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Potential Mouthworks of Celery Leaf Extractin Inhibiting the Growth of Streptococcus Mutants Bacteria (Laboratory Test)

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Abstract:- Caries is caused by microbial activity of a fermented carbohydrate. The results of Basic Health Research (RISKESDAS) state that the largest proportion of dental problems in Indonesia are damaged/cavities/sick teeth (45.3%). Preventive efforts gargling mouthwash contains antibacterial, long-term use has side effects, so we need herbal ingredients that have antibacterial properties, one of which is celery leaves. This study aims to prove the potential of celery leaf extract (Apium graveolens L.) as a mouthwash ingredient in inhibiting the growth of caries-causing bacteria and being able to reduce the number of bacterial colonies compared to formulary controls without active ingredients. This type of research used laboratory and field experiments with pretest-posttest consisting of the intervention group of celery leaf extract mouthwash formulation concentrations of 30%, 15% and control formulary without active ingredients, the sample consisted of 39 people, the sample was rinsed for 1 minute. Saliva collection before and after gargling. The variables studied the inhibition of bacteria. The results of the inhibition of Streptococcus mutans bacteria carried out by the ANOVA test at a concentration of 15% showed a mean of 5 mm and a concentration of 30% with a mean of 6.2 mm with a p-value of 0.000 which means that there was a significant difference in inhibiting Streptococcus mutans bacteria, while the control had a mean of 0. 0 mm which means there is no resistance formed. The conclusion of this study is that giving celery leaf extract mouthwash Calculate the inhibition zone with a concentration of 15%, 30% being able to inhibit bacteria while the control has no inhibition on Streptococcus mutans bacteria.

Keywords:- *Antibacterial, Bacteria that Cause Dental Caries, Celery Leaf Extract.*

I. INTRODUCTION

Dental caries is one of the diseases in the oral cavity whose prevalence in Indonesia is still quite high. Dental caries is an infectious disease of the hard tissues of the teeth, namely email, dentin and cementum. Dental caries is caused by microbial activity on a fermented carbohydrate.[1]Factors that cause dental caries include host, substrate, microorganisms, and time. According to survey data from the World Health Organization (WHO), it is noted that around the world 60–90% of children experience dental caries.[2]The results of Basic Health Research (RISKESDAS) state that the largest proportion of dental problems in Indonesia are damaged/cavities/sick teeth (45.3%).[34] The prevalence of caries in the province of Central Java at the age of 12 years and over is 67.8% and 43.1% is active dental caries with the highest prevalence, namely in Semarang City as much as 74%.[5]

Dental caries is initially marked by an increase in the activity of microorganisms in the oral cavity. Bacteria play an important role in the process of dental caries. The number of microorganisms in a person's mouth depends on the condition of oral hygiene and health with different species of bacteria in several areas of the oral cavity.[6] Various kinds of microorganisms that multiply in an intercellular matrix consisting of extracellular polysaccharides and salivary proteins. Approximately 80% of the weight of plaque is water, while the number of microorganisms is ± 250 million per mg of wet weight. Bacteria are transparent, small in size so they cannot be seen directly and can be done with special techniques. Various kinds of microorganisms that multiply in an intercellular matrix consisting of extracellular polysaccharides and salivary proteins.[7]Some microorganisms found in the oral cavity are Streptococcus mutans, staphylococcus, lactobacillus.[6] Preventive efforts that are carried out mechanically, such as brushing the teeth at the right time in the right way, however, the act of cleaning by brushing the teeth is often not able to reach the entire surface of the teeth, so other efforts are needed, such as using antibacterial ingredients.[8]Chemical methods can be done by applying a fluorine solution, rinsing the mouth using a mouthwash that contains an antiseptic or you can also use herbal ingredients with plant extracts that contain antibacterials.[9] Continuous use of antibiotics can increase the possibility of resistance.[9]Various side effects arising from the use of chemicals in mouthwash are quite a lot and significant[10]such as discolored teeth, irritation of the mouth and tongue, dry mouth and decreased taste or change in taste.[11]So we need another alternative as a raw material for making mouthwash with minimal side effects, economical and efficacious.[10] Alternatives that meet these requirements

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are herbal ingredients and one of them is celery leaves and a plant safety limit test has been carried out with LD50 safety data. orally in rats> 5 g/kg BW and declared non-toxic.[12] Celery (Apium graveolens L. var secalinum Alef) is a plant that is easy to find in Indonesia, can live in the highlands and lowlands,[13]Celery (Apium graveolens L.) is better known by Indonesian people as a vegetable. However, it turns out that celery can be useful for lowering cholesterol, as an antibacterial, antioxidant and anti-inflammatory. Ingredients in celery that can be useful as an antibacterial include essential oils, flavonoids, saponins and tannins.

II. MATERIALS AND METHODS

A. Location and Place of Research

This research was conducted at the Campus 1 Dormitory of the Semarang Poltekkes, the Microbiology and Parasitology Laboratory of the Faculty of Medicine UNISSULA Semarang for the manufacture of extracts, mouthwashes and the campus laboratory of the Polytechnic of the Ministry of Health Semarang

B. Types and Research Design

The type of research used in this research is quasy experimental with pretest and posttest with control group design, with a sampling technique that is purposive sampling. consisting of 2 (two) groups, namely the intervention group and 1 control group. The design in this study was chosen because it was carried out pretest before treatment and posttest after treatment. The intervention in this study was mouthwash with celery leaf extract with a concentration of 15% and 30%. Meanwhile, the control group was given control in the form of a formulary without active ingredients.

C. Preparation of Celery Extract (Apium Graveolens L.)

The preparation of celery leaf extract was made according to Oktaviani (2021). As much as 5 kg of fresh celery leaves are washed thoroughly with running water and drained using a tampah. Then the celery leaves were baked at 50°C for 20 hours. The dried simplicia was weighed and then crushed using a blender and sieved using a 50 mesh sieve. Fine simplicia was soaked in 5 liters of 96% ethanol in a glass jar with a lid at a ratio of 1:10 b/v for 24 hours with the first 6 hours of occasional stirring and the next 18 hours hushed up. The results of the soaking are then filtered. The filtrate from the soaking was re-macerated using 96% ethanol according to the previous maceration process. All macerate was evaporated at 40-50 °C for 3 hours using a rotary evaporator and 300 grams of thick celery leaf extract was produced.[14]

D. Formulation of Celery Leaf Extract Mouthwash Formula

The formulation of a celery leaf mouthwash formulation was made based on a modification from Oktaviani (2021).

Table 1. Formulation of celery leaf mouthwash preparations.[14]

Material	Utility	Formulation	
		F1	F2
Extract	Active substance	15%	30%
Benzoic acid	Sweetener	0.01gr	0.01gr
Glycerin	humectants	5 gr	5 gr
Xylitol	Flavors	10 gr	10 gr
Oleum Menthe	humectants	1 gr	1 gr
Aquadest	Solvent	AD 100 ml	AD 100 ml

The first preparation of the mouthwash is to prepare the tools to be used, all the materials provided are weighed as needed, then the benzoic acid is put into the mortar, then the oleum menthe is added and then mixed until homogeneous. Next, add xylitol and stir until homogeneous, then add distilled water little by little until all is dissolved, then add glycerin. Next, add the celery leaf extract and then stir until everything is completely dissolved, then the solution is filtered and put into a bottle container.[14]

E. Sample Preparation of Research Respondents

Respondents used in this study were dormitory students aged 18 years and over as many as 39 students who experienced dental caries. The sampling procedure for research respondents is as follows:

The research subjects were divided into three groups, namely the 15% and 30% celery leaf extract treatment group and the formulary control group without active ingredients, each group consisting of 13 samples.

- The researcher instructed the patient to sit in the chair provided.
- The researcher used a handscoon first to remain sterile when taking samples.
- Researchers took samples by collecting saliva using a sterile pot before gargling celery leaf mouthwash.
- Furthermore, research subjects were instructed to rinse their mouths using the mouthwash that had been prepared for 1 minute for each treatment group, namely: the group with a concentration of 15% and 30% with 10ml of mouthwash for each respondent.
- the control group used a formulary without an active ingredient of 10 ml per respondent and gargling for 1 minute.
- Furthermore, the researcher instructed the respondent to collect the saliva in a sterile pot that had been provided after gargling the celery leaf extract mouthwash according to the respondent's treatment group.
- Samples that have been obtained from each respondent are then taken to the laboratory to be tested for bacteria.

F. Preparation of Streptococcus mutans bacteria samples

The Streptococcus Mutans bacteria sample used in this study was obtained from the sample through the respondent's saliva with as much as 0.1 ml of 0.5 Mc Farland. The bacteria were put in a test tube containing liquid medium and incubated at 37°C for 24 hours.

G. Preparation of Muller Hinton Agar (MHA) Media

Mueller Hinton Agar (MHA) media was prepared by weighing 34 grams of material and then dissolving it in 75 ml of distilled water and then heating while stirring using a magnetic stirrer until homogeneous. Then the media was sterilized in an autoclave for 15 minutes at a temperature of 1210C and a pressure of 1.5 atm. After the sterilization process, 15 ml of the media was put into a petri dish and then stored in the refrigerator.[15]

H. Standard Mc. Farland No. 0.5

Standard turbidity Mc. Farland was prepared by mixing 0.5 ml of 1% BaCl2 and 9.95 ml of 1% H2SO4 in a test tube and shaking until homogeneous. Turbidity is used as a standard for the turbidity of the test bacterial suspension.[15]

I. Antibacterial test of the inhibition of the clear zone of bacteria using the disc diffusion method

Antibacterial testing was carried out using the agar diffusion method (Kirby-Bauer). Prepare a petri dish for each sample of the respondent, write on the bottom of each petri dish using a marker that reads: F30 is a celery leaf extract mouthwash with a concentration of 30%, and F15% is a drug gargling celery leaf extract with a concentration of 15%. Control is Formulary without active ingredients.

In each petri dish, Muller Hintom Agar media was poured, 25 ml each in each petri dish. A petri dish containing Muller Hinton Agar (MHA) media was spread with a bacterial suspension in an amount of about 106cfu/ml to the entire surface of the agar until it was evenly distributed, let it dry for about 1 hour. Each paper disk or disc paper is immersed in the sample, namely celery leaf mouthwash for each concentration, namely 15%, 30% and Formulary without the active ingredient and placed in a petri dish containing sterile MHA, then the petri dish is incubated at 37oC for 24 hours. Furthermore, observations and measurements were made after 24 hours of incubation period. The clear zone is an indication of the sensitivity of the bacteria to the antibacterial agent used as the test material which is expressed by the width of the diameter of the inhibition zone,[16]

The results are entered in the table and analyzed using the SPSS program, which is a normality test. The data shows a normal distribution, so the One Way Anova test is continued.

III. RESULTS AND DISCUSSION

Based on the research conducted, namely the inhibition of Streptococcus mutans bacteria and the calculation of the number of bacterial colonies, the average size of the inhibition zone in celery leaf extract concentrations of 15%, 30% and the control, namely formulary without active ingredients. Can be seen in table 2.

Table 2. *Difference* inhibition of bacteria in the intervention and control groups

Variable	Test group	Mean+SD	P-values
S. mutans bacteria	concentration 15%	5.00+0.00	0.000
	concentration 30%	6.20+0.27	
	Formulary without active ingredients	0.00+0.00	

Based on table 2, the results of the One Way Anova test obtained a p value of 0.000 (p <0.05) which indicated that there was a significant difference between the three intervention groups of celery leaf mouthwash and the control group. % was proven by an average value of 6.2, whereas at a concentration of 15% an average value of 5.0 was obtained in the control group which did not show any inhibition against Streptococcus Mutans bacteria as evidenced by an average value of 0.0.

Measurement of the inhibition zone is the determination and measurement of the sensitivity of a bacterium to a drug where low concentration levels still show an inhibition zone.[17] The zone of inhibition of bacterial growth is the clear area around the disc. the stronger the antibacterial activity, the wider the inhibition area.[18]

Antibacterial activity was indicated by the presence of a zone of inhibition in the form of a clear area around the paper disk containing celery leaf extract with an average of 5 mm at a concentration of 15%, while at a concentration of 30% an average of 6.2 mm was obtained for both groups belonging to the power zone. inhibition of bacteria in the category of medium inhibition ability or quite resistant bacteria in inhibiting bacteria. This has also been proven in Madjidah's research (2014) that celery leaf mouthwash has an inhibitory power against Streptococcus Mutans bacteria. The formation of inhibition zones at each concentration of celery leaf extract is influenced by the active substances contained therein, namely essential oils, flavanoids, saponins and tannins, each of which has different antibacterial activity.[15]

The mechanism of action of these active compounds are:

- *Essential Oil*

The essential oil components that are thought to play an active role as antibacterials are sabinen, β-mirsen, α-pinen, α-tuyan, trans-caryophyllene, β-pinen. The compounds α-pinene and β-pinene are terpenoid compounds which are known to have antimicrobial effects.[19] The mechanism of action of essential oils in killing bacteria is by changing the permeability of cell membranes, removing ions in cells, blocking the proton-pump, and reducing the production of adenosine triphosphate (ATP). Essential oils are lipophilic which can pass through the bacterial wall because the bacterial wall consists of polysaccharides, fatty acids and

phospholipids. This can result in damage to the cell wall so that it can kill bacteria.[20]

- *Flavanoids*

Flavanoids, namely bioactive compounds present in these compounds, are thought to have potential as antibacterial compounds.[21]

Flavanoids inhibit the function of the bacterial cell membrane through complex bonds with soluble extracellular proteins that can disrupt the integrity of the bacterial cell membrane. Any disturbance in the permeability of the cell membrane will affect the electrochemical gradient of protons across the membrane which is very important for bacteria in synthesizing ATP, membrane transport and movement of bacteria. activity of absorption of metabolites and biosynthesis of bacterial macromolecules.[20,22]

- *Saponins*

Saponins have antibacterial ability by providing protection against potential pathogens besides that saponins will interfere with the surface tension of the cell wall.[22] The active content of saponins is a compound that can form foam and damage cell membranes because it can form bonds with lipids from cell membranes. Saponins work as an antibacterial by interfering with the stability of the bacterial cell membrane, causing bacterial cell lysis or saponin compounds that damage the cytoplasmic membrane and kill the cytoplasmic membrane cells. the active ingredient compounds enter the bacterial cell, resulting in leakage of essential metabolites formed by the bacteria and damaging the permeability of the cell membrane.[23]

- *Tannins*

Tannins have antibacterial activity by means of bacterial walls that have been lysed due to saponins and flavonoids, causing tannins to easily enter the bacterial cell and coagulate the protoplasm of the bacterial cell.[24] Tannin is a compound that has antimicrobial activity against Streptococcus Mutans.

In line with the research of Marani Suwito et al (2017) that celery leaf mouthwash has antibacterial properties with its compounds capable of inhibiting Streptococcus Mutans bacteria.[13]In general, the predicted mechanism is as follows: tannin toxicity can damage bacterial cell membranes, tannin compounds can induce the formation of bonding complexes to enzymes or microbial substrates and the formation of tannin bond complexes to metal ions which can increase the toxicity of the tannins themselves. The antibacterial effects of tannins include: reactions with cell membranes, enzyme inactivation, and destruction or inactivation of material functions.[25]

In addition to antibacterial agents, the structure and composition of bacterial cells also have an important role in the antibacterial mechanism. The walls of Gram positive bacteria have teichoic acid contained in the peptidoglycan while Gram negative bacteria do not have teichoic acid. This teichoic acid functions as a way for ions to enter and exit from and into the bacterial cell. Lipoteichoic acid, which is a type of teichoic acid found in peptidoglycan, can bind to tannins, so that bacterial growth will be more easily inhibited by antibacterial components.[24]

According to S. Adrian Gombart et al (2020) that biochemical compounds found in celery extract, such as alkaloids and saponins, reduce oxidative stress and redox-dependent pathways to prevent infection severity. The reduction in oxidative stress triggers a more specific immune response to fight infection. Furthermore, exogenous antioxidant activity derived from celery extract induces a balance of endogenous antioxidants in the pericytes to maintain membrane integrity and inhibit bacterial cell attachment.[26]

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The administration of celery leaf extract mouthwash with concentrations of 15% and 30% proved to be effective in inhibiting Streptococcus mutans compared to formularies without active ingredients. Formulation of mouthwash preparations that have the highest antibacterial, namely at a concentration of 30%.

Suggestions for this study include that future researchers are able to avoid things that can interfere with research results such as room temperature, time and air contamination in research samples. Celery leaf mouthwash preparations can be combined with other plants that have the same active substance so that they have the potential to maximally inhibit the bacteria that cause dental caries. Further researchers are advised to carry out an immunological test on the respondent's saliva in order to obtain good results for the research conducted. Researchers can then carry out organoleptic tests on celery leaf mouthwash which includes taste tests, color tests, aroma tests and texture tests as well as pH and viscosity of the mouthwash used.

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