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Article

Antibacterial Activity of Mouthwash with Celery and Cinnamon Extracts Against *Streptococcus mutans* and *Staphylococcus aureus*

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Abstract. Dental caries remains a major oral health problem in Indonesia and is closely associated with dental plaque accumulation caused by pathogenic bacteria such as *Streptococcus mutans* and *Staphylococcus aureus*. The prolonged use of chemical mouthwashes may cause undesirable side effects, encouraging the development of safer herbal alternatives. Celery (*Apium graveolens*) and cinnamon (*Cinnamomum burmanni*) contain flavonoids, phenolics, and essential oils with antibacterial activity. This study aimed to evaluate the physical properties, antibacterial activity, and optimal combination ratio of celery and cinnamon extracts in mouthwash formulations. Ethanol extracts obtained by maceration were formulated into three formulations, F1 (5%:10%), F2 (7.5%:7.5%), and F3 (10%:5%). Physical evaluations included organoleptic properties, pH, homogeneity, and viscosity tests. Antibacterial activity was evaluated using the disc diffusion method and analyzed using one-way ANOVA and multiple linear regression. All formulations met the required physical evaluation criteria. F1 exhibited the highest antibacterial activity, with inhibition zones of 15.17 mm against *S. mutans* and 18.29 mm against *S. aureus*. Regression analysis revealed that cinnamon extract was the dominant factor, while statistical analysis showed significant differences among formulations ($p < 0.05$). In conclusion, F1 was identified as the optimal mouthwash formulation.

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1. Introduction

Oral and dental health disorders remain a significant public health concern in Indonesia. Data from the Riset Kesehatan Dasar (Riskesdas) 2018 indicate that 57.6% of the population experience oral and dental health problems, while only approximately 10% receive medical treatment [1]. Low awareness of oral hygiene practices may contribute to plaque accumulation, halitosis, and various oral infectious diseases [2]. Dental caries and periodontal diseases are among the most common conditions, primarily resulting from the activity of bacteria such as *Streptococcus mutans*, which plays a role in the demineralisation of enamel and dentin [3]. In addition, an imbalance in salivary pH may promote the growth of opportunistic pathogenic bacteria such as *Staphylococcus aureus*, which can potentially cause soft tissue infections and gingival inflammation [4].

The use of mouthwash constitutes an additional measure in maintaining oral hygiene. Although routine tooth brushing is primary method for preserving dental health, it cannot effectively reach all tooth surfaces and interdental spaces. Therefore, mouthwash is used as an adjunct because it can access areas that are difficult to clean, inhibit bacterial growth, reduce plaque accumulation, and freshen the oral cavity [5]. One commonly used synthetic agent in mouthwash is chlorhexidine (Chx), which is effective in controlling plaque and pathogenic bacteria. However, its long-term use may lead to adverse effects such as tooth discolouration, taste disturbances, xerostomia, and a burning sensation [6].

The use of herbal materials presents a safer and promising alternative. One plant known for its antibacterial activity is celery (*Apium graveolens*), which contains flavonoids, saponins, tannins, and essential oils such as terpenes and phenolic compounds [7]. Celery extract inhibited *S. mutans* with an inhibition zone of 5 mm at a concentration of 15% [8]. An inhibition zone of 0.6 mm at a concentration of 30% against *S. aureus* [9]. In addition to celery leaves, cinnamon bark (*Cinnamomum burmanni*) is also known to exhibit antibacterial activity derived from compounds such as flavonoids, tannins, and the essential oil cinnamaldehyde [10]. Cinnamon extract inhibited *S. mutans* with an inhibition zone ranging from 7.33 to 9.33 mm at concentrations of 10–15% [11]. Furthermore, cinnamon extract exhibited inhibition zones of 3.7–12.7 mm at concentrations of 30–75% against *S. aureus* [12].

Although the antibacterial activities of celery and cinnamon extracts have been reported individually, studies evaluating the combination of these extracts in a mouthwash formulation against *S. mutans* and *S. aureus* remain limited. The combination of these two plants is considered potentially synergistic due to their different mechanisms of action. Apigenin in celery acts as an antibiofilm agent by inhibiting bacterial adhesion and biofilm formation [13], while cinnamaldehyde in cinnamon exhibits a more aggressive mechanism through direct bactericidal effects as well as anti-quorum sensing activity [14]. Therefore, this study aims to evaluate the effectiveness of the combined extracts of celery and cinnamon and to determine the optimal concentration ratio of the combination.

2. Experimental Section

2.1. Materials and Methods

The instruments used in this study included a blender (Philips), a moisture balance analyser (MB60), Petri dishes, an analytical balance (HWH-DJ203A), an autoclave (Alamerican), an incubator (Memmert IN 55), an oven (Getra), a rotary evaporator (RE-2010), a water bath (DFS), a laminar airflow (LAF) cabinet (Sugold), a pH meter, a Brookfield viscometer (Welhome), a micropipette (Ecopippette), filter paper, an 18-mesh sieve, an inoculating loop, paper discs, a mortar and pestle, a UV–Vis spectrophotometer, and glassware (Pyrex). The materials used in this study included celery, cinnamon, *Streptococcus mutans* and *Staphylococcus aureus*, nutrient agar (NA), 96% ethanol, glycerin, sorbitol, peppermint oil, sodium benzoate, Tween 80, distilled water, 1% H_2SO_4, 1% $BaCl_2$, 0.9% NaCl, and Minosep (0.2% chlorhexidine).

2.2. Experimental Procedures

2.2.1 Preparation and Determination of Materials

A total of 5000 g of celery samples used in this study were obtained from Sigedang Village, Kejajar District, Wonosobo Regency. Meanwhile, 600 g of cinnamon bark samples were procured from Kesambi Market, Margasari District, Tegal Regency. The determination of celery and cinnamon was conducted at the Natural Materials Laboratory, Department of Pharmacy Program, Bhamada University Slawi, to verify the authenticity of the plant materials used in accordance with the literature.

2.2.2 Preparation of Simplicia Powder

Simplicia powder from celery leaves and cinnamon bark was prepared by first weighing the raw materials, followed by cleaning and cutting them into small pieces. The materials were dried in an oven at a temperature of 40–50°C until completely dry. The dried simplicia were subsequently ground into a fine powder and sieved using an 18-mesh sieve [15].

2.2.3 Preparation of Extracts

The extraction of celery leaves and cinnamon bark was carried out using the maceration method with 96% ethanol at a ratio of 1:10 for three days. The macerate was filtered and concentrated using a rotary evaporator at a temperature of 50–60°C until a viscous extract was obtained [16-17].

2.2.4 Non-Specific Parameters of the Extract

2.2.4.1 Organoleptic Test

The viscous extract was evaluated using the sensory organs, including colour, form, aroma, and texture, to describe its physical characteristics [18].

2.2.4.2 Loss on Drying Test

A total of 1–2 g of the extract was placed in a pre-heated weighing bottle, evenly distributed, and then dried in an oven at 105°C until a constant weight was achieved. The loss on drying met the specified requirement if the value was ≤10% [19].

2.2.4.3 Moisture Content Test

A total of 0.5 g of the extract was introduced into a Moisture Balance Analyzer set at 105°C. The moisture content of the extract complied with the standard if the value was <10% [19].

2.2.4.4 Total Ash Content Test

A total of 2 g of the extract was placed into a pre-ignited silica crucible, evenly spread, and then incinerated at 600°C for 2 hours until complete combustion was achieved. The total ash content met the requirement if the value was < 10% [19].

2.2.4.5 Acid-Insoluble Ash Content Test

The total ash was treated with 25 mL of 2 N HCl, heated for approximately 5 minutes, filtered, and washed with hot water. The residue was then dried and incinerated. The acid-insoluble ash content complied with the requirement if the value was ≤1% [19].

2.2.5 Phytochemical Screening

The extract was qualitatively analysed to determine the presence of alkaloids, flavonoids, tannins, saponins, as well as steroids and triterpenoids, following standard phytochemical screening procedures. Alkaloids were tested using Mayer and Dragendorff reagents, flavonoids using the Wilstätter test, tannins using ferric chloride ($FeCl_3$), saponins by foam formation, and

steroids/triterpenoids using the Liebermann–Burchard reaction. The presence of phytochemical constituents was indicated by characteristic precipitate or colour changes according to each test [20].

2.2.6 Preparation of Mouthwash Formulation

The mouthwash formulation consisting of a combination of celery leaf extract (*Apium graveolens*) and cinnamon bark extract (*Cinnamomum burmanni*) was prepared in a total volume of 100 mL with fixed concentration ratios of the extracts based on the studies conducted by Yardha *et al* [7] and Rakhmayanti *et al* [18], with slight modifications. The composition of the mouthwash formulation is presented in Table 1.

Table 1. Mouthwash formulations

Materials	Concentration %w/v					Function	Range % (Rowe, 2009)
	K (+)	F0	F1	F2	F3		
Minosep	0.2%	-	-	-	-	Control (+)	-
Extract Celery	-	-	5	7.5	10	Active substance	-
Extract Cinnamon	-	-	10	7.5	5	Active Substance	-
Glycerin	-	15	15	15	15	Humectant	≤ 30
Sorbitol	-	10	10	10	10	Sweetener	3-15
Peppermint oil	-	0.045	0.045	0.045	0.045	Flavouring agent	0.04-0.2
Sodium Benzoate	-	0.4	0.4	0.4	0.4	Preservative	0.02-0.5
Tween 80	-	0.3	0.3	0.3	0.3	Emulsifier	0.1-2
Aquadest ad	-	100	100	100	100	Solvent	-

The preparation of the mouthwash began with accurately weighing all ingredients and preparing a calibrated 100 mL volumetric flask. Distilled water was heated, and 60 mL was used to dissolve sodium benzoate. Subsequently, glycerin, sorbitol, and Tween 80 were added, followed by the addition of one drop of peppermint oil, and the mixture was homogenised. Thereafter, celery and cinnamon extracts were incorporated gradually at the final stage to maintain the stability of the active compounds. The resulting solution was filtered, transferred into the bottle, and diluted with distilled water up to the calibration mark, then homogenised thoroughly.

2.2.7 Physical Evaluation of Mouthwash

2.2.7.1 Organoleptic Test

Sensory evaluation was conducted using the human senses to assess the appearance, colour, aroma, and taste of the mouthwash [7].

2.2.7.2 pH Test

The pH of the mouthwash was measured using a calibrated pH meter with standard buffer solutions of pH 4 and 7. The electrode was immersed in the mouthwash sample for approximately 2 seconds. The acceptable pH range for mouthwash formulations is 5–7, ensuring antibacterial efficacy without causing irritation to the oral cavity [21].

2.2.7.3 Viscosity Test

The viscosity of the mouthwash was determined using a Brookfield viscometer (spindle no. 4, rotor 1, 30 RPM) to evaluate its thickness, which may influence user comfort and the physical stability of the formulation [21].

2.2.7.4 Homogeneity Test

The homogeneity of the mouthwash was assessed visually under adequate lighting conditions. The preparation was considered homogeneous if it appeared clear, uniform, and stable [22].

2.2.8 Antibacterial Activity Test

All instruments and materials were sterilised using an autoclave at 121°C for 15 minutes. Nutrient Agar (NA) was prepared by dissolving 2.8 g of the medium in 100 mL of distilled water, heated until completely dissolved, and then poured into slant tubes (3 mL) and Petri dishes (10 mL) and allowed to solidify. The bacterial suspension was prepared by transferring one loopful of rejuvenated bacterial culture into 5 mL of 0.9% NaCl solution, with turbidity adjusted to the McFarland standard. The antibacterial activity of the mouthwash was evaluated using the disc diffusion (*Kirby–Bauer*) method. Sterile discs were immersed in the mouthwash formulation, positive control, and negative control, and then incubated at 37°C for 24 hours. The inhibition zones formed were measured using a vernier caliper [21].

2.2.9 Data Analysis

The physical evaluation data of the mouthwash were analysed descriptively. Meanwhile, the inhibition zone data were analysed using SPSS with a one-way ANOVA test, followed by Tukey's post hoc test and multiple linear regression analysis.

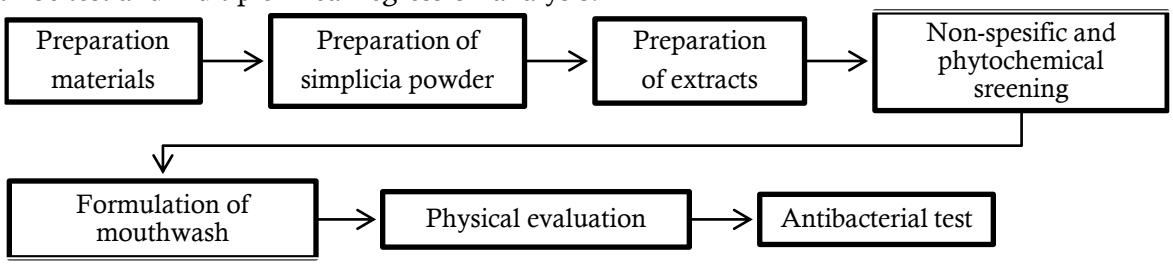

Figure 1. Research flow diagram

3. Results and Discussion

3.1 Extract Yield

Extraction was carried out using the maceration method, as it is relatively simple and effective for extracting secondary metabolite compounds ranging from polar to semi-polar without the application of high temperatures that may potentially degrade active constituents [23]. A 96% ethanol solvent was used due to its ability to dissolve flavonoid and phenolic compounds found in celery leaves [7], as well as aromatic compounds in cinnamon bark, such as cinnamaldehyde and essential oils [10]. Fresh plant materials were washed, cut, and dried using an oven at controlled temperature until constant weight was achieved. After drying, the materials were used for the extraction process. The extract yield results are presented in Table 2.

Table 2. Extract yield results

Sample	Initial weight (g)	Final weight (g)	Yield (%)
Celery leaf extract	500.03	81.16	16.23
Cinnamon bark extract	500.13	116.63	23.31

The extraction results showed that the celery leaf extract exhibited a green colour, whereas the cinnamon bark extract appeared light brown. The extract yields met the acceptable requirement of greater than 10% (19). The variation in yield values is presumed to be influenced by differences in chemical composition, where cinnamon bark contains higher levels of essential oils and phenolic compounds, making it more easily extracted in ethanol solvent [10].

3.2 Non-Specific Parameters and Phytochemical Screening of Extracts

The results of the non-specific parameters test of the extracts are presented in Table 3.

Table 3. Non-specific parameter results

Parameters	Sample	Result (%)	Requirement (Depkes, 2022)	Remarks
Moisture Content	CE	9.24	<10%	Complies
	CNE	5.00		
Loss on Drying	CE	1.03	<10%	
	CNE	1.38		
Total Ash Content	CE	9.00	<10%	
	CNE	7.50		
Acid-insoluble Ash Content	CE	0.60	<1%	
	CNE	0.68		

Descriptions: CE = Celery leaf extract
CNE = Cinnamon bark extract

The results of the phytochemical screening of the extracts are presented in Table 4.

Table 4. Phytochemical screening result

Secondary Metabolites	Celery Extract	Cinnamon bark Extract	Description
Flavonoids	+	+	Yellow-orange solution
Alkaloids	+	+	Formation of precipitate
Tannins	+	+	Darkened solution
Saponins	+	+	Formation of stable foam
Triterpenoids	-	+	Reddish-brown solution
Steroids	+	-	Yellow to green solution

All non-specific parameters indicated that the extracts met the standard requirements, suggesting good quality raw materials and extract stability, thereby making them suitable for further development into mouthwash formulations. The phytochemical screening results revealed that the celery leaf extract contains flavonoids, alkaloids, tannins, saponins, and steroids [7]. Meanwhile, the cinnamon bark extract contains flavonoids, alkaloids, tannins, saponins, and triterpenoids [12]. These secondary metabolites exhibit antibacterial activity, thereby supporting the potential of both extracts as active ingredients in mouthwash formulations.

3.3 Formulation and Physical Evaluation of Mouthwash

In the formulation of the mouthwash preparation, celery leaf extract and cinnamon bark extract were combined at a total concentration of 15%. The resulting mouthwash formulations are presented in Figure 2.

(a) (b) (c) (d)

Figure 2. Mouthwash preparations

(a) Formulation 0, (b) Formulation 1, (c) Formulation 2, (d) Formulation 3

Based on organoleptic and homogeneity observations, the mint aroma and taste of the mouthwash are derived from peppermint oil, while the bitter taste originates from the combination of celery leaf and cinnamon bark extracts. Visually, F0 appears clear, whereas F1–F3 become progressively more concentrated with increasing extract concentration [7], with F3 exhibiting a deep brownish-orange colour due to the presence chlorophyll pigment of celery leaves and the presence of tannins and cinnamaldehyde from cinnamon bark. This indicates the contribution of each extract to the overall characteristics of the mouthwash [21]. The results of pH and viscosity evaluations of the mouthwash are presented in Table 5.

Table 5. Physical Evaluation Results of Mouthwash

Parameters	Formula	Result ± SD	Requirement	Remarks
pH	F0	6.36±0.05	pH 5-7 (Depkes RI, 1995)	Complies
	F1	5.03±0.05		Complies
	F2	5.3±0		Complies
	F3	5.36±0.05		Complies
Viscosity (mPa.s)	F0	2.33±0.11	Viscosity 3-8 mPa.s (Rakhmayanti, 2023)	Doesn't Complies
	F1	3.66±0.11		Complies
	F2	3.33±0.11		Complies
	F3	3.13±0.11		Complies

The mouthwash formulations exhibited pH values within the acceptable range of 5–7. The results indicated that F0 tended to be neutral (6.36 ± 0.05), whereas F1–F3 were more acidic with increasing concentrations of cinnamon bark extract. This variation in acidity is influenced by the proportion of extracts, particularly cinnamon bark extract, which has a naturally lower pH (4.5–5.5), thereby contributing to a gradual decrease in the pH of the formulations [24]. A pH value within the physiological range of the oral cavity is essential to prevent mucosal irritation and to maintain the balance of normal flora, while still being effective in inhibiting the growth of pathogenic bacteria [7].

In terms of viscosity, F0 did not meet the required standard of 3–8 mPa.s, with a value of 2.33 ± 0.11 mPa·s, indicating a relatively low viscosity approaching that of water. Low viscosity may reduce the contact time between the formulation and the oral mucosal surface, potentially decreasing its antibacterial effectiveness. In contrast, the viscosity of F1–F3 increased in line with the concentration of cinnamon bark extract, with F1 showing the highest value (3.66 ± 0.11 mPa·s). This is likely attributable to the high content of polysaccharides, pectin, and tannins present in cinnamon bark, which influence the thickness of the formulation [12].

3.4 Antibacterial Activity of Mouthwash

The antibacterial activity test was conducted to evaluate the ability of the mouthwash formulations to inhibit bacterial growth [25]. The bacterial suspensions were standardized against the McFarland (0.08–0.12 nm) to ensure an appropriate inoculum density. The absorbance values obtained for *Streptococcus mutans* (0.101 nm) and *Staphylococcus aureus* (0.098 nm) were within the acceptable range, indicating that the suspensions met the required standard [9]. The results of the inhibition zone test are presented in Table 6 and Figure 3.

Table 6. Inhibitory activity against *Streptococcus mutans* and *Staphylococcus aureus* result

Bacteria	Formula	Mean (mm)	±SD (mm)	Bacteria	Formula	Mean (mm)	±SD (mm)
Streptococcus mutans	Control (-)	-	-	*Staphylococcus aureus*	Control (-)	-	-
	Control (+)	24.57	2.01		Control (+)	20.02	7.95
	F1	15.17	1.77		F1	18.29	4.92
	F2	5.53	0.06		F2	11.75	2.67
	F3	3.17	0.85		F3	6.72	0.98

The antibacterial testing of the mouthwash formulated with a combination of celery leaf extract and cinnamon bark extract demonstrated that F0 produced no inhibition zone due to the absence of active compounds. In contrast, formulations F1–F3 exhibited inhibition zones with varying diameters. This indicates that the combination of celery leaf and cinnamon bark extracts possesses effective antibacterial activity, whereby variations in the compositional ratio of the two extracts influence the resulting inhibition zone diameter.

Based on the inhibition zone classification by David Stout (1971), the combined extract mouthwash is categorized as weak to strong against *Streptococcus mutans* and moderate to strong against *Staphylococcus aureus* [26]. These differences are influenced by factors such as bacterial load, temperature, incubation time, pH, and the presence of dissolved organic substances. The larger inhibition zone observed against *S. aureus* is attributed to its cell wall structure, which is more permeable and more susceptible to antibacterial compounds. In contrast, *S. mutans* is capable of forming thick biofilms, rendering it more resistant to antibacterial agents [27].

The positive control exhibited a larger inhibition zone against *S. mutans*, which may be attributed to the broad-spectrum activity of chlorhexidine against oral pathogenic bacteria [21]. The highest antibacterial activity was observed in formulation F1, which exhibited the highest viscosity and the most acidic pH. Increased viscosity enhances the contact time between active compounds and bacterial cells [21], while a more acidic pH contributes to bacterial growth inhibition by disrupting cellular environmental balance. The combination of these factors, together with an optimal extract composition, is presumed to be responsible for the enhanced antibacterial effectiveness of F1 compared to other formulations.

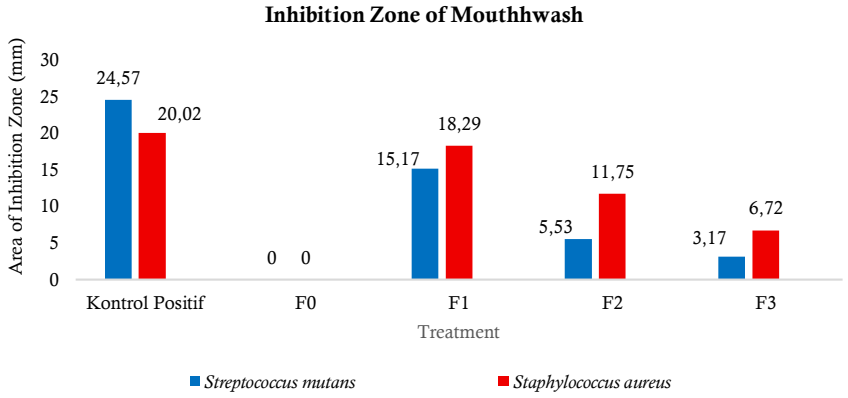

Figure 3. Diagram inhibition zone of mouthwash

The antibacterial activity of the combined extract mouthwash is attributed to the presence of secondary metabolites. Flavonoids inhibit bacterial growth by interfering with nucleic acid synthesis and essential enzymatic activity [28]. Terpenoids and steroids reduce bacterial cell wall permeability [12]. Alkaloids act by inhibiting bacterial cell wall synthesis, saponins increase membrane permeability, and tannins precipitate proteins and damage bacterial cell membranes [9]. Furthermore, cinnamaldehyde present in cinnamon bark suppresses bacterial cell division, ATPase enzyme activity, biofilm formation, and alters bacterial membrane lipid profiles through an anti-quorum sensing mechanism [14].

The inhibition zone diameters against *Streptococcus mutans* and *Staphylococcus aureus* observed in this study were generally higher than those reported in previous studies employing single extracts. Celery extract produced an inhibition zone of approximately 5 mm against *S. mutans* [8]. Meanwhile, cinnamon bark extract exhibited antibacterial activity with inhibition zones of approximately 8 mm against both *S. mutans* and *S. aureus* [11-12]. These findings suggest a potential for increased antibacterial activity through a combination of celery leaf and cinnamon bark extracts, indicating their suitability for development as a herbal mouthwash formulation, while further studies are required to evaluate stability and in vivo effectiveness.

3.5 Data Analysis

The inhibition zone data were analysed using SPSS version 27 with a one-way ANOVA test. Prior to analysis, normality was assessed using the Shapiro–Wilk test ($p > 0.05$) and homogeneity of variance was evaluated using Levene's test ($p > 0.05$), indicating that the data were normally distributed and homogeneous. The ANOVA results revealed a statistically significant difference ($p < 0.05$), indicating that variations in extract concentration significantly affected antibacterial activity. Post hoc Tukey HSD analysis for *S. mutans* showed that the positive control differed significantly from all formulations ($p < 0.05$), whereas no significant difference was observed between F2 and F3 ($p > 0.05$), indicating comparable inhibitory effectiveness. For *S. aureus*, significant differences were observed only between the positive control and F3 ($p < 0.05$), with no significant difference between F1 and F2 ($p > 0.05$), suggesting similar effectiveness between these formulations.

Multiple linear regression analysis was conducted to determine the effect of extract concentration on the increase in inhibition zone diameter. A fixed ratio of 15% for both extracts resulted in perfect linearity (Tolerance = 0.000), thus the analysis focused on cinnamon bark extract. The results indicated that cinnamon bark extract was the dominant component, contributing more significantly than celery leaf extract to antibacterial activity. Each 1% increase in concentration resulted in an increase in inhibition zone diameter of 2.40 mm against *S. mutans* and 2.31 mm against *S. aureus*. This is likely associated with bioactive compounds such as cinnamaldehyde, eugenol, and phenolic compounds, which exhibit strong antibacterial activity through anti-quorum sensing mechanisms [14],[29]. In contrast, apigenin flavonoids present in celery leaf extract exhibited relatively weaker antibacterial activity, functioning primarily as anti-biofilm agents by inhibiting biofilm formation and adhesion rather than acting as bactericidal agents that directly kill bacterial cells [30-31]

4. Conclusion

Based on the findings of this study, the mouthwash formulation containing a combination of celery leaf extract and cinnamon bark extract met the required physical quality standards across all formulations. Antibacterial activity evaluation demonstrated that formulation F1 exhibited the highest effectiveness, with strong inhibitory activity against *Streptococcus mutans* and *Staphylococcus aureus*. This is attributed to the higher concentration of cinnamon bark extract, which resulted in optimal antibacterial activity.

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