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Antibacterial potential of celery (*Apium graveolens* L.) extract gel against *Staphylococcus aureus*

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ABSTRACT

Background: *Staphylococcus aureus* can cause furuncles or boils, painful nodules on the skin. Conventional topical therapies often lead to side effects such as contact dermatitis and pruritus, compounded by the increasing problem of antibiotic resistance. Natural ingredients like celery (*Apium graveolens L.*) offer promising alternatives due to their antibacterial properties and gentler effects.

Objective: This study aimed to formulate a celery extract gel with ideal physical characteristics and evaluate its antibacterial potency against *Staphylococcus aureus*.

Methods: Celery extract gel was prepared using HPMC, celery extract, methylparaben, propylparaben, and propylene glycol. Formulations with various celery extract concentrations were tested alongside mupirocin ointment (positive control) and gel base (negative control) against *Staphylococcus aureus* using the well-diffusion method. The results were analyzed using the One-Way ANOVA statistical test.

Results: Celery extract gel demonstrated strong antibacterial potency at 25% (F1), 50% (F2), and 75% (F3) concentrations and very strong potency at 100% (F4). The One-Way ANOVA analysis showed a significant difference in the mean inhibitory zone diameter between treatment groups ($p < 0.05$).

Conclusion: Celery extract gel exhibits significant antibacterial activity against *Staphylococcus aureus*, suggesting its potential as a natural alternative for topical therapy in managing skin infections.

Keywords: antibacterial, celery, gel, Staphylococcus aureus

Introduction

Infections can occur throughout the human body, including the skin [1]. Among skin infections, those caused by Gram-positive bacteria, particularly *Staphylococcus aureus*, are common [2–4]. Normally part of the skin's flora, *S. aureus* can invade the dermis through cuts or abrasions, triggering acute inflammation and forming painful nodules known as boils or furuncles [5]. Skin infections caused by *S. aureus* are among the most prevalent infectious diseases, with over 1.5 million cases of furunculosis reported annually in the United States [6]. In Indonesia, a pyoderma profile study at the Department of Dermatology, Sanglah Hospital Denpasar (2016–2017), identified furuncles as the dominant type of pyoderma [7]. Treatment often involves incision, drainage, and antibiotics to expedite healing [8].

For minor furuncles, topical antibiotics such as 2% fusidic acid cream, 2% clindamycin gel, or 2% mupirocin ointment are often prescribed, with or without topical antiseptics like 2–10% benzoyl peroxide gel or 3–5% hypochlorite cream or soap. However, these treatments may cause

side effects such as contact dermatitis, dry skin, and pruritus. More severe cases, particularly those with systemic symptoms, require systemic antibiotics like dicloxacillin or cephalosporins [9]. Unfortunately, community-associated methicillin-resistant *S. aureus* (CA-MRSA) often renders empirical therapy with penicillins or cephalosporins ineffective [10]. MRSA strains have demonstrated resistance to a wide range of antibiotics, including penicillins, cephalosporins, chloramphenicol, aminoglycosides, macrolides, quinolones, sulfonamides, and rifampicins [11].

The irrational use of antibiotics further exacerbates bacterial resistance, weakening their efficacy and increasing treatment costs, morbidity, mortality, and the likelihood of adverse effects from multidrug use and high dosages [12,13]. According to the Global Antimicrobial Resistance and Use Surveillance System (GLASS) in 2019, Indonesia faces a relatively high level of antimicrobial resistance [14]. The growing incidence of antibiotic resistance and associated challenges highlights the need for alternative therapies using natural ingredients [15]. The World Health Organization (WHO) supports this approach, reporting that herbal medicines are widely used in Asia, Africa, and the Americas for disease treatment [16].

Celery (*Apium graveolens* L.) is a medicinal plant with known pharmacological properties, including antimicrobial activity [17]. It contains antibacterial compounds such as alkaloids, flavonoids, saponins, and tannins [18,19]. Studies have demonstrated celery extract's antibacterial efficacy, particularly against *S. aureus* [3,20,21].

For skin infections, drug formulations that enhance skin application efficacy, such as gels, are essential. Hydroxypropyl methylcellulose (HPMC) is commonly used in pharmaceutical gel formulations to achieve optimal consistency and performance [22]. Therefore, this study aims to evaluate the antibacterial properties of celery ethanol extract gel preparations against *S. aureus*, offering a potential natural alternative for treating skin infections.

Method

Sample

The test bacterium used in this study was *Staphylococcus aureus* ATCC 25923, obtained from the Surabaya Health Laboratory Center. Celery plants were collected from plantations in Oben Village, Nekamese District, Kupang Regency, East Nusa Tenggara. The study included six groups: four groups treated with varying concentrations of celery extract (25%, 50%, 75%, and 100%), determined based on prior research [3], and two control groups (mupirocin as a positive control and the gel base as a negative control). Each sample group underwent four repetitions.

Celery extraction and phytochemical screening

Fresh green celery leaves and stems were sorted, washed thoroughly, air-dried, and pulverized into powder. Celery powder was extracted via maceration with 70% ethanol in a 1:5 ratio in a sealed glass jar. The mixture was allowed to stand for three days with occasional stirring, then filtered to obtain the macerate, which was concentrated using a rotary evaporator to produce a thick extract.

Phytochemical screening was conducted to identify secondary bioactive metabolites, such as alkaloids, flavonoids, saponins, and tannins.

Gel formulation

The celery extract gel was prepared starting with the gel base. Hydroxypropylmethylcellulose (HPMC) powder was diluted in approximately 50 mL of distilled water heated to 80°C. Methylparaben and propylparaben were dissolved in propylene glycol. Once the HPMC expanded, it was mixed with the dissolved parabens. The ethanol extract of celery was then incorporated into the gel base, followed by the addition of distilled water, and the mixture was stirred until homogeneous [22,23]. The final gel was stored in 100 mL labeled tubes according to the extract concentration.

The gel was evaluated for physical characteristics to ensure usability and comfort, including organoleptic properties, homogeneity, pH, and spreadability tests.

Table 1. Formulation of celery extract gel preparation

Material	Formulation (%)					Function
	Negative control	F1	F2	F3	F4	
Celery extract	-	25	50	75	100	Active substance
HPMC	2	2	2	2	2	Gelling agent
Propylenglycol	15	15	15	15	15	Humectants
Metylparaben	0.075	0.075	0.075	0.075	0.075	Preservatives
Propylparaben	0.025	0.025	0.025	0.025	0.025	Preservatives
Aquades ad	100	100	100	100	100	Solvent

Antibacterial test

The bacterial species were confirmed via Gram staining, catalase tests, and mannitol fermentation tests to ensure purity and the absence of contamination. Bacterial suspensions were prepared by mixing 0.9% NaCl, 1–2 streaks of rejuvenated bacteria, 0.05 mL of 1.175% $BaCl_2.2H_2O$, and 1% H_2SO_4 to achieve turbidity equivalent to 0.5 McFarland, corresponding to 1–2 × 10^8 CFU/mL [24].

A 1 mL bacterial suspension was spread onto nutrient agar (NA) test media (20% b/v), allowing the media to absorb. Wells were prepared in the agar, and 100 µL of celery ethanol extract at different concentrations (25%, 50%, 75%, 100%), as well as positive and negative controls, were added to the wells. Plates were incubated at 37°C for 18–24 hours, and the inhibition zone diameter (clear zone around the wells) was measured and categorized based on Davis and Stout's (1971) criteria [25].

Data analysis

Data were analyzed using the One-Way ANOVA statistical test, followed by Dunnett T3 Post Hoc analysis. A p-value of < 0.05 was considered statistically significant.

Results

Celery extraction

A total of 10 kg of celery plants were sorted, washed, drained, and air-dried at room temperature for seven days. After being mashed and sieved, 790 grams of celery powder was obtained. The powder was macerated in 3,950 mL of 70% ethanol (1:5 b/v) and stirred intermittently for three days. The resulting mixture was filtered, and the extract was concentrated using a rotary evaporator, yielding 169 grams of viscous celery extract with a 21.4% yield.

Ethanol free test and phytochemical screening

An ethanol-free test confirmed the absence of ethanol in the final extract (Figure 1), ensuring that any observed antibacterial activity was attributable to the celery extract. Phytochemical screening identified the presence of secondary metabolites, including alkaloids, flavonoids, saponins, and tannins (Figure 2).

Evaluation of gel's physical characteristics

The physical characteristic evaluation of the celery extract gel formulations showed that formulations with 25% (Formulation 1), 50% (Formulation 2), and 75% (Formulation 3) extract met optimal standard values (Figure 3, Table 2).

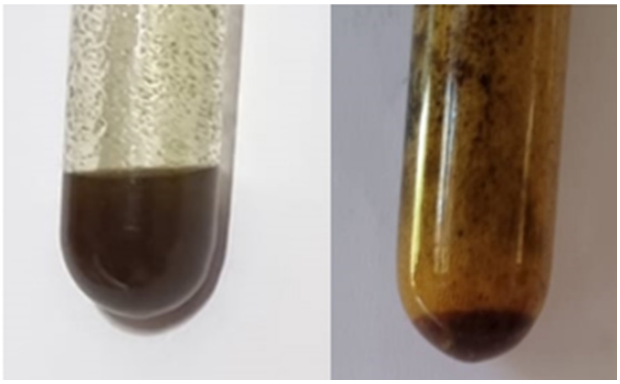

Figure 1. The result of ethanol-free test

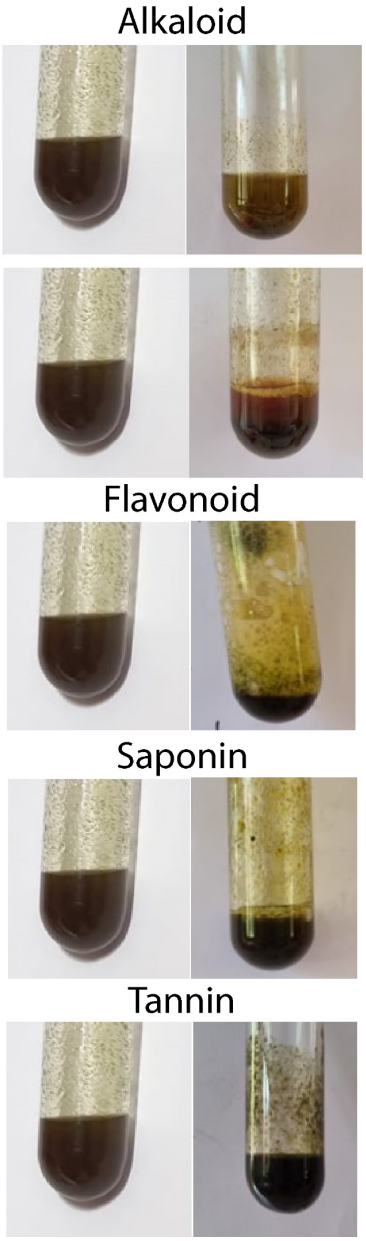

Figure 2. The result of phytochemical screening

Antibacterial test

Gram staining revealed cocci arranged in clusters resembling purplish grapes, confirming that the bacteria were Gram-positive. The catalase test produced gas bubbles (O_2) upon the addition of H_2O_2, and the mannitol fermentation test on Mannitol Salt Agar (MSA) showed a color change from red to yellow after 24–48 hours of incubation.

The antibacterial potency test revealed that celery extract gel effectively inhibited the growth of *S. aureus*, with larger inhibition zones observed as extract concentrations increased. Formulations F1 (25%), F2 (50%), and F3 (75%) were categorized as having strong antibacterial activity, while F4 (100%) and the positive control exhibited very strong antibacterial activity (Figures 4, 5). Bivariate analysis using the One-Way ANOVA test and Post Hoc Dunnett T3 test revealed significant differences between treatment groups. The normality test indicated that the data were normally distributed ($P > 0.05$). The One-Way ANOVA test yielded a p-value of 0.000 (<0.05), confirming a statistically significant difference in the mean inhibition zone diameters among the groups.

Figure 3. Preparation of celery extract gel in tube packaging

Table 2. Results of physical characteristic evaluation of celery extract gel

Evaluation	Negative control	F1 (25%)	F2 (50%)	F3 (75%)	F4 (100%)	Standard value
Organoleptic:						
Smell	Typical gel	Typical celery	Typical celery	Typical celery	Typical celery	-
Color	Clear	Green	Blackish green	Blackish green	Blackish green	-
Consistency	Semisolid	Semisolid	Semisolid	Semisolid	Liquid	Semisolid
Homogenity	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	6.91	5.67	5.48	5.40	5.28	4.5-6.5
Spreadability	5.55 cm	5.85 cm	6.50 cm	6.65 cm	9.80 cm	5-7 cm
Interpretation	Not optimal	Optimal	Optimal	Optimal	Not optimal	Optimal

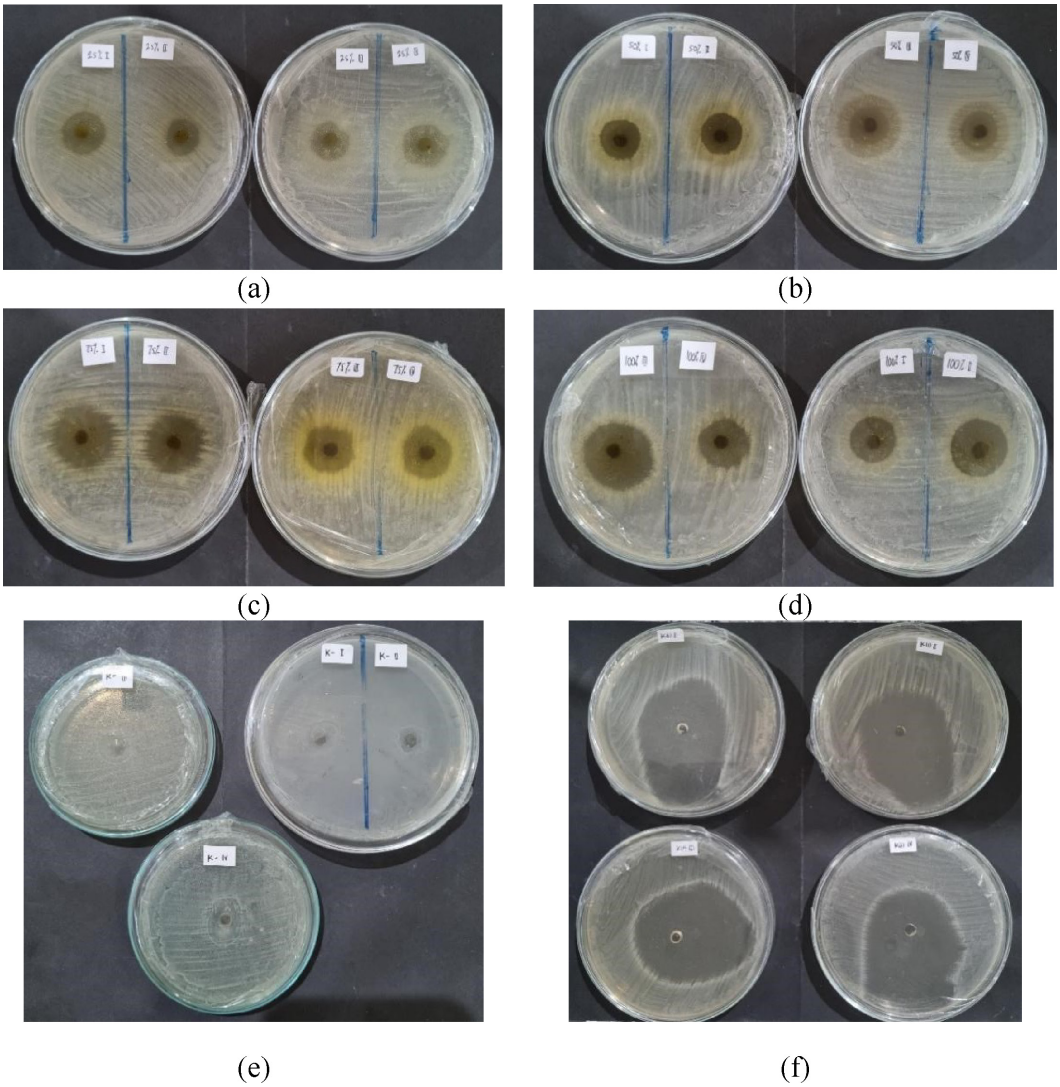

Figure 4. Antibacterial test results for celery ethanol extract gel concentrations: (a) 25% (F1), (b) 50% (F2), (c) 75% (F3), (d) 100% (F4), (e) negative control, (f) positive control

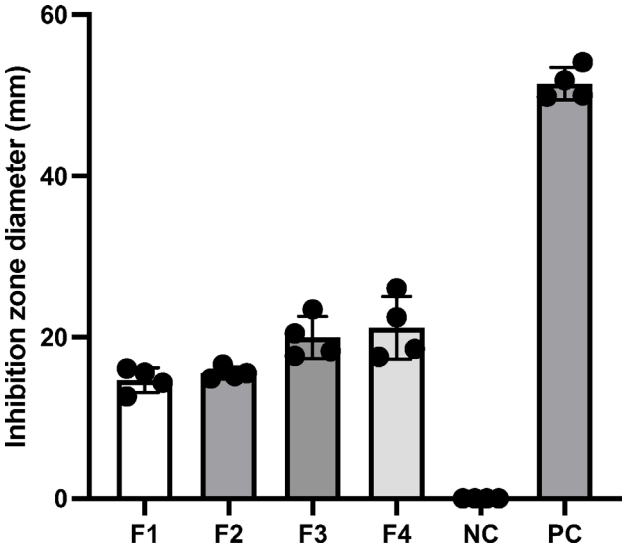

Figure 5. Inhibition zone diameters of celery ethanol extract gel against *S. aureus* growth

Discussion

This study demonstrated the antibacterial potential of celery (*Apium graveolens L.*) ethanol extract gel against *Staphylococcus aureus*. The maceration method yielded a thick extract with a 21.4% yield, considered optimal (>10%), indicating a high content of bioactive components [26]. The ethanol-free test confirmed that ethanol was absent, ensuring that the observed antibacterial activity was attributed solely to the extract. Phytochemical screening identified the presence of alkaloids, flavonoids, saponins, and tannins, consistent with prior studies on celery's bioactive properties [19,27].

The physical evaluation of the gel formulations revealed that odor, color, and consistency were

influenced by the concentration of celery extract. While formulations containing 25%, 50%, and 75% extract had semisolid consistency and met optimal spreadability standards, the 100% extract formulation had a liquid consistency, failing the spreadability test. The pH of all formulations fell within the safe range for skin application, and the homogeneity test confirmed that all formulations were free from lumps or coarse grains.

The antibacterial test revealed a strong correlation between extract concentration and inhibition zone size, supporting findings from previous research [3,28]. Based on Davis and Stout's classification [25], formulations with 25%, 50%, and 75% celery extract exhibited strong antibacterial activity (<20 mm inhibition zone), while the 100% extract formulation (21.16 mm inhibition zone) and positive control (51.45 mm inhibition zone) demonstrated very strong antibacterial activity.

The antibacterial effects of celery extract can be attributed to its secondary metabolites. Alkaloids disrupt bacterial cell walls by interfering with peptidoglycan synthesis, leading to cell death [29]. Flavonoids damage cell membranes through lipophilicity, inhibit nucleic acid synthesis, and disrupt bacterial respiratory chains [30]. Saponins perforate cell membranes by interacting with sterols, leading to biofilm collapse and membrane rupture [31]. Tannins inhibit bacterial attachment, sugar and amino acid absorption, and bacterial growth, ultimately causing cell death [32].

Mupirocin, used as the positive control, demonstrated its broad-spectrum antibacterial and anti-biofilm properties, effectively treating *S. aureus* skin infections by inhibiting bacterial RNA and protein synthesis [33,34]. The absence of inhibition zones in the negative control confirmed that the gel base did not contribute to the antibacterial effects.

This study has several limitations. The antibacterial efficacy of the gel was not tested in vivo, which would provide valuable insights into its practical effectiveness, comfort, and usability when applied to the skin. While the F4 formula demonstrated the highest inhibition zone diameter, it did not meet the spreadability requirements. Optimization of this formulation is needed, potentially by using different excipients to achieve the desired consistency and usability.

Conclusion

Celery ethanol extract gel demonstrated strong antibacterial activity against *S. aureus* at concentrations of 25%, 50%, and 75%, and very strong activity at 100%. Formulations containing 25%, 50%, and 75% celery extract met the optimal physical characteristics for gel preparation, making them suitable for topical application.

Funding

None.

Declaration of interest

The authors declare no conflict of interest.

Author contributions

MES: conceptualization, investigation, methodology, writing – original draft, supervision, project administration; ALSA: investigation, data curation, writing – review & editing; AST: validation, formal analysis, visualization; PDP: resources, writing – review & editing.

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