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ANTIBACTERIAL ACTIVITY TEST OF CUCUMBER PEEL EXTRACT (*Cucumis sativus L.*) AGAINST *Streptococcus mutans* BACTERIA

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ABSTRACT
Background: *Dental caries is a process of tooth demineralization caused by acids produced from food metabolism in bacteria. One of the main etiological factors of dental caries is the microorganisms known as Streptococcus mutans bacteria. S. mutans is one of the components of human dental plaque that is associated with sucrose diet and high caries activity. Cucumber peel extract (Cucumis sativus L.) is known to have an antibacterial activity due to the presence of secondary metabolites such as flavonoids, steroids, alkaloids, saponins and phenols found in cucumber peel.* ***Purpose:*** *This study aimed to determine the antibacterial activity at the lowest concentration of cucumber peel extract (Cucumis sativus L.) against Streptococcus mutans bacteria.* ***Method:*** *This study employed the disc diffusion method with a post-test only control group design. Streptococcus mutans bacteria were cultured on Mueller Hinton Agar media, and cucumber peel extract (Cucumis sativus L.) was applied as the antibacterial agent at concentrations of 0.01 g/ml, 0.05 g/ml, 0.1 g/ml, 0.2 g/ml, 0.4 g/ml and 0.5 g/ml with seven repetitions.* ***Results:*** *The results of the study showed that cucumber peel extract (Cucumis sativus L.) formed inhibition zones around the paper discs against the growth of Streptococcus mutans bacteria at concentrations of 0.4 g/ml and 0.5 g/ml.* ***Conclusion****: The concentrations of 0,4 g/ml and 0,5 g/ml are the lowest concentrations of cucumber peel extract (Cucumis sativus L.) that can inhibit the growth of Streptococcus mutans bacteria.*

Keywords *: Antibacterial, Cucumber peel, Dental Caries, Streptococcus mutans*
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INTRODUCTION

Dental caries is an oral disease characterized by the demineralization of teeth caused by bacterial metabolism. Dental caries are mostly caused by infection with cariogenic bacteria. Some evidence suggests that streptococcus is associated with the development of caries. One of the bacteria involved is *Streptococcus mutans.*[1] *S. mutans* is a component of dental plaque, especially in individuals with a high sucrose diet and high caries activity. The cariogenicity of *S. mutans* depends on the formation of insoluble extracellular glucans and its ability to produce acid.[2.]

There were approximately 64.6 million cases of dental caries in adults and 62.9 million cases in children in 2019 in global.[3] In Indonesia alone, dental caries cases accounted for the highest percentage of oral diseases in 2018, at 45.3%.[4] Based on this data, it is evident that dental caries is a significant oral health issue that requires attention and intervention.

Mouthwash is one of the products used for the prevention and treatment of oral cavity diseases. Over-the-counter mouthwashes contain chemical ingredients, one of which is chlorhexidine gluconate mouthwash. The use of chlorhexidine gluconate has shown good efficacy and strength correlation, but it has side effects when used in high concentrations, such as brown discoloration of teeth and restorations, decreased taste sensation, and changes in the color of oral mucosa.[5] This has increased the awareness among researchers to search for alternative ingredients for mouthwash by utilizing plants as natural herbal ingredients.

Cucumber (*Cucumis sativus L.*) is a plant species originating from the Cucurbitaceae family. Cucumber is rich in nutrients and bioactive compounds. Cucumber has been utilized as a food ingredient, medicine, and beauty material since ancient times.[6] The peel of cucumber contains phytochemical compounds such as flavonoids, steroids, alkaloids, saponins, and phenols. These compounds provide antibacterial effects on

cucumber peel.[7,8] Flavonoids are the most abundant compounds in cucumber peel (*Cucumis sativus L.*), followed by steroid content.[9] Flavonoids play an active role in inhibiting bacterial growth. Flavonoids work by inhibiting bacterial growth through cell wall destruction, enzyme inhibition, adhesion binding, and cell membrane destruction.[10] The antibacterial mechanism of flavonoids works by inhibiting bacterial metabolism, nucleic acid synthesis, bacterial attachment, and biofilm formation.[11] Steroids are also antibacterial compounds. The antibacterial mechanism of steroid compounds involves inhibiting membrane cell function and forming complex extracellular protein compounds. Steroid compounds can cause damage to the cell membrane, leading to the release of intracellular components.[11]

In research on cucumber peel extract (*Cucumis sativus L.*) as an antibacterial agent, it has been found to inhibit various gram-positive and gram-negative bacteria such as *B. cereus, S. aureus, Lactobacillus*, (*Shigella flexneri, E. coli, Proteus vulgaris*, as well as fungi.[12,13] Studies on the antibacterial properties of cucumber juice extract (*Cucumis sativus L.*) have been conducted extensively, but the use of cucumber peel as a material is rarely investigated. Researchers are interested in studying the antibacterial properties of cucumber peel extract to observe its inhibitory effects on the growth of bacteria such as *S. mutans*.

Cucumber peel extract in the study is being used in low concentrations. This aims to determine the lowest concentration concentration of cucumber peel extract (*Cucumis sativus L.*) that can inhibit the *Streptococcus mutans* bacteria and considering it as an alternative mouthwash ingredient if it exhibits stronger inhibitory effect than 0.2% chlorhexidine gluconate.

MATERIALS AND METHODS

The research was conducted after obtaining approval and ethical clearance No. 67 / KEPK-FK / VI / 2022 issued by the Health Research Ethics Commission of the Faculty of Medicine, Mulawarman University. This study is of the laboratory experimental type. The research design used was a post-test only control group design. The research method to be employed is the antibacterial activity test using the disc diffusion method in vitro to observe the inhibition zones generated by cucumber peel extract (*Cucumis sativus L.*) against the growth of *Streptococcus mutans* bacteria.

Tools and Materials

The tools used in the study were fruit peeler, balance scale, tray, drying cabinet, blender, maceration container, measuring glass, funnel, Büchner funnel, rotary evaporator, Whatman filter paper no. 42, dropper pipette, single-channel pipette, microtube, petri dish, ose, tweezers, Bunsen burner, Erlenmeyer flask, test tube, laboratory glass/beaker, hot plate, vortex mixer, Biosafety Cabinet Class II, turbidimeter, incubator, and calipers.

The sample materials used in the study were peel of fresh cucumber (*Cucumis sativus L.*) totaling 20 kg of cucumbers that grown in one of the vegetable gardens located at L1 Teluk Dalam, Tenggarong Sebrang District, Kutai Kartanegara Regency, East Kalimantan Province. The *Streptococcus mutans* ATCC® 25175 bacterial strain was obtained from the Microbiology Laboratory of the Faculty of Agriculture, Mulawarman University. Other materials used in this study include 96% ethanol solution, filter paper discs, cotton swabs, 0.2% chlorhexidine gluconate, sterile distilled water, 0.9% NaCl (saline solution), Mueller-Hinton Agar (MHA), Nutrient Agar (NA), and Nutrient Broth (NB).

Time and Place of Research

The research was conducted over a period of 2 months, from May to June 2022. The research took place at the Phytopharmaceutical Laboratory of the Faculty of Medicine, Mulawarman University, and at the Health Laboratory Unit of the East Kalimantan Provincial Government..

Preparation of Cucumber Peel Extract

Cucumbers are washed under running water, and then the cucumber is peeled off from the fruit. The cucumber peel is dried in a drying cabinet for 3 days. The dried cucumber peel is then pulverized using a laboratory blender. The finely ground cucumber peel is placed in a maceration container for extraction with 96% ethanol solution for 3 days using the maceration extraction technique.[9] The extract obtained is filtered using Whatman filter paper no. 42. The collected filtrate is then evaporated using a rotary evaporator at 50°C until a thick cucumber peel extract is obtained.

Dilution of Cucumber Peel Extract

The cucumber peel extract is diluted with a solvent to obtain the desired concentrations of cucumber peel extract (*Cucumis sativus L.*). The concentrations of cucumber peel extract (*Cucumis sativus L.*) that were produced were 0.01 g/ml, 0.05 g/ml, 0.1 g/ml, 0.2 g/ml, 0.4 g/ml, and 0.5 g/ml. The formula $V_1 \times C_1 = V_2 \times C_2$ is used to achieve these concentrations. The diluted solutions then stirred until homogeneous.

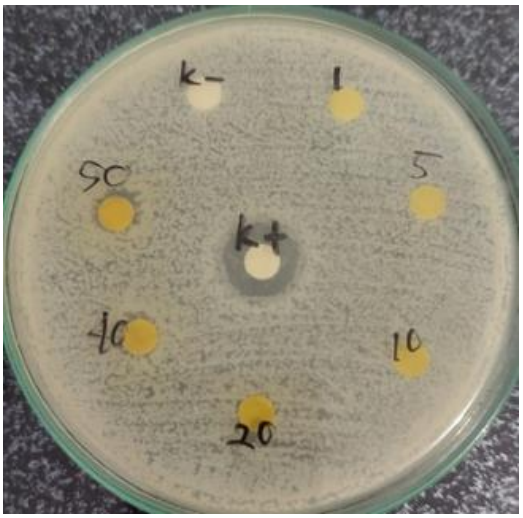
Testing of Antibacterial Activity Using the Disc Diffusion Method

The antibacterial activity of cucumber peel extract was tested using the disc diffusion method on Mueller Hinton Agar (MHA) medium. A total of 38 grams of MHA powder was dissolved in 1 L

of distilled water, then autoclaved at 121°C for 15 minutes.[14] Bacterial suspension was prepared and diluted with sterile 0.9% NaCl solution to a turbidity equivalent to McFarland standard 0.5, which is approximately 1.5 x 10^8 CFU/ml.[15] The bacterial suspension is inoculated evenly onto the entire surface of the agar medium by streaking it using a sterile swab. Filter paper discs were dipped with cucumber peel extract at concentrations of 0.01 g/ml, 0.05 g/ml, 0.1 g/ml, 0.2 g/ml, 0.4 g/ml, and 0.5 g/ml, while 0.2% chlorhexidine gluconate was used as a positive control and distilled water as a negative control. The discs dipped with cucumber peel extract were placed on the surface of the MHA agar previously inoculated with S. mutans, then incubated for 24 hours at 37°C.[16] Clear zones of inhibition formed around the discs were observed.

RESULTS

The results of testing the antibacterial activity of cucumber peel extract (Cucumis sativus L.) at concentrations of 0.01 g/ml, 0.05 g/ml, 0.1 g/ml, 0.2 g/ml, 0.4 g/ml, and 0.5 g/ml, along with



0.2% chlorhexidine gluconate as a positive control and distilled water as a negative control, against bacterial growth with 7 replications can be seen in Figure 1.

Figure 1. Results of Antibacterial Testing of Cucumber Peel Extract against *S. mutans* Bacteria on MHA Media

The research results indicate that cucumber peel extract (*Cucumis sativus L.*) at concentrations of 0.01 g/ml – 0.2 g/ml does not exhibit antibacterial activity in inhibiting the growth of *S. mutans* bacteria. This is evidenced by the absence of inhibition zones around the filter paper discs at these concentrations. Inhibition zones against *Streptococcus mutans* bacteria began to form at concentrations of 0.4 g/ml and 0.5 g/ml, with average values of inhibition zone diameter 6.72 mm and 7.52 mm, which are lower than the average inhibition zone diameter value of the positive control, which is 13.05 mm. Distilled water did not show any inhibition zones. The interpretation of the average diameter values of the inhibition zones for cucumber peel extract (*Cucumis sativus L.*) concentrations of 0.4 g/ml and 0.5 g/ml falls into the moderate inhibition classification. Meanwhile, 0.2% chlorhexidine gluconate falls into the strong inhibition classification. Table 1 shows the results of the measurement of inhibition zone diameters in this study, and Figure 2 shows a bar graph of the average values and standard deviations of the inhibition zone diameters produced by cucumber peel extract (*Cucumis sativus L.*) as well as the positive and negative controls.

Table 1. Average diameter values of inhibition zones (mm) and standard deviation of *C. sativus* extract against *S. mutans* bacteria..

Concentration (g/ml)	**Inhibition Zone Diameter**	**Category**	***Index Inhibition***
	Mean (mm) ± SD		
0,01	-	-	-
0,05	-	-	-
0,1	-	-	-
0,2	-	-	-
0,4	6,72 ± 0,26	Moderate	0,06-0,18
0,5	7,52 ± 0,24	Moderate	0,21-0,33
Control +	13,05 ± 0.68	Strong	0,97-1,29
Control -	-	-	-

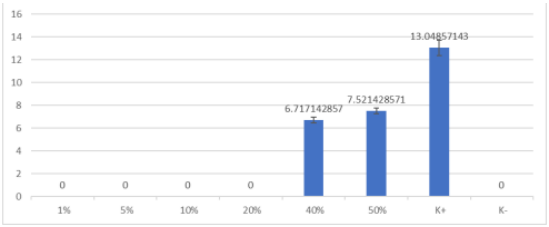


Figure 2. Average Inhibition Zone Diameter

The normality test and homogeneity test on the data results of the inhibition zone values of cucumber peel extract (*Cucumis sativus L.*) against *S. mutans* bacteria have p-values > 0.05, indicating that the data meets the assumptions for proceeding with the One-Way ANOVA test.

The One-Way ANOVA test was conducted to determine the significance of the differences between cucumber peel extract concentrations and the resulting inhibition zone diameters. Based on the One-Way ANOVA results, a p-value = 0.00 was obtained, which is less than 0.05. Therefore, there is a significant difference in the inhibition zone diameters between cucumber peel extract (*Cucumis sativus L.*) and 0.2% chlorhexidine gluconate as the positive control in inhibiting the growth of *Streptococcus mutans* bacteria.

To determine the differences between treatment groups of cucumber peel extract concentrations (*Cucumis sativus L.*) and the control against S. mutans bacteria, a post-hoc test with Tukey was conducted. Significant differences between groups are obtained if $p < 0.05$. The results of the post-hoc test show that among all treatment groups of cucumber peel extract concentrations (*Cucumis sativus L.*) and the positive control group, significant differences are observed in Table 2, as indicated by p-values < 0.05. Table 3 also explains that all treatment groups of cucumber peel extract concentrations (*Cucumis sativus L.*) and the positive control do not have significantly different results ($p > 0.05$), as seen by observing the subset column in the table.

Table 2. Results of post-hoc test with Tukey for inhibition zone diameter of cucumber peel extract (*Cucumis sativus L.*) against *S. mutans* bacteria

No	Concentration	Difference Means	*P-value*
1.	0,4 g/ml	-0,80	0,008
	0,5 g/ml		
2.	0,4 g/ml	-6,33	0,000
	K+		
3.	0,5 g/ml	0,80	0,008
	0,4 g/ml		
4.	0,5 g/ml	-5,53	0,000
	K+		
5.	K+	6,33	0,000
	0,4 g/ml		
6.	K+	5,53	0,000
	0,5 g/ml		

Description :

K+ : The positive control in the study is Chlorhexidine Gluconate 0.2g/ml.

K- : The negative control in the study is distilled water.

Table 3. Post Hoc Homogeneous Subset of average inhibition zone diameter values of cucumber peel extract (*Cucumis sativus L.*)

Treatment	N	*Subset for alpha* = 0,05		
		1	2	3
0,4 g/ml	7	6,72		
0,5 g/ml	7		7,522	
K+	7			13,05
Sig.		1,000	1,000	1,000

DISCUSSION

Cucumber peel extract (*Cucumis sativus L*) can inhibit *S. mutans* at concentrations of 0.4 g/ml and 0.5 g/ml with average inhibition zone diameters of 6.72 mm and 7.52 mm, respectively. However, the inhibitory effect of cucumber peel extract (*Cucumis sativus L.*) is considered weak because the average inhibition zone diameter falls between more than 5 mm and less than 9 mm. According to research by Piatek Jacek et al., inhibition zones ranging from 6 mm to 8 mm in diameter in in vitro inhibition tests indicate weak inhibitory activity.[17]

Cucumber peel extract (*Cucumis sativus L.*) can inhibit the growth and development of S. mutans microorganisms depending on its secondary metabolite content. Phytochemicals in plants can act against microbes. Phytochemical analysis of cucumber peel extract (*Cucumis sativus L.*) reveals the presence of flavonoids, alkaloids, steroids, phenolics, saponins, diterpenes, and glycosides, which function as antibacterial agents.[7,8] The secondary metabolite content in cucumber peel extract (*Cucumis sativus L.*) is predominantly composed of flavonoids, steroids, and alkaloids, which likely play a significant role in producing the antibacterial effect.[9] Flavonoids exert antibacterial effects by forming complexes with sophoraflavan G and epigallocatechin gallate to inhibit the function of bacterial cytoplasmic membranes, thereby damaging bacterial cells and inhibiting their growth.[18] Steroids also belong to antibacterial compounds. The antibacterial mechanism of steroid compounds involves inhibiting the function of cell membranes and forming complex extracellular protein compounds. Steroid compounds can cause destruction of the cell membrane, leading to the release of intracellular components.[11] Alkaloids can inhibit the action of transpeptidase enzymes involved in the synthesis of peptidoglycan in bacterial cells. By disrupting the formation of peptidoglycan, it can lead to suboptimal bacterial cell wall formation and cell destruction.[18]

In the treatment with a concentration of 0.5 g/ml, the average inhibition zone is higher

compared to the treatment with a concentration of 0.4 g/ml. The size of the inhibition zone is proportional to the concentration given. As the concentration of plant extract increases, the resulting inhibition zone also becomes wider. With increased dilution of the extract, the amount of active metabolite compounds contained decreases. This is because the solvent in the dilution can dissolve and neutralize the active metabolite compounds present in cucumber peel extract (*Cucumis sativus L.*).[18] The concentrations of 0.4 g/ml and 0.5 g/ml are the lowest concentrations of cucumber peel extract (*Cucumis sativus L.*) that can inhibit the growth of *Streptococcus mutans* bacteria, with average inhibition zone diameters of 6.72 mm and 7.52 mm, respectively. The inhibitory strength of cucumber peel extract (*Cucumis sativus L.*) is considered weak and lower than that of 0.2% chlorhexidine gluconate. Further research is needed to investigate the combination of cucumber peel extract with other extracts or antibacterial agents to produce stronger antibacterial activity against *Streptococcus mutans* bacteria.

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