leaf ethanol extract has inhibitory activity because of the compounds contained in papaya leaf extract (*Carica papaya* L) such as flavonoids, saponins and tannins which have antibacterial effects on the growth of *Staphylococus epidermidis* bacteria. There is a difference in the average diameter of the inhibition zone at each concentration. At a concentration of 5% (7.32 mm), at a concentration of 10% (8.44 mm), at a concentration of 15% (9.44 mm) and at a concentration of 20% (10.3 mm). The higher the concentration of papaya leaf ethanol extract (*Carica papaya* L), the greater the inhibition zone produced. The minimum inhibition zone produced which was still able to inhibit the growth of *Staphylococus epidermidis* bacteria was at a concentration of 5% and produced an inhibition zone of 7.32%.

REFERENCES

[1] Adnan, M. (1997). Teknik Kromatografi Untuk Analisis Bahan Makanan. Yogyakarta : Penerbit Andi. Page. 10, 15-16.

[2] Barus, (2008). Budi Daya Tanaman Pepaya. Edisi ke-2. Yogyakarta: Pustaka Baru Press
[3] Depkes, RI. (1979). *Farmakope Indonesia*. Edisi Ketiga. Jakarta: Departemen Kesehatan RI.
[4] DitjenPOM RI, 1995. *Farmakope Indonesia*. Edisi Keempat. Jakarta: Direktorat Jendral Pengawasan obat dan Makanan Departemen Kesehatan. Halaman 81-82
[5] Depkes RI.(2000). Parameter Standar Umum Ekstrak Tumbuhan Obat. Edisi I. Jakarta: Departemen Kesehatan Republik Indonesia. Hal 10-11.