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Original Article

Antibacterial Activity Test of Combined Extracts of Guava Leaves (*Psidium guajava* L.) and Celery Stalks (*Apium graveolens* L. *var. dulce*) Against *Staphylococcus aureus* and *Escherichia coli*

Uji Aktivitas Antibakteri Ekstrak Gabungan Daun Jambu Biji (Psidium guajava L.) dan Batang Seledri (*Apium graveolens* L. *var. dulce*) Terhadap *Staphylococcus aureus* dan *Escherichia coli*

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Abstract

Antibiotic resistance in pathogenic bacteria such as *Staphylococcus aureus* and *Escherichia coli* has become a critical global health concern. Flavonoids, tannins, and saponins in guava leaves (*Psidium guajava* L.) and celery stalks (*Apium graveolens* L. *var. dulce*) suggest their potential as natural antibacterial agents, but studies on their combined effectiveness remain limited. This study aimed to evaluate the synergistic effect, optimal concentration, and antibacterial activity of combined guava leaf and celery stalk extracts against gram-positive and gram-negative bacteria. This laboratory-based experimental study used the maceration method with 96% ethanol as the solvent. Antibacterial activity was assessed through the disc diffusion method against *S. aureus* and *E. coli*. The study tested 40% single extracts and combinations in ratios of 1:1, 1:2, and 2:1. The findings showed that the combination did not improve antibacterial activity compared with the most active single extract under the disc diffusion assay. The highest antibacterial activity against *S. aureus* was observed in the 2:1 combination, producing an inhibition zone of 11.06 mm, although still lower than the 40% guava leaf extract (11.75 mm). In *E. coli*, all treatments demonstrated weak activity, with the 1:1 combination yielding the highest inhibition zone of 3.74 mm. Statistical analysis using ANOVA and Games-Howell indicated a significant difference for *S. aureus*, while the Kruskal-Wallis test showed no significant difference for *E. coli* ($p > 0.05$). The combination of guava leaf and celery stalk extracts did not exhibit a synergistic effect. The 2:1 combination was the most effective against *S. aureus*, while the 1:1 combination showed limited activity against *E. coli*.

Abstrak

Resistensi antibiotik pada bakteri patogen seperti *Staphylococcus aureus* dan *Escherichia coli* telah menjadi masalah kesehatan global yang kritis. Flavonoid, tanin, dan saponin dalam daun jambu biji (*Psidium guajava* L.) dan batang seledri (*Apium graveolens* L. *var. dulce*) menunjukkan potensi mereka sebagai agen antibakteri alami, tetapi studi tentang efektivitas gabungan mereka masih terbatas. Studi ini bertujuan untuk mengevaluasi efek sinergis, konsentrasi optimal, dan aktivitas antibakteri dari ekstrak daun jambu biji dan batang seledri gabungan terhadap bakteri gram positif dan gram negatif. Studi eksperimental berbasis laboratorium ini menggunakan metode maserasi dengan etanol 96% sebagai pelarut. Aktivitas antibakteri dinilai melalui metode difusi cakram terhadap *Staphylococcus aureus* and *Escherichia coli*. Studi ini menguji 40% ekstrak tunggal dan kombinasi dalam rasio 1:1, 1:2, dan 2:1. Hasil penelitian menunjukkan bahwa kombinasi tersebut tidak meningkatkan aktivitas antibakteri dibandingkan dengan ekstrak tunggal yang paling aktif dalam uji difusi cakram. Aktivitas antibakteri tertinggi terhadap *Staphylococcus aureus* diamati pada kombinasi 2:1, menghasilkan zona inhibisi sebesar 11,06 mm, meskipun masih lebih rendah daripada ekstrak daun jambu biji 40% (11,75 mm). Pada E. coli, semua perlakuan menunjukkan aktivitas yang lemah, dengan kombinasi 1:1 menghasilkan zona inhibisi tertinggi sebesar 3,74 mm. Analisis statistik menggunakan ANOVA dan Games-Howell menunjukkan perbedaan yang signifikan untuk *Staphylococcus aureus*, sedangkan uji Kruskal-Wallis menunjukkan tidak ada perbedaan yang signifikan untuk E. coli ($p > 0,05$). Kombinasi ekstrak daun jambu biji dan batang seledri tidak menunjukkan efek sinergis. Kombinasi 2:1 adalah yang paling efektif terhadap S. aureus,

sedangkan kombinasi 1:1 menunjukkan aktivitas terbatas terhadap E. coli.

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INTRODUCTION

Bacterial infections are one of the main factors causing disease worldwide. In 2019, there were 7.7 million deaths due to bacterial infections caused by 33 different types of bacteria.[1] Antibiotics are used for the treatment of bacterial infections by inhibiting and killing bacteria. However, inappropriate use of antibiotics can lead to bacterial resistance.[2]

S. aureus is a major cause of death in 135 countries. In 2019, more than 1 million deaths occurred, with 299,000 cases associated with fatal bloodstream infections.[1] Based on a 2022 survey by the *World Health Organization* (WHO) on the distribution of priority pathogens in 70 hospitals across 15 provinces in Indonesia, the percentage of *Methicillin-Resistant S. aureus* (MRSA) in Papua was 100%, higher than in DKI Jakarta at 30%.[3] A total of 290,000 deaths occurred due to fatal peritoneal and intra-abdominal infections caused by *E. coli*. Deaths due to *E. coli* occurred in 37 cities across various countries.[1] The percentage of *E. coli* resistance to cephalosporin antibiotics was highest in Sumatra at 75%, followed by DKI Jakarta at 68%. Meanwhile, *E. coli* resistance to carbapenem antibiotics in DKI Jakarta was 13%.[3]

Secondary metabolite compounds contained in guava leaf (*Psidium guajava* L.) and celery stalk (*Apium graveolens* L. *var. dulce*) are known to contain flavonoids, saponins, and tannins, which are effective as antibacterial agents.[4,5] Based on research, *Psidium guajava* L. leaf extract effectively inhibits the growth of *S. aureus* at a concentration of 15% with an inhibition zone of 6.75 mm.[6] Meanwhile, against *E. coli*, a concentration of 50% produces an inhibition zone of 10.42 mm with strong effectiveness.[7] *Apium graveolens* L. *var. dulce* has the potential to inhibit the growth of *S. aureus* at a concentration of 20% with an inhibition zone of 2.8 mm.[5] The maceration method was chosen because it is effective in extracting secondary metabolite compounds such as flavonoids, tannins, alkaloids, steroids, and terpenoids.[8]

Guava leaf (*Psidium guava* L.) and celery stalk (*Apium graveolens* L. var. *dulce*) were selected due to their well-documented antibacterial properties, particularly flavonoids, saponins, and tannins, which work through distinct mechanisms: flavonoids disrupt cell membrane integrity, tannins precipitate bacterial proteins, and saponins increase membrane permeablitiy [9] Combining the two extracts is expected to produce a synergistic antibacterial effect by simultaneously targeting multiple bacterial mechanisms. [16] *S. aureus* and *E. coli* were chosen as bacterial models because they represent Gram-positive and Gram-negative bacteria with structurally different cell walls, making them suitable for a comprehensive evaluation of antibacterial activity. The combination ratios of 1:1, 1:2, and 2:1 were selected to systematically determine the most effective proportion of each extract and to identify the optimal ratio for synergistic activity. [16] To date, no study has investigated the antibacterial activity of the combined extract of *Psidium guava* L. leaf and *Apium graveolens* L. var. *dulce* against both *S. aureus* and *E. coli*, representing a research gap that this study aims to address.

METHODS

The study was conducted using an experimental laboratory method. It aims to determine the synergistic effect, potential concentration, and antibacterial effectiveness of the combination of guava leaf extract (*Psidium guajava* L.) and celery stalk (*Apium graveolens* L. *var. dulce*) against Gram-positive and Gram-negative bacteria. The sample extraction method used the maceration principle with 96% ethanol pro analysis as the solvent. The antibacterial activity test was carried out using the disc diffusion method. The study was conducted at the Microbiology and Phytochemistry Laboratory of STIKes IKIFA from December 2024 to August 2025.

Tools : Analytical balance (OHAUS), evaporating dish (Pyrex), beaker glass (Pyrex), 60-mesh sieve, grinder, rotary evaporator (IKA RV 8), maceration vessel, glass funnel (Pyrex), stirring rod, test tubes (Pyrex), measuring cylinder (Iwaki), beaker glass (Pyrex), test tube rack, Bunsen burner, Petri dish (Norma), Erlenmeyer flask (Pyrex), hot plate (Maspion), autoclave (Hirayama), incubator, oven (Shimadem), forceps, vortex mixer (Dragon Lab), micropipette (Dragon Lab) and tips, vernier caliper, spatula, inoculating loop, laminar air flow cabinet, microscope slide.

Materials: *Psidium guajava* L. leaves, *Apium graveolens* L. *var. dulce*, 96% ethanol pro analysis, distilled water, Nutrient Agar (NA) medium (Millipore), filter paper, aluminum foil, HVS paper, magnesium powder (Mg), concentrated HCl, 10% $FeCl_3$ solution, ciprofloxacin 500 mg tablets (Hexapharm Jaya), 0.9% NaCl, disc paper, 0.5 McFarland solution, sterile cotton swab, Eosin Methylene Blue agar (Millipore), Gram staining kit (Indoreagen).

Research Procedure

1. **Plant collection**

   A total of 2 kg of dried *Psidium guajava* L. leaves were obtained from UPT Laboratorium Herbal Materia Medica Batu, and 4 kg of fresh *Apium graveolens* L. *var. dulce* were obtained from a plantation area in Lembang, Bandung.

2. **Plant Determination**

   Plant determination was carried out at Yayasan Generasi Biologi Indonesia for *Apium graveolens* L. *var. dulce* and at UPT Laboratorium Herbal Materia Medica Batu for *Psidium guajava* L. leaves.

3. **Sample preparation**

   Fresh simplicia were wet-sorted, washed, cut, and oven-dried at 50°C. The dried simplicia were dry-sorted, ground, and sieved through a 60-mesh sieve. The resulting powder was stored in a tightly closed container protected from light.

4. **Extract preparation**[9]

   A total of 200 g of *Psidium guajava* L. leaf simplicia powder and 227 g of *Apium graveolens* L. *var. dulce* simplicia powder were weighed and macerated with 96% ethanol (4000 mL and 4540 mL, respectively) at a 1:10 (w/v) ratio. The samples were soaked for 6 hours with occasional stirring and then left to stand for 18 hours. The extraction was repeated using half of the initial solvent volume. All macerates were collected and filtered, and the filtrates were evaporated under reduced pressure using a rotary evaporator to obtain a thick extract. The extracts were stored in brown containers, and the extraction yield was calculated.

$$\text{Extract yield} = \frac{\text{Weight of thick extract}}{\text{Weight of dry powder}} \times 100\%$$

5. **Extract evaluation test**

   a. Organoleptic test[9]

Psidium guajava L. extract showed a thick consistency, dark brown color, characteristic odor, and astringent taste, while *Apium graveolens* L. *var. dulce* extract showed a thick consistency, dark green color, and characteristic odor and taste.

b. Phytochemical screening

1) Flavonoid Shinoda test[4]

Mix 1 mL of diluted extract with 0.5 g magnesium powder, then add 2 to 3 drops of concentrated hydrochloric acid. A positive result is indicated by a red or yellow color.

2) Flavonoid alkaline reagent test[10]

Add a few drops of sodium hydroxide solution to 1 mL of extract. A positive result is indicated by a yellow color.

3) Tannin test[4]

Add 2 to 4 drops of 10 percent ferric chloride solution to 1 mL of diluted extract. A positive result is indicated by a bluish black or green color.

4) Saponin test[11]

Mix 1 g of extract with 10 mL of hot water, allow to cool, and shake for 10 minutes. A positive result is indicated by stable foam with a height of 1 to 10 cm that persists for at least 10 minutes and remains after the addition of 2N hydrochloric acid.

c. Total flavonoid content[9]

Dissolve approximately 0.2 g of extract in 25 mL of ethanol, stir for 30 minutes, filter, and adjust to volume. Prepare standard solutions in ethanol at concentrations of 100, 75, 50, and 25 µg per mL. Mix 0.5 mL of sample or standard with ethanol, aluminum chloride, sodium acetate, and water, then incubate for 30 minutes at room temperature. Measure absorbance at the maximum wavelength using a blank without aluminum chloride, construct a calibration curve, and calculate the flavonoid content

6. Bacterial indentification

Gram staining was carried out by inoculating bacterial culture onto a glass slide containing physiological NaCl, followed by heat fixation and sequential staining with crystal violet, Lugol's iodine, alcohol decolorization, and safranin as a counterstain; Gram-positive bacteria were identified by their purple coloration and coccal morphology, whereas Gram-negative bacteria appeared pink to red with rod-shaped morphology when observed under a microscope.[12] Identification of Escherichia coli was performed by streaking a bacterial isolate onto Eosin Methylene Blue (EMB) agar and incubating at 35°C for 24 hours, with positive results indicated by the formation of characteristic metallic green colonies.[13]

7. Antibiotic and extract sensitivity test[5]

Antibacterial activity was evaluated using the disc diffusion method by preparing ciprofloxacin solutions (2 and 4 µg/mL) and plant extracts of guava leaves and celery stalks at concentrations of 10%, 20%, 30%, 40%, and 50%. Nutrient Agar (NA) was poured into Petri dishes and allowed to solidify, followed by inoculation of bacterial suspension using a sterile cotton swab. Sterile paper discs (6 mm diameter) were impregnated with 20 µL of each test solution, placed on the inoculated media, and experiments were conducted in triplicate for each concentration. Plates were incubated at 37°C for 24 hours, after which the inhibition zones were observed and

measured. All used materials were decontaminated by immersion in disinfectant solution for approximately 30 ± 5 minutes, followed by boiling for 30 ± 5 minutes.

8. **Antibacterial activity test**[5]

Antibacterial activity of guava leaf (Psidium guajava L.) extract (40%; F1), celery stalk (Apium graveolens L. var. dulce) extract (40%; F2), and their combinations at ratios of 1:1 (F3), 1:2 (F4), and 2:1 (F5) was evaluated using the disc diffusion method. Bacterial suspensions were spread onto solidified Nutrient Agar (NA), followed by placement of 6 mm sterile discs impregnated with 20 µL of each test solution, including ciprofloxacin (4 µg/mL) as a positive control and 96% ethanol as a negative control. All treatments were performed in triplicate and incubated at 37°C for 24 h, after which inhibition zones were measured. All materials were decontaminated by immersion in disinfectant and subsequent boiling (30 ± 5 min each).

Data Analysis

Data analysis was performed by measuring the Diameter of Inhibition Zone (DIZ) using a caliper (mm), calculated as[14]:

$$\frac{(a-c)+(b-c)}{2}$$

Description:

a = vertical diameter

b = horizontal diameter

c = paper disc diameter

The data were further analyzed using one-way ANOVA with Statistical Product and Service Solutions (SPSS) version 26.0 for Windows.

RESULT AND DISCUSSION

1. **Plant Determination**

Fresh celery stalks was collected from a plantation in Lembang, Bandung and identified as *Apium graveolens* subsp. *dulce* (Mill.) Schübl. & G.Martens Dried guava leaf was obtained from UPT Laboratorium Herbal Materia Medica Batu and identified as *Psidium guajava* L.

2. **Extract Preparation**

The maceration method was employed to preserve thermolabile antibacterial constituents, including flavonoids, tannins, and saponins.[15] The extraction yield of celery stem was 14.27%, while that of guava leaf extract was 13.29%. The yields complied with the Indonesian Herbal Pharmacopoeia (FHI) 2nd edition requirement of not less than 12.3%.[9] However, specific standards for celery stem yield remain unavailable. Yield may be influenced by factors including plant variety, harvest age, geographical conditions, extraction parameters, solvent type, agitation intensity, extraction time, and temperature.[8]

3. **Extract evaluation test**

Guava leaf extract was a viscous, dark brown extract with a characteristic odor. In contrast, celery stem extract was dark green, exhibited a characteristic celery odor, and was viscous in form. The organoleptic properties of guava leaf extract complied with the Indonesian Herbal Pharmacopoeia (FHI) 2nd edition.[9] However,

specific standards for the organoleptic evaluation of celery stem have not been reported. This evaluation was conducted as a preliminary identification based on sensory characteristics perceived by the senses.

Table 1. Results of Phytochemical Screening

No.	Simplicia	Identification	Result	Remark
1	Guava Leaf	Flavonoid (shinoda)	Yellow	Positive (+)
2		Flavonoid (alkaline reagent)	Orange	Positive (+)
3		Tannin	Greenish-black	Positive (+)
4		Saponin	Foam height 5 cm within ≥10 minutes and persists after addition of 2N HCl	Positive (+)
5	Celery Stalk	Flavonoid (shinoda)	Yellow	Positive (+)
6		Flavonoid (alkaline reagent)	Yellow	Positive (+)
7		Tannin	Orange	Negative (-)
8		Saponin	Foam height 1 cm within ≥10 minutes and persists after addition of 2N HCl	Positive (+)

In Table 1. Phytochemical screening was performed to confirm antibacterial secondary metabolites, namely flavonoids, tannins, and saponins, using specific reagents. Guava leaf extract was positive for all three compounds, whereas celery stem extract contained flavonoids and saponins only. Flavonoids were identified using Shinoda (concentrated HCl + Mg) and NaOH reagents. The Shinoda reaction produces flavilium salts, indicated by an orange color due to formation of a flavilium cation.[16] The NaOH test confirmed flavonoids through formation of yellow–brown acetophenone derivatives following C-ring cleavage.[16] Flavonids contribute to antibacterial activity by disrupting bacterial cell membrane integrity and inhibiting nucleic acid synthesis, thereby impairing bacterial growth and replication. [17] Tannins reacted with $FeCl_3$ to form blue-black or dark green complexes via interaction with phenolic hydroxyl groups and Fe^{3+}.[16] Tannins precipitate bacterial proteins and inhibit bacterial enzymes, leading to disruption of metabolic process essential for bacterial survival. [9] Saponins showed positive results by stable foam formation due to their amphiphilic properties and micelle formation.[5] This amphiphilic nature enables saponins to interact with and increase bacterial membrane permeability, causing leakage of intracellular contents and ultimately bacterial cell death. [9]

Total flavonoid content was determined to quantify antibacterial bioactive compounds and was analyzed at Agricultural Post-Harvest Assembly and Modernization Agency. Guava leaf extract contained 600.05 mg/100 g, while celery stalks extract contained 1851.83 mg/100 g. Differences may reflect variation in flavonoid types, as the method does not distinguish individual compounds. Guava leaves are rich in quercetin, which exhibits antibacterial activity by disrupting cell permeability, inhibiting nucleic acid synthesis, and reducing enzyme activity.[9,17] In contrast, celery stem predominantly contains apigenin, which shows antibacterial effects through

membrane damage and intracellular disruption.[9,14] Despite its lower total flavonoid content, guava leaf extract demonstrated stronger antibacterial activity, likely due to the presence of quercetin.[17]

4. **Bacterial identification**

Bacterial identification was performed to confirm the species used in this study. Gram staining is based on the ability of bacterial cell walls to retain or release the crystal violet–iodine (CV–I) complex during alcohol decolorization.[12] Gram-positive bacteria such as *S. aureus* possess a thick peptidoglycan layer that retains the CV–I complex, resulting in a purple color.[12] In contrast, Gram-negative bacteria such as *E. coli* have a thin peptidoglycan layer and an outer membrane, allowing alcohol to extract the CV–I complex, after which cells take up safranin and appear red.[12] Microscopic observation showed *S. aureus* as purple cocci in clusters, consistent with reported characteristics.[12] *E. coli* appeared as red rod-shaped cells, also in agreement with the literature.[12]

To confirm *E. coli* identification, a biochemical test was performed using selective differential agar. This medium inhibits Gram-positive bacteria and differentiates Gram-negative bacteria based on metabolic activity. After incubation on EMB agar, *E. coli* produced colonies with a characteristic metallic green sheen.[13] This feature is attributed to strong lactose fermentation, leading to acid production and precipitation of eosin Y and methylene blue, forming the metallic green complex on colony surfaces.[12,13]

5. **Antimicrobial Susceptibility Testing and Antibacterial Activity**

Table 2a. Antibacterial Activity of Single Extracts at Various Concentrations

Bacteria	Treatment	Concentration	Inhibition Zone Diameter (mm) Mean ± SD	n	Activity Category	Sig.
S. aureus	Ciprofloxacin	2 µg/mL	8.07 ± 0.180	3	Moderate	a
		4 µg/mL	9.43 ± 1.038	3	Moderate	a
	Guava Leaf	10%	11.53 ± 1.153	3	Strong	a
		20%	13.23 ± 1.565	3	Strong	ab
		30%	12.47 ± 1.056	3	Strong	ab
		40%	13.23 ± 1.276	3	Strong	b
		50%	13.56 ± 1.165	3	Strong	b
	Celery Stalk	10%	2.67 ± 0.227	3	Weak	a
		20%	2.73 ± 0.455	3	Weak	a
		30%	3.76 ± 0.322	3	Weak	a
		40%	5.27 ± 0.719	3	Moderate	b
		50%	5.87 ± 0.681	3	Moderate	b
E. coli	Ciprofloxacin	2 µg/mL	9.28 ± 1.678	3	Moderate	a
		4 µg/mL	10.07 ± 0.840	3	Strong	a
	Guava Leaf	10%	0.99 ± 0.191	3	Weak	a
		20%	1.82 ± 0.250	3	Weak	a

Bacteria	Treatment	Concentration	Inhibition Zone Diameter (mm) Mean ± SD	n	Activity Category	Sig.
		30%	3.75 ± 0.844	3	Weak	b
		40%	4.21 ± 0.514	3	Weak	b
		50%	4.09 ± 0.399	3	Weak	b
		10%	0 ± 0	3	—	a
		20%	1.19 ± 0.382	3	Weak	ab
	Celery Stalk	30%	0.86 ± 0.889	3	Weak	ab
		40%	1.73 ± 0.201	3	Weak	b
		50%	1.94 ± 0.326	3	Weak	b

Note: Different letters within each treatment group indicate significant differences ($p < 0.05$). Ciprofloxacin: one-way ANOVA, $p = 0.506$ (E. coli), $p = 0.088$ (S. aureus). Guava leaf vs. S. aureus: one-way ANOVA, $p = 0.345$ (ns). Guava leaf vs. E. coli: one-way ANOVA, $p = 0.000$, Post-hoc Tukey. Celery stalk vs. S. aureus: one-way ANOVA, $p = 0.000$, Post-hoc Tukey. Celery stalk vs. E. coli: Kruskal-Wallis, $p = 0.039$. ns = not significant.

Table 2b. Antibacterial Activity of 40% Single Extracts and Their Combination

Bacteria	Treatment	Ratio/Concentration	Inhibition Zone Diameter (mm) Mean ± SD	n	Activity Category	Sig.
	Guava Leaf	40%	11.75 ± 0.368	3	Strong	a
	Celery Stalk	40%	4.58 ± 0.438	3	Weak	b
		1:1	10.26 ± 0.463	3	Strong	a
S. aureus	Combination	1:2	8.09 ± 2.124	3	Moderate	ab
		2:1	11.06 ± 1.094	3	Strong	a
	Control (+)	4 µg/mL	9.95–13.74	3	Moderate–Strong	—
	Control (−)	—	0 ± 0	3	—	—
	Guava Leaf	40%	3.25 ± 0.498	3	Weak	a
	Celery Stalk	40%	2.47 ± 0.788	3	Weak	a
		1:1	3.74 ± 1.769	3	Weak	a
E. coli	Combination	1:2	3.28 ± 0.958	3	Weak	a
		2:1	2.43 ± 0.672	3	Weak	a
	Control (+)	4 µg/mL	13.28–17.45	3	Strong	—
	Control (−)	—	0 ± 0	3	—	—

Note: Different letters within each bacterial group indicate significant differences ($p < 0.05$). S. aureus: one-way ANOVA, $p = 0.000$, Post-hoc Games-Howell (data not homogeneous, Levene $p = 0.033$). E. coli: Kruskal-Wallis, $p = 0.335$ (no significant difference among treatments). Variation in positive control values reflects independent experimental runs conducted on different days. ns = not significant.

Following bacterial identification, antibiotic sensitivity and extract activity were evaluated using inhibition zone diameter as the primary parameter. Ciprofloxacin was used as a positive control and exhibited antibacterial activity against both bacteria, with the highest inhibition observed against *E. coli* at 4 µg/mL, measuring 10.07 mm and categorized as strong, compared to S. aureus, measuring 9.43 mm and categorized as moderate.

Guava leaf extract demonstrated strong antibacterial activity against *S. aureus*, with the highest inhibition at 50% concentration, measuring 13.56 mm, followed closely by 40% at 13.23 mm. In contrast, activity against *E. coli* was markedly lower, with the highest inhibition observed at 40%, measuring 4.21 mm and categorized as weak. The lower activity against *E. coli* compared to *S. aureus* is likely attributable to structural differences in their cell walls. As a Gram-negative bacterium, *E. coli* possesses an outer membrane composed of lipopolysaccharides (LPS) that acts as an additional permeability barrier, limiting the penetration of hydrophilic antibacterial compounds such as tannins and saponins into the cell. [12] This outer membrane reduces the accessibility of bioactive compounds to their intracellular targets, resulting in smaller inhibition zones compared to *S. aureus*, which lacks this outer membrane and is therefore more susceptible to tested extracts.

Celery stalk extract showed moderate activity against *S. aureus* at higher concentrations, with the greatest inhibition at 50%, measuring 5.87 mm, and at 40%, measuring 5.27 mm. However, its activity against *E. coli* was weak to negligible, with the highest inhibition at 50%, measuring 1.94 mm.

Overall, both extracts exhibited increased antibacterial activity with increasing concentration. However, the difference between 40% and 50% concentrations was minimal, indicating a plateau effect.[12] Therefore, 40% concentration was considered the most optimal and efficient for antibacterial activity testing.

Antibacterial testing of 40% guava leaf extract, 40% celery stem extract, and their combinations showed that against *S. aureus*, guava leaf extract alone produced the highest inhibition of 11.75 mm, while celery stalk showed the lowest at 4.58 mm. No combination exceeded the activity of guava leaf extract, although the 2:1 ratio showed the closest effect at 11.06 mm.

Against *E. coli*, all treatments exhibited weak activity. The highest inhibition was observed in the 1:1 combination at 3.74 mm, slightly higher than guava leaf extract at 3.25 mm and celery stalk extract at 2.47 mm. Overall, combinations did not markedly improve antibacterial activity compared to single extracts. Differences in inhibition zone results between single extracts and their combinations may be influenced by several factors, including extract stability and homogeneity, variations in bacterial suspension density, and agar medium thickness.[18]

LIMITATION

This study is subject to several limitations. The antibacterial evaluation relied on the disc diffusion method, which primarily reflects inhibition zone formation and may not accurately quantify the minimum effective concentration of the extracts. The absence of MIC and MBC determination limits the interpretation of antibacterial potency. In addition, only a single extract concentration was selected for combination testing, which may not fully capture the potential interaction profile between the two extracts. The study was also restricted to two representative bacterial strains, thereby limiting broader applicability. Future studies should incorporate quantitative

antibacterial assays, evaluate a wider range of extract concentrations and ratios, and include additional microbial strains to better characterize the antibacterial potential and interaction effects.

CONCLUSION

The combination of *Psidium guava* L. leaf extract and *Apium graveolens* L. var. *dulce* stalk extract did not enhance antibacterial activity compared with the most active single extract. Against *S. aureus*, the 2:1 combination showed the highest inhibition among the combinations, with an inhibition zone of 11.06 mm, but it remained lower than 40% guava leaf extract. Against E. *coli*, all combination showed weak antibacterial activity, with the 1:1 combiantion producing the highest inhibition zone of 3.74 mm.

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AUTHOR CONTRIBUTION STATEMENT

DIS: Conceptualization, Methodology, Validation, Investigation, Resources, Writing-Original draft, Writing-review & editing, ; **PES**: Software, Formal analysis, Data curation, Visualization ; **IAH**: Project administration, Funding acquisition, Supervision.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest

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