FRACTINATION OF ANTIBACTERIAL COMPOUNDS ON THE PEEL OF SWEET FRAGRANT MANGO(*Mangifera indica* L) AND ITS ANTIBACTERIAL ACTIVITIES AGAINST BACTERIAL *Methicillin Resistant Staphylococcus aureus* (MRSA)

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ABSTRACT

Sweet fragrant mango is a fruit that is often found in Indonesia. Indonesian people use mango fruit only on the pulp, while the potential of the fruit peel has not been explored. Antibacterial compound fractionation in mango fruit peel contains steroid / triterpenoid compounds in the n-hexane fraction, ethyl acetate fraction contains flavonoids, tannins, steroids / triterpenoids and saponins. The water fraction contains flavonoids, tannins, steroids / triterpenoids.

The results of the antibacterial activity of the n-hexane fraction carried out at a concentration of 5%, 10%, 15%, 20%, 25% were 0.466 cm; 0.769 cm; 0.865 cm; 0.961 cm and 1.044 cm. The antibacterial activity of the ethyl acetate fraction with a concentration of 5%, 10%, 15%, 20%, 25% was 1.127 cm; 1,458 cm; 1,563 cm; 1,638 cm and 1,776 cm while the water fraction with a concentration of 5%, 10%, 15%, 20% 25% was 0.473 cm respectively; 0.662 cm; 0.839 cm; 1,033 cm and 1,220 cm.

The conclusion of this research is that compound fractionation from the peel of sweet fragrant mango has the potential as antibacterial agent against MRSA bacteria.

Keywords: sweet fragrant mango peel, compound fractionation, *Methicillin Resistant Staphylococcus aureus*

BACKGROUND

Mango (*Mangifera indica* L.) is a fruit that is well known in Indonesia, this plant is cultivated by the community for the main purpose of harvesting the fruit only. Mango fruit has a lot of interest because of its sweet taste. The potential waste obtained from mangoes is the skin of the fruit. Mango rind contains secondary metabolites such as flavonoids, tannins, alkaloids, saponins, triterpenoids / steroids which have antibacterial activity [1].

Methicillin resistant Staphylococcus aureus is a strain of *Staphylococcus aureus* that has been resistant to the β-lactam class of antibiotics, including 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, cotrimoxasol, and ciprofloxacin [2].

The test for the antibacterial activity of the peel of the sweet fragrant mango is still limited to the extract. Fractionation needs to be done to separate the compounds in the extract based on the level of polarity of each compound.

The formulation of this study was to determine the antibacterial potential of the n-hexane fraction, ethyl acetate fraction and water fraction from the skin of the sweet fragrant mango against MRSA bacteria. This study aims to determine whether the n-hexane fraction, ethyl acetate fraction and water fraction from mango peel have potential as antibacterial properties against MRSA bacteria.

The formulation of this study was to determine the antibacterial potential of n-hexane fraction, ethyl acetate fraction and water fraction from mango peel against MRSA bacteria. This study aims to determine the n-hexane fraction, ethyl acetate fraction and water fraction from mango peel have potential as antibacterial against MRSA bacteria.

METHODS

Tool

The equipment used in this study were glass tools, separating funnel, waterbath, porcelain plates, petri dishes, round ose, spirit lamps, cylinder cups, incubators, calipers, autoclaves, Laminar Air Flow (LAF), micropipettes, spectrophotometers.

Material

The materials used in this study were the peel of sweet fragrant mango (*Mangifera indica* L), 70% ethanol, n-hexane, ethyl acetate, water, Nutrient Broth media, Nutrient Agar and Mannitol Salt Agar, *Methicillin resistant Staphylococcus aureus* bacteria, ciprofloxacin and Dimethylsulfoxide (DMSO).

Sampling and Processing of Samples

The sample of sweet fragrant mango peel used was obtained from the Gubug area, Grobogan district, Central Java. The collected samples were cleaned and dried. The mango peel that has dried is smoothed and ready to be extracted and fractionated.

Extraction and Fractionation

The peel powder of the sweet fragrant mango is weighed as much as 600 grams and remacerated 3 x 24 hours using 70% ethanol solvent with a powder: solvent ratio (1: 5) in a closed vessel. Let the mixture sit for 24 hours while stirring occasionally. The mixture of powder and filter is filtered with a cola cloth to obtain macerate. The obtained maserate is combined and then evaporated with the help of a rotary evaporator. The liquid extract obtained was concentrated again on a water bath at a temperature of 40ºC - 50ºC until a thick extract was obtained.

The extract obtained was then fractionated using the separating funnel method. The ethanol extract of the sweet fragrant mango peel as much as 10 grams was added with 100 ml of water until all the thick extract dissolved, then put it in a separating funnel and fractionated with 100 ml of n-hexane and 100 ml of ethyl acetate. Then each fraction was evaporated until a more concentrated fraction was obtained.

Sterilization Equipment

The tools used such as erlenmeyer flasks, test tubes, petri dishes and media are sterilized in an autoclave at a temperature of 121ºC for 15 minutes at a pressure of 15 psi / 1 atm.

Uji Phytochemical Screening

a. Flavonoid Test

The sample was added with 0.1 mg of Mg powder and 5 drops of concentrated HCl, shake it. Positive samples contain flavonoids when a yellow, orange or red color is formed [3].

b. Tannin Test

The sample was added with distilled water, then boiled for 5 minutes. The solution is then filtered and the filtrate is added with 1% $FeCl_3$ solution [4].

Further identification is carried out by dissolving the sample in aquadest and heating it, then adding a 10% NaCl solution and 1% gelatin if a sediment occurs indicating the presence of tannins [5].

c. Steroid / Triterpenoid Test

The sample was added with 5 ml of chloroform then filtered and evaporated. The filtrate is added with anhydrous acetic acid and H_2SO_4. Positive samples contain steroids / triterpenoids if they produce a reddish brown or green color [4].

d. Alkaloid Test

The sample was mixed with 1 ml of 2N HCl and 9 ml of heat aquadest. The solution was heated for 2 minutes, then cooled and filtered. The filtrate is divided into 3 parts, each added with Dragendorff, Mayer and Bouchardat reagents. Positive samples containing alkaloids are indicated by the presence of brick-red deposits for Dragendorff's reagent, white or yellowish deposits for Mayer's reagent and brown to black sediment for Bouchardat's reagent [6].

e. Saponin Test

The sample was added with 20 ml of distilled water and then shaken in a test tube for 15 minutes. The presence of saponins is shown to form a stable foam [7].

Antibacterial Activity Test with the Well Method

10 ml of MSA media was put into a petri dish and left to stand, let it solidify. The petri dishes that already contain MSA media are placed in 7 sterile cylinder cups to make holes in the pitting method. MSA media as much as 20 ml in a liquid state temperature of 40-500C mixed with a suspension of Escherichia coli bacteria with a concentration of 1.0 x 108 CFU / ml of 5 µl. The media containing the bacteria is poured into a petri dish on top of the base layer without filling the inside of the cylinder cup. The media is allowed to solidify at room temperature.

Each cylinder cup was removed and the well formed was given 50 µl of n-hexane ethyl acetate fraction and water from mango peel with a concentration of 5%, 10%, 15%, 20% and 25%. The negative control used was DMSO, and the positive control used was ciprofloxacin 0.005%. The treated media was incubated for 24 hours at 37˚C. Inhibition, which is the diameter of the clear zone where bacteria is not overgrown, was measured using a caliper.

RESULTS AND DISCUSSION

Previous research conducted by Wulandari and Sulistyarini (2018) stated that the extract of the sweet fragrant mango peel has the potential to be antibacterial. This study aims to determine the antibacterial activity of the fraction of n-hexane, ethyl acetate and water from the skin of sweet fragrant mango (*Mangifera indica* L) at concentrations of 5%, 10%, 15%, 20% and 25% against the growth of *Methicillin Resistant Staphylococcus aureus* (MRSA).

The fractionation process is carried out to separate the main content from one main group from the other from the secondary metabolite compounds in the skin of the sweet fragrant mango fruit. The separation of these components is based on differences in polarity of the types of compounds contained. The results of the phytochemical screening of each fraction are presented in Table 1.

Table 1.The results of phytochemical screening for n-hexane, ethyl acetate, water from the peel of sweet fragrant mango

Compound	Reagent	Test results		
		n-Heksan	Etil asetat	Air
Flavonoid	Sample + powder Mg + HCl (p) + amyl alcohol	Clear (-)	Color Orange (+)	Color Orange (+)
Tannin	Sample + 2-3 drops $FeCl_3$	Does not change color (-)	Blue - black (+)	Blue- black (+)
	Sample+ 1% gelatin solution in 10% NaCl	No precipitate (-)	There precipitate (+)	There precipitate (+)
Steroid/ Triterpenoid	Sample + 5 ml Cloroform → filter and then steam Filtrate + asam asetat anhidrat + H_2SO_4 (p)	Reddish brown (triterpenoid) (+)	Reddish brown (triterpenoid) (+)	Reddish brown (triterpenoid) (+)
Alkaloid	Sampel+ 1 ml HCl 2 N +9 ml aquadest panas, dinginkan, dan disaring + *Dragendorff*	Tidak terbentuk endapan (-)	Tidak terbentuk endapan (-)	Tidak terbentuk endapan (-)
	Sample+ 1 ml HCl 2 N + 9 ml heat aquadest, chill and filtered + *Mayer*	No precipitate (-)	No precipitate (-)	No precipitate (-)
	Sample+ 1 ml HCl 2 N +9 ml heat aquadest, chill and filtered + *Bouchardat*	No precipitate (-)	No precipitate (-)	No precipitate (-)
Saponin	Sample + heat aquadest, Lighten and shake until foam / foam appears. Shut up 10'+ HCl 2 N	No precipitate (-)	The foam is stable (+)	There is no foam (-)

Table 1 shows that the n-hexane fraction in the skin of the sweet fragrant mango contains steroid / triterpenoid compounds. Ethyl acetate fraction contains flavonoids, tannins, steroids / triterpenoids and saponins. The water fraction contains flavonoids, tannins, steroids / triterpenoids.

After knowing the active compounds in each fraction of the peel of the sweet fragrant mango, then the test was continued on the antibacterial activity of *Methicillin Resistant Staphylococcus aureus* (MRSA) using the well diffusion method. The results of several replications of antibacterial activity are presented in Table 2.

Table 2. Antibacterial Test of Sweet Fragrant Mango Peel Fraction

Sample	Average Inhibition Zone Diameter (cm)						
	5 %	10 %	15 %	20 %	25 %	Control +	Control -
Fraksi n-Heksan	0.466	0.769	0.865	0.961	1,044	2,687	0,000
Fraksi Etil asetat	1,127	1,458	1,563	1,638	1,776	2,684	0,000
Fraksi Air	0.473	0.662	0.839	1,033	1,220	2,690	0,000

Table 2 shows that the compounds in the peel fraction of sweet fragrant mango have antibacterial activity which is indicated by the formation of clear zones at various fraction concentrations. The clear zone that is formed is getting bigger with an increase in the concentration of the fraction. This is in line with Purwanto's (2015) statement which states that the diameter of the clear zone is influenced by the size of the concentration in the sample.

Based on clear zone data, the ethyl acetate fraction has the greatest antibacterial activity compared to other fractions, this can be due to the fact that the active compound content in this fraction is more than the n-hexane fraction and the water fraction.

The antibacterial activity test used in this study is the well diffusion method. This method has several advantages including the antibacterial activity of the sample that can be observed in all directions on the surface, middle and lower layers and can accommodate a larger number of samples. The media used in this study was Mannitol Salt Agar (MSA). Mannitol Salt Agar contains very high NaCl, namely 7.5% - 10%.

Staphylococcus aureus bacteria are able to adapt to environments with high salt levels, while other bacteria are not able to survive in environments with high salt levels. In addition, MSA contains mannitol and phenol red indicators. The presence of Staphylococcus aureus will ferment mannitol to produce acid which changes the color of the phenol red indicator medium from red to yellow, so MSA is a selective medium for the growth of *Staphylococcus aureus* [8].

Planting the MRSA bacterial suspension using the pour plate method so that the bacteria added to the MSA media can evenly diffuse throughout the media, from the surface to the MSA media. The solvent used to dissolve the fraction to be tested for antibacterial is Dimethyl Sulfoxide (DMSO). This solvent is a solvent that can dissolve almost all compounds, both polar and non-polar. This solvent also does not provide an inhibitory power against bacteria in the final result so that it can be ascertained that the inhibition zone formed in the antibacterial activity test comes from compounds in the peel fraction of sweet arum mango, not from the solvent. Therefore DMSO was also used as a negative control.

The positive control used in this study was ciprofloxacin. Ciprofloxacin has bactericidal activity in the bacterial growth phase based on inhibition of bacterial DNA enzymes, so that bacterial DNA synthesis can be inhibited [9]. Ciprofloxacin is effective against bacteria that are resistant to other antibiotics such as penicillins, aminoglycosides, cephalosporins and tetracyclines. Ciprofloxacin is effective against gram-negative and positive bacteria [10].

CONCLUSION

The conclusion that can be drawn from this study is that the compounds in the peel fraction of sweet fragrant mango has the ability to inhibit the growth of *Methicillin Resistant Staphylococcus aureus* (MRSA) bacteria.

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