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Antibacterial Activity of Pare Fruit (*Momordica Charantia* L.) Against Bacteria *Staphylococus Aureus*

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ABSTRACT

Bitter Gourd Fruit (*Momordica charantia* L). contains alkaloids, flavonoids, saponins, tannins and terpenoids. The compounds contained in bitter melon have anti-biofilm activity. Biofilm is a collection of microorganisms that stick to the surface of objects and form a matrix. This research aims to determine the potential of the active fraction of n-hexane, ethyl acetate and water in antibacterial and biofilm activity in the form of inhibiting biofilm formation, biofilm degradation, bacteria *S. Aureus* Bitter melon fruit was extracted using the maceration method with 96% ethanol solvent. The thick extract was then fractionated using n-hexane, ethyl acetate and water as solvents.

INTRODUCTION

Infectious disease is a very important health problem and is one of the main causes of disease and death in humans. This infectious disease is caused by microorganisms, one of which is pathogenic bacteria. Over the past few years, there has been an increase in the incidence of infectious diseases caused by bacteria along with the increase in the human population (1). Plants generally produce many compounds for self-defense against microbial infections. Compounds produced by plants include secondary metabolites where many of these compounds have antibacterial properties, namely phenols, phenolics, terpenoids, flavonoids, saponins, alkaloids, tannins, polyacetylenes, polyamines, isothiocyanates, thiosulfinates, and glucosides (2) .

THEORETICAL REVIEW

Bitter gourd contains secondary metabolic compounds, namely alkaloids, tannins, polyphenols, saponins, cardenolin/bufadienol and flavonoids. Several compounds from the groups of alkaloid compounds, tannins, polyphenols, saponins and flavonoids that can inhibit bacterial growth, it is possible that bitter melon has antibacterial activity. (3). Antibacterial mechanism is damage to cell walls and cell membranes. Antimicrobial potential test of bitter gourd on several clinical isolates of pathogens. Ethanol extract and aqueous extract of bitter gourd can inhibit the growth of bacteria in clinical pathogen isolates. Pare fruit is widely known by people in various regions. The benefits of breadfruit plants for the treatment of various diseases such as in several countries the use of bitter gourd for health, including for the people of southern Japan as a laxative, laxative, and anthelmintic. In India, bitter melon extract is used as a diabetes medicine, rheumatic medicine, gout medicine, liver disease medicine and lymph disease medicine(4). Bitter gourd plant parts, such as the fruit, are known to be rich inalkaloids, tannins, polyphenols, saponins and flavonoids which can inhibit bacterial growth (5). Activity antibacterial compounds saponins, tannins, flavonoids that are inin Pare fruit has a fairly high effectiveness compared to antibacterialfamous such as tetracycline (6). These antibacterial compounds are known as non-volatile compounds sample drying process, If evaporation occurs during drying at high temperatures, contained antibacterial activitytherein does not cause change/decrease in activity valueantibacterial.

Compound phytochemicals in bitter melon (*Artocarpus altilis* F.) can be obtained by extraction. Extraction is a process of separating a substance from a mixture using a suitable solvent (7). The type of solvent and the level of polarity of the solvent greatly affect the antioxidant activity obtained (8). Biofilms area collection of microorganism cells, especially bacteria, attached to a biological surface or inanimate object and covered by a carbohydrate adhesive produced by bacteria. (9).

Biofilm inhibition process based on the reduction of antibiofilm treatment, when the treatment inhibits the cell wall and encounterselectronic, biofilm will be inhibited, then biofilm degradation occurs so that it can destroy the biofilm that is formed (10). The antibacterial activity of bitter melon using methanol extract is different from the research we did, namely using three fractions,

namely the n-hexane fraction, ethyl acetate fraction, and ethanol fraction, which obtained very strong antibacterial results (11). This study aims to determine the best antibacterial and anti-biofilm activity of the n-hexane, ethyl acetate, and water fractions of bitter melon (*Momordica charantia* L.)

METHODOLOGY

A. Determination of Bitter Gourd

The first stage of this research was to determine the correctness of the bitter melon sample based on its morphological characteristics in the bitter melon fruit. The determination was carried out at the Laboratory of the Faculty of Pharmacy, Setia Budi University, Surakarta.

B. Material Preparation

Bitter gourds were obtained from BPTO Tawamangu, Surakarta Regency, Bitter gourds were sorted to select leaves that fit the criteria then washed to remove dirt on the leaves, after that the leaves were washed first, then sliced and dried, then the final step was grinding to get bitter gourd powder. The pare fruit used is selected fresh, clean and free of impurities. The prepared leaves are then washed using running water until clean. The leaves are finely chopped and dried in the hot sun to dry. Dried bitter melon fruit was crushed into powder and sieved using a 40 Mesh sieve, bitter melon fruit was measured for moisture using a*moisture balance*.

C. Measurement of Drying Shrinkage

Determination of the drying shrinkage by the drying shrinkage method according to FHI, weighing 2 g of the sample is put in*moisture balance* at 105^{o}C wait until the heating is complete. Drying shrinkage is not more than 10 % (12).

$$\text{Drying shrinkage} = \frac{weight\ before\ drying - weight\ after\ drying}{weight\ before\ drying} \times 100\% \quad \text{.....(1)}$$

D. Determination of Water Level on Powder

Determination of the water content in 20 g bitter melon ethanol powder was carried out by distillation using a tool*Bidwell Sterling*. The powder is put in a round base flask*Bidwell Sterling* 200 ml of toluene is added and the toluene is saturated with 20 ml of toluene and then heated for 15 minutes, when the toluene boils the distillation is adjusted to 2 drops per second so that more water is distilled. Increase speed again to 4 drops per second. Clean the cooler parts and tubes connected to the wires using a brush and water-saturated toluene when all the water is distilled off. Distillation was carried out for 5 minutes. The receiving tube is cooled to room temperature, if there are still droplets attached, then scrubbing the cooling tube is carried out, affix the receiving tube with copper wire, then wet it with water-saturated toluene until the water droplets sticking to it are saturated. The volume of water after water

and toluene are completely separated can be calculated for its water content in units of % v/w using formula (13):

$$Water\ level = \frac{read\ volume}{Material\ weight} x100\% \quad \text{.....(2)}$$

E. Preparation of Bitter Gourd Extract

Making Bitter gourd fruit extract was carried out using the maceration extraction method with 70% ethanol solvent. Put 1 part of the simplicia powder/bitter melon fruit into the maceration vessel then add 10 parts of the solventethanol 70% (1:10). Leave the simplicia for the first 6 hours while stirring occasionally, then let the simplicia sit for 18 hours. Filter the macerate results obtained and then repeat the extraction process with half the volume of solvent from the first extraction. The macerate results obtained were collected and then evaporated using a tool*rotary evaporator* until a fairly thick extract is obtained. The results obtained are calculated in yield by the formula:

$$\text{Yield} = \frac{extract\ weight}{simple\ weight} \text{ x } 100\% \quad \text{.....(3)}$$

F. Test the Water Level of the Extract

Testing the water content of the extract has the goal of knowing the constant weight of the water content of the extract obtained using the gravimetric method. The initial step, weigh the empty exchange rate then put it in the oven for 15 minutes, put it in the desiccator to cool for 15 minutes. The next step is weighing 10 grams of extract into the exchange rate, in the oven again for 5 hours after that it is cooled using a desiccator for 1 hour then weighed, and the results are recorded (14).

G. Making Bitter Gourd Extract Fractionation

Fractionation was carried out by weighing 20 grams of bitter melon extract. Fractionation was carried out 3 times with n-hexane, ethyl acetate and water as solvents. The fractionation procedure was carried out using a separatory funnel to form two separate liquid layers, a lower water layer and an upper hexane layer. The n-hexane fraction obtained was evaporated at*rotary evaporator*. The residue obtained from the n-hexane fraction was further treated with the same amount of ethyl acetate as the solvent used for the dispersion until two clearly separated layers were formed, the lower water layer and the upper ethyl acetate layer. The ethyl acetate and water fractions were evaporated at 40°C using*rotary evaporator* (15).

H. Extract identification test

Pare fruit extract is done by looking at a shape, smell, color, from somethingextract bitter melon. Screening for flavonoid compounds was carried out by weighing 0.1 g of bitter melon powder in a test tube and then adding 0.1 g of magnesium powder and 1 ml of concentrated HCl and 2 ml of amyl alcohol, then shaking vigorously and allowing it to separate. Positive results of flavonoids are indicated by the formation of red, yellow or orange on the amyl alcohol layer (11); saponin test by weighing 0.1 g of bitter melon powder in a test tube, then adding 10 ml of hot water, shaking vigorously for 10 seconds. A positive saponin result is indicated by the formation of froth or foam for approximately 10 minutes as high as 1-10 cm which is then added 1 drop of HCl 2 N, if the froth does not disappear indicates the presence of saponins (11); tannin test by weighing 0.1 g of bitter melon powder in a test tube, then adding 2-3 drops of $FeCl_3$. A positive tannin result is indicated by the formation of a blackish blue or green color (11); Alkaloid test by weighing 0.1 g of bitter melon powder in a test tube, then dropping 2 drops of Dragendroff reagent to produce a brick red precipitate, adding 2 drops of Mayer reagent produces a white or yellow precipitate, while adding 2 drops of Bouchardat reagent produces a precipitate dark brown. A positive alkaloid result is indicated if there is at least two or three precipitates from the experiment (11); The phenolic test was carried out by taking 0.1 g of bitter melon powder in a test tube, then adding 2 drops of $FeCl._3$ 1%. The result is positive if it turns blue-black (11).

I. Antibacterial Activity Testing

The test controls used were extracts and fractions. Concentrations of 2, 4, 6 and 8 mg/mL were prepared from the extract and each of the water, ethyl acetate and*n*- bitter melon hexane. Then the concentration required for testing is taken. The negative control is bacteria*S aureus* BHI in the media (8). MIC determination was carried out using the liquid dilution method*broth dilution test (Serial dilution)*. The test medium used is*Brain Heart Infusion* (BHI). This method uses 1 row of test tubes consisting of 12 sterile tubes containing the active fraction of bitter gourd with a concentration of 100; 75; 50%; 25%; 12.5%; 6.25%; 3.13%; 1.56%; 0.73%; 0.39%; positive control (+) and negative control (-). Tube 1 which is the negative control contains 2 ml of the most active fraction of bitter melon. Tubes 2 to 11 contain each concentration of 0.5 ml with multilevel dilution, then 0.5 ml of bacterial suspension is added aseptically. Tube 12 as a positive control only contained bacterial suspension, then incubated at 37°C for 24 hours. (16). The determination of MBC was carried out using the solid dilution method. Minimal Kill Concentration (MBC) was determined by inoculating a clear tube from the results of MIC by streaking, then incubating at 37°C for 24-48 hours. Observation of the results of the solid dilution method can be seen from the presence or absence of black colonies and around yellow colonies on the surface of the media. Minimal Kill Concentration (MBC) is

shown at the lowest concentration that does not show growing bacterial colonies.

RESULTS AND DISCUSSION

A. Results of Determination and Identification

The purpose of the determination is to see the correctness of the bitter melon sample, avoiding errors in plant determination by matching the morphological characteristics of bitter melon plants. Determination of plants in this study was carried out in the laboratory of Setia Budi University, Surakarta. The results of the determination based on number 195/DmaseET/UPT-LAB/21.01.2023 can be ascertained that the plant samples used in this study were bitter melon (*momordica chrantia* L.).

B. Collection of Materials and Production of Pare Fruit Powder

The sample used in this study was bitter melon obtained from BPTO Tawangmangu, Karanganyar, Central Java. The results obtained from the dry weight of bitter melon fruit were 850 grams, then the percent yield calculation was carried out and the yield of bitter melon fruit was obtained at 21%. The results meet the requirements because the yield is> 10%. The results of the yield of bitter melon powder can be seen in table 1.

Table 1. Yield of bitter gourd fruit

Sample	Wet weight (g)	Dry weight (g)	Yield % b/b
bitter gourd	4.000	850	21%

C. Powder Water Rate Results

Determination of water content is said to be good if the percentage of water content is <10%. The results of calculating the water content of bitter melon powder can be seen in table 2.

Table 2. The result of determining the water content of pare fruit powder

Replication	Powder weight (g)	Volume air (ml)	Water content (%)
1	20	1	5
2	20	1,2	6
3	20	1,6	8
	Rate-rate		6,3

It was concluded that based on the test results above, the results of determining the moisture content of bitter gourd powder obtained an average

of around 6.3%. According to the 2017 FHI, it was explained that the requirements for the results of the bitter melon powder moisture content test were no more than 10%. This aims to prevent the growth of mold in the powder. The results of the water content of bitter melon powder, namely 6.3%, meet the requirements for powder moisture content because it is <10%.

D. Results of Determination of Pare Fruit Powder Drying Shrinkage

Drying shrinkage is the content of the volatile part of a substance. Drying shrinkage is the content of the volatile part of a substance. Drying shrinkage is different from the determination of moisture content. Determination of the water content that is calculated is the percentage of water, while in determining the water content, the calculated drying shrinkage is the volatile substances present in the simplicia, including water. The purpose of knowing the drying shrinkage is to provide a maximum limit for the amount of compound lost in the drying process (17). The results of determining the shrinkage content of powder drying can be seen in table 3.

Table 3. The result of determining the drying rate of pare fruit powder

Sample	Replication	Powder weight (g)	% drying loss
Powder	1	2	9,9
Powder	2	2	9,5
Powder	3	2	9,1
Rate-rate			9,5

Determination of the drying shrinkage content of bitter melon powder obtained results of 9.5%. These results said that bitter melon powder meets the requirements. The requirement for drying shrinkage of simplicia powder is that it should not exceed 10% (18).

E. Preparation of Bitter Gourd Extract

Pare fruit is extracted using ethanol solvent. Ethanol is a universal solvent that can dissolve polar and non-polar analytes. The results of maceration are concentrated using a tool*rotary evaporator* at a temperature of 40-50^{O}C, then after it becomes concentrated and thickens the extract is weighed and a total of 62 grams of viscous extract is obtained, then calculating the yield value, the yield is 12.4%, the yield is said to be good if the value is more than 9.9%. The results of making ethanol extract from bitter gourd can be seen in table 4.

Table 4. Percentage of bitter melon extract yield

Powder weight (g)	Empty container weight(g)	Weight of bottle+extract (g)	Extract weight (g)	Yield %
500 g	163	225	62	12,4

F. Results of Fractionation of Bitter Gourd Ethanol Extract

This study used ethanol extract of bitter gourd which was fractionated using 3 kinds of solvents including solvent*n*-hexane serves as a non-polar solvent, ethyl acetate serves as a semi-polar solvent, and water functions as a polar solvent.

Table 5. Percentage of bitter melon extract yield

Fraction	Extract weight	Fraction weight	yield
N-hexane	20	1	5
Ethyl acetate	20	5	25
Air	20	13	65

The table above shows the yields obtained with each solvent where the water fraction is greater than the n-hexane and ethyl-acetate fractions, and the ethyl-acetate fraction is greater than the n-hexane fraction. Water is a polar solvent, it can be seen that the compounds contained in bitter melon contain many polar compounds.

G. Identification Results of Bitter Gourd Extract

In this study, the organoleptic test of bitter melon extract was carried out in the form of shape, color, smell. The purpose of this examination is to see the characteristics or physical properties of bitter melon extract in accordance with existing guidelines which can be seen in table 6.

Table 6. Results of organoleptic examination of bitter melon extract

Inspection	Results
Form	Thick
Color	Dark chocolate
Smell	Special extract

H. Results of Determination of Water Content of Pare Fruit Extract

The results of the bitter melon extract water content test were 4.64%, the results were categorized as late fulfilling the requirements because they were <10% according to FHI (2017). The condition for the water content of the extract is <10%. If > 10% can cause the sample to be damaged due to the presence of microbes. The purpose of determining the water content of bitter melon extract is to provide a maximum limit or range regarding the amount of water content in the extract. Determination of the water content of the extract can be seen in table 7.

Table 7. The result of extract water content

Replication	Initial extract weight (gram)	Final extract weight (gram)	Much (%)
1	10,0067	9,5205	4,85
2	10,0051	9,5113	4,93
3	10,0031	9,5885	4,14
	Rate – rate		4,64

I. Antibacterial Activity Test Results

Antibacterial activity testing was carried out by dilution method or serial dilution test. This method is useful for knowing the minimum dose of drugs that are bacteriostatic and bactericidal. The Minimum Kill Concentration (MBC) can be known from the lowest concentration which indicates no growth of bacterial colonies in the media. Determination of the MIC value in the antibacterial activity test is determined by looking at the turbidity of the bacterial suspension which has been incubated for 3 days so that the bacteria already have a biofilm and then treated with the fraction bitter melon fruit water The MIC results were observed by looking at the turbidity in the tube. Turbidity in the tube due to the growth of test bacteria.

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