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Fitricia Lely Milleningrum
Faculty of Dentistry, Jember University, Jember, Indonesia

Achmad Gunadi
Ph.D, Prosthodontia, Jember University, Jember, Indonesia

Yuliana MD Arina
Periodontics, Jember University, Jember, Indonesia

Corresponding Author:
Fitricia Lely Milleningrum
Faculty of Dentistry, Jember University, Jember, Indonesia

Antibacterial effect of celery leaf extract (*Apium graveolens* L.) against *Staphylococcus aureus in vitro*

Fitricia Lely Milleningrum, Achmad Gunadi and Yuliana MD Arina

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Abstract
Staphylococcus aureus is a bacterium that causes various oral infections. Infections caused by *S. aureus* are difficult to treat because they are susceptible to becoming resistant to antibiotics. Oral infection caused by *S. aureus* can be prevented by preventing plaque buildup with mouthwash, one of which is chlorhexidine. Chlorhexidine is reported to have side effects in the short and long term. Therefore, it is necessary to search for natural ingredients that are easy to obtain and cheap as an alternative mouthwash in the prevention of oral plaque, one of which is celery leaf. Celery leaves contain flavonoids, saponins, and tannins which are antibacterial compounds.
Purpose: This study aimed to analyze the antibacterial activity of celery leaf extract against the growth of *S. aureus*.
Methods: Celery leaves were extracted using maceration method with 96% ethanol. Inhibition test using disc diffusion method on MHA media that has been overgrown with *S. aureus*. The concentrations used were 5%, 10%, 20%, and 40%. The research data were analyzed using SPSS.
Results: The study showed that there was an inhibition zone formed around the paper disk at a concentration of 20% and 40% celery leaf extract. The results of data analysis showed that there were significant differences (p>0.05) in all treatment groups except between negative control, 5% celery leaf extract group, and 10% celery leaf extract group.
Conclusion: Celery leaf extract (*Apium graveolens* L.) has antibacterial activity against *S. aureus* and the extract with 40% concentration has the greatest antibacterial activity against *S. aureus* but still lower than chlorhexidine.

Keywords: Antibacterial, *Staphylococcus aureus*, celery leaf extract

1. Introduction
Dental caries and periodontal disease are diseases that often occur and cause dental and oral health problems [1]. One of the bacteria that forms plaque and plays a role in causing various oral infections is *Staphylococcus aureus* (*S. aureus*) [2, 3]. *S. aureus* can cause periapical infections, osteomyelitis and abscesses as a continuation of caries and periodontal disease [4, 5]. Infections caused by *S. aureus* are difficult to treat because they are susceptible to becoming resistant to antibiotics [6].
Oral cavity infection due to *S. aureus* can be prevented by controlling plaque so as to prevent caries and periodontal disease. Plaque control can be done mechanically and chemically. Chemically, plaque control uses mouthwash which aims to remove bacteria in parts that are not reached by a toothbrush [7]. Long-term use of mouthwashes is reported to have side effects such as dry mouth, reduced saliva production, tooth decay, erosion of the oral mucosa, and risk of cancer. mouth. In short-term use it is reported to cause a burning feeling in the oral mucosa, discolored teeth and tongue, loss of taste when eating but no irritation is found [8, 9, 10]. In addition, in some communities, mouthwash is considered relatively expensive and in some areas there are limitations in getting it, for example in rural areas. Therefore, it is necessary to search for natural ingredients that are easily available and inexpensive as alternative mouthwashes, one of which is celery leaves.
Celery leaves have been reported to have antibacterial properties against *E. coli*, *S. mutans*, and *S. aureus* which are oral bacteria [11, 12, 13]. Research by Majidah *et al*. (2014) and Suwito (2017) showed that the phytochemical content of celery leaves has the potential as an

antibacterial on *S. mutans*. Khaerat & Ihwan (2011) [13] stated that the content of ethanol extract of celery leaves with a concentration of 4% has the ability to have antibacterial power on *S. aureus*. Although it was reported that the inhibitory power of celery leaf extract was at a concentration of 4%, this study had not been compared with existing mouthwashes, so further research is needed to look for the potential concentration of celery leaf extract which has the largest inhibition area equivalent to chlorhexidine in inhibiting *S. aureus*.

Materials and Methods

This research is a type of experimental laboratory research with the post test only control group design. The size of the study sample was 24 samples divided into 6 groups, namely 5%, 10%, 20%, 40% celery leaf extract as the treatment group and K(+) chlohexidine 0.2%, control (-) sterile aquadest as the control group.

The celery leaves used in this study were celery leaves from the Kalisat garden, Sukowono, Jember. Celery leaves were extracted using the maceration method with 96% ethanol.[11,14] Suspension of *S. aureus* ATCC 6538/PK 5 was made on MHB media and inoculation of the *S. aureus* bacterial suspension was carried out on the surface of the MHA media in petridishes. The petridish was left open for 3-15 minutes until the inoculum seeped into the surface of the agar.

The inhibition test of celery leaf extract against *S. aureus* was carried out using the disc diffusion method. The celery leaf extract that was tested was dripped onto a disk and placed on a medium that had been overgrown with *S. aureus*. Then it was put in a desiccator and incubated for 24 hours at 37 ºC.15 The diameter of the inhibition zone was measured by inverting the petridish so that a clear area around the disc paper was visible. The diameter of the inhibition zone was measured using a digital caliper from the edge (break point) to the edge (break point) of the inhibition zone opposite the center of the paper disk.

The research data were then analyzed using SPSS 22.0. Normality test was carried out using the Shapiro Wilk test and homogeneity test using the Levene test. The data showed normal distribution but not homogeneous, so a non-parametric statistical test was performed with the Kruskal Wallis test and the Mann Whitney test to see the difference between the two sample groups.

Results

The results showed that the average inhibition zone value of celery leaf extract (*Apium graveolens* L.) for S. aureus was the largest in the 40% concentration of celery leaf extract group, which was 16.88 mm. The 5% concentration of celery leaf extract and 10% concentration of celery leaf extract group did not show any inhibition zones (Table 1).

Tabel 1: The average inhibition zone (mm) of celery leaf extract (*Apium graveolens* L.) against *S. Aureus*

Groups	n	$\bar{X}$±SD (mm)
Control	4	0
Negative control	4	20,18±11,44
Celery leaf extract concentration 5%	4	0
Celery leaf extract concentration 10%	4	0
Celery leaf extract concentration 20%	4	13,15±6,47
Celery leaf extract concentration 40%	4	16,88±9,11

n : Number of samples
$\bar{X}$: Average number of *S. aureus* colonies
SD : Standard deviation

The results of the research data analysis showed that the data were normally distributed but not homogeneous, so a non-parametric statistical test was carried out with the Kruskal Wallis test. The results showed a p value <0.05 so that there were differences between the study groups and followed by the Mann Whitney test. The results showed that there were significant differences between the study samples as indicated by the significance value of p<0.05 in the 20% and 40% concentration treatment groups with negative controls but lower than the positive controls. This shows that this group has the ability to inhibit the growth of *S. aureus*. Whereas in the negative control group with 5% celery leaf extract group and 10% celery leaf extract group there was no significant difference (Table 2).

Tabel 2. Mann Whitney test results of. Elery leaf extract in various concentrations

Groups	K-	K+	5%	10%	20%	40%
K-	-	0,014*	1,000	1,000	0,013*	0,014*
K+			0,014*	0,014*	0,020*	0,021*
5%				1,000	0,013*	0,014*
10%					0,013*	0,014*
20%						0,020*
40%						

K(-) : Negative control (aquades)
K(+): Positive control (*chlorhexidine*)
5%: Celery leaf extract concentration 5%
10%: Celery leaf extract concentration 10%
20%: Celery leaf extract concentration 20%
40%: Celery leaf extract concentration 40%
*: There were significant differences between groups

Discussion

This research is a laboratory experimental research. Preparation of celery leaf extract using the maceration method with 96% ethanol solvent. The maceration method was chosen because it is easy, simple, and without heating. 16 Celery leaves contain compounds that are thermolabile, namely compounds that are easily damaged by heating and polar compounds such as phenolic compounds of flavonoids, saponins, and tannins so that the maceration method with ethanol solvent can be used to optimize withdrawal. these compounds.

The results showed that celery leaf extract had antibacterial activity against *S. aureus* compared to distilled water (negative control). This is evidenced by the concentration of 20% and 40% celery leaf extract which has an average value of the inhibition zone which increases with increasing concentration. This is in accordance with research conducted by Khaerat and Ihwan (2011) [13] which stated that the higher the concentration of celery leaf extract, the greater the diameter of the inhibition zone, indicating that the stronger the antibacterial activity against *S. aureus*. In addition, the increase in the resulting inhibition zone may be due to the active substance content of celery leaves, the higher the concentration, the higher the active substance content contained in the extract so that the larger the inhibition zone is formed.

The existence of antibacterial power from celery leaves is probably due to the active substances contained in celery leaves, including flavonoids, saponins and tannins which are compounds with antibacterial properties. Flavonoids have the ability to inhibit bacterial nucleic acid synthesis by forming complex compounds with proteins through hydrogen bonds so that the structure of proteins in bacteria is disrupted, and proteins cannot function anymore resulting in damage or

denaturation of proteins and nucleic acids. The denaturation of proteins and nucleic acids causes coagulation of proteins and disrupts bacterial metabolism and physiological functions of bacteria and inhibits the function of the bacterial cytoplasmic membrane.

Saponins as antibacterials diffuse through the outer membrane and vulnerable cell walls and then bind to the cytoplasmic membrane and disrupt and reduce the stability of surface tension resulting in increased permeability or cell leakage and result in intracellular compounds being released. This causes cytoplasmic lysis out of the cell resulting in cell death

Tannins are able to form strong polymer bonds that can cause damage to porins (gates for entry and exit of compounds) which will reduce the permeability of the bacterial cell membrane and can cause bacterial cells to lack bacterial nutrition. 22, 23 The cell wall layer of Gram-positive bacteria contains peptidoglycan and also teichoic acid. and teicuronic acid. Therefore the cell walls of Gram positive bacteria are mostly polysaccharides. Cell wall denaturation occurs most easily in cell walls composed of polysaccharides compared to cell walls composed of phospholipids. S. aureus has a cell wall consisting of 50% peptidoglycan layer and has a compact wall structure that makes it sensitive to antibacterial substances such as flavonoids, saponins and tannins.

However, 5% and 10% concentrations of celery leaf extract did not show any inhibition as indicated by the absence of a clear zone around the disc paper (inhibition zone = 0). In this study, the absence of an inhibition zone at small concentrations was possible because the active substance in celery leaves also did not appear completely so that the active ingredient inhibiting bacteria did not work optimally. Soil conditions and their elements greatly affect the content of secondary metabolites in plants but it is difficult to determine specifically the nutrient factors that can affect the production of secondary metabolites. 25 This causes differences in results with previous studies which stated that at a concentration of 4% it could inhibit *S. aureus* bacteria, but In this study, *S. aureus* could only be inhibited at a concentration of 20% which was possible because the content of the antibacterial active substance in celery leaf extract was different from previous studies which were influenced by soil growth conditions so that it was unable to damage cell membranes and was unable to interfere with cell physiological processes which resulted in non-forming zone of inhibition at small concentrations.

In addition, the size of the inhibition area is affected by the growth rate of microorganisms, the ability and rate of diffusion of the active ingredients in the medium, the sensitivity of microorganisms to the active substances and the thickness and viscosity of the medium.

The results showed that the antibacterial activity of celery leaf extract (*Apium graveolens* L.) at a concentration of 40% showed the greatest antibacterial activity compared to other concentrations. However, the antibacterial activity of celery leaf extract at a concentration of 40% was still lower than that of chlorhexidine (positive control). This may be due to the fact that the 40% concentration used in research has not shown the optimal antibacterial effect. Therefore, further research is needed to determine the optimal concentration of celery leaf extract (*Apium graveolens* L.) in inhibiting *S. aureus.*

The potential antibacterial ingredients contained in celery leaf extract are expected to reduce the growth of *S. aureus* so that it can reduce infectious diseases in the oral cavity.

Conclusion

Celery leaf extract (*Apium graveolens* L.) has antibacterial activity against *S. aureus* and the extract with 40% concentration has the greatest antibacterial activity against *S. aureus* but still lower than chlorhexidine.

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Author's Contribution

Not available

Conflict of Interest

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