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Potential Mouthworks of Celery Leaf Extract (Apium graveolens L.) as an Antibacterial Alternativein Reducing the Number of Bacteria Collies in the Mouth

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Abstract:- Caries is caused by microbial activity of a fermented carbohydrate. 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 reducing 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 variable studied was the number of bacterial colonies. The results of the Paired t-test for the number of bacterial colonies at a concentration of 15% showed a Δ -105.846 p-value of 0.025 meaning that there were differences in bacterial colonies before and after rinsing, a 30% concentration had a Δ value of 17.846 p-value 0.351 and a control value of 7.385 p-value 0.183 meaning that there is no significant difference but there is a decrease in the number of bacterial colonies. The conclusion of this study is that giving celery leaf extract mouthwash with a concentration of 30% for 1 minute is effective in reducing the number of bacterial colonies compared to formularies without active ingredients. 183 means there is no significant difference but there is a decrease in the number of bacterial colonies. The conclusion of this study is that giving celery leaf extract mouthwash with a concentration of 30% for 1 minute is effective in reducing the number of bacterial colonies compared to formularies without active ingredients. 183 means there is no significant difference but there is a decrease in the number of bacterial colonies. The conclusion of this study is that giving celery leaf extract mouthwash with a concentration of 30% for 1 minute is effective in reducing the number of bacterial colonies compared to formularies without active ingredients.

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

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%).[3,4] 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. 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.[7]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.[8] 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 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. *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.

B. *Preparation of Celery Extract (Apium Graveolens L.)*

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.

C. *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]

D. *Organoleptic Test of Celery Leaf Extract Mouthwash*

Organoleptic test of bay leaf extract mouthwash was carried out directly covering the observation parameters: texture, color, aroma and taste.

E. *Research Respondent Sample Preparation*

Respondents who will be used in this study are dormitory students aged 18 years and over as many as 39 students who experience dental caries. The sampling procedure for research respondents is as follows: the research subjects were divided into two groups, namely the treatment group and the control group, the number of samples based on the formula obtained was 39 samples, the samples were divided into 3 groups, each group namely 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. *Sample management and testing*

➢ *Dilution of the number of bacterial colonies*

Calculation of the number of colonies was done by dilution. A 10-1-10-3 dilution series was made. Next prepare 5 test tubes that have been filled with 9 ml of NaCl into each tube. Then, from the tube that was measured with the spectrophotometer, 1 ml of the bacterial suspension was taken and then mixed with the dilution tube 1 (10-1) and homogenized. From tube 1, 1 ml was taken using a micropipette and then transferred to dilution tube 2 (10-2) and then homogenized. From tube 2, 1 ml was taken with a micropipette then transferred to dilution tube 3 (10-3) and then homogenized.

➢ *Isolation of oral bacteria*

- Take 0.1 ml of the bacterial suspension using a micropipette in each dilution tube (10-1 - 10-3) and drop it into several petri dishes containing Muller Hinton Agar (MHA) media. Then it was carried out using the spread plate method using L rods. Then incubated in an incubator for 24 hours at 370C.

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- Prepare three test tubes and then each tube is marked according to the concentration to be used. Tube 1 was filled with Formulary without active ingredient (control), tube 2 was filled with 15% concentration extract, tube 3 was filled with 30% concentration extract, each tube was filled with 3.5 ml of celery leaf extract and added 1 ml of TSB (Tryptic Soy Broth). Then each tube was filled with 0.5 ml of bacterial suspension which had been diluted using the serial dilution method and then shaken until homogeneous.
- Then from each tube 0.1 ml of the suspension was taken using a micropipette and dripped on MHA media (Muller Hinton Agar) to be planted using the spread plate method. Then it was leveled by scattering method using L rods and incubated for 48 hours at 37oC. After 48 hours, observations were made by counting the number of colonies that had grown using a colony counter.[15]

G. Antibacterial Test of Celery Leaf Mouthwash

The dilution method used a concentration of 15% and 30% and then observed the number of bacterial colonies in the oral cavity.

Prepare a petri dish for each sample from the respondent, each bottom of the petri dish is written using a marker that reads:

- 30% is the 30% concentration group and is given the respondent's code and 15% is the 15% concentration group and is given the respondent's code.
- K is the control, namely the formulary without active ingredients and is coded as a respondent.

✓ Each sample in each concentration group has 3 petri dishes containing MHA media (Muller Hinton Agar) then takes 0.1 ml of bacterial suspension using a micropipette, in a test tube containing 10-1 dilution to drip on 1 petri dish, the same thing is done up to a 10-3 dilution. Each petri dish was then carried out using the spread plate method using an L rod. Then incubated in an incubator for 24 hours at 370C. Furthermore, gram staining was performed on each petri dish to identify the bacteria present in the petri dish. Next, count the bacterial colonies in the petri dish using a colony counter with units of CFU/ml. The results are entered in the table and analyzed using the SPSS program, namely the normality test, the data showed a normal distribution, so the Paired T-Test was continued for the paired test and the One Way Anova test for unpaired data.

III. RESULTS AND DISCUSSION

Based on the research conducted, namely the calculation of the number of bacterial colonies in the oral cavity. The results of counting the number of bacterial colonies can be seen from table 2.

Table 2. Differences in the Number of Bacterial Colonies Before and After Gargling in the Intervention Group and the Control Group

Test group	**Paired Data Test***		
		Mean±SD	***p-values***
15%	Pre	118.31±52.12	0.025
	Post	224.15±124.13	
30%	Pre	94.15±50.54	0.351
	Post	76.31±40.21	
Control	Pre	99.85±21.40	0.183
	Post	91.85±27.89	
Unpaired Data Test(Δ)**			
		Mean±SD Pre-Postest (Δ)	***p-values***
15%		-105.85±149.10	
30%		17.85±66.24	
Control		7.38±18.85	0.000
Unpaired Data Post Hoc Test(Δ)***			
	15%	15%	30%
	30%	Control	Control
p-values	**0.002**	**0.004**	**0.780**

*Paired T-Test **One Way Anova ***PostHoc LSD

Table 3 shows that the results of the test for the number of bacterial colonies in the oral cavity in the paired data of the 15% concentration intervention group had a p-value of 0.025 (p <0.05) meaning that there was a significant difference in the 15% group but the change that occurred was an increase in the number bacterial colonies. In the 30% group, the p-value was 0.351 and the formulary without the active ingredient was 0.183, meaning that there was no significant change, but at the 30% concentration and the formulary without the active ingredient, there was a decrease in the number of bacterial colonies in the oral cavity.

The results of the test for the number of bacterial colonies in the oral cavity unpaired data on the pre-post value (Δ) showed that the p-value between the intervention group and the control group was 0.003 (p <0.05) meaning that there were differences in each group but at a concentration of 15% there is a difference in the increase in the number of bacterial colonies while at a concentration of 30% there is a difference in reducing the number of bacterial colonies in the oral cavity more effectively than the control group this is evidenced by the mean change (Δ) value of the 30% group better than the control group without ingredients active namely 17.85 and 7.38.

Post Hoc test results of unpaired data showed that the p-value between the 15% concentration and 30% concentration groups was 0.002 (p <0.05) meaning that the 15% concentration had a significant difference but in increasing the number of bacterial colonies, the p-value between groups 15% and formulary without active ingredients 0.004 (p <0.05) meaning that the 15% concentration is a significant difference but in increasing the number of bacterial colonies, the p-value between groups 30% formulary without active ingredients is 0.780 (p> 0 .05) means that there is no significant difference in reducing the number of bacterial colonies in the oral cavity.

➢ *Organoleptic Test Results for Celery Leaf Mouthwash*

The results of the organoleptic test were carried out by direct visual observation of the preparation of celery leaf mouthwash including texture, color, aroma and taste of the preparation as shown in Table 3.

Table 3. Organoleptic test results for mouthwash preparations

No	Formulation of mouthwash preparations	Texture	Color	Aroma	Flavor
1	F1	Liquid	Brownish green	Mint + typical celery leaves	A little sour and a little spicy
2	F2	Liquid		Mint + typical celery leaves	Slightly bitter, sour and a little spicy
3	K	Liquid	Clear white	Mint	Slightly sweet, fresh

Information
F1: formulation of 15% concentration of celery leaf mouthwash mouthwash
F2: formulation of 30% concentration of celery leaf mouthwash mouthwash
K: control (formulary without active ingredient)

➢ *pH Test Results for Mouthwash Celery Leaf Extract*

The results of testing the pH of the bay leaf extract mouthwash can be seen in Table 4.

No.	Formulation of celery leaf mouthwash	pH
1.	Concentration 15%	5,2
2.	Concentration 30%	5,2

Table 4 shows that the 15% concentration formulation and 30% concentration obtained the same pH, namely 5.2 for the pH requirements of the mouthwash, namely 5-7.

Celery leaves (Apium graveolens L.) is a plant that is easy to find in Indonesia, can live in the highlands and lowlands and contains active compounds such as essential oils, flavanoids, saponins and tannins which are antibacterial.[13]

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.[16] 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.[17]

- *Flavanoids*

Flavanoids, namely bioactive compounds present in these compounds, are thought to have potential as antibacterial compounds.[18] 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.[17,19]

- *Saponins*

Saponins have antibacterial ability by providing protection against potential pathogens besides that saponins will interfere with the surface tension of the cell wall.[19] 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.[20]

- *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.[21] Tannin is a compound that has antimicrobial activity.

In line with the research of Marani Suwito et al (2017) that celery leaf mouthwash has antibacterial properties due to its compounds.[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.[22]

Celery leaf extract mouthwash at a concentration of 15% has an effect on changes in the number of bacterial colonies in the oral cavity but the change that occurs is an increase in the number of bacterial colonies, this is due to air temperature contamination in the sample testing process so that the bacteria can adapt to live and grow. According to Arivo et al (2017), that is a factor in increasing the number of bacterial colonies, one of which is temperature, chemical reactions will increase with increasing temperature, because an increase in temperature causes an increase in the kinetic energy of the reactants. Growth is essentially the result of metabolism, a directed chemical reaction that takes place in cells catalyzed by enzymes.[23]

In line with the research by Luthfiyani et al (2019) that the factors influencing the results of the study showed an increase in the number of bacteria because bacteria experienced resistance due to environmental changes. An unfavorable environment such as bacteria can die, inhibit growth or even be able to adapt to the environment to grow. Factors that can influence bacteria to adapt include changes in temperature, pH and ion concentrations such as sodium. One example of adaptation in gram-negative bacteria is the change in a molecule called lipopolysaccharide. Lipopolysaccharide can turn out to be more water resistant or even less water resistant.[24]

The factors that affect the growth of microorganisms, namely: 1) Nutrition Microbes require a supply of nutrients for energy sources and cell growth. 2) Temperature / temperature If the temperature rises it will increase metabolic processes and accelerate growth. If the temperature drops will lower metabolism and slow growth. If the temperature rises and falls drastically, the growth rate will stop, the cell components become inactive and damaged so that the cells die. 3) pH / microbial acidity has an optimum pH that varies depending on the type or species. 4) Availability of oxygen Microbes have different levels of oxygen demand. 5) Water Plays a role in metabolic reactions in cells and as a means of transporting nutrients into cells or metabolites outside cells.[25]

In line with Wardhani et al's research (2020), the factors that increase growth will provide different conditions for each microbe according to their respective living environments, thereby affecting the kinetics of their fermentation. In addition, each bacterium will show differences in growth patterns, the time period needed to grow or adapt, and the metabolites produced.[26]

At a concentration of 30% there was no significant effect, but a change occurred, namely a decrease in the number of bacterial colonies in the oral cavity after gargling with 30% concentration of celery leaf extract. In line with Kurniawati's research (2020) that the decrease in the number of bacterial colonies in saliva was due to the presence of antibacterial substances contained in celery leaf mouthwash such as essential oils, flavanoids, saponins and tannins. The process of protein denaturation results in protein coagulation in the bacterial cytoplasmic membrane followed by the release of intracellular compounds, the bacterial cytoplasm is limited by the cell membrane which acts as a selective permeability barrier carrying the function of active transport and controlling the internal composition of the cell, if the integrity of the cytoplasmic membrane is damaged, maromolecules and intracellular ions will come out of the cell. cells leading to bacterial death.[27]

In formularies without active ingredients there was no significant effect but there was a decrease in the number of bacterial colonies, this was due to the absence of antibacterial substances in formularies without active ingredients. in line with Kurniawati's research (2020) that there is a decrease but there is a mechanical effect of rinsing which can dissolve a small amount of plaque, rinsing can stimulate salivary secretion, clean debris and desquamate oral epithelial cells thereby reducing the ability of bacteria tocolonize.

Organoleptic tests were carried out to see the physical appearance characteristics of a mouthwash including texture, color, and aroma. The results of the organoleptic test of celery leaf extract mouthwash with a concentration of 15% had a liquid texture, brownish green color, a distinctive mixed mint aroma of celery leaves and a slightly sour and slightly spicy taste. At a concentration of 30%, it has a liquid color texture which is brownish green with a distinctive minty aroma mixed with celery leaves and a slightly bitter, sour and slightly spicy taste. Whereas in the formulary without active ingredients, it has a liquid texture, the color that is formed is clear white with a minty aroma, a slightly sweet and fresh taste. Various types of plants can affect and give a distinctive color and aroma to the preparation according to the color and aroma contained in the extract of the plant parts. The addition of plant extracts will affect various kinds of formulations, such as the formulation of bay leaf extract mouthwash in this study. Organoleptic properties will affect a person's interest in consuming mouthwash, therefore the resulting mouthwash preparations should have attractive colors, pleasant aromas and taste good and fresh.[28]

Measurement of pH in mouthwash preparations of celery leaf extract was carried out using a pH meter. The pH test that was carried out on each celery leaf mouthwash preparation obtained the same pH value of 5.2. Several types of plants are known to be able to lower or increase the pH of various preparations, this is because each type of plant has a different pH. Testing the pH is one of the requirements for mouthwash, because it will come into direct contact with the lining of the oral cavity and can cause irritation problems if the pH does not match the pH of the lining of the oral cavity. The pH value is a value that indicates the degree of acidity of a material. The pH test for mouthwash is carried out using a pH meter. According to SNI, the permissible pH of mouthwash ranges from pH 6.0 to 7.5.[28]

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Administration of celery leaf extract mouthwash with a concentration of 30% was proven to be effective in reducing the number of colonies compared to formularies without active ingredients. In the celery leaf extract mouthwash formulation with a concentration of 15%, there was an increase in the number of bacterial colonies due to factors that

influenced bacterial growth, one of which was temperature. Whereas at a concentration of 30% there was a decrease in the number of bacterial colonies, this was due to the presence of antibacterial substances that were able to disrupt the cell membrane and internal cell ions would come out of the cell which would lead to the death of the bacteria.

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. 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

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