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Antibacterial activity test of sweet arum mango peel extract gel preparation (Mangifera indica L.) against growth methicillin resistant staphylococcus aureus

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ABSTRACT

Inappropriate use of antibiotics can make bacteria's reistance that cause skin diseases, the example is acne. Acne tretment usually uses synthethic drugs that causes side effects, so the alternative are used by plants that come from nature. On of the plats that has the potential as an antibacterial is the arum manis mango (Mangifera indica L.) because contains compounds that can inhibit the growth of Mathicillin Resistant Staphylococcus aureus. The research of this study was to determine the antibacterial activity of extract gel's arum manis mango peel to c. Extract of arum manis mango peel was obtained remaserated using 70% ethanol solvent. The extract obtined were phytochemicel screening and affirmation tests using the TLC (Thin Layer Chromatography) method to ensure the compounds contained. Arum manis mango peel is preparation of gel with a concentration of 5%, 10%, 15%, 20%, 25%. The results showed that extract of arum manis mango peel preparation gel with concentrations of 5%, 10%, 15%, 20%, 25%. The results showed that extract of arum manis peel preparation gel with concentration 5%, 10%, 15%, 20%, 25% showed significant difference in potential of antibacterial.

1. Introduction

Human skin is the outer layer of the human body. Human skin is the largest organ in the body and is a blanket that covers the surface of the body. The main function of human skin is as a protector from various kinds of disorders and external stimuli [1]. Many microorga-

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nisms live on the skin because they are located on the outside of the body. Staphylococcus aureus bacteria are the bacteria most commonly found on human skin [2]. Acne usually appears on the surface of the skin of the face, neck, chest and pungggung when the oil glands on the skin are too active so that the pores of the skin will be blocked by excessive fat deposits. Acne treatment is usually done by administering antibiotics and chemicals, but the drugs also have side effects such as resistance to antibiotics and skin irritation. Therefore, it is necessary to search for antibacterials from natural ingredients that are known to be safe compared to chemical drugs, one of the sources of natural medicine is sweet arum mango peel (Mangifera indica L.).

Methicillin Resistant Staphylococcus aureus is a strain of Staphylococcus aureus that has been resistant to the antibiotic activity of the β-lactam group, including the penicillinase-resistant penicillins (oxcacillin, methicillin, nafcillin, cloxacillin, dicloxacillin), cephalosporin and carbapenem. In addition, cross-resistance also occurs in non-β-lactam antibiotics such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin [3].

The gel was chosen because it does not contain oil so it will not worsen the condition of acne, clear, easy to dry out forming an easy-to-wash film layer also gel dosage form suitable for topical therapy in acne, especially patients with oily skin types [4].

2. Method

The object of this study was the antibacterial activity of the gel preparation of sweet arum bark extract (Mangifera indica L.) against the growth of Methicillin Resistant Staphylococcus aureus bacteria differences in the antibacterial activity of sweet arum mango peel extract (Mangifera indica L.) gel preparations at a concentration of 5%,10%,15%, 20%, 25%. Sampling based on random sampling techniques. Free variables are variables that influence the bound variables, can be manipulated, changed or replaced, and arranged according to the researcher's wishes. The free variable in this study was the gel concentration of sweet arum mango peel extract (Mangifera indica L.) 5%, 10%, 15%, 20%, 25%. The bound variable in this study was a clear zone that showed the antibacterial activity of the sweet arum mango peel extract gel preparation (Mangifera indica L.) against the growth of Methicillin Resistant Staphylococcus aureus bacteria, the difference in antibacterial activity of the gel preparation of sweet arum mango peel extract (Mangifera indica L.) at a concentration of 5%, 10%, 15%, 20%, 25% against the growth of Methicillin Resistant Staphylococcus aureus bacteria and measured using calipers. The control variables used in the study are materials and tools used to make gel preparations. Anti-acne gel sweet arum mango peel extract 5%, 10%, 15%, 20%, 25% positive control ciprofloxacin and negative control gel base. The method used in testing antibacterial activity is the well method. The medium used is MSA (Manitol Salt Agar). The type of bacteria used is Methicillin Resistant Staphylococcus aureus with 37oC incubation and 1x24 hours incubation time. Extraction using 70% ethanol.

The skin of the sweet arum mango (Mangifera indica L.) is dried and reduced in size with a blender, then sifted with a mesh sieve number 30/40. 1500 g of dried mango arum powder (Mangifera indica L.) is put into beakerglass and added with 70% ethanol filtering

liquid for k 5000 ml, soaked for 3 days and covered with black plastic with the aim of protecting the pigment contained in the skin of sweet arum mango (Mangifera indica L.) from direct sunlight. Then filtered obtained maserate (1) then the pulp was soaked again with 70% ethanol for 1 day, re-filtered and obtained macerate (2) and carried out again the next day until a macerate color was obtained that was close to clear and no more compounds were attracted by the solvent. Maserat is collected together and concentrated using waterbath (±70oC) until a thick extract is obtained. Then in the phytochemical screening test (preliminary test and affirmation test) and made a concentration of 5%, 10%, 15%, 20%, 25% for antibacterial test.

Table 1. Gel Dosage Formula

Material Name	Formula						Material Functions
	Gel Base	I	II	III	IV	V	
Sweet arum mango peel extract	-	5 g	10 g	15 g	20 g	25 g	Active subtances
CMC Na	4 g	4 g	4 g	4 g	4 g	4 g	Gel base
Glycerin	10 g	10 g	10 g	10 g	10 g	10 g	Emollient
Propilenglikol	5 g	5 g	5 g	5 g	5 g	5 g	Humectants
Aquadest	Ad 100 g	Ad 100 g	Ad 100 g	Ad 100 g	Ad 100 g	Ad 100 g	Solvent

The data obtained from the research data was analyzed quantitatively using SPSS statistics with a confidence level of 95%. Normal and homogeneous distributed data are carried out one-way Anova Test, and if there are significant differences, the Post-Anava Test or Scheffe Test is carried out. Data that are neither normally distributed nor homogeneous are carried out the Kruskal-Wallis Test, if there are significant differences, the Mann-Whiteney Test continues.

3. Results and Discussion

This study aims to determine the antibakery activity of the gel preparation of sweet arum mango peel extract (Mangifera indica L.) against the growth of Methicillin Resistant Staphylococcus aureus bacteria. Simplisia used in antibacterial preparations is a sweet arum mango peel obtained from a home plant in Pucang Gading taken sweet arum mango peel in the same place to ensure uniformity of chemical content contained in sweet arum mango peel because it has the same geographical factors. Plant determination is carried out in advance in the biological laboratory of the College of Pharmaceutical Sciences "Yayasan Pharmasi Semarang". Determination is carried out in order to obtain certainty that the plants used in the study are derived from the plants in question.

The selection of samples of mango peel arum is sweet because so far people have only consumed the flesh of the fruit while the skin of the fruit is simply thrown away as waste. Whereas in the mango peel contained active compounds that can be used as a treatment, especially as an antibacterial. Mango peel arum sweet before being tested for antibacterial activity, mango peel is extracted for easy testing. Before extraction, the sweet arum mango peel is dried, this drying aims to reduce the moisture content, prevent the emergence of mold and mold, as well as to prevent enzymatic reactions that decompose the content of the active substance, so that simplicia is not easily damaged and can be stored for a long time.

The sweet arum mango peel that has been dried in a blender until it becomes powder and then sifted. Converting into powder is carried out to reduce the particle size so that the area of the material in contact with the solvent is larger, so it is expected that the content of dissolved compounds will be greater. This sifting aims to equalize the particle size of sweet arum mango peel powder. Sweet arum mango peel that has become a powder in the remasceration with 70% ethanol. Thus, it is expected that the amendments obtained will be even greater. From 2.5 kg of dried powder of mango peel, 657 g of extract were obtained.

Sweet arum mango peel extract before use needs to be tested ethanol-free. The ethanol-free test aims to find out that the extract really no longer contains ethanol for use in testing antibacterial activity. That ethanol has antibacterial properties so that the presence of ethanol in the sample will make it ambiguous whether it works as an antibacterial ethanol in the sample or the content of active compounds in the sample of sweet arum mango peel. The results of the ethanol-free test can be seen in Table 2.

Table 2. Ethanol-Free Test Results

Sample	Procedure	Result	Information
Extrak sweet arum mango peel	Extract+ astatic acid+ H2SO4, heated	Ester smell	Negative (ethanol-free)
Extrak sweet arum mango peel	Sulfanilic acid+HCL+$NaNO_2$, heated	Brown Color	Negative (ethanol-free)

The sweet arum mango peel extract obtained was followed by phytochemical screening tests. This test is a preliminary test to determine the content of active substances contained in sweet arum mango peel extract. The results of phytochemical screening of arummanis mango peel powder and extract in Table 3 and KLT results in Table 4.

Table 3. Phytochemical Screening Results of Powders and Extracts of Sweet Arum Mango Peel

Compound	Reagents	Positive results based on literature	Result	
			Powder	Extract
Flavonoids	Mg powder + amyl alcohol	Red, yellow, and orange colors on the amyl alcohol coating [5]	Formed yellow color (+)	Formed brownish-yellow color on the amyl alcohol layer(+)
Tanin	HCL + gelatin 0,5%	Formed precipitate [6]	Formed white precipitate (+)	Formed white precipitate (+)
Saponin	HCL 1% (shake strongly)	Froth does not go away [6]	Constant froth (+)	Constant froth (+)
Steroid/ Triterpenoid	Evaporated with 20ml n-hexane+ 2tts acetic acid	Bluish-green [7]	Green (+)	Green (+)
Alkaloids	Sample + 1ml HCL + 9ml hot aquadest, cooled and strain+Dragendroff	Formed brick red deposits [8]	Brick-red precipitate (+)	Brick-red precipitate (+)
	Sample + 1ml HCL 2N + 9ml hot aquadest, cooled and filtered+Mayer	White or yellow precipitate formed [8]	No precipitate (-)	No deposits (-)

	Sample + 1ml HCL 2N + 9ml hot aquadest, cool and strain +Bouchardat	Brown to black deposits are formed [8]	Black precipitate (+)	Black precipitate (+)

Table 4. KLT Results of Sweet Arum Mango Peel Extract

Compound	Phases of Motion	Spotting Appearance	Positive results based on literature	Result		Conclusioon
				Rf	Spotting	
Flavonoid	n-butanol:asam asetat:air (4:1:5)	ALCL2 5%	Green, Yellow [7]	0,18 0,87	Yellow Yellow	Positive
Alkaloid	Metanol: amonia (200:3)	Pereaksi dragendroff	Brown with a yellow background [7]	0,18	Brown	Positive
Saponin	Kloroform: metanol:air (5:4:1)	Vanilin-asam sulfat	Pink mauve [7]	0,62 0,81	Lembayung Crimson	Positive
Tannins	n-butanol:asam asetat:air (14:1:5)	FeCl3 10%	Blue-Black [5]	0,06 0,68 0,81	Blue-black Blue-black Blue-black	Positive
Steroid/triterpenoid	n-heksan:etil asetat (1:1)	Lieberman-Burchadat	Various colors [7]	0,93 1,06 1,18	Yellow Yellow Yellow	Positive

Flavonoid tests were carried out to ascertain whether the mango peel of the sweet arum variety contains this compound. Mg and HCl metals in this test reduce the benzopiron core contained in the flavonoid structure so that a color change is formed to red, yellow or orange (Prashant, 2011). The tannin test was carried out with a 1% $FeCl_3$ reagent characterized by the formation of a blackish-green or blue-black color because tannins reacted with Fe^{3+} ions to form complex compounds [7]. The second method with the addition of NaCl and gelatin is characterized by the onset of a yellowish-white precipitate of tannins reacting with gelatin to form a steady copolymer that is insoluble in water [7].

Tripenoid compounds will be dehydrated with the addition of strong acids and form salts that give a number of color reactions [9]. The positive result of the tripenoid preliminary test is the formation of orange or purple. The addition of anhydrous acetic acid aims to form acetyl derivatives of tripenoids [7]. In the alkaloid test, the addition of HCl aims to increase the solubility of alkaloids because alkaloids are alkaline, when extracted with acidic solvents, alkaloids will form in the form of salts that are more easily soluble in polar solvents [7].

Saponins are compounds that have polar and non-polar groups that are active on the surface so that when shaken with water, saponins can form micelles. In the micelle structure, the polar and non-polar groups face outwards while the non-polar groups face

inward. This state of affairs is what looks like foam [9]. Based on preliminary tests and KLT listed in the table above, it is confirmed that sweet arum mango peel positively contains flavonoids, alkaloids, tannins, saponins, steroids/tripenoids.

After the active compounds in mango peel extract arum seminal were known, the test continued on the antibacterial activity on *Methicillin Resistant Staphylococcus aureus.* The results of the antibacterial activity test of the sweet arum mango peel extract gel preparation can be seen in Table 5.

Table 5. Antibacterial Activity of Sweet Arum Mango Peel Extract Gel Preparation

Replication of vials to-	Clear Zone Diameter					Positive control (+)	Negative control (-)
	Acne Gel Sweet Arum Mango Peel Extract						
	F1	F2	F3	F4	F5		
1	1,437	1,617	1,667	1,512	1,992	2,647	0,000
2	1,525	1,607	1,822	1,852	1,892	2,315	0,000
3	1,075	1,657	1,777	1,942	1,967	2,192	0,000
4	1,362	1,495	1,692	1,752	1,872	2,175	0,000
5	1,432	1,595	1,682	1,875	1,842	2,032	0,000
Average	**1,366**	**1,594**	**1,728**	**1,786**	**1,913**	**2,272**	**0,000**
SD	**0,172**	**0,060**	**0,067**	**0,167**	**0,063**	**0,232**	**0,000**
Average ±SD	**1,366± 0,172**	**1,594± 0,060**	**1,728± 0,067**	**1,786± 0,167**	**1,913± 0,063**	**2,272±0,232**	

Antibacterial power testing using the well method. The well method was chosen because with this method the sample can diffuse not only on the surface of the media but also in the media, so that the antibacterial activity caused will be maximized. The planting technique used is sprinkling (Pour Plate Method), namely by mixing media and suspension until homogeneous so that bacterial growth in the test media can be spread evenly. Methicillin Resistant Staphylococcus aureus bacterial suspension planting uses a pour plate because this method can spread bacteria throughout the media from the surface to the medium. The media used in this study were MSA (Mannitol Salt Agar) and NB (Nutrient broth) media. MSA media contains high NaCl, which is 7.5%-10% Methicillin Resistant Staphylococcus aureus bacteria able to adapt to an environment with high salt content while other bacteria are unable to survive in the environment with a high salt content. In addition, MSA contains mannitol to produce acids that change the color of the phenol indicator media red. The presence of Methicillin Resistant Staphylococcus aureus will

ferment mannitol to produce acid that changes the color of the phenol red indicator media from red to yellow, so MSA is a selective medium for the growth of Methicillin Resistant Staphylococcus aureus [10]. The positive control used in this study was ciprofloxacin tablets, ciprofloxacin has bactericidal activity in the bacterial growth phase based on bacterial DNA enzyme inhibition, so that bacterial DNA synthesis can be inhibited [11]. Ciprofloxacin is effective against bacteria resistant to other antibiotics e.g. penicillins, aminoglycosides, cephalosporins and tetracyclines. Ciprofloxacin is effective against gram-negative and positive bacteria [12].

The gel preparation formulation uses antibacterial ingredients of sweet arum mango peel extract with various concentrations that have been tested to have antibacterial activity against Methicillin Resistant Staphylococcus aureus. The selection of making gel preparations because the gel does not contain oil so it will not aggravate acne, clear dries easily to form a film that is easy to wash. The process of making gel preparations is carried out according to the manufacturing method that has been described, namely by mixing each of the existing components. The finished gel preparation is then carried out a test of the physical characteristics of the preparation which includes organoleptic test, homogeneity test, pH test, adhesion test, viscosity and dispersion test. The results of the test of the physical characteristics of the preparation can be seen in Table 6.

Table 6. Test Results of Physical Characteristics of Sweet Arum Mango Peel Gel

No	Test Parameters	Formulation						Condition
		F0	F1	F2	F3	F4	F5	
1.	Organoleptis							
	Shape	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
	Color	Brown	Brown	Brown	Brown	Brown	Brown	Brown
	Smell	Typical mango aroma	Typical mango aroma	Typical mango aroma	Typical mango aroma	Typical mango aroma	Typical mango aroma	Distinctive
2.	Ph	5,31	5,12	4,95	4,82	4,79	4,59	4,5-6,5 [13]
3.	Homogeneity Test	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
4.	Spread Power Test	6,867	6,679	5,975	5,775	5,664	5,483	5-7 cm [14]
5.	Adhesion Test	3,76	1,35	1,57	2,15	2,49	3,55	>1 second [15]
6.	Viscosity Test	29,860	21,451	22,260	23,650	24,324	25,641	4000-40000 cps [16]

Explanation :
F0 = Acne Gel Formula Without Mango Peel Extract Arum Manis
F1 = Acne Gel Formula Mango Peel Extract Arum Sweet Concentration 5%
F2 = Acne Gel Formula Mango Peel Extract Arum Sweet Concentration 10%
F3 = Acne Gel Formula Skin Extract Mangga Arum Sweet Concentration 15%
F4 = Acne Gel Formula Mango Peel Extract Arum Sweet Concentration 20%
F5 = Acne Gel Formula Mango Peel Extract Arum Sweet Concentration 25%

Table 7. Parametic Test Results One Way Anova Gel Mango Peel Extract Arum Manis

Contrast	Sig.	Information
Positive Vs Formula 1 Control	0,000	Significant Differences
Positive Vs Formula 2 Control	0,000	Significant Differences
Positive Vs Formula 3 Control	0,000	Significant Differences
Positive Vs Formula 4 Control	0,000	Significant Differences
Positive Vs Formula 5 Control	0,000	Significant Differences
Formula 1 Vs Formula 2	0,000	Significant Differences
Formula 1 Vs Formula 3	0,000	Significant Differences
Formula 1 Vs Formula 4	0,000	Significant Differences
Formula 1 Vs Formula 5	0,000	Significant Differences
Formula 2 Vs Formula 3	0,000	Significant Differences
Formula 2 Vs Formula 4	0,000	Significant Differences
Formula 2 Vs Formula 5	0,000	Significant Differences
Formula 3 Vs Formula 4	0,000	Significant Differences
Formula 3 Vs Formula 5	0,000	Significant Differences
Formula 4 Vs Formula 5	0,000	Significant Differences

Of the five concentrations of sweet arum mango peel extract gel preparations 5%, 10%, 15%, 20%, 25% have an effect on the antibacterial power of the growth of Methicillin Resistant Staphylococcus aureus bacteria. Data in Table 7. It can be seen that the results show significant differences so that it can be concluded that there are differences in antibacterial power between groups.

The one-way anova parametic test obtained significant difference results. This is because there is a difference in concentration, the higher the concentration of sweet arum mango

peel extract (Mangifera indica L.) then the clearer zone will be, this happens because the active compound content is more. The average results of the clear zone can be seen that the antibacterial power of the sweet arum mango peel acne gel is still low compared to the positive control of ciprofloxacin tablets.

4. Conclusion

The anti-acne gel preparation of sweet arum mango peel extract (Mangifera indica L.) has antibacterial activity against Methicillin Resistant Staphylococcus aureus. There was a significant difference in the antibacterial activity of the anti-acne gel preparation of sweet arum mango peel extract (Mangifera indica L.) concentration of 5%, 10%, 15%, 20%, 25% against the growth of Methicillin Resistant Staphylococcus aureus bacteria.

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