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Antibacterial activity of active fraction of sweet corn hair extract (zea mays f saccharata kornicke & werner) against methicillin resistant staphylococcus aureus (MRSA) growth

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ABSTRACT

Methicillin-resistant Staphylococcus aureus (MRSA) is a Staphylococcus aureus bacterium that is resistant to methicillin-type antibiotics. This study aims to determine the antibacterial activity of the active fraction of sweet corn hair extract on the growth of Methicillin-Resistant Staphylococcus aureus (MRSA) bacteria. Extraction was carried out by the remuneration method in 70% ethanol. In fractionation, liquid vacuum column chromatography was used with n-hexane, ethyl acetate, and methanol solvents. The fraction with the largest clear zone of methanol (100%) has a clear zone diameter of 0.752 cm. Testing the antibacterial activity of ethanol extract at concentrations of 10%, 15%, and 20% obtained by clear zone with an average of 1,013; 1,073; 1,159 cm and in the active fraction with an average of 0,703; 0.903; 1,004 cm. Ciprofloxacin 0.005% was used as a positive control and DMSO as a negative control. The results showed that ethanol extract and an active fraction of sweet corn hair (Zea mays f saccharata Kornicke & Werner) had antibacterial activity against Methicillin-Resistant Staphylococcus aureus (MRSA). One-way ANAVA test results showed that there were significant differences in providing antibacterial activity (p> 0.05) between ethanol extract and active fractions at concentrations of 10%, 15%, and 20%. Flavonoid compounds, alkaloids, saponins, tannins, and triterpenoids in the active fraction of corn hair have antibacterial activity against MRSA bacteria.

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1. Introduction

Infectious diseases can be caused by various microorganisms such as bacteria,one of which is Staphylococcus aureus. Staphylococcus aureus bacteria are reported to have experienced resistance to methicillin-type antibiotics and are known as Methicillin Resistant Staphylococcus aureus (MRSA) [1]. WHO recommends looking for new antibacterials and other treatment alternatives. Natural ingredients can be used as an alternative to finding new antibacterials which are known to provide minimal side effects and are safer than synthetic drugs.

A sweet great (Zea mays f saccharata Kornicke & Werner) is on the sweet corn hair that has the potential as an antibacterial. Sweet corn hair contains the bioactive components flavonoids, saponins, tannins, phlobatanin, phenols, alkaloids, and cardiac glycosides. The component suggests that compounds found in sweet corn hair extract contribute to the pharmaceutical field [2].

Research that has been carried out by Dhinarty [3] says that ethanol, ethyl acetate and petroleum ether extracts of sweet corn hair have antibacterial activity against Staphylococcus aureus bacteria. The research is only limited to extracts that need to be fractionated to separate compounds based on their level of polarity. Based on this background, a study was conducted on the test of the antibacterial activity of fractional extracts of sweet corn hair (Zea mays f saccharata Kornicke & Werner) against the growth of Methicillin Resistant Staphylococcus aureus (MRSA) bacteria.

2. Method

In this section, it is explained the results of the research and at the same time is given the comprehensive discussion. Results can be presented in figures, graphs, tables and others that make the reader understand easily [4], [5]. The discussion can be made in several sub-sections.

2.1. Research Object

The object of study is the antibacterial activity of the active fraction of sweet corn hair extract (Zea mays f saccharata Kornicke & Werner) against the growth of Methicillin Resistant Staphylococcus aureus (MRSA) bacteria.

2.2. Tools and Materials

Tools used for research cover the smoothing machine, erlenmeyer, water bath, vacuum rotary evaporator, split funnel, chamber, capillary pipe, mercury tube, test tube rack, petri dish, UV lamp 254, bunsen, autoclave, aluminum foil, spray bottle, cotton, volume pipette, spatula, analytical scales, incubator, ose needle, Laminar Air Flow (LAF), cylinder cup, micropipette, tweezers, calipers, yellow tip, blue tip, mask, gloves, scissors, label paper, spectrophotometer. The research ingredients are sweet corn hair, ethanol 70%, methanol, ethyl acetate, N-hexane, NaOH, NaNO2, sulfanilic acid, glacial acetic acid, concentrated H2SO4, HCl, dragendroff, Mg powder, amyl alcohol, $FeCl_3$, gelatin, aquadest, ether, H 2 SO 4, n-butanol, acetic acid, ammonia, chloroform, dragendroff, chloroform, methanol, anisaldehyde, H2SO4, FeCl3, toluene, Methicillin Resistant bacteria Staphylococcus aureus

(MRSA), MSA media (Mannitol Salt Agar), NA(Nutrient Agar) media, NB media (Nutrient Broth), ciprofloxacin, Dimethyl Sulphoxide (DMSO).

2.3. Extraction process

Making extracts by re-maceration using 70% ethanol solvent. Jagung manis hair powder of 100 g is soaked in 750 ml of 70% ethanol solvent, then pouredand squeezed, the pulp is re-macerated with 70% ethanol. During the remaceration process, the vessel is closed, solvent replacement every 1 x 24 hours, while stirring ses, is carried out for 3 days protected from light [4]. The extract is then concentrated with vacuum rotary evaporator.

2.4. Fractionation process

Thick extract of corn hair weighed 5 grams then with ethanol impregnated with silica gel 60 until dry. It is further fractionated with kvc with n-hexane solvent, ethyl acetate, methanol. Each fraction of the kvc yield is evaporated above the water bath so that a viscous fraction is obtained.

2.5. Ethanol Free Test

An ethanol-free test is performed for extracts obtained from the extraction results. The color test was carried out by adding a solution of sulfanilic acid HCl, $NaNO_2$ solution and NaOH to the sweet corn hair extract. A positive result if a red color of frambors is formed. The odor test is carried out by adding acetic acid and concentrated H_2SO_4. Positive result if there is a banana smell [5].

2.6. Preliminary test

Flavonoid samples were dissolved in ethanol, and Mg powder and concentrated HCl were added. These results are added amyl alcohol, shaken vigorously, and left to separate. The flavonoid-positive extract when red, yellow, or orange is formed on the amyl alcohol layer [6]. The sample saponin is mixed with 10 ml of hot water and then cooled and shaken vigorously until foam appears. Filtrate is allowed to stand for 2 minutes, then dripped HCl 2N. Positive saponin compounds when the foam formed steady for 10 minutes [7]. The sample tannins are mixed with 10 ml of hot aquadest and heated for approximately 1 hour, then cool and strain. Filtrate plus reagent $FeCl_3$1%, positive result if a blue-black / greenish-brown color is formed [8]. The next tannin test that is best known is the precipitation of gelatin. The addition of 1% gelatin and 10% NaCl solution (1:1) resulted in a white precipitate indicating the presence of tannins [9].

The sample alkaloids were mixed with 1 ml of HCl 2 N and 9 ml of hot aquadest. The filtrate is heated 2 minutes, then cool and strain and then the filtrate is divided into three. The first filtrate plus a Dragendroff reagent, a positive sample when a brick-red precipitate is formed. The second filtrate plus Mayer reagent, the sample is positive when a white precipitate forms. The third filtrate plus Bouchardat reagent, a positive sample when brown to black deposits form [7]. Triterpenoids and steroid samples dissolved 0.5 ml of chloroform and added Lieberman-Burchard reagent, if green color arises shows positive results in the presence of steroids, while if reddish brown color arises indicates a positive result of triterpenoids [10].

2.7. KLT

The extract and active fraction of sweet corn hair obtained were then identified by means of KLT . The sample is placed on the GF254 silica gel plate and then put into a chamber that has been saturated with eluent vapor and tightly closed. After the elution is completed the plate is removed from the chamber, dried and then sprayed with spotting. The color of the spotting is observed and calculated its Rf price. Flavonoids use n-butanol eluent: acetic acid: water (4:1:5) and are steamed using concentrated ammonia vapors. The formation of light yellow to orange colors indicates the presence of flavonoids [11]. Saponins using chloroform eluents: methanol: water (64:50:10) and the appearance of Lieberman-Burchard spots forming a green to blue color indicates the presence of saponin content [9]. Tannins using n-butanol eluent: acetic acid: water (4:1:5) and the appearance of AlCl3 spots 1%, the formation of reddish-purple or greenish-brown stains indicates the presence of tannin content [12]. Alkaloids using methanol eluents: NH4OH concentrated (200:3) and dragendroff spotting displays, the formation of orange brown spots indicates the presence of alkaloid content [11]. Triterpenoids and steroids using toluene eluent: ethyl acetate (93:7) and Lieberman-Burchard spotting display, positively containing triterpenoids if purple and orange stains appear [13].

2.8. Antibacterial Activity Test

Test using the well method by placing the cylinder cup on top of the first 10 ml layer of M S A media that has been compacted, then poured the MRSA suspension mixture of 5 µl into 20 ml of MSA media and left to compact. The cylinder cup is lifted, inserted as much as 50 µl of sample into the well with a concentration of 10%, 15% and 20%. 0.00 5% ciprofloxacin solution as positive control while DMSO solution as negatif control. The cup was incubated 1 x 24 hours at a temperature of 37oC tomudian measured in a meter clear zone of growth a n MRSA with a caliper device. Based on the results of KLT screening and testing, the compounds contained in the extract and the active fraction, each compound is carried out a contact bioautographic test. The results of the KLT that have been diluted, are attached to the media that has been suspended with ama cell bacteria for 15 minutes, then removed the KLT plate and the media is incubated for 1 x 24 hours. If the location of the stain on the chromatogram plate is the same as the location of the clear zone of the bioautography results, it can be concluded that the compounds on the chromatogram plate have diffused into the media and have antibacterial activity.

2.9. Data Analysis

Antibacterial activity test results are statistically analyzed. Normal and homogeneous distributed data, used uji parametric statistics anava 1 way, followed by Post hoc test to find out which groups have differences.

3. Results and Discussion

The drying process uses the remaceration method with 70% ethanol solvent for three days. The remaceration method is carried out solvent replacement to prevent solvent saturation and stirring so that the difference in concentration between the inner and outer solution of the simplician powder cell is maintained. The number of active compounds contained in the solvent affects the resulting amendment [14]. The fractionation process is carried out using the liquid vacuum column chromatography (KVC) method with a gradiently increased polarity motion phase. The active fraction is selected from the fraction that

exerts antibacterial activity at the same yamg concentration measured based on the largest clear zone produced. Based on the antibacterial test, the fraction that has the largest antibacterial activity is the methanol fraction (100%) with a clear zone of 0.752 cm.

Phytochemical screening to determine the content of compounds contained in the powder, extract, and active fraction of sweet corn hair. The results of phytochemical screening are presented in Table 1.

Table 1. Phytochemical Screening Results

Compound class	Reagents	Positive results	Research results		
			Powder	Extract	Active Fraction
Flavonoids	Powder Mg + HCl p + amyl alcohol	Red, yellow, or orange color on the amyl alcohol coating [6]	(+) **The red color of the amyl alcohol layer**	(+) **The red color of the amyl alcohol layer**	(+) **The red color of the amyl alcohol layer**
Alkaloids	HCl 2N + Dragen-dorff	Red brick deposits [7]	(+) **The red precipitate (Dragendorff reagent)**	(+) **The red precipitate (Dragendorff reagent)**	(+) **The red precipitate (Dragendorff reagent)**
	HCl 2N + Mayer	White precipitate [7]	(+) **White precipitate (Mayer reagent)**	(+) **White precipitate (Mayer reagent)**	(+) **White precipitate (Mayer reagent)**
Saponins	Shaken + HCl 2N	Steady foam 10 minutes [7]	(+) **Stable foam**	(+) **Stable foam**	(+) **Stable foam**
Tannins	Aquadest + $FeCl_3$ 1%	Positive greenish-brown color of condensed tannins, and positive blue-black color of hydrolyzed tannins [8]	(+) **Greenish-brown (condensed tannins)**	(+) **Greenish-brown (condensed tannins)**	(+) **Greenish-brown (condensed tannins)**
	+ 1% gelatin: NaCl 10% (1:1)	White precipitate [9]	(+) **White precipitate**	(+) **White precipitate**	(+) **White precipitate**
Triterpenoids and Steroids	Chloroph ysome + anhydro us acetic acid $+_{H2SO4}$p	The positive green color of steroids, positive reddish brown color of triterpenoids [10]	(+) **Reddish brown (Triterpenoid)**	(+) **Reddish brown (Triterpenoid)**	(+) **Reddish brown (Triterpenoid)**

The affirmation test using KLT obtained positive extract results and active fractions of sweet corn hair containing flavonoid compounds, alkaloids, saponins, tannins and oid triterpenes. KLT results are presented in Table 2.

Table 2. KLT Results Sweet Corn Hair

Compound Group	Phases of Motion	Spotting Appearance	Extract Results	Active Fraction Results	Description (Library)
Flavonoids	Butanol:glacial acetic acid:water (4:1:5)	Ammonia vapor	(+) **Rf 0.63 Greenish yellow**	(+) **Rf 0.63 Greenish yellow**	Brown, yellow-green after ammonia steamed [15]
Alkaloids	Chloroform: ethyl acetate (70:30)	*Dragendorff*	(+) **Rf 0.38 Light brown**	(+) **Rf 0.39 Light brown**	Formed brown color after spraying *dragendorff* spotting [16]
Saponins	Chloroform:methanol:water (64:50:10)	Anisaldehyde -sulfuric acid	(+) **Rf 0.39 Dark blue**	(+) **Rf 0.38 Dark blue**	Formed red, yellow, dark blue, purple, green, and brownish-yellow after spraying the appearance of annisaldehydehyde -acid sulphuric spots [17]
Tannins	Ethyl acetate: methanol: water (100:13,5:10)	$FeCl_3$	(+) **Rf 0.46 Blackish green**	(+) **Rf 0.46 Blackish green**	Formed a blackish-green color after spraying the appearance of $FeCl_3$ spots [18]
Triterpenoids	Toluene: ethyl acetate (93:7)	Anisaldehyde -sulfuric acid	(+) **Rf 0.13 Rf 0.25 blue**	(-)	Formed blue or purple [19]

Antibacterial activity tests were carried out on the extract and active fraction of sweet corn hair concentrations of 10%, 15%, and 20%. The test method of antibacterial activity performed is the diffusion of the well. The medium used is Manitol Salt Agar (MSA) because it selectively grows Staphylococcus bacteria. MSA contains high salt content (7.5% NaCl) so bacteria other than Staphylococcus are not able to grow in high salt conditions. DMSO (Dimethyl Sulphoxide) is used as a solvent and negative control because it is able to dissolve all compounds both polar and semipolar and is not bacteriostatic. Ciprofloxacin was chosen as a positive control because it is a broad-spectrum antibacterial with its mechanism of action inhibiting the synthesis of nucleic acids by means of inhibition of the transcription process and replication of microorganisms [20]. The test results of the antibacterial activity of extract and the active fraction of sweet corn hair are shown in Tables 3 and 4.

Table 3. Results of Antibacterial Activity Test of Sweet Corn Hair Extract

Replication	**Inhibitory** Zone Diameter **(cm)**				
	Ethanol Extract			**Control**	
	10%	**15%**	**20%**	**Control (+)**	**Control (-)**
1	1,011	1,071	1,160	1,318	0,000

2	1,012	1,072	1,158	1,319	0,000
3	1,014	1,075	1,157	1,320	0,000
4	1,015	1,074	1,161	1,316	0,000
5	1,013	1,073	1,159	1,317	0,000
Average	1,013	1,073	1,159	1,318	0,000

Table 4. Test Results of Antibacterial Activity of Active Fraction of Sweet Corn Hair

Replication	Inhibitory Zone Diameter (cm)				
	Active Fraction			Control	
	10%	15%	20%	Control (+)	Control (-)
1	0,701	0,901	1,002	1,309	0,000
2	0,704	0,902	1,004	1,306	0,000
3	0,703	0,904	1,005	1,308	0,000
4	0,702	0,905	1,003	1,310	0,000
5	0,706	0,903	1,006	1,307	0,000
Average	0,703	0,903	1,004	1,308	0,000

Testing of ethanol extract and ethyl acetate fraction obtained different results. The diameter of the inhibitory zone in the extract and the active fraction of corn hair have increased according to the increase in concentration. Testing of antibacterial activity using the contact bioautography method using a chromatogram of KLT results. The results of the bioautographies of contacts are presented in Table 5.

Table 5.Bioautographic Test Results of Sweet Corn Hair Extract and Active Fraction

Compound	Extract	Active Fraction
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+
Tannins	+	+
Triterpenoids	+	-

Description:
(-) Negative results
(+) Positive results

Based on the results of the Post Hoc test, all data in each group showed significant differences. This shows that significant differences in each group have different effects in inhibiting bacterial growth, and their ability is different from the positive control, namely ciprofloxacin.

4. Conclusion

The active fraction of the ethanol extract of sweet corn hair has antibacterial activity against the growth of MRSA bacteria. The methanol fraction (100%) of sweet corn hair

extract has the greatest antibacterial activity against the growth of MRSA bacteria. There are differences in the antibacterial activity of the active fraction of sweet corn silk at concentrations of 10%, 15%, and 20% against the growth of MRSA bacteria. Compounds contained in the active fraction of sweet corn hair that positively has antibacterial activity against the growth of MRSA bacteria by the contact bioautographic method are flavonoids, alkaloids, saponins, and tannins.

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