

ANTIBACTERIAL EFFECTIVENESS OF MOUTHWASHES MADE FROM AJWA AND SUKARI DATE SEED EXTRACTS AGAINST STREPTOCOCCUS MUTANS

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ABSTRACT

Background: Maintaining oral health is essential to prevent dental diseases, including dental caries, which affects the hard tissues of the teeth and is primarily caused by *Streptococcus mutans*. Herbal mouthwash containing Ajwa and Sukari date seed extracts has the potential to inhibit bacterial growth due to its bioactive compounds, including flavonoids, phenolic compounds, and tannins. This study aimed to compare the antibacterial effectiveness of mouthwash containing Ajwa and Sukari date seed extracts against *Streptococcus mutans*.

Methods: This in vitro laboratory experimental study employed a post-test only control group design. The samples were divided into ten groups with six replications each, consisting of a positive control (0.2% chlorhexidine), a negative control (distilled water), and Ajwa and Sukari date seed extract mouthwashes at concentrations of 3.25%, 6.5%, and 12.5%. Antibacterial activity was evaluated by measuring the inhibition zone diameter after 24 hours of incubation.

Results: All extract groups demonstrated strong antibacterial activity, with the largest inhibition zone observed in the 12.5% Ajwa extract group. The Kruskal–Wallis test showed a significant difference among the groups ($p < 0.05$). The Mann–Whitney test revealed significant differences between Ajwa and Sukari extracts at concentrations of 3.25% and 6.5% ($p = 0.004$), while no significant difference was found at 12.5% ($p = 0.336$).

Conclusion: Ajwa and Sukari date seed extract mouthwashes exhibit antibacterial activity against *Streptococcus mutans*. Ajwa date seed extract mouthwash demonstrates greater antibacterial activity than Sukari date seed extract mouthwash at concentrations of 3.25% and 6.5%.

Keywords: Date seed, Mouthwash, *Streptococcus mutans*

INTRODUCTION

The oral cavity plays an essential role in supporting daily functions, including breathing, speech, and mastication. In addition, psychosocial aspects such as self-confidence and comfort enable individuals to carry out daily activities and social interactions effectively. The Indonesian Health Survey (SKI) 2023 reports that 43.6% of the population aged ≥ 3 years experienced dental problems, with a prevalence of 42.8% in Central Java⁽¹⁾. Dental caries is one of the most common oral diseases and is caused by the activity of *Streptococcus mutans*, a bacterium capable of converting sucrose into acidogenic organic acids⁽²⁾. This bacterium produces the enzymes glucosyltransferase and fructosyltransferase, which convert sucrose into glucans and fructans⁽³⁾. *Streptococcus mutans* is a Gram-positive, facultative anaerobic bacterium that resides in the oral cavity⁽⁴⁾. It is characterized by its spherical shape and its ability to ferment carbohydrates, resulting in acid production⁽⁵⁾. The production of these acids lowers the pH of the oral environment, which can trigger the demineralization of tooth enamel and initiate the development of dental caries⁽²⁾.

Dental caries is a multifactorial disease influenced by various factors, including environmental conditions, host factors,

duration of exposure, and the presence of bacterial colonies. Two major factors contributing to the development of dental caries are the frequent consumption of free sugars and the metabolic activity of bacteria adhering to the tooth surface⁽⁶⁾. Mouthwash is a liquid oral hygiene preparation containing antimicrobial agents that is used as an adjunct to mechanical plaque control through rinsing⁽⁷⁾. According to another definition, mouthwash is a solution containing ingredients such as breath fresheners, astringents, demulcents, surfactants, or antibacterial agents that helps clean and freshen the oral cavity while preventing the growth of the Gram-positive bacteria such as *Streptococcus mutans*, one of the primary causative agents of dental caries⁽⁸⁾. Various active ingredients can be incorporated into mouthwash formulations, including chlorhexidine and herbal plants that possess antimicrobial properties⁽⁹⁾. Chlorhexidine (CHX) possesses antiplaque properties and exhibits bactericidal, bacteriostatic, and non-toxic effects. However, its use may cause side effects, including staining of the teeth, restorative materials, oral mucosa, and tongue⁽¹⁰⁾. Herbal mouthwashes are considered safer because they are derived from natural ingredients and generally produce fewer side effects than chemically based mouthwashes⁽¹¹⁾. One approach to maintaining oral health is the use of mouthwash, which is intended to prevent dental caries and

suppress the growth of pathogenic microorganisms⁽²⁾. Several herbal plants possess antimicrobial, antibacterial, and anti-inflammatory properties that can inhibit the growth of *Streptococcus mutans*⁽¹²⁾.

Date fruit is a promising natural source of phytochemicals and possesses antibacterial activity, making it a safe alternative for maintaining oral health. In the date-processing industry, date seeds are generated as waste in considerable quantities, accounting for approximately 6.10–11.47% of the total fruit weight⁽¹³⁾. Ajwa dates are characterized by their dark color, which approaches a reddish-black hue, accompanied by fine wrinkles across the entire surface. They are generally smaller in size. In contrast, Sukari dates exhibit a light yellowish-brown color when ripe and have a crispier flesh texture compared to Ajwa dates⁽¹⁴⁾. Numerous studies have reported that the dominant compounds in date seeds are phenolic compounds and flavonoids, which play important roles in their biological activities⁽¹⁵⁾. Date seeds are known to contain dietary fiber and exhibit high antioxidant activity, largely attributed to their phenolic and flavonoid content, which is higher than that found in the fruit flesh. Ajwa date seeds are a rich source of flavonoids and various phenolic compounds, including gallic acid, p-hydroxybenzoic acid, o-coumaric acid, and m-coumaric acid. These compounds contribute to their antioxidant and anti-inflammatory properties⁽¹⁶⁾. Previous studies have demonstrated that Ajwa date seed extract at a concentration of 3.25% and Ajwa date flesh extract at a concentration of 12.5% strongly inhibited the growth of *Streptococcus mutans*. Furthermore, both Ajwa date seed extract and Ajwa date flesh extract at a concentration of 3.25% were shown to inhibit the growth of *Candida albicans*. These findings indicate that Ajwa dates possess antibacterial, antifungal, antioxidant, and anti-inflammatory properties that may be beneficial for oral health⁽¹⁷⁾. Furthermore, inhibition tests have shown that the higher the concentration of the extract, the larger the inhibition zone produced, increasing from 12.09 mm at a concentration of 3.25% to 17.57 mm at a concentration of 50%⁽¹⁸⁾. Ajwa date extracts obtained using acetone and methanol as solvents demonstrate inhibitory activity against both Gram-positive and Gram-negative bacteria⁽¹⁹⁾.

In addition to Ajwa date seeds, other varieties such as Sukari date seeds are known to contain high levels of phenolic compounds, flavonoids, and antioxidants. Their flavonoid content varies depending on the extraction method used. The phenolic acid compounds present in Sukari dates have also demonstrated stronger antibacterial activity against Gram-positive bacteria than against Gram-negative bacteria⁽²⁰⁾. However, the phenolic and flavonoid contents of Sukari dates are lower than those of Ajwa dates, indicating that their antioxidant activity is comparatively weaker⁽²¹⁾. Date fruits are known to contain phenolic compounds in both aqueous and ethanolic extracts, which exhibit considerable antibacterial activity. Research findings have shown that the aqueous extract produces an inhibition zone of 8 mm, which is larger than that of the ethanolic extract, measuring 5 mm. The concentration of active compounds in plant materials is influenced

by the type of solvent used during the extraction process; therefore, solvent selection should be adjusted according to the polarity of the compounds to be extracted⁽¹³⁾. Based on the above findings, this study aims to investigate and compare the antibacterial effectiveness of mouthwashes containing Ajwa date seed extract and Sukari date seed extract against *Streptococcus mutans*.

MATERIALS AND METHODS

This study employed a laboratory experimental method using a post-test only control group design. The research was conducted from February to March 2026 at the Cendekia Nanotech Hutama Laboratory and the Laboratory of the College of Pharmaceutical Sciences (STIFAR), Semarang. The treatment groups consisted of Ajwa date seed extract and Sukari date seed extract at concentrations of 3.25%, 6.5%, and 12.5%, respectively. The positive control group (K+) consisted of 0.2% chlorhexidine, while the negative control group (K-) consisted of distilled water. Each group was tested with six replications.

The instruments used in this study included beakers, graduated cylinders, test tubes, spatulas, an oven, a rotary evaporator, a magnetic stirrer, an autoclave, a drying cabinet, an analytical balance, droppers, a blender, trays, forceps, micropipettes, a mortar and pestle, a Bunsen burner, an incubator, a spectrophotometer, Petri dishes, a digital caliper, and markers. The materials used in this study included Mueller–Hinton Agar (MHA), sulfuric acid (H_2SO_4), barium chloride ($BaCl_2$), McFarland standard solution, 0.9% sodium chloride (NaCl), paper discs, *Streptococcus mutans* cultures obtained from the STIFAR Laboratory, Ajwa date seeds, Sukari date seeds, glycerin, sodium lauryl sulfate, menthol, and distilled water.

The Ajwa and Sukari date seeds were weighed and manually crushed using a hammer. Subsequently, the crushed seeds were ground into a fine powder using a blender. Extraction was performed using the maceration method by soaking the powdered date seeds in 96% ethanol at room temperature for 3 × 24 hours. The mixture was stirred once daily to facilitate the dissolution of active compounds into the solvent. The filtrate obtained from the maceration process was then concentrated using a rotary evaporator at 50°C to separate the ethanol solvent from the extract. The resulting semi-solid extract was transferred to a tray and dried overnight in a drying cabinet at 50°C. The dried extract was subsequently weighed and stored in a refrigerator to maintain its stability and prevent degradation. The preparation of the mouthwash formulation began with weighing the Ajwa date seed extract and Sukari date seed extract separately and transferring each extract into a beaker. Distilled water was then added, and the mixture was stirred until homogeneous. Subsequently, glycerin, sodium lauryl sulfate, and menthol were added. Additional distilled water was added to achieve the desired final volume, and the mixture was homogenized using a magnetic stirrer until a homogeneous mouthwash formulation was obtained.

Table 1. Formulation of Ajwa Date Seed Extract Mouthwash

Ingredient			Function	Composition			Standard %
				F1	F2	F3	
Ajwa date seed extract			Active substance	3.25%	6.5%	12.5%	-
Glycerin			Cosolvent	10 ml	10 ml	10 ml	< 20
Sodium lauryl sulfate			Foaming agent	1 ml	1 ml	1 ml	1-2
Menthol			Flavoring agent	0.5 ml	0.5 ml	0.5 ml	0,1-2
Distilled water			Solvent	Add 100 ml	Add 100 ml	Add 100 ml	-

Table 1 presented the formulations of Ajwa date seed extract mouthwash with three different extract concentrations: F1 (3.25%), F2 (6.5%), and F3 (12.5%). All formulations contained 10 ml of glycerin as a co-solvent, 1

ml of sodium lauryl sulfate as a foaming agent, and 0.5 ml of menthol as a flavoring agent. Distilled water was added to obtain a final volume of 100 ml.

Table 2. Formulation of Sukari Date Seed Extract Mouthwash

Ingredient			Function	Composition			Standard %
				F1	F2	F3	
Sukari date seed extract			Active substance	3.25%	6.5%	12.5%	-
Glycerin			Cosolvent	10 ml	10 ml	10 ml	< 20
Sodium lauryl sulfate			Foaming agent	1 ml	1 ml	1 ml	1-2
Menthol			Flavoring agent	0.5 ml	0.5 ml	0.5 ml	0.1-2
Distilled water			Solvent	Add 100 ml	Add 100 ml	Add 100 ml	-

Table 2 presented the formulations of Sukari date seed extract mouthwash with three different extract concentrations: F1 (3.25%), F2 (6.5%), and F3 (12.5%). All formulations contained 10 ml of glycerin as a co-solvent, 1 ml of sodium lauryl sulfate as a foaming agent, and 0.5 ml of menthol as a flavoring agent. Distilled water was added to obtain a final volume of 100 ml.

The antibacterial activity test was initiated by preparing and sterilizing all instruments and materials. The assay was then performed using the disc diffusion method, with all instruments were sterilized in an oven at 180°C for 2 hours. A total of 3.8 g of Mueller–Hinton Agar (MHA) was dissolved in 100 ml of distilled water in an Erlenmeyer flask until the solution was completely dissolved. The medium was subsequently sterilized in an autoclave at 121°C for 15 minutes.

A bacterial suspension with turbidity equivalent to the 0.5 McFarland standard was prepared by mixing 9.95 ml of H₂ SO₄ solution with 0.05 ml of 1% BaCl₂ solution. The resulting standard suspension was used as a reference for adjusting the turbidity of the bacterial suspension. A pure bacterial culture was transferred onto an agar slant and incubated at 37°C for 24 hours. Subsequently, the bacterial suspension was prepared by inoculated the culture into 10 ml of 0.9% NaCl solution until a turbidity equivalent to that of the McFarland standard was achieved. Bacterial inoculation was performed using the pour plate technique. A volume of 5 µL of the bacterial suspension was inoculated into a Petri dish, followed by the addition of 25 mL of Mueller–Hinton Agar (MHA) medium. The medium was then allowed to solidify.

The antibacterial activity of the Ajwa and Sukari date seed extract mouthwashes was evaluated using the disc

diffusion method with the pour plate technique. Each paper disc was impregnated with 15 µL of the test solution using a micropipette, including Ajwa and Sukari date seed extract mouthwashes at concentrations of 3.25%, 6.5%, and 12.5%, as well as the positive and negative controls. The paper discs were placed on the surface of the agar medium in Petri dishes using sterile forceps and gently pressed to ensure proper contact with the agar surface. Each disc was labeled according to its respective treatment group, including Ajwa and Sukari date seed extract mouthwashes at concentrations of 3.25%, 6.5%, and 12.5%, as well as the control groups. Subsequently, the Petri dishes were incubated at 37°C for 24 hours.

The inhibition zones were observed, and the diameter of the inhibition zone was measured in millimeters using a

digital caliper. The inhibition zone diameter was determined by measuring the clear zone surrounding the paper disc. The caliper was positioned parallel to the inhibition zone during measurement. When the inhibition zone was not perfectly circular, its diameter was determined by calculating the mean of the measured diameters using the following formula ⁽²²⁾.

$$\text{Mean inhibition zone diameter (d)} = \frac{(d_1 + d_2 + \dots + d_n)}{n}$$

Where:

d = Mean inhibition zone diameter

d₁ = Inhibition zone diameter measured along the first axis

d₂ = Inhibition zone diameter measured along the second axis

n = Number of measurements

RESULTS

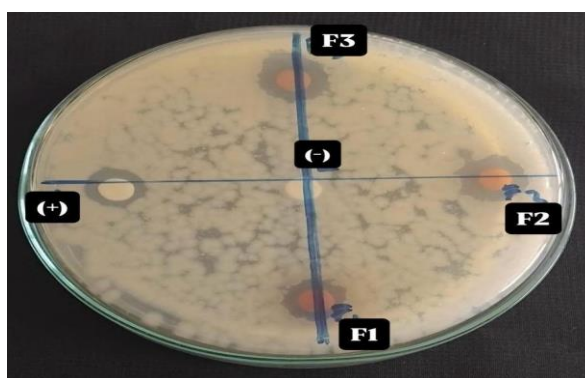


Figure 1. Inhibition zones produced by Sukari date seed extract mouthwash against *Streptococcus mutans* (F1: 3.25% extract concentration; F2: 6.5% extract concentration; F3: 12.5% extract concentration); (+): positive control; (-): negative control.

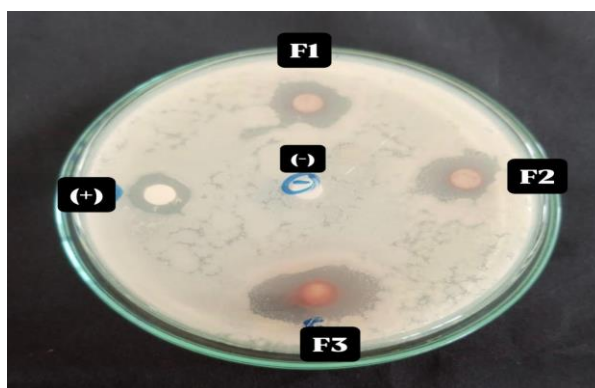


Figure 2. Inhibition zones produced by Ajwa date seed extract mouthwash against *Streptococcus mutans* (F1: 3.25% extract concentration; F2: 6.5% extract concentration; F3: 12.5% extract concentration); (+): positive control; (-): negative control.

Based on Table 3, the data represented the inhibition zone diameters for each treatment group. The results showed that the negative control group did not produce any inhibition zone (0.00 mm). The highest mean inhibition zone was

observed in the mouthwash containing 12.5% Ajwa date seed extract, with a diameter of 14.85 mm.

Tabel 3. Results of Inhibition Zone Diameter Measurements for Each Treatment Group (mm).

Replication	Ajwa			K+	K-	Sukari			K+	K-
	3.25%	6.5%	12.5%			3.25%	6.5%	12.5%		
1	13.90	13.45	14.70	11.22	0.00	10.68	11.84	12.96	9.45	0.00
2	13.95	13.58	12.18	13.54	0.00	11.25	12.30	13.06	10.35	0.00
3	14.48	15.06	15.15	12.50	0.00	10.88	11.22	12.94	7.13	0.00
4	15.83	14.95	19.55	11.91	0.00	11.12	12.42	13.12	10.20	0.00
5	12.27	16.02	15.08	14.35	0.00	10.52	12.18	12.90	8.31	0.00
6	14.51	13.43	12.44	13.54	0.00	10.35	11.72	12.94	10.20	0.00
Mean	14.16	14.42	14.85	12.84	0.00	10.80	11.95	12.99	9.27	0.00

As shown in Table 3, the inhibition zone diameters were measured for each treatment group. The results indicated that the negative control group did not produce any inhibition zone (0.00 mm). The highest mean inhibition zone diameter was observed in the 12.5% Ajwa date seed extract mouthwash group, measuring 14.85 mm.

The results of the normality test using the Shapiro-Wilk method showed that the significance values (p-values) for all treatment groups were greater than 0.05 ($p > 0.05$), indicating that the data were normally distributed. The results of the homogeneity test using Levene's test revealed that the inhibition

zone data from the ten treatment groups did not show homogeneous variances ($p < 0.05$). The significance value obtained was $p = 0.001$, indicating that the assumption of homogeneity of variance was not met. The Kruskal-Wallis test yielded a p-value of 0.000, indicating a statistically significant difference in antibacterial activity among the treatment groups. The results demonstrated that different concentrations of Ajwa date seed extract mouthwash and Sukari date seed extract mouthwash exerted significantly different effects on inhibiting the growth of *Streptococcus mutans* growth ($p < 0.05$).

Tabel 4. Mann-Whitney U Test Results for Pairwise Comparisons Among the Ten Treatment Groups.

Group	KA+	KA-	A1	A2	A3	KS+	KS-	S1	S2	S3
KA+	-	0.002*	0.054	0.078	0.109	0.004*	0.002*	0.006*	0.127	1.000
KA-	0.002*	-	0.002*	0.002*	0.002*	0.002*	1.000	0.002*	0.002*	0.002*
A1	0.054	0.002*	-	0.873	0.522	0.004*	0.002*	0.004*	0.010*	0.054
A2	0.078	0.002*	0.873	-	0.873	0.004*	0.002*	0.004*	0.004*	0.004*
A3	0.109	0.002*	0.522	0.873	-	0.004*	0.002*	0.004*	0.013*	0.336
KS+	0.004*	0.002*	0.004*	0.004*	0.004*	-	0.002*	0.005*	0.004*	0.004*
KS-	0.002*	1.000	0.002*	0.002*	0.002*	0.002*	-	0.002*	0.002*	0.002*
S1	0.006*	0.002*	0.004*	0.004*	0.004*	0.005*	0.002*	-	0.006*	0.004*
S2	0.127	0.002*	0.010*	0.004*	0.013*	0.004*	0.002*	0.006*	-	0.004*
S3	1.000	0.002*	0.054	0.004*	0.336	0.004*	0.002*	0.004*	0.004*	-

Note: An asterisk (*) indicates a statistically significant difference ($p < 0.05$). KA+ = Ajwa positive control; KA- = Ajwa negative control; A1 = 3.25% Ajwa extract; A2 = 6.5% Ajwa extract; A3 = 12.5% Ajwa extract; KS+ = Sukari positive control; KS- = Sukari negative control; S1 = 3.25% Sukari extract; S2 = 6.5% Sukari extract; S3 = 12.5% Sukari extract.

DISCUSSION

The Ajwa and Sukari date seed extract mouthwashes at all tested concentrations (3.25%, 6.5%, and 12.5%) exhibited antibacterial activity against *Streptococcus mutans*, as demonstrated by the formation of inhibition zones surrounding the paper discs. Based on the sensitivity criteria proposed by Greenwood⁽¹³⁾, the antibacterial activity of Ajwa and Sukari date seed extract mouthwashes was classified according to the diameter of the inhibition zones observed during testing. According to these criteria, antibacterial activity is categorized as weak when the

inhibition zone diameter is less than 5 mm, moderate when it ranges from 5 to 10 mm, strong when it is between 10 and 20 mm, and very strong when the inhibition zone diameter exceeds 20 mm⁽¹³⁾.

As presented in Table 3, the Ajwa date seed extract mouthwash exhibited a strong average inhibitory zone against *Streptococcus mutans*. The mean inhibition zones were 14.16 mm at a concentration of 3.25%, 14.42 mm at 6.5%, and 14.85 mm at 12.5%. Meanwhile, the positive control (0.2% chlorhexidine) produced a mean inhibition

zone of 12.84 mm, whereas the negative control (distilled water) produced 0.00 mm, which indicated the absence of an inhibitory effect. Based on the results of the Mann–Whitney test presented in Table 4, several significant differences were observed among the treatment groups. However, the Ajwa date seed extract mouthwash at all tested concentrations (3.25%, 6.5%, and 12.5%) did not show a statistically significant difference in antibacterial effectiveness compared with the positive control (0.2% chlorhexidine) ($p > 0.05$).

These findings indicated that the Ajwa date seed extract mouthwash had antibacterial potential comparable to that of chlorhexidine and could therefore be considered a potential natural alternative for mouthwash formulations. The absence of statistically significant differences among the concentrations of the Ajwa date seed extract mouthwash indicated that its antibacterial activity had reached an optimal level at the lowest concentration (3.25%). The comparison between the 12.5% concentration (A3) and the 6.5% concentration (A2) yielded a p value of 0.873 ($p > 0.05$), whereas the comparison between the 12.5% concentration (A3) and the 3.25% concentration (A1) yielded a p value of 0.522 ($p > 0.05$). Therefore, no statistically significant differences were found among the three concentrations.

As presented in Table 3, the Sukari date seed extract mouthwash exhibited a strong average inhibitory zone against *Streptococcus mutans*. The mean inhibition zones were 10.80 mm at a concentration of 3.25%, 11.95 mm at 6.5%, and 12.99 mm at 12.5%. Meanwhile, the positive control (0.2% chlorhexidine) exhibited a moderate inhibitory zone of 9.27 mm, whereas the negative control (distilled water) did not demonstrate antibacterial activity. Based on the results of the Mann–Whitney test presented in Table 4, the Sukari date seed extract mouthwash at concentrations of 3.25%, 6.5%, and 12.5% showed p -values of < 0.05 when compared with the positive control, which indicated statistically significant differences in the inhibition of *Streptococcus mutans* growth. Significant differences were also observed between S1 and S2 ($p = 0.006$), S1 and S3 ($p = 0.004$), and S2 and S3 ($p = 0.004$), as all p values were < 0.05 . These findings indicated that increasing the concentration of the Sukari date seed extract mouthwash resulted in a more optimal antibacterial effect. Furthermore, the diameter of the inhibition zone produced by the Sukari date seed extract mouthwash was influenced by the concentration of the date seed extract used. Higher extract concentrations tended to produce greater inhibitory activity against the growth of *Streptococcus mutans*, as evidenced by the statistically significant differences among the concentration groups ($p < 0.05$).

Based on the mean inhibition zone diameters, the Ajwa date seed extract mouthwash exhibited greater inhibitory activity than the Sukari date seed extract mouthwash at each concentration tested. At the 3.25% concentration, the Ajwa date seed extract mouthwash produced a mean inhibition zone of 14.16 mm, whereas the Sukari date seed extract

mouthwash produced a mean inhibition zone of 10.80 mm. The Mann–Whitney test yielded a p value of 0.004 ($p < 0.05$), which indicated a statistically significant difference between the Ajwa and Sukari date seed extract mouthwashes at this concentration. At the 6.5% concentration, the mean inhibition zones of the Ajwa and Sukari date seed extract mouthwashes were 14.42 mm and 11.95 mm, respectively. The Mann–Whitney test yielded a p value of 0.004 ($p < 0.05$), which indicated a statistically significant difference between the two treatment groups. Meanwhile, at the 12.5% concentration, the mean inhibition zones of the Ajwa and Sukari date seed extract mouthwashes were 14.85 mm and 12.99 mm, respectively. The Mann–Whitney test yielded a p value of 0.336 ($p > 0.05$), indicating that no statistically significant difference was observed between the Ajwa and Sukari date seed extract mouthwashes at the 12.5% concentration.

These findings indicated that the Ajwa date seed extract mouthwash exhibited higher antibacterial activity than the Sukari date seed extract mouthwash at low and moderate concentrations. However, at the highest concentration, both mouthwash formulations demonstrated relatively similar effectiveness in inhibiting the growth of *Streptococcus mutans*. As the concentration of the extract mouthwash increased, the content of bioactive compounds, such as alkaloids, flavonoids, tannins, and saponins, also increased, thereby enhancing their antibacterial activity. Higher extract concentrations were associated with greater inhibitory effects on bacterial growth. The antibacterial activity of an extract was determined not only by its concentration but also by the content and types of antibacterial active compounds present. In addition, the ability of the extract to diffuse through the test medium and the type of bacteria used in the assay were important factors that influenced the magnitude of the antibacterial activity produced⁽¹³⁾.

The phytochemical composition of date fruits was influenced by their variety. The analysis showed that the total phenolic content (TPC) of Ajwa dates was 93.37 mg GAE/g, whereas that of Sukari dates was only 39.01 mg GAE/g. These findings indicated that Ajwa dates contained substantially higher levels of phenolic compounds than Sukari dates. Similarly, the flavonoid content followed the same pattern, with the Safawi variety exhibiting the highest flavonoid content, followed by Ajwa, while Sukari had the lowest flavonoid content among the three varieties. Phenolic compounds and flavonoids exhibited strong antibacterial activity. These compounds caused damage to the bacterial cell wall, disrupted cell membrane permeability, and inactivated essential enzymes and proteins within bacterial cells. Flavonoids also inhibited nucleic acid synthesis and damaged the structure of the bacterial cytoplasmic membrane. Therefore, higher levels of phenolic compounds and flavonoids were associated with greater antibacterial activity. Furthermore, the study demonstrated that Ajwa dates exhibited higher antioxidant activity than Sukari dates. Antioxidant activity was closely associated with the

presence of bioactive compounds that contributed to antibacterial activity. Therefore, the higher antioxidant activity of Ajwa dates also supported their effectiveness in inhibiting bacterial proliferation⁽¹⁹⁾.

Based on the phytochemical screening results of the methanolic extracts of the Ajwa date flesh and seeds, the total phenolic contents were 245.30 ± 0.046 and 195.54 ± 0.046 mg GAE/g dry extract, respectively. The total flavonoid contents (TFC) of the ethanolic extracts of the Ajwa date flesh and seeds were 43.58 and 35.28 mg QE/g dry extract, respectively. The Ajwa date seeds exhibited a higher extraction efficiency than the date flesh, with an extraction yield of 21.19%, whereas the date flesh yielded 18.79% from 100 g of dried powder. The preliminary phytochemical screening showed that both the Ajwa date flesh and seed extracts contained flavonoids and phenolic compounds, as indicated by positive (+) test results. Further phytochemical screening demonstrated that both the Ajwa date flesh and seed extracts contained various bioactive compounds, including alkaloids, saponins, flavonoids, glycosides, terpenoids, and phenolic compounds⁽²³⁾.

The phytochemical composition of date seed extracts differed among the Ajwa, Zaghloul, and Sukari varieties. The highest total phenolic content (TPC) was found in the Zaghloul variety at 168.66 mg GAE/100 g dry weight, followed by the Ajwa date seed extract at 115.05 mg GAE/100 g dry weight, while the lowest TPC was observed in the Sukari date seed extract at 65.88 mg GAE/100 g dry weight. These findings indicated that the phenolic compound content was strongly influenced by differences in date variety. A similar pattern was observed for the total flavonoid content (TFC). The Zaghloul variety exhibited the highest TFC at 112.33 mg CAE/100 g, followed by the Ajwa variety at 55.33 mg CAE/100 g, whereas the Sukari variety had the lowest TFC at 28.95 mg CAE/100 g. These results indicated that the Sukari date seed extract contained the lowest flavonoid content among the three varieties⁽²¹⁾.

Date seeds were known to contain high levels of dietary fiber and exhibited high antioxidant activity, which was influenced by their phenolic and flavonoid contents when compared with date flesh⁽¹⁶⁾. Ajwa date extracts obtained using acetone and methanol as extraction solvents demonstrated the ability to inhibit the growth of both Gram-positive and Gram-negative bacteria. In addition, the date fruit extracts exhibited antiviral activity by inhibiting the replication of certain viruses⁽¹⁹⁾.

CONCLUSION

Both Ajwa and Sukari date seed extract mouthwashes exhibit antibacterial activity against *Streptococcus mutans*. Ajwa date seed extract mouthwash at all concentrations (3.25%, 6.5%, and 12.5%) showed no significant difference compared to the positive control chlorhexidine 0.2% ($p > 0.05$), indicating that it is comparable to chlorhexidine and has the potential to be used as a natural alternative mouthwash. In contrast, Sukari date seed extract mouthwash

at all concentrations (3.25%, 6.5%, and 12.5%) showed a significant difference compared to the positive control chlorhexidine 0.2% and had lower effectiveness than chlorhexidine ($p < 0.05$). Ajwa date seed extract mouthwash demonstrated superior effectiveness at concentrations of 3.25% and 6.5%, whereas at a concentration of 12.5%, both extracts showed comparable effectiveness.

SUGGESTION

Future studies are recommended to conduct toxicity testing, formulation stability testing, and the development of more optimal formulations. This is to enable the mouthwash to be further developed as a natural alternative agent for the prevention of dental caries.

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