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ANTIBACTERIAL ACTIVITY TEST OF SWEET CORN (*ZEA MAYS L.*) ETHANOL EXTRACT ON *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS EPIDERMIDIS* BACTERIA

Muhammad Andry[1], Tetty Noverita Khairani[1], Rida Evalina Tarigan[1], Nursafwati[1], Muhammad Amin Nasution[2], Ika Julianti Tambunan[3], Muhammad Faizal Fathurrohim[4], Firman Rezaldi[5]

[1]Program Studi Farmasi, Institut Kesehatan Helvetia Medan, Indonesia
[2]Program Studi Farmasi, Universitas Muslim Nusantara al Washliyah Medan, Indonesia
[3]Program Studi Farmasi, Universitas Tjut Nyak Dhien Medan, Indonesia
[4]Program Studi Pendidikan Biologi, Universitas Sali Al-Aitaam, Indonesia
[5]Program Studi Farmasi, STIKes Bhakti Husada Mulia Madiun, Indonesia

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ABSTRACT

Sweet corn fruit peel (*Zea mays L.*) is one of the plant species belonging to the Poaceae family. The aim of the study was to determine the antibacterial activity and the most effective concentration of sweet corn rind in inhibiting the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria. Types of experimental research in the form of sample collection, manufacture of simplicia, screening of phytochemicals, characteristics of simplicia, preparation of ethanol extract of sweet corn husks with various concentrations of 10%, 15%, 20%, extract characteristics, rejuvenation of *Escherichia coli* bacteria with EMBA media and *Staphylococcus epidermidis* with media MSA, as well as testing the antibacterial activity using MHA media for both bacteria with the disc diffusion method in inhibiting the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria with 1% *Chloramphenicol* positive control and DMSO negative control. The results of the ethanol extract of sweet corn rind were able to inhibit the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria. The antibacterial activity test of the ethanol extract of sweet corn rind on *Escherichia coli* bacteria showed an average inhibition zone of each concentration, namely 10% (11.18 mm), 15% (12 mm), 20% (13.42 mm). And the *Staphylococcus epidermidis* bacteria showed an average inhibition zone of each concentration, namely 10% (10.72 mm), 15% (12.37 mm), 20% (14.67 mm). In conclusion, the ethanol extract of corn husks has antibacterial activity against *Escherichia coli* and *Staphylococcus epidermidis* bacteria.

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Keywords: Antibacterial, Ethanol Extract, *Escherichia coli*, *Staphylococcus epidermidis*, Sweet Corn Peel.

INTRODUCTION

Indonesia is a tropical country with various types of plants that are efficacious as traditional medicines. The use of medicinal plants is based on the properties believed by their ancestors for generations. The presence of secondary metabolites in plants has been reported by several studies to be an important factor in the selection of potential medicinal plants (Andry & Winata, 2022).

Traditional medicine has been used widely in the world and about 80% of the population in several countries use traditional medicine to protect their health. Several factors such as the increasing prevalence of chronic diseases and the failure to use modern medicine for certain diseases and the wide access to information on herbal medicines are the reasons increasing use of herbal medicines in developed countries. Traditional medicine has been well

Correspondance Address
E-mail: muhammadandry874@yahoo.co.id

received in almost all countries in the world, both in developing and developed countries (Dewi et al. 2019).

Escherichia coli is a coliform bacterium that belongs to the Enterobacteriaceae family. Enterobacteriaceae are enteric bacteria or bacteria that can live and survive in the digestive tract. *Escherichia coli* is a gram-negative rod-shaped bacterium, facultative anaerobe, does not form spores, and is a natural flora in the intestines of mammals (Winiati et al. 2021). Under normal circumstances *Escherichia coli* can grow in the digestive tract but can be pathogenic and capable of attacking animals and humans in certain circumstances such as digestive disorders (Kartikasari et al., 2019).

Infection is the most common disease suffered by people in developing countries including Indonesia, one of which is caused by the bacterium *Staphylococcus epidermidis*. *Staphylococcus epidermidis* is a gram-positive bacterium, found on human skin, respiratory tract and digestive tract. These bacteria cause infections, swelling like pimples, wound infections, urinary tract infections and kidney infections (Rahminiwati et al. 2020). The increase in sufferers of diarrhea causes the use of antibiotics also increases. Most of the antibiotics are made from synthetic compounds which if consumed continuously can cause side effects and bacteria become resistant. Various efforts have been made to reduce the negative impact of using synthetic antibiotics such as the development of research on secondary metabolites that have the potential as antibacterials from natural ingredients (Winata et al. 2023).

Widespread and irrational use of antimicrobials such as antibiotics can lead to a state of resistance. The phenomenon of bacterial resistance to antibacterial drugs currently tends to increase and is detrimental, both socially and financially. Phytochemical compounds, namely saponins, alkaloids, flavonoids, and polyphenols which have the potential as antibacterial sources (Andry et al. 2022). Corn is one of the carbohydrate-producing food crops. Corn is also an important component for animal feed, apart from being a staple food source, corn is also taken for its oil and can also be processed into flour. Various derivative products resulting from corn processing are also raw materials for a number of food industry products (Aidah, 2020). Almost all parts of it can be used, such as the skin, leaves, straw, stems, cobs, hair and seeds (Fauziah et al. 2021).

One of the plants that has potential as an antibacterial is corn husk (*Zea mays L.*) based on previous research reports by Safitri and Luluk (2019) that secondary metabolites in corn husk (*Zea mays L.*) show antimicrobial properties including secondary metabolites of alkaloids, flavonoids, and saponins (Hamidah safitri & Jubaidah, 2019).

The purpose of this study was to determine the antibacterial activity of the ethanol extract of sweet corn (*Zea mays L.*) peels in inhibiting the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria. To determine the most effective concentration of the ethanol extract of sweet corn (*Zea mays L.*) peels in inhibiting the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria.

MATERIALS AND METHODS

This research was conducted using experimental research through testing in the laboratory. This research was conducted in July-August 2022. This research was conducted at the Phytochemistry and Microbiology Laboratory of the University of North Sumatra. The objects used in this study were *Escherichia coli* and *Staphylococcus epidermidis* obtained at the Microbiology Laboratory, University of North Sumatra. Sampling was carried out purposively, sampling that is without comparing with similar plants from other areas. The sample for this study was the skin of sweet corn (*Zea mays L.*) obtained from Ikulung Village, Jeumpa District, Southwest Aceh District, Aceh.

Tool

The tools to be used are autoclave, stir bar, blender, beaker glass, petri dish, glass deck, erlenmeyer, measuring cup, incubator, caliper, loop needle, object glass, disc paper, label paper, parchment paper, ash-free filter paper , tripod, bunsen, digital scale, oven, tweezers, tube rack, rotary evaporator, spatula, test tube, vortex, 40 mesh sieve, water bath, drying cabinet, desiccator, dropping pipette, hotplate, volumetric flask, porcelain crucible, bottle weighing, evaporating cup, aluminum foil, magnetic stirrer, microscope, object glass, deck glass.

Material

The materials to be used are sweet corn rind, *Escherichia coli* bacteria, *Staphylococcus epidermidis* bacteria, distilled water, Eosin Methylene Blue Agar (EMBA), Mannitol Salt Agar (MSA), Mueller Hinton Agar (MHA), NaCl

0.9%, ethanol 70 %, 96% ethanol, Mc standard suspension. Farland, HCL, Mayer's reagent, Wagner's reagent, Dragendrof's reagent, ethyl acetate, acetic anhydrous acid, concentrated sulfuric acid, hydrochloric acid, 2N HCl, $FeCl_3$, NaCl, 1% $BaCl_2$, 1% H_2SO_4, chloroform, magnesium powder, amyl alcohol, 2N hydrochloric acid

Preparation of Ethanol Extract Test Solution from Sweet Corn (*Zea mays L.*) Peel

The preparation of the test solution for the ethanol extract of sweet corn (*Zea mays L.*) peels was carried out by dilution. The mother liquor is made from a concentration of 20% by dissolving 1 gram of extract which has previously been weighed in 5 ml of distilled water, for a concentration of 15%, 1.5 ml of the 20% extract is taken and then added in 2 ml of distilled water, for a concentration of 10% taken 1 ml of 20% extract and added to 2 ml of distilled water. *Chloramphenicol* powder was weighed as much as 0.10 grams and dissolved in 10 ml of solvent as a positive control, where as DMSO as a negative control.

Inhibition Zone Measurement

In this study, the antibacterial activity test was carried out using the disc diffusion method. Sweet corn peel extract (*Zea mays L.*) was diluted with a concentration of 10%, 15% and 20% by adding distilled water. Furthermore, 100 µl of bacterial suspension was spread into a petri dish, then added EMBA media for testing Echerichia coli bacteria and MSA media for testing *Staphylococcus epidermidis* bacteria as much as 15 ml, stir to form a number 8 until homogeneous and let stand until it solidifies, then place the disc that has been filled with concentration solutions of 10%, 15% and 20%. Discs for the positive control of *Chloramphenicol* and negative control discs containing DMSO.

The discs were placed on the surface of the solid media that had been inoculated with bacteria, then incubated for 1x24 hours at 40oC, then observed and measured the inhibition zone formed at 24 hours. Then the Inhibitory Diameter (DDH) formed was measured using a caliper (Hamidah safitri & Jubaidah, 2019).

RESULT AND DISCUSSION

Phytochemical Screening Test Results

The results of the phytochemical screening test for sweet corn (*Zea mays L.*) rind simplicia were carried out to show the class of secondary metabolites contained therein. The examination carried out on sweet corn rind simplicia is an examination of the class of alkaloid compounds, flavonoids, saponins, glycosides, tannins, and steroids/terpenoids. The results of the phytochemical screening test can be seen in **Table 1** below:

Table 1 Results of the Phytochemical Screening of Sweet Corn Fruit Skins

No	Secondary Metabolites	Reactor	Result
1.	Alkaloids	Mayer	-
		Bouchardat	+
		Dragdroft	+
2.	Flavonoids	Mg powder + HCl P	_
3.	Saponins	HCl 2N	+
4.	Glycosides	Acetic anhydrous P + sulfuric acid P	+
5.	tannins	$FeCl_3$ 1%	_
6.	Steroids/terpenoids	Liberman/ Bouchardt	+

Note: (+) contains the substance being examined, (-) does not contain the substance being examined

Based on the results of the phytochemical screening test for sweet corn (*Zea mays L.*) rind simplicia shown in **Table 1**, it shows that sweet corn rind contains alkaloids, saponins, glycosides, and terpenoids.

Simplicia Characteristics Examination Results

The results of examining the simplicia characteristics of sweet corn (*Zea mays L.*) peels can be seen in **Table 2**.

Table 2 Examination of Characteristics of Sweet Corn Fruit Peel Simplicia

No	Parameter	Result
1.	Macroscopic examination	Elongated shape, sharp skin tip, light green to dark green color, characteristic weak odor, tasteless.
2.	Microscopic examination	Cover hair, epidermis, parenchyma.

3.	Determination of water content	6.87%
4.	Determination of total ash content	2.19%
5.	Determination of water-soluble essence content	10.62%
6.	Determination of the content of soluble extracts in ethanol	8.22%
7.	Determination of acid insoluble ash content	0.93%

Extraction Results

The yield of sweet corn (*Zea mays L.*) peel extract can be seen in **Table 3**.

Table 3 Yield of Sweet Corn Fruit Peel Extract

Yield of Sweet Corn Fruit Peel	Sample Weight		Yield Value
	Before	After	
Extract yield	500g	33g	6.6%

Extract Characteristic Examination Results

The results of examining the characteristics of the peel extract of sweet corn (*Zea mays L.*) can be seen in **Table 4**.

Table 4 Examination Results of Characteristics of Sweet Corn Fruit Peel Extract

No	Parameter	Result
1.	Determination of drying shrinkage	31.29%
2.	Determination of water content	6.1%
3.	Determination of ash content	3.75%
4.	Determination of acid insoluble ash content	0.76%

Antibacterial Activity Test Results

The results of measuring the diameter of the inhibition zone from sweet corn fruit peel extract can be seen in **Table 5**.

Table 5 Measurement Results of Inhibition Zone Diameter (mm)

Test Bacteria	Formula	Growth Inhibition Zone Diameter Bacteria (mm)				
		Repetition			The average value of the inhibition zone	Inhibition zone category
		I	II	III		
Escherichia coli	F_0	0	0	0	0	Nothing
	F_1	11.65	11.95	9.95	11.18	Strong
	F_2	12.45	12.3	11.25	12	Strong
	F_3	14.25	12.7	13.3	13.42	Strong
	F_4	13.5	14.1	13.1	13.57	Strong
Staphylococcus epidermidis	F_0	0	0	0	0	Nothing
	F_1	11.65	10.55	9.95	10.72	Strong
	F_2	12.85	12.25	12	12.37	Strong
	F_3	15.55	13.45	15	14.67	Strong
	F_4	24.3	24.8	22.85	23.98	Very strong

Description: (F_0) DMSO negative control, (F_1) Sweet corn peel extract (*Zea mays L.*) 10%, (F_2) Sweet corn peel extract (*Zea mays L.*) 15%, (F_3) Corn fruit peel extract sweet (*Zea mays L.*) 20%, (F_4) *Chloramphenicol* positive control

Results of Data Analysis

The results of the ANOVA test of sweet corn peel extract (*Zea mays L.*) diameter of the inhibition zone for *Escherichia coli* bacteria can be seen in **Table 6** below:

Table 6 Statistical Test Results of One Way Anova Zone of Inhibition of *Escherichia coli* Bacteria

Formulasi	Shapiro-Wilk Data Normality Test (sig.)	Homogeneous Test (Sig.)	ANOVA (sig.)
F_1	0.266	0.57	0.000
F_2	0.220		
F_3	0.752		
F_4	0.780		

The results of the ANOVA test of sweet corn peel extract (*Zea mays L.*) diameter of the inhibition zone of *Staphylococcus epidermidis* bacteria can be seen in **Table 7** below:

Table 7 Statistical Test Results of One Way Anova Zone of Inhibition of *Staphylococcus epidermidis* Bacteria

Formulasi	Shapiro-Wilk Data Normality Test (sig.)	Homogeneous Test (Sig.)	ANOVA (sig.)
F_1	0.679	0.70	0.000
F_2	0.554		
F_3	0.488		
F_4	0.476		

Phytochemical Screening Test Results

Examination of alkaloid compounds with the addition of Mayer's reagent gave negative results because it did not produce a white to yellowish precipitate, while the bouchardat reagent gave a positive result indicated by the formation of a brown precipitate, whereas in the dragendroft reagent the alkaloid compounds were shown to form a brick red precipitate (Sulistyarini et al. 2020). Alkaloids have the ability as an antibacterial, namely by interfering with the peptidoglycan component in bacterial cells so that the cell wall layer is not formed completely and causes cell death (Chairani & Harfiani, 2018).

In the positive saponin examination because the sample tested formed foam 1-10 cm high with an interval of ± 10 minutes, the resulting foam was stable with the addition of HCL (Sulistyarini et al., 2020). Saponins have antibacterial activity, namely by interfering with the permeability of microbial cell membranes by interfering with the stability of bacterial cell membranes, causing bacterial cell lysis, which results in damage to the cell membrane and causes the release of various important components of microbial cells, namely proteins, nucleic acids, nucleotides and others (Chairani & Harfiani, 2018).

In the positive glycoside examination because the test sample formed a purple ring at the liquid boundary on the tube wall (Jannah et al 2017). Glycosides have antimicrobial activity, the mechanism of action of glycosides is the penetration of compounds into the bacterial cell membrane which causes coagulation of proteins and bacterial cell membranes, so that the bacterial cells experience lysis (Soeka et al. 2007).

In the positive terpenoid examination because the test sample produces a purple color (Habibi et al. 2018). Terpenoids have the potential as antibacterials. The mechanism of action of terpenoids as antibacterials is by damaging the membrane by lipophilic compounds, terpenoids can react with transmembrane proteins, namely on the outer membrane of the bacterial cell wall so that the bacterial cell lacks nutrients, the growth of the bacteria becomes inhibited or dies (Ratulangi, 2020).

Examination of Simplicia Characteristics

Examination of simplicia characteristics aims to ensure uniform quality of simplicia in order to meet the simplicia standard requirements (Diana Febriani et al. 2015). It was carried out by observing simplicia macroscopically and microscopically and also determining the water content, determining the total ash content, determining the water-soluble extract content, determining the soluble extract content in ethanol, and determining the acid-insoluble ash content (Mayasari et al. 2018). There are several factors that can influence the examination of the characteristics of simplicia

including the raw materials for simplicia, the method of manufacture and storage of simplicia (Diana Febriani et al., 2015).

The macroscopic test aims to determine the characteristics of simplicia by direct observation based on the simplicia form of sweet corn (*Zea mays L.*) peel (Mayasari et al. 2018). The macroscopic results of the skin of sweet corn (*Zea mays L.*) are elongated in shape, pointed at the tip of the skin, light green to dark green in color, characteristic weak odor, and tasteless. Meanwhile, the microscopic test aims to see identifying fragments in the form of cells, cell contents or plant tissue of the simplicia powder of sweet corn (*Zea mays L.*) fruit skin (Mayasari et al. 2018). Microscopic results of sweet corn (*Zea mays L.*) rind simplicia include covering hairs, epidermis, and parenchyma. Determination of the simplicia's water content is carried out to provide a maximum limit for the water content in the simplicia, a high amount of water can become a medium for the growth of bacteria or fungi and can damage the compounds contained in the simplicia (K. Fitri et al., 2023). The results of the water content test for sweet corn rind simplicia were 6.87%.

Determination of the total ash content of simplicia was carried out to provide an overview of internal and external mineral content originating from the initial process until the formation of simplicia, the total ash content is related to minerals, both organic and inorganic compounds obtained internally and externally (Diana Febriani et al., 2015). The test results for the total ash content for sweet corn husk simplicia were 2.19%. Meanwhile, the determination of the acid-insoluble ash content was carried out to determine the amount of ash obtained from external factors, namely from impurities originating from sand or silicate soil (Winata et al., 2023). The test results for acid insoluble ash content for sweet corn rind simplicia were 0.93%.

Determination of water- and ethanol-soluble extracts was carried out to provide an initial description of the number of compounds that could be extracted with water and ethanol solvents from a simplicia (Pamungkas et al., 2022). From the test results for sweet corn rind simplicia, the water-soluble extract content was 10.62%. While the content of the soluble essence in ethanol is 8.22%. This shows that the number of polar compounds that can be dissolved in water is greater than the number of less polar (semi-polar or non-polar) that can be dissolved in ethanol (Fitri et al. 2023).

Extraction Results

Extraction is carried out with the aim of dissolving all substances contained in the sample using an appropriate solvent and preventing damage to the metabolite compounds. Besides that, the advantage of the extraction process by maceration of the simplicia powder is soaked in a solvent until it is absorbed and will soften the cell composition, so that substances that are easily soluble will be dissolved (Diana Febriani et al., 2015).

In this study, extraction was carried out using the maceration method, soaked using 70% ethanol solvent. the use of ethanol as a solvent because ethanol is a universal organic solvent that is safe, is expected to attract polar, semi-polar or non-polar compounds. Remaceration aims to prevent saturation of the solvent, so that it can redissolve the desired compound (Andry et al., 2022). A lot of sweet corn rind simplicia powder was used for maceration, namely 500 grams with 5 liters of solvent and produced 3810 mL of filtrate, then 33 grams of viscous extract was produced to obtain a yield of 6.6%.

Extract Characteristics Results

The characteristics of the extract tested included determination of drying shrinkage, determination of water content, determination of ash content, and determination of acid insoluble ash content. Determination of drying shrinkage aims to provide a maximum limit or range of the amount of compounds lost in the drying process (Ministry of Health of the Republic of Indonesia, 2000). From the test results of sweet corn peel extract for drying shrinkage was obtained at 31.29%. Determination of water content is carried out aiming to provide or range the amount of water content in the material, measurement of the water content in the material is carried out by the gravimetric method (Ministry of Health of the Republic of Indonesia, 2000).

From the results of testing the sweet corn rind extract, the determination of the water content was obtained at 6.1%. Determination of ash content aims to provide an overview of internal and external mineral content from the initial process until the extract is formed (Ministry of Health of the Republic of Indonesia, 2000). From the test results of the sweet corn rind extract on the determination of the ash content, it was obtained at 3.75%, while the results of the sweet corn rind test on the determination of the acid insoluble ash content were obtained at 0.76%.

Antibacterial Activity Test Results

Antibacterial activity test aims to determine the ability of sweet corn peel extract (*Zea mays L.*) to inhibit the growth of the tested bacteria, namely *Escherichia coli* and *Staphylococcus epidermidis*. The inhibition ability was stated to be positive which was indicated by the formation of a clear zone of inhibition around the disc, this clear zone which indicated the antibacterial activity of the extract being tested and its diameter was measured with a caliper (Eulis Reni Sundari, 2022).

In this study using the disc diffusion method, where the working principle of the diffusion method is the diffusion of antibacterial compounds into the solid media where the test microbes have been inoculated. The diffusion method using discs is carried out by means of disc paper to absorb the extract as a test material after that the disc paper is placed on the surface of the agar media which has been inoculated with the test microbial culture, then incubated for 24 hours at 350C, the area or clear zone around the disc paper is observed to indicate the presence or absence of microbial growth. The advantage of the disc method is that it can be tested more quickly by preparing paper discs (Nurhayati et al. 2020). Determination of the strength category of antibacterial activity was grouped into four categories, namely weak activity (<5 mm), moderate (5-10 mm), strong (11-20 mm), and very strong (>20) (Fiana et al. 2020).

Based on the data on the diameter of the inhibition zone, the results of the antibacterial effectiveness test observed for 1 x 24 hours showed that there was an inhibition zone at concentrations of 10%, 15% and 20%. Marked by the presence of clear areas around the disc paper, in the first, second, and third repetitions. For the inhibition zone of *Escherichia coli* bacteria with an extract concentration of 10% it has an average inhibition zone of 10.18 mm with a strong inhibition category, at a concentration of 15% it has an average inhibition zone of 12 mm with a strong inhibition category, and a 20% concentration has an inhibition zone an average of 13.42 mm in the category of strong inhibition, on the positive control *Chloramphenicol* gave an average inhibition zone of 13.57 mm in the category of strong inhibition, and on the negative control DMSO did not show any inhibition zone. Based on the inhibition zone diameter data, it can be seen that the inhibition zone diameter increases with an increase in concentration.

Whereas in the test bacteria *Staphylococcus epidermidis* with an extract concentration of 10% had an average inhibition zone of 10.72 mm with a strong inhibition category, at a concentration of 15% it had an average inhibition zone of 12.37 mm with a strong inhibition category, and a concentration of 20% had an inhibition zone an average of 14.67 mm in the category of strong inhibition, the positive control of *Chloramphenicol* gave an average inhibition zone of 23.98 mm with the category of very strong inhibition, and the negative control of DMSO did not show any inhibition zone at all. Based on the inhibition zone diameter data, it can be seen that the inhibition zone diameter increases with an increase in concentration.

The positive control used in this study was *Chloramphenicol*. *Chloramphenicol* antibiotics belong to antibiotics that only inhibit the growth of microorganisms (bacteriostatic) and belong to broad spectrum antibiotics. Broad spectrum antibiotics can inhibit or kill bacteria from the gram-positive and gram-positive groups. negative. These antibiotics work by inducing binding and transfer to mRNA without inducing peptide bonds. When *Chloramphenicol* binds to ribosomes, distortion occurs in the ribosome components, preventing the formation of peptide bonds and ribosome migration (Eulis et al. 2022).

In this study, there was a difference in the inhibition zone between *Escherichia coli* and *Staphylococcus epidermidis* towards the positive control of *Chloramphenicol* where *Escherichia coli* bacteria were categorized as strong while *Staphylococcus epidermidis* bacteria were categorized as very strong. The growth that is formed in each bacterium is due to differences in inhibitory activity which is influenced by the type of bacterial cell wall that is inhibited (Hamidah, et a. 2019).

CONCLUSION

The ethanol extract of sweet corn peel (*Zea mays L.*) has antibacterial activity in inhibiting the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria which are characterized by the presence of clear areas around the disc paper. The results of the antibacterial activity test of sweet corn peel extract (*Zea mays L.*) in inhibiting the growth of *Escherichia coli* and *Staphylococcus epidermidis* bacteria showed differences in antibacterial activity with the use of several concentrations, namely 10%, 15%, and 20%. Which provides a more effective inhibition zone against *Escherichia coli* bacteria, namely at a

concentration of 20% with a diameter of 13.42 mm, while for *Staphylococcus epidermidis* bacteria, namely at a concentration of 20% with a diameter of 14.67 mm.

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