ORIGINAL ARTICLE

Antibacterial potential of celery leaf extract toothpaste on the growth of *Streptococcus mutans* ATCC 25175

Maulida Hayati[1*]
Shifa Khumaria Savitri[1]
Fitria Meiliza[2]

[1]Departmentof Periodontology, University Baiturrahmah, Padang, Indonesia
[2]Department of Oral Medicine, University Baiturrahmah, Padang, Indonesia

* Correspondence:
maulidahayati@fkg.unbrah.ac.id

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ABSTRACT

Introduction: Celery (*Apium graveolens* L.) is a member of the *Apiaceae* family that can grow everywhere. Celery contains flavonoids, saponins, tannins, essential oils, apigenin, choline, vitamins A, B, C, asparagine bitter substances. Celery contains flavonoids, saponins, and tannins which are antibacterial compounds. *Streptococcus mutans* is a Gram-positive bacterium creating an acidic atmosphere in the oral cavity, which causes insufficient saliva to prevent enamel demineralisation which can lead to dental caries. The purpose of this study was to analyze the inhibition effect of celery leaf extract (*Apium graveolens* L.) on the growth of *Streptococcus mutans*. **Methods:** This type of experimental laboratory research uses the Post Test Only Control Group Design. The samples were *Apium graveolens* L. and *Streptococcus mutans* ATCC 25175. The number of treatment groups was 24 with concentrations of 20, 40, 60, and 80%. Toothpaste containing antibacterial ingredients was used as positive control and for negative control, a basic toothpaste formula without antibacterial ingredients, namely carboxymethyl cellulose (CMC), xylitol, calcium carbonate ($CaCO_3$), sorbitol, sodium lauryl sulphate (SLS), natrium benzoate. Data analysis using one-way ANOVA test with normality test using Shapiro-Wilk and homogeneity test using Levene test. The results at a concentration of 20, 40, 60 and 80% with a positive control there was no significant difference because the sig value was >0.05. **Results:** The results showed that Celery containing toothpaste inhibits *Streptococcus mutans* growth (p value <0.05) that there is a significant inhibition effect of *Apium graveolens* L. extract toothpaste on the growth of *Streptococcus mutans*. **Conclusion:** There is an inhibition effect of celery leaf (*Apium graveolens* L.) extract toothpaste on the growth of *Streptococcus mutans.*

KEYWORDS
streptococcus mutans ATCC 25175, inhibition, toothpaste, celery, *apium graveolens*

INTRODUCTION

Dental caries has generally been considered the most important problem of global oral health. Health facilities and dental health education counseling have been carried out, but public knowledge about dental caries is still low.[1] According to World Health Organization (WHO) survey data, it is noted that worldwide 60–90% of children experience dental caries. The highest prevalence of dental caries in children in America and Europe, the index is somewhat lower than the East Mediterranean and western Pacific regions, while the lowest prevalence is Southeast Asia and Africa. According to WHO global oral health, it estimates that oral diseases affect at least 3.58 billion people worldwide, with permanent dental caries being the most common of all conditions assessed. Globally, an estimated 2.4 billion people suffer from permanent dental caries. and 486 million children suffering from primary tooth caries. According to the National Health Research (Riskesdas) 2018 data, it was noted that 56.7% in Indonesia experienced oral and dental problems.[1,2]

Streptococcus mutans is a Gram-positive bacteria that creates an acidic environment in the oral cavity. There are two enzyme systems in the cell wall of *Streptococcus mutans* which can form two extracellular polysaccharides from sucrose. Sucrose will be hydrolyzed into fructose (levan) and glucose (dextran). Fructose is hydrolyzed by the enzyme fructosyltransferase and glucose is hydrolyzed by glucosyltransferase. Sucrose fermentation will result in a decrease in the pH of the saliva to 5.0 or even lower. If the plaque pH is below 5.0, the salivary state is disturbed and it can cause the release of calcium and phosphate ions from the hydroxyapatite crystals. The increase in lactic acid causes insufficient saliva to prevent the dissolution of enamel, resulting in demineralization of enamel which can lead to the process of dental caries.[3]

Dental caries can be prevented by maintaining oral hygiene both chemically and mechanically. The chemical method uses antibacterial ingredients, while mechanically brushing your teeth using a toothbrush and toothpaste that contains antibacterial properties.[4] Toothpaste generally contains abrasive ingredients to clean the tooth surface, a moisturizer to prevent water evaporation, a binder to hold all the ingredients together and give the paste texture, *peppermint* to cover the taste of unpleasant ingredients, water as a solvent, desensitizing agent to reduce and eliminate dentin sensitivity, *fluoride* as an anti-caries agent to control dental caries caused by bacteria *Streptococcus mutans* and *triclosan* are non-ionic chemicals from the synthetic bisphenol group which has antibacterial properties that can inhibit the growth of Gram positive bacteria such as *Streptococcus mutans*.[5]

Toothpaste containing *fluoride* is recognized as the best, *fluoride* most effectively protects primary and permanent teeth from caries. However, *fluoride* and antibacterial ingredients such as *triclosan* will cause the risk of *fluorosis*, toxicity, demineralization of teeth, and discoloration of the enamel so that an alternative option like using herbal ingredients is preferable.[6] In Indonesia, celery leaf are widely used as vegetable. Celery *(Apium graveolens* L.) is a member of the *Apiaceae* family that can live in the highlands and lowlands. Celery is a plant that is easy to find, one of which is because the warm climate is suitable for celery growth. Celery contains flavonoids, saponins, tannins, apiin, essential oil, apigenin, choline, vitamins A, B, C, the bitter substance asparagine. Among the ingredients in celery, flavonoids, saponins, and tannins are compounds that are antibacterial.[7,8]

The antibacterial mechanism of flavonoids consists of three types, namely by inhibiting nucleic acid synthesis, inhibiting the function of the cytoplasmic membrane, and inhibiting energy metabolism. Saponins have antibacterial properties by providing protection against potential pathogens. Saponins also disrupt the surface tension of the cell walls. Tannins have antibacterial activity by taking advantage of bacterial walls that have been lysed due to saponin and flavonoid compounds, thus causing tannin compounds to easily enter bacterial cells and coagulate the protoplasm of bacterial cells.[9]

Celery (*Apium graveolens* L.) is a member of the *Apiaceae* family that can grow everywhere. Celery contains flavonoids, saponins, tannins, essential oils, apigenin, choline, vitamins A, B, C, asparagine bitter substances. Among the ingredients owned by celery, flavonoids, saponins, and tannins are antibacterial compounds. Celery is useful for maintaining oral hygiene and dental health, especially for the elderly. Raw celery can induce saliva production so that it can inactivate the activity of germs that can cause cavities.

Based on the search results, previous research was conducted by Rachmawati (2014), the research was carried out with variations in the concentration of ethanol extract 20, 40, 60 and 80%. The results showed that the ethanol extract of celery leaf (*Apium graveolens* L.) had an inhibition against the growth of *Candida albicans* and there is an effect of the celery leaf ethanol extract's concentration on the inhibition. The 80% concentration of ethanol extract of celery leaf was the most effective against the growth of *Candida albicans*. The chemical content in this extract that has the potential to be an antimicrobial is 1.7% flavonoids, 0.36% saponins, 1% tannins.[10] Among the ingredients owned by celery, flavonoids, saponins, and tannins are antibacterial compounds. *Streptococcus mutans* is a Gram-positive bacterium creating an acidic atmosphere in the oral cavity, which causes insufficient saliva to prevent enamel demineralisation which can lead to dental caries. The purpose of this study was to analyze the effect of the inhibition of celery leaf extract on the growth of *Streptococcus mutans*.[14,15]

METHODS

Type of experimental laboratory research uses the Post Test Only Control Group Design. The samples were *Apium graveolens* L. and *Streptococcus mutans* ATCC 25175. The number of treatment groups was 24 with concentrations of 20, 40, 60, and 80%. Toothpaste containing antibacterial ingredients was used as positive control and for negative control, a basic toothpaste formula without antibacterial ingredients, namely carboxymethyl cellulose (CMC), xylitol, calcium carbonate ($CaCO_3$), sorbitol, sodium lauryl sulphate (SLS), natrium benzoate.

Identification test of celery leaf which was carried out at the Herbarium Laboratory of Andalas University. Celery leaves have gone through the phytochemical test stage. Preparation of celery leaf extract (*Apium graveolens* L.) are washed, dried at room temperature, weighed as much as 1 kg, then soaked with 96% ethanol and stirred until homogeneous. Mixture soaking results are sealed for 5 days. After 5 days, the mixture is then filtered using filtering paper. The filtrate obtained is mixed for 2 days in a cool place and protected from light. The precipitate was separated and vacuum distilled to obtain a thick extract of celery leaf.[11]

Concentrations of extracts of *Apium graveolens* L. was prepared in various concentrations to be tested on bacteria *Streptococcus mutans* at 20, 40, 60, and 80% (water/volume) instead. The concentration of the solution carried out in this study used a general formula. Preparation of celery leaf extract toothpaste as basic formula of toothpaste by combining *menthol crystal* and mint oil as flavoring, *calcium carbonate*, glycerin, *sodium lauryl sulphate*, triethanolamine. The concentration of celery extract is 100% diluted to 20, 40, 60 and 80%, then mixed with 3 grams of toothpaste formula and mixing until homogeneous.[12]

Inhibition test of antibacterial efficacy in this study used the well diffusion method. Petri dishes that have been rubbed with the bacterial suspension *Streptococcus mutans* using a sterile cotton stick are then a well hole was made using a perforator. The diameter of the pit is 6 mm. Each well was filled with celery leaf extract toothpaste test material, 20, 40, 60, and 80%. Positive control was made with commercial toothpaste, negative control was the basic toothpaste. The sample in this study were 6 treatment groups with replication 4 times. The petri dishes were then put in an incubator and incubated at 37° C for 24 hours. The diameter of the inhibition zone that is formed around the well is measured using sliding calipers three dimensions were vertical, horizontal and diagonal, then the results are averaged.[13]

Data analysis was carried out using the SPSS program, then proceeded by using the *One Way Anova* test. This type of experimental laboratory research uses the *Post Test Only Control Group Design.*

RESULTS

Inhibition zone based on the research that has been conducted to determine the inhibition effect of celery leaf extract toothpaste (*Apium graveolens* L.) on the growth of *Streptococcus mutans*. The following results can be shown at table 1.

Table 1. Inhibition of celery leaf extract toothpaste (*Apium graveolens* L.) on the growth of *Streptococcus mutans*

Concentration of celery leaf extract (%)	Mean ± Deviation Standard
20	6.225 ± 0.125
40	6.350 ± 0.238
60	6.725 ± 0.287
80	7.450 ± 0.526

The results presented in table 1 show that the average inhibition power of celery leaf extract toothpaste (*Apium graveolens* L.) against the growth of *Streptococcus mutans* bacteria is the highest at an extract concentration of 80%, namely 7.450. Thus, in line with the positive control, the inhibition of growth of *Streptococcus mutans* bacteria was 7.450. So it can be concluded that the 80% celery leaf extract toothpaste (*Apium graveolens* L.) has a greater effect than the other concentrations.

The Minimum Inhibitory Concentration (MIC) test in this study was to determine the minimum concentration value of the active compound extract of celery leaf toothpaste (*Apium graveolens* L.) which can inhibit the growth of *Streptococcus mutans* ATCC 25175 bacteria. MIC is a micro dilution. Micro-dilution is a method of gradual dilution in each well that is carried out on the microplate, then scanned using a multimode ELISA reader. The concentration of 80% is the initial concentration for diluting so that at a concentration of 40% you can see the results of the dilution. From the table, the MIC value was obtained at a concentration of 10%, which is the smallest concentration that inhibits the growth of *Streptococcus mutans* ATCC 25175. The normality test used is the Shapiro-Wilk test because the data is less than 50.

DISCUSSION

The results of study about the inhibition effect of celery leaf (*Apium graveolens* L.) extract toothpaste on the growth of *Streptococcus mutans* showed that the concentration of 80% of celery leaf extract toothpaste (*Apium graveolens* L.) was the extract with the highest average of 7.450. Previous research by Majidah et al, illustrated that all toothpastes studied at various concentrations of celery leaves extract exhibited antibacterial activities. Maximum inhibition zone in antibacterial activity test was shown by F2 (12.5%). Therefore, we can use these toothpastes as natural antibacterial to prevent the occurrence of dental caries caused by *Streptococcus mutans*.[7] This shows that the higher the concentration of the sample used, the more active substance contained in celery leaf extract (*Apium graveolens* L.), so the greater the inhibition against the growth of *Streptococcus mutans*. On the contrary, the lower the concentration used, the smaller it is the resistance obtained.[8]

Apart from 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 which is present in peptidoglycan while Gram negative bacteria do not have teichoic acid. This teichoic acid serves as a way to leave and enter ions from and into bacterial cells. 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.[14,15]

The positive control group (commercial toothpaste) had the second greater antibacterial inhibition than various concentrations of celery leaf extract except for 80% extract. Differences with herbal toothpaste results showed there was a significant difference of inhibition effect among those three herbal toothpaste ($p<0.05$). The average diameter of the inhibition zone of clove, miswak (*siwak)* and betel leaves containing toothpaste were 16.075 mm, 13.375 mm and 11.080 mm respectively. Hence, it can be concluded that herbal toothpaste has shown antibacterial effect toward the growth of *Streptococcus mutans* in which the strongest antibacterial effect were shown by clove containing toothpaste.[15] This is because the fluorine content in commercial toothpaste has the ability to stimulate early caries remineralization and reduce the ability of bacteria to produce acid. Eucalyptus oil has antibacterial properties and is used as an antiseptic. Clove in toothpaste is a mild, temporary anesthetic. Tea tree oil contains mild antiseptic properties that can control bacterial growth.[7]

The results at the concentration of 20, 40, 60 and 80% extract with a positive control there was no significant difference because the significance value was <0.05. Compared with previous research Inhibition test of the growth of bacteria was done by using diffusion method and was said to be a positive result when a clear zone surrounding the colony of *Streptococcus mutans* was identified. One way ANOVA test result showed there was a significant difference of inhibition effect among those three herbal toothpaste ($p<0.05$). There was no significant difference between the groups that had the same average inhibition against *Streptococcus mutans*. Previous research showed an alternative solution by utilizing celery extract (*Apium graveolens* L. var *secalinum Alef*) containing flavonoids, saponins, and tannins which are antibacterial compounds. This research is designed as an experimental laboratory with dilution method to determine Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC). This study used 6 tubes and 2 control tubes with concentrations of 100, 50, 25, 12.5, 6.25, and 3.125%. The Minimal Inhibition Concentration (MIC) is 3.125%, while for Minimum Bactericidal Concentration (MBC) there is no result.[8] The similarity of

the average inhibitory power can be interpreted that the extract group of 20, 40, 60 and 80% with positive control contains almost the same amount of active substances. Previous Research by Suwito et al, Based on the result, celery extract (*Apium graveolens* L. var *secalinum Alef*) able to inhibit the growth of *Streptococcus mutans* bacteria but can not kill the bacteria.[8] 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 present in peptidoglycan while Gram negative bacteria do not have teichoic acid. This teichoic acid serves as a pathway for ions to enter and leave 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.[14] Pharmacodynamic interactions between two or more substances used simultaneously have several effects, including additive, synergistic and antagonistic effects. An additive effect is an interaction between two or more ingredients that has the same amount of effect when each ingredient is administered separately. A synergistic effect is an interaction between two or more ingredients that has a greater number of effects than each of the ingredients given separately because the first ingredient benefits the other, while an antagonistic effect is an interaction between two or more ingredients that have a lower number of effects because one material reduces the function of the other.[15]

CONCLUSION

Based on the results of the research that has been conducted, it can be concluded that there is an inhibition effect of *Apium graveolens* L. extract toothpaste on the growth of S*treptococcus mutans.*

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