ORIGINAL ARTICLE

Screening in Vitro Antimicrobial Activity of Celery (*Apium Graveolens*) Against *Staphylococcus* sp.

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ABSTRACT

Introduction: Many herbal antimicrobials have been developed to treat various diseases. Celery (*Apium graveolens*) has antibacterial potential because it contains flavonoids, saponins, and tannins The purpose of the current research was to determine the extract celery potential as an agent of antibacterial *Staphylococcus* sp. **Methods:** This research was conducted by two methods, namely the well diffusion test and the dilution test. The parameters measured for the well diffusion test were the diameter of the inhibition zone, and for the dilution test with Total Plate Count (TPC), for the determination of Minimum Inhibitory Concenntration (MIC) and Minimum Bactericidal Concentration (MBC). The inhibition zone diameter data were analyzed statistically using SPSS 20.0, while the dilution test data were analyzed descriptively. **Results:** The results of the well diffusion test showed that the concentration of celery extract affected inhibiting bacterial growth at all concentrations. The highest value of the inhibition zone diameter of celery extract was found at a concentration of 100%, for *Staphylococcus aureus* there (11.67 ± 0.57 mm), and *Staphylococcus epidermidis* (11.67 ± 0.57mm). The MIC value of celery extract on the *Staphylococcus aureus* and *Staphylococcus epidermidis* growth was at 25 %. Meanwhile, the MBC could not be found because at the highest concentration of the extract there was still bacterial growth. **Conclusion:** In general, celery extract (*Apium graveolens*) had an effect in impeding the *Staphylococcus aureus* and *Staphylococcus epidermidis* growth.

Keywords: *Apium graveolens*, MBC, MIC, *Staphylococcus* sp.

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INTRODUCTION

Antimicrobials are compounds that kill microorganisms by inhibiting bacterial growth. The widespread availability of antibiotics and inappropriate use of antibiotics have led to a phenomenon of bacterial resistance The case of antibiotic resistance has constantly occurred in both humans and animals for a period of time (1). For that reason, it is urgently needed to develop alternative medicines to treat diseases.

Current developments regarding bacterial resistance to antibiotics necessitate the need to find new alternatives, an antibacterial agent. Gram-positive bacteria such as *Staphylococcus* sp. are responsible for several infections, including respiratory and skin infections, urogenital diseases, and wound contamination. *Staphylococcus aureus* is considered to be the main cause of nosocomial infection. It has resistance to a range of antimicrobial agents (2). A member of the coagulase-negative Staphylococci, *Staphylococcus epidermidis*, is an important commensal organism of human skin and mucous membranes. *Staphylococcus epidermidis* is known as the bacteria most often isolated in clinical culture and has emerged as the main nosocomial pathogen (3).

As an alternative, many herbal antimicrobials have been developed to treat various diseases. Antimicrobials are found mostly in natural ingredients such as plants and spices (4). Several plants that are often used as traditional medicines are celery leaves, betel leaves, papaya leaves, soursop leaves, gambier leaves, and others (5). One of the plants that have been used as an antimicrobial agent is celery (*Apium graveolens*) (6).

A celery seed extract has been commonly used as an anti-rheumatic treatment, rheumatic pain reliever, rheumatic conditions treatment, and gout treatment.

Apart from playing a role in relieving rheumatism, celery seeds have proven their uses in the treatments of asthma, bronchitis, and inflammatory conditions (7). Research on the ability of celery extract as antibacterial has been carried out. Celery leaf extract has antibacterial power against the growth of *Streptococcus mutans* (12,5 %) (8). The results of other studies show that celery essential oil has antibacterial properties against *Escherichia coli*, *Salmonella*, *Bacillus cereus*, *Pseudomonas aeruginosa* (9). Therefore, it is necessary to study the use of celery extract as an antibiotic made from herbs that can be used as an alternative to antibiotics for gram-positive bacteria.

MATERIALS AND METHODS

Preparations of extracts

The extraction procedure was carried out with 750 grams of dry celery extracted by maceration method using 96% ethanol solvent. Celery powder soaked in 96% ethanol for 3x24 hours, stored in a dark room. To attain a semi-solid mass, a rotary evaporator is utilized to entirely remove the solvent in the extract. The rotator carried out the concentration at 40^{0}C. The fractions were stored in CMC-Na (Carboxymethyl Cellulose–Natrium) at 4^{0}C until being tested.

Bacterial Preparation

The bacteria which consisted of two Gram-positive strains, S. *aureus* (ATCC 29213) and S. *epidermidis* (ATCC 14990, were maintained at the Laboratory of Center for Health, Surabaya. To prepare the deferral of the cell, they were cultured overnight (+18 h) at 37^{o}C in the broth of nutrients. The deferral of the bacteria cell was homogenized. Then, the adjustment to 0.5 McFarland standards (1.0 x 10^8 CFU/mL) using spectrophotometry was performed.

Antimicrobial Susceptibility Assays

The antibacterial assay of crude extracts was conducted using Wells diffusion method. 15 ml of Muller Hinton Agar (MHA) (pH 7.2 ± 0.2, at 25°C) which has been sterilized were applied into the sterile Petri dishes surface (diameter of 9 cm). This stage allowed them to settle the preparation of the base plate. In the surface base plate, 10 ml of bacterial test suspension with 1.0 x 10^8 CFU/ml (0.5 McFarland standards) were poured with a cotton swab to the base plate surface. The well diffusion method uses a concentration of 250 ppm, 500 ppm, 750 ppm, and 1000 ppm celery extract. Separately, the distinguished concentrations were equipped by dissolving the extract with CMC-Na (Carboxymethyl Cellulose–Natrium). On the MHA surface, the wells were made of a sterile cork borer by punching aseptically (6 mm in diameter). Approximately 50 µl of extract concentration were loaded into the wells (equivalent with a diameter of 6 mm and thickness of ± 4 mm of wells). The negative control was filled with 50 µl of sterile aquadest and the positive control used chloramphenicol 30 mg/disc concentration, and the palte was incubated at 37 ± 2°C for 24 hours. After the incubation, the measurement in mm around the disc in the inhibition zones of growth around discs was performed. All of the experiments were conducted in triplicate. Then, the calculation of the mean value of each measurement was carried out.

Determining the Minimum Inhibitory Concentration (MICs) and Minimum Bactericidal Concentration (MBCs)

The determination of MIC values was carried out by utilizing the method of broth dilution. Concisely, the cultures of microbial were set by suspending one colony of isolates on agar media into 5ml Nutrient Broth After 24 hours of incubation, the suspension was diluted to obtain the inoculum population according to the standard of 0.5 Mac Farland (1.0 x 10^8 CFU / ml). Then, it was diluted according to the concentration of the extract (250 ppm, 500 ppm, 750 ppm, and 1000 ppm celery extract) using broth media and control (media and bacteria without celery extract). The addition of an equal volume of bacterial inoculum was carried out in every tube with celery extract. It was then incubated at 37 ± 2 0C for 24 hours. MIC value refers to the lowest concentration of a substance that obstructs the growth of bacterial seen in the media. MBC is determined by looking at the growth of bacteria on agar media. Planting streaks on agar was carried out to determine MIC and MBC values by the TPC method. It was then incubated for 48 hours at 37°C. The MBC was determined from the plant extract concentration with 100% inhibition value.

Statistical Analysis

The collected data were described as mean Inhibition zone diameter ± SEM. One-way ANOVA was performed to analyze the documented results. SPSS version 20.0 was utilized in the data analysis.

RESULTS

Antibacterial Activity with Well Diffusion Method

Figure 1 depicts the inhibition zones which were indicated by the extract of celery at distinguished concentrations against *Staphylococcus* sp. The test results showed that the higher the concentration of celery extracts, the greater the inhibitory power. Based on the data in Table I, the inhibition zone in the *Staphylococcus aureus* (7,67± 0,57 mm) and *Staphylococcus epidermidis* (7,33± 1,15 mm) had been seen from a concentration of 25%. At a concentration of 100%, they had the highest average value compared to the other concentrations, *Staphylococcus aureus* (11,67± 0,57 mm) and *Staphylococcus epidermidis* (11,67± 0,57 mm). The zone of inhibition diameter has

a relation to the extract concentration. The higher the extract concentration, the wider the inhibition zone was formed. This also shows the stronger antibacterial power possessed by the celery extract.

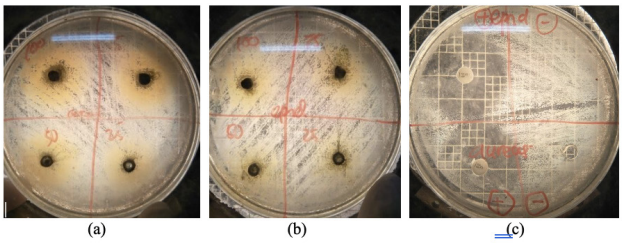

Figure 1 : Result of celery extract inhibition test on bacteria (a) *Staphylococcus aureus* (b) *Staphylococcus epidermdis*, and (c) Positive control (Chloramphenicol 30 µg) and negative control (disc antibiotic blank).

Tabel I : Zone of inhibition of the plant extracts

Bacteria	Concen-tration	Diameter (mm)	CI		p-value
			lower	Upper	
Staphylococcus aureus	25	7.67 ± 0.58	6.23	9.10	0.00
	50	9.00 ± 0.00	9.00	9.00	
	75	9.67 ± 0.58	10.00	10.00	
	100	11.67 ± 0.58	10.23	13.10	
	Control (+)	30.33 ± 0.58	28.90	31.77	
	Control (-)	0.00 ± 0.00	0.00	0.00	
Staphylococcus epidermidis	25	7.33 ± 1.15	6.23	9.10	0.00
	50	8.00 ± 1.73	3.70	12.30	
	75	9.00 ± 1.00	6.52	11.48	
	100	11.67 ± 0.58	10.23	13.10	
	Control (+)	31.00 ± 1.73	26.70	35.30	
	Control (-)	0.00 + 0.00	0.00	0.00	

Data are means of three replicates (n = 3) ± standard error, statistic with ANOVA Sig <0.05

Based on statistical analysis, celery extract has an effect in inhibiting the growth of the bacteria of *Staphylococcus aureus* and *Staphylococcus epidermidis*. The analysis results of variant (ANOVA test) showed that the effect of celery extract on the test bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* has a significant difference in the inhibition zone formed between treatments ($p < 0.05$) The concentration of 25% showed the lowest area diameter value in the two tested bacteria. Furthermore, the concentration of 100% showed the highest area diameter value in the two tested bacteria.

IC (Minimum inhibitory concentrations) and MBC (Minimum bactericidal concentrations) of the effective plants extract

To assess the bacteriostatic and bactericidal properties, the MIC and MBC values of celery extract were used. The determination of MIC and MBC values was carried out by dilution method of celery extract, which then determined the TPC value for bacterial culture at each extract concentration (Table II). MIC and MBC values were determined based on bacterial growth on MHA (Muller Hinton Agar) (Figure 2 and Figure 3). The plate count method was used to count the number of living bacterial colonies. The MIC value was determined with lower bacterial growth compared to the control, and the MBC value was determined in the absence of bacterial growth on the agar medium.

In the nonappearance of the growth of the bacteria from the strain which was verified using the determination of the lowest MIC value, the MBC was confirmed. Celery extract has not shown the potential of the activity of bactericidal in contradiction of the pathogenic bacteria, S. *aureus*, and S. *Epidermidis*. This is indicated by the growth of bacteria at a 100% extract concentration. The MIC and MBC results of this study suggested that the use of celery extract could only inhibit the growth of the tested bacteria, and could be used to control and prevent bacterial growth.

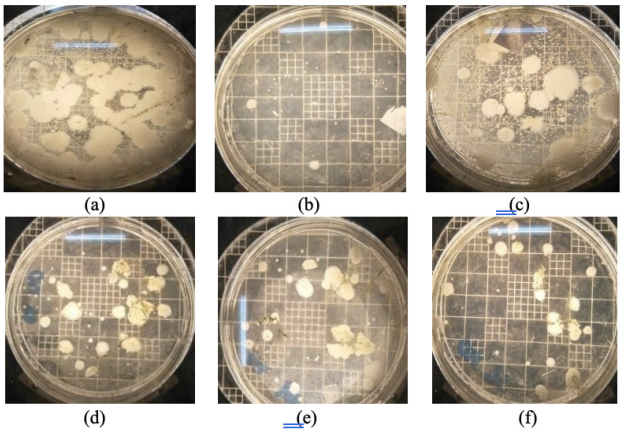

Figure 2 : MBC test result of celery extract against Staphylococcus aureus (a-f) positive control (Chloramphenicol 30 µg), negative control (CMC-Na), 250 ppm celery extract, 500 ppm celery extract, 750 ppm celery extract, and 1000 ppm celery extract.

Table II : Visualization of Dilution Test of variations in the concentration of celery extract (*Apium graveolens*)

Concentration extract (%)	Turbidity of media	
	Staphylococcus aureus	*Staphylococcus epidermidis*
12.5	Turbidity (+) with yellow layer	Turbidity (+) with yellow layer
25	Turbidity (+) with yellow layer	Turbidity (+) with yellow layer
50	Turbidity (++) with yellow layer	Turbidity (++) with yellow layer
100	Turbidity (+++) without yellow layer	Turbidity (+++) without yellow layer
control	Turbidity (++) without yellow layer	Turbidity (++) without yellow layer

Note : (+) : level of turbidity; Control : nutrient broth with bacteria test

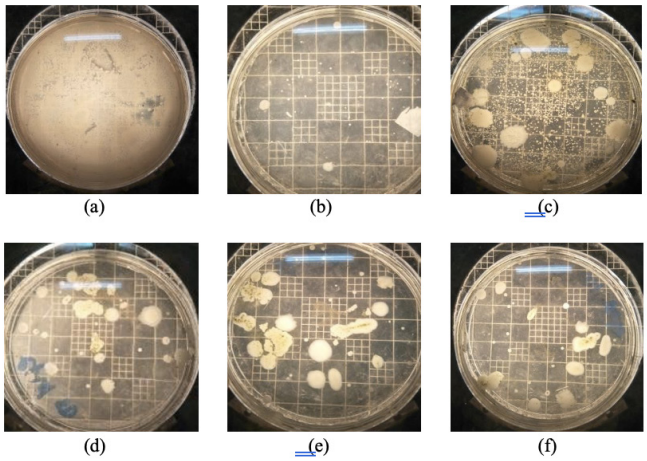

Figure 3 : MBC test result of celery extract against *Staphylococcus epidermidis* (a-f) positive control (Chloramphenicol 30 µg), negative control (CMC-Na), 250 ppm celery extract, 50o ppm celery extract, 750 ppm celery extract, and 1000 ppm celery extract.

Table III : Total Plate Count (TPC) of the extract celery leaf extracts against *Staphylococcus* sp.

Concentration extract (%)	*Staphylococcus aureus*	*Staphylococcus epidermidis*
12.5	3,89 x 10^3 CFU/ml	2,49 x 10^4 CFU/ml
25	2,49 x 10^3 CFU/ml	3,57 x 10^3 CFU/ml
50	2,10 x 10^2 CFU/ml	2,00 x 10^2 CFU/ml
100	8,00 x 10^1 CFU/ml	4,00 x 10^1 CFU/ml
control	TNTC	TNTC

Data are means of three replicates (n = 3), TNTC: Too Numerous To Count

DISCUSSION

Antibacterial Activity with Well Diffusion Method

In the diffusion method, the determination of antibacterial activity was determined by the presence or absence of clear areas around the well. The clear zone showed the inhibition of the extract against the tested bacteria (10). It is seen in Table I that, in the inhibition zone, there was an increase of diameter in the extract concentration of *Staphylococcus aureus* and *Staphylococcus epidermidis* growth. This shows that the higher the celery extract concentration extract, the greater the toxic effect, marked by a higher inhibition zone.

The active substances contained in celery extract have an antibacterial function, including essential oils, flavonoids, tannins, and saponins (6). The extraction method greatly affects the levels of secondary metabolite compounds from a plant. The maceration extraction method can produce higher total flavonoid levels than the infundation method. The average total flavonoid content in the macerate extract of 5.32% w/w (11).

The solvent will affect the active compounds that can be dissolved and can affect the results of antibacterial tests. The CMC-Na solvent in the extracted test aims to increase the viscosity of the extract and will not settle due to the influence of gravity. The important properties in choosing a solvent are the polarity and polar groups of a compound (12). In another study conducted by Wirantika in 2000 (13) about celery extract with ethanol solvent against *Staphylococcus aureus* and *Escherichia coli* showed the highest inhibition power was found at a concentration of 100%. A study from Wirantika shows that celery extract has the potential to inhibit *Staphylococcus aureus*. So, we conducted a study with celery extract to determine the MIC and MBC values showing potential against *Staphylococcus aureus* and other gram-positive ones which can also be common causes of nosocomial infections.

The positive control with Chloramphenicol 30 mg had more excellent antibacterial activity than various concentrations of celery extract. The antibacterial mechanism of chloramphenicol is to inhibit the protein synthesis by binding to the 50S ribosome subunit. Therefore, Chloramphenicol is a class of antibiotics that inhibits protein synthesis and has antibacterial power that is effective against Gram-positive bacteria (14).

MIC (Minimum inhibitory concentrations) and MBC (Minimum bactericidal concentrations) of the effective plants extract

Celery extract is potential as an antibacterial against the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria showed the same results. When it was compared to the treatment in the control group, celery extract concentration of 25% was able to inhibit bacterial growth. Meanwhile, the extract's highest concentration, which was 100%, had not shown a bactericidal effect for both types of bacteria. The greater the concentration of the extract used provided higher inhibition against bacterial growth but did not have a bactericidal effect. This follows the statement of Sukmawati in 2018 (15) which stated that the higher the value of the extract concentration, the fewer the life of microorganism.

This dilution method was also carried out by Suwito et al in 2017 (16) which resulted in the Minimal Inhibitory Concentration (MIC) at a concentration of 25%. The Minimal Bactericidal Concentration (MBC) could not be determined because it was suspected to be related to the low active compound in the research sample. It was suspected that there was a degradation of active compounds in celery extract due to exposure to sunlight, heat, and pH. In conclusion, celery extract can prevent Streptococcus mutants' bacteria' growth, but it cannot kill these bacteria.

The celery extract's antibacterial activity is related to the content of phytochemicals found in celery leaves. There is a relationship between phytochemical constituents plants and antimicrobial activity (17). The content of celery extract includes flavonoids, alkaloids, and saponins, which are related to antibacterial effect in various studies using plant extracts (18). The activity of flavonoids as antibacterial can be found in several mechanisms such as cytoplasmic membrane function inhibition, nucleic acids synthesis inhibition, and energy metabolism (19). Saponins are connected to bacterial cell membranes' penetrability (20). Celery extract (A. buttonens) has a significant effect as an antibacterial and a source of antioxidants. It also has the potential to enhance wound healing promoters by increasing fibroblast proliferation and reepithelialization (21).

Factors that affect the inhibition and eradication of microorganisms by an antimicrobial agent include the concentration of antimicrobial substances, the number of microorganisms, the type of test microorganisms, the temperature and pH of the antimicrobial material. The mechanism of attack of an antimicrobial agent is by knowing the structure and composition of the microbes. Damage to one of its constituent components can initiate changes that lead to cell death (4).

CONCLUSION

Celery extract (*Apium graveolens*) has an effect in inhibiting the growth of *Staphylococcus aureus* and *Staphylococcus epidermidis* bacteria. The extract concentration used only had the ability to inhibit (MIC), and had not shown bactericidal ability (MBC) against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

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