JMU
Jurnal Medika Udayana
ISSN: 2597-8012 JURNAL MEDIKA UDAYANA, VOL. 15 NO.10, OKTOBER, 2026
Received: 2026-05-11Revision: 2026-06-14 Accepted: 30-08-2026

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

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ABSTRACT

Background: Maintaining oral and dental health is essential for preventing diseases that can damage tooth structures, one of which is dental caries. Dental caries is a disease affecting the hard tissues of the teeth, including enamel, dentin, and pulp, primarily caused by *Streptococcus mutans*, a major pathogen that produces organic acids. One alternative approach for caries prevention is the use of herbal mouthwash derived from date seeds, particularly Ajwa and Sukari varieties, which contain bioactive compounds such as flavonoids, phenolics, and tannins that exhibit antibacterial properties. This study aimed to compare the antibacterial effectiveness of Ajwa and Sukari date seed extract mouthwashes against the growth of *Streptococcus mutans*.
Methods: This study was an in vitro laboratory experimental study employing 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%. The measured parameter was the diameter of the inhibition zone after 24 hours of incubation.
Results: The results demonstrated that all extract groups exhibited antibacterial activity, categorized as strong. The highest inhibition zone was observed in the 12,5% Ajwa date seed extract group. The Kruskal–Wallis test revealed a statistically significant difference among the groups ($p < 0,05$). Furthermore, the Mann–Whitney test showed that Ajwa date seed extract was more effective than Sukari date seed extract at concentrations of 3,25% and 6,5% ($p = 0,004$), whereas no significant difference was found at the 12,5% concentration ($p = 0,336$).
Conclusion: In conclusion, mouthwashes containing Ajwa and Sukari date seed extracts demonstrated antibacterial activity against *Streptococcus mutans*, with Ajwa date seed extract showing superior effectiveness at concentrations of 3,25% and 6,5%.

Keywords: Date seed, Mouthwash, *Streptococcus mutans*

INTRODUCTION

The oral cavity is a part of the human body that supports essential daily functions such as breathing, speaking, and chewing food. 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 reported 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 of the oral cavity [(7)]. According to another definition, mouthwash is a solution containing ingredients such as breath fresheners,

astringents, demulcents, surfactants, or antibacterial agents that help clean and freshen the oral cavity while preventing the growth of the Gram-positive bacterium *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 aims 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 have been shown to inhibit the growth of 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 produced an inhibition zone of 8 mm, which was 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 was conducted to further 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 then crushed using a hammer. Subsequently, they were ground into a fine powder using a blender. Extraction was carried out using the maceration method by soaking the powdered date seeds in 96% ethanol at room temperature for 3 × 24 hours. The mixture was stirred 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

doi:10.24843.MU.2026.V15.i10.03

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

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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 presents 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

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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 presents 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 with the preparation and sterilization of all instruments and materials. The assay was then performed using the disc diffusion method, with all instruments 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 a homogeneous solution was obtained. 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 H_2SO_4 solution with 0,05 ml of 1% $BaCl_2$ solution. The resulting standard suspension was used as a reference for comparing the turbidity of the bacterial suspension. A pure bacterial culture was inoculated onto an agar slant and incubated at 37°C for 24 hours. Subsequently, the bacterial suspension was prepared by transferring 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

doi:10.24843.MU.2026.V15.i10.03

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.

Bacterial growth was 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. If the inhibition zone was not perfectly circular, its diameter was determined by calculating the mean of the measured diameters using the following formula [22].

$$\textit{Mean inhibition zone diameter } (d) = \frac{(d1 + d2 + \ldots + dn)}{n}$$

Where:
d = Mean inhibition zone diameter
d_1 = Inhibition zone diameter measured along the first axis
d_2 = Inhibition zone diameter measured along the second axis
n = Number of measurements

RESULTS

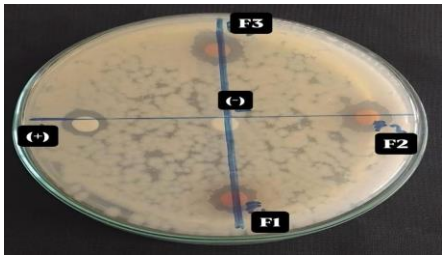


Figure 1. Inhibition zones produced by Sukari date seed extract mouthwash against *Streptococcus mutans* (F1: 3,25% concentration; F2: 6,5% concentration; F3: 12,5% concentration); (+): positive control; (-): negative control.

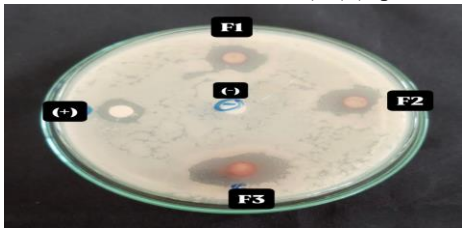


Figure 2. Inhibition zones produced by Ajwa date seed extract mouthwash against *Streptococcus mutans* (F1: 3,25% concentration; F2: 6,5% concentration; F3: 12,5% concentration); (+): positive control; (-): negative control.

Based on Table 3, the data represent 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

doi:10.24843.MU.2026.V15.i10.03

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 have 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 the inhibition 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 strong antibacterial activity against *Streptococcus mutans*. The mean inhibition zone diameters were 14,16 mm, 14,42 mm, and 14,85 mm at concentrations of 3,25%, 6,5%, and 12,5%, respectively. In comparison, the positive control group (0,2% chlorhexidine) produced a mean inhibition zone diameter of 12,84 mm, whereas the negative control group (distilled water) did not produce any inhibition zone (0,00 mm). Based on the results of the Mann–Whitney U test presented in Table 4, several significant differences were observed among the treatment groups. However, the Ajwa date seed extract mouthwash formulations 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 group containing 0,2% chlorhexidine (p > 0,05). The antibacterial activity of the extract may be attributed to the presence of bioactive compounds that disrupt bacterial cell walls and cell membranes, resulting in changes in membrane permeability and interference with the structure and function of proteins and nucleic acids [23].

These findings indicate that Ajwa date seed extract mouthwash possesses antibacterial activity comparable to that of chlorhexidine and may therefore be considered a natural alternative for mouthwash formulations. The absence of significant differences among the concentrations of Ajwa date seed extract mouthwash suggests that its antibacterial activity had already reached an optimal level at the lowest concentration tested (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), while 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 observed among the three concentrations.

As presented in Table 3, the Sukari date seed extract mouthwash exhibited strong antibacterial activity against *Streptococcus mutans*. The mean inhibition zone diameters were 10,80 mm, 11,95 mm, and 12,99 mm at concentrations of 3,25%, 6,5%, and 12,5%, respectively. The positive control group (0,2% chlorhexidine) exhibited moderate

doi:10.24843.MU.2026.V15.i10.03

antibacterial activity, with a mean inhibition zone diameter of 9,27 mm, whereas the negative control group (distilled water) exhibited weak antibacterial activity.

Based on the Mann–Whitney U test results presented in Table 4, the Sukari date seed extract mouthwash formulations at concentrations of 3,25%, 6,5%, and 12,5% showed p-values of less than 0,05 when compared with the positive control group, indicating significant differences in their effects on the growth of *Streptococcus mutans*. 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 less than 0,05. These findings indicate that, for Sukari date seed extract mouthwash, higher extract concentrations resulted in greater antibacterial activity. Furthermore, the size of the inhibition zone produced by Sukari date seed extract mouthwash was influenced by the concentration of the extract used. Increasing the concentration of the date seed extract was associated with enhanced inhibitory activity against the growth of *Streptococcus mutans*, as evidenced by the significant differences observed among the concentration groups ($p < 0,05$).

Based on the mean inhibition zone diameter results, Ajwa date seed extract mouthwash showed a higher inhibitory effect compared to Sukari date seed extract mouthwash at all concentrations. At a concentration of 3,25%, Ajwa exhibited an inhibition zone of 14,16 mm, while Sukari showed 10,80 mm. The Mann-Whitney test result showed a p-value of 0,004 ($p < 0,05$), indicating a significant difference between Ajwa and Sukari at this concentration. At a concentration of 6,5%, the mean inhibition zone of Ajwa was 14,42 mm, whereas Sukari showed 11,95 mm. The Mann-Whitney test resulted in a p-value of 0,013 ($p < 0,05$), indicating a significant difference between the two treatment groups. Meanwhile, at a concentration of 12,5%, the mean inhibition zone of Ajwa was 14,85 mm and Sukari was 12,99 mm. The Mann-Whitney test showed a p-value of 0,336 ($p > 0,05$), indicating that there was no significant difference between Ajwa and Sukari date seed extract mouthwash at the 12,5% concentration.

This indicates that Ajwa date seed extract mouthwash has higher antibacterial activity compared to Sukari at low and moderate concentrations. However, at higher concentration, both show relatively similar effectiveness in inhibiting the growth of *Streptococcus mutans*. An increase in the concentration of the extract used leads to a higher content of bioactive compounds such as alkaloids, flavonoids, tannins, and saponins, which act as antibacterial agents. The higher the concentration of the extract, the greater its potential inhibitory effect on bacterial growth. The antibacterial activity of an extract is not only determined by its concentration but is also influenced by the type and content of active antibacterial compounds present in it. In addition, the ability of the extract to diffuse into the test medium and the type of bacteria used in the assay are also important factors that may affect the magnitude of the resulting inhibitory activity [13].

The phytochemical content of dates is influenced by their variety. Analysis results showed that the total phenolic content (TPC) of Ajwa dates was 93,37 mg GAE/g, whereas Sukari dates contained only 39,01 mg GAE/g. This indicates that Ajwa dates have a significantly higher phenolic compound content compared to Sukari dates. In addition, flavonoid content showed a similar pattern, where the Safawi variety had the highest flavonoid content, followed by Ajwa, while Sukari had the lowest flavonoid content among the three varieties. Phenolic and flavonoid compounds exhibit strong antibacterial activity. These compounds act by damaging bacterial cell walls, disrupting cell membrane permeability, and inactivating essential bacterial enzymes and proteins. Flavonoids also inhibit nucleic acid synthesis and damage the structure of the bacterial cytoplasmic membrane. Therefore, a higher content of phenolic and flavonoid compounds corresponds to stronger antibacterial activity. Furthermore, research findings also show that the antioxidant activity of Ajwa dates is higher than that of Sukari dates. Antioxidant activity is closely related to the presence of bioactive compounds that also contribute to antibacterial effects. Thus, the higher antioxidant activity of Ajwa dates further supports its effectiveness in inhibiting bacterial proliferation [19].

Based on phytochemical screening results of Ajwa date flesh and seed extracts using methanol extraction, the total phenolic content was found to be 245,30 ± 0,046 and 195,54 ± 0,046 mg GAE/g of dry extract weight, respectively. The total flavonoid content (TFC) in the ethanol extracts of Ajwa date flesh and seed was 43,58 and 35,28 mg QE/g of dry extract weight, respectively. Ajwa date seeds showed higher extraction efficiency compared to the flesh, with a value of 21,19%, while the flesh showed 18,79% from 100 grams of dry powder weight. Preliminary phytochemical tests indicated that both the flesh and seed of Ajwa dates contained flavonoids and phenolic compounds, as shown by positive (+) results. Further phytochemical screening revealed that Ajwa date flesh and seed extracts contained various bioactive compounds, including alkaloids, saponins, flavonoids, glycosides, terpenoids, and phenolic compounds [24].

The phytochemical content of date seed extracts shows differences among varieties, namely Ajwa, Zaghloul, and Sukari. The highest total phenolic content (TPC) was found in the Zaghloul variety at 168,66 mg GAE/100 g dry weight, followed by Ajwa date seed extract at 115,05 mg GAE/100 g, while the lowest was observed in Sukari date seed extract at 65,88 mg GAE/100 g. These results indicate that phenolic compound content is strongly influenced by date variety differences. A similar pattern was also observed for total flavonoid content (TFC), where the Zaghloul variety showed the highest value at 112,33 mg CAE/100 g, followed by Ajwa at 55,33 mg CAE/100 g, while Sukari had the lowest content at 28,95 mg CAE/100 g. This indicates

doi:10.24843.MU.2026.V15.i10.03

that Sukari date seed extract has the lowest flavonoid content compared to the other two varieties [21].

Date seeds are known to contain dietary fiber and exhibit high antioxidant activity, which is influenced by their phenolic and flavonoid content when compared to the fruit flesh [16]. Ajwa date extracts obtained using acetone and methanol solvents have the ability to inhibit the growth of both Gram-positive and Gram-negative bacteria. In addition, date fruit extracts also demonstrate 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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