

Comparison of the Effectiveness of Ajwa Date (*Phoenix dactylifera* L.) Flesh and Seed Extracts against *Aggregatibacter actinomycetemcomitans* Bacteria with 70% and 100% Concentrates

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Abstrak. *Aggregatibacter actinomycetemcomitans* is a primary etiological agent in aggressive periodontitis, driving the need for alternative antibacterial agents. This experimental study aimed to compare the antibacterial effectiveness of Ajwa date (*Phoenix dactylifera* L.) flesh and seed extracts against this pathogen at concentrations of 70% and 100%. Utilizing a posttest-only control group design, the disc diffusion method was employed to assess inhibitory activity. Data were analyzed using the non-parametric Kruskal-Wallis test, with Mann-Whitney tests for post-hoc comparisons. Results indicated mean inhibition zones of 8.82 ± 1.02 mm and 10.51 ± 1.28 mm for flesh extracts at 70% and 100% concentrations, respectively. Seed extracts demonstrated significantly greater activity, producing zones of 15.61 ± 0.72 mm (70%) and 16.84 ± 0.57 mm (100%). The positive control, 0.2% chlorhexidine, yielded the largest inhibition zone (19.65 ± 0.38 mm), while DMSO as a negative control showed no activity. Kruskal-Wallis analysis confirmed statistically significant differences among all treatment groups ($p = 0.000$). Subsequent Mann-Whitney tests revealed significant differences in most pairwise comparisons, except between the two concentrations of flesh extract ($p = 0.055$). In conclusion, both Ajwa date flesh and seed extracts possess antibacterial properties against *A. actinomycetemcomitans*, with seed extracts exhibiting superior inhibitory potential. These findings support the therapeutic promise of Ajwa date components as natural alternatives for periodontal infection management.

Keywords: *Aggregatibacter actinomycetemcomitans*, ajwa dates, antibacterial, periodontitis.

INTRODUCTION

Indonesia's Basic Health Data in 2018 shows that the main problem related to teeth is caries or toothache, which accounts for around 45.3%. According to the Indonesian Ministry of Health (2020), inflammatory gingival conditions and abscesses are among the most frequent oral health problems, with a prevalence rate of around 14%. These findings highlight an urgent public health concern, emphasizing the necessity for timely intervention to prevent the escalation of oral disease incidence (Zavera Adam et al., 2022).

A common inflammatory condition among Indonesians, periodontal disease results from bacterial infections that damage the structures supporting the teeth, classified as either gingivitis or periodontitis (Gani et al., 2020). Its development is strongly linked to the accumulation of pathogenic bacterial communities, known as biofilms, which form dental plaque. The area beneath the gums harbors a diverse group of anaerobic, Gram-negative

bacteria responsible for the disease. This group includes bacteria such as *Porphyromonas gingivalis*, *Actinobacillus* species, *Prevotella intermedia*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, along with *Capnocytophaga* and *Veillonella* species (Primasari & Syaharani, 2023).

According to the American Academy of Periodontology (AAP), periodontitis is categorized into distinct types, notably chronic and aggressive periodontitis. Chronic periodontitis, which occurs more frequently in adults over 35 (Cahyani & Putri, 2021), represents the most prevalent form. Aggressive periodontitis is characterized by rapid progression and significant alveolar bone destruction, often with subtle initial symptoms and no clear association with localized factors (Salsabila et al., 2024). Treatment strategies for patients with aggressive periodontitis include mechanical plaque removal, scaling and root planing, adjunctive antibiotic use, and antimicrobial therapies (Cangara & Thahir, 2024).

Associated with aggressive periodontitis, *Aggregatibacter actinomycetemcomitans* is a facultatively anaerobic, non-motile, Gram-negative coccobacillus measuring 0.4–1 µm (Alibasyah et al., 2020). Preventive and therapeutic measures for oral bacterial infections commonly involve antiseptics and antibiotics, but extended use poses risks of microbial resistance and tissue irritation. Chlorhexidine 0.2%, a cationic broad-spectrum antiseptic, adheres effectively to bacterial surfaces (Oktaviani et al., 2022) and is utilized as an antiplaque mouthwash (Dumitriu et al., 2023). Despite its efficacy, chronic use may result in tooth discoloration, taste disturbances, allergic responses, mucosal irritation, calculus buildup, and glandular swelling (Sari, 2023; Primasari et al., 2023). Further complications such as xerostomia, oral pain, or numbness have been reported. Consequently, identifying natural antibacterial agents with favorable safety profiles is increasingly important in dental practice (Tobaq et al., 2023).

Ajwa dates (*Phoenix dactylifera* L) have long been known to have various health benefits, including as a natural antibacterial agent. The content of active compounds in Ajwa date meat extract, such as flavonoids, tannins, and saponins, has been shown to have antibacterial activity against various pathogens, including bacteria that cause periodontal disease (Rachmi Bachtiar et al., 2023). Based on the results of research, the date plant Ajwa (*Phoenix dactylifera* L.) has secondary metabolite compounds of flavonoids, phenolics, tannins, alkaloids and terpenoids (Attala et al., n.d., 2022). Meanwhile, Ajwa date seeds contain flavonoids, terpenoids, tannins, and saponins. The mechanism of action of saponins as antibacterial, namely can cause the leakage of proteins and enzymes from within the cell. Tannins are able to form metal ion bonds with iron that are indispensable for bacteria to reduce DNA ribonucleotide precursors (Fikayuniar et al., 2022).

Prior research has substantiated the antimicrobial potential of Ajwa date extracts. Bachtiar et al. (2023) demonstrated that Ajwa date extract effectively inhibits *Porphyromonas gingivalis* at concentrations of 50%, 70%, and 100%. Further investigations by Amiruddin & Rusyd (2024) confirmed that both flesh and seed extracts of Ajwa dates exhibit antibacterial activity against oral microorganisms, including *Candida albicans* and *Streptococcus mutans*. Additionally, Ajwa date flesh extract prepared with aquadest solvent has been shown to inhibit *Staphylococcus aureus* (Albab et al., 2020). Similarly, seed extracts at 25% concentration demonstrated inhibitory effects against *S. aureus* (Fikayuniar et al., 2022). Broader studies on date seed (*Phoenix dactylifera* L.) extracts have reported antibacterial

efficacy against *Lactobacillus casei*, *Streptococcus mutans*, and *Staphylococcus aureus*, with a minimum inhibitory concentration (MIC) of 20 mg/ml, a minimum bactericidal concentration (MBC) of 80 mg/ml, and optimal activity at 100 mg/ml (Adedayo et al., 2020).

This study uses the disc diffusion method because the diffusion method is used to evaluate the sensitivity of the test material to bacteria, while the dilution method serves to determine the values of the Minimum Inhibition Concentration (KHM) and Minimum Bactericidal Concentration (KBM) (Prisnanda & Wulandari, n.d., 2022). This diffusion method has various advantages, including practicality, easy to carry out tests, and able to measure sensitivity to aerobic bacteria and facultative anaerobic bacteria. In addition, the results of this method can also be read quickly (Fatimah et al., 2022).

Research on the comparison of Ajwa date meat and seed extracts has never been researched, therefore this researcher wants to prove "Comparison of the effectiveness of Ajwa date meat and seed extract against *Aggregatibacter actinomycetemcomitans* bacteria with a concentration of 70% and 100%" which is one of the bacteria that causes aggressive periodontitis. Thus, the researchers hope that the results tested can contribute to developing natural ingredients that can be used in the prevention and treatment of periodontal disease.

The objective of this research is to evaluate and compare the antibacterial effectiveness of Ajwa date flesh and seed extracts at 70% and 100% concentrations against *Aggregatibacter actinomycetemcomitans*, hypothesizing a measurable difference between extract types. The methodology includes phytochemical screening and antibacterial testing, with chlorhexidine as a comparative control. Expected contributions encompass multiple domains: theoretical advancement in the study of natural antimicrobials; clinical relevance as a potential adjunctive natural treatment for periodontal disease; academic enrichment by providing foundational data for future investigations in oral health; and societal benefit through public education on the oral health potential of Ajwa dates.

MATERIALS AND METHODS

An experimental approach with a posttest-only control design was implemented to examine the antibacterial effectiveness of Ajwa date (*Phoenix dactylifera* L.) flesh and seed extracts against *Aggregatibacter actinomycetemcomitans*. The disc diffusion technique was applied, wherein antibacterial activity was indicated by the presence and size of inhibition halos around discs containing the test substances. Experimental groups included 70% and 100% concentrations of both flesh and seed extracts, complemented by a 0.2% chlorhexidine positive control and a DMSO negative control.

The research timeline spanned September to October 2025 and involved several specialized locations: extract production occurred at the Medicinal Plant Research and Development Laboratory (ASPETRI) in Medan; bacterial propagation and antibacterial assays were conducted at the Integrated Laboratory of Universitas Prima Indonesia; and botanical authentication of Ajwa dates was performed at the University of North Sumatra Herbarium. The study utilized a pure culture of *A. actinomycetemcomitans* as its population, with a total of 36 experimental units allocated across six treatment groups each replicated six times as determined by standard experimental replication guidelines.

This study defined the independent variables as Ajwa date flesh extract (70%, 100%),

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seed extract (70%, 100%), 0.2% chlorhexidine (positive control), and DMSO (negative control). The dependent variable was the inhibitory effect on *Aggregatibacter actinomycetemcomitans*. Methodologically, Ajwa dates were collected and authenticated, then extracted with 70% ethanol via maceration and concentrated by evaporation. Extracts were screened for flavonoids, tannins, saponins, alkaloids, and terpenoids.

For antibacterial testing, a uniform lawn of *A. actinomycetemcomitans* was prepared on MHA plates. Sterile discs infused with extracts or controls were applied, and plates were incubated for 24 hours at 37°C in a CO₂ incubator. Zone diameters were measured with a caliper. Data were analyzed using the Kruskal-Wallis test; if significant, Mann-Whitney post-hoc testing was performed to differentiate between specific groups.

RESULTS AND DISCUSSION

Screening Phytochemistry

Table 1. Phytochemical Screening Results of Ajwa Dates

Metabolite seconds	Results	
	Ajwa Date Meat Extract	Ajwa Date Seed Extract
Flavonoid	+	+
Alkaloid	+	+
Terpenoid	+	+
Tannins	+	+
Saponin	+	+

Note: (+) indicates the presence of the compound.

Source: Primary data from laboratory analysis (2025)

Based on the results of the study, it can be stated that ajwa dates contain several secondary metabolites including flavonoids, saponins, triterpenoids, alkaloids, and tannins.

Average Diameter of Inhibition Zone

The assessment of antibacterial activity involved measuring the clear zones formed around discs impregnated with Ajwa date extracts when tested against *Aggregatibacter actinomycetemcomitans*. Diameters were recorded with a calibrated sliding caliper. To strengthen the validity of findings, six replicate trials were executed for every treatment condition. A complete summary of the derived measurements is consolidated in Table 2.

Table 2. Average Diameter of Inhibition Zone

Groups	N	Jamming Zone Diameter (mm)
		Mean ± SD
Ajwa Date Meat Extract 70%	6	8,82 ± 1,02
100% Ajwa Date Meat Extract	6	10,51 ± 1,28
Ajwa Date Seed Extract 70%	6	15,61 ± 0,72
100% Ajwa Date Seed Extract	6	16,84 ± 0,57
Positive Control	6	19,65 ± 0,38
Negative Control	6	0,0 ± 0,0

Source: Primary data from antibacterial assay using disc diffusion method (2025)

A comparative evaluation of the inhibition data showed significant variation in antibacterial performance. For Ajwa date flesh extract, the mean zone diameters were 8.82 ± 1.023 mm (70%) and

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10.51 ± 1.28 mm (100%). The corresponding seed extract groups produced larger zones of 15.61 ± 0.72 mm and 16.84 ± 0.57 mm, respectively. The reference antiseptic, 0.2% chlorhexidine, generated the maximum inhibition zone (19.65 ± 0.38 mm), while DMSO, serving as the negative control, failed to inhibit bacterial growth.

Normality and Homogeneity Test Results

Normality and homogeneity are the requirements for the statistical test to be used. The full results of the study can be seen in Table 4.3 below.

Table 3. Normality and Homogeneity Test Results

Groups	Shapiro-Wilk	Levene Test
Ajwa Date Meat Extract 70%	0,52	0,000
100% Ajwa Date Meat Extract	0,37	
Ajwa Date Seed Extract 70%	0,08	
100% Ajwa Date Seed Extract	0,88	
Positive Control	0,86	
Negative Control	-	

Source: Statistical analysis using SPSS version 26 (2025)

Testing for normality using the Shapiro-Wilk procedure confirmed that the data were normally distributed ($p > 0.05$). In contrast, Levene's test for homogeneity of variance indicated significant heterogeneity across groups ($p < 0.05$). Due to the unmet assumption of equal variances, non-parametric analyses were performed. The Kruskal-Wallis test assessed the global differences between groups, followed by Mann-Whitney tests for specific group comparisons where indicated.

Difference in Diameter of the Inhibition Zone of the Whole Group

The test of the difference in the diameter of the inhibition zone of the whole group used the Kruskal-Wallis statistical test which can be seen in the following table 3.4.

Table 4. Difference in Diameter of Inhibition Zones of All Groups

Groups	Red ± SD	Kruskal-Valais
Ajwa Date Meat Extract 70%	8,82 ± 1,02	0,000*
100% Ajwa Date Meat Extract	10,51 ± 1,28	
Ajwa Date Seed Extract 70%	15,61 ± 0,72	
100% Ajwa Date Seed Extract	16,84 ± 0,57	
Positive Control	19,65 ± 0,38	
Negative Control	0 ± 0	

Source: Statistical analysis using SPSS version 26 (2025)

*Signifikan

According to the results displayed in Table 4, the Kruskal-Wallis test identified a statistically significant difference in the inhibition zone diameters among the treatment groups ($p = 0.000$). This finding substantiates that both the type of extract (flesh versus seed) and the concentration (70% and 100%) significantly influence the antibacterial activity against *A. actinomycetemcomitans*.

Difference in Inhibition Zone Diameter Between Two Different Groups

The diameter of the barrier zone between two different groups in this study was tested using the *Mann-Whitney* test. The full results of the research can be seen in Table 3.5 below.

Table 5. Difference in Inhibition Zone Diameter between Two Different Groups

Groups	EDKA 70%	EDKA 100%	EBKA 70%	EBKA 100%	K+	K-
EDKA 70%		0,055	0,004*	0,004*	0,004*	0,002*
EDKA 100%	0,055		0,004*	0,004*	0,004*	0,002*
EBKA 70%	0,004*	0,004*		0,010*	0,004*	0,002*
EBKA 100%	0,004*	0,004*	0,010*		0,004*	0,002*
K+	0,004*	0,004*	0,004*	0,004*		0,002*
K-	0,002*	0,002*	0,002*	0,002*	0,002*	

Source: Statistical analysis using SPSS version 26 (2025)

*Signifikan

Description:

EDKA: Ajwa Date Meat Extract

EBKA: Ajwa Date Seed Extract

K+: Kontrol Positif (*Chlorhexidine* 0.2%)

K-: Negative Control (DMSO)

The analysis presented in Table 5 indicates no statistically significant variance in the inhibition zone diameter between the 70% and 100% ajwa date flesh extract groups ($p=0.055$). In contrast, a significant difference was observed when comparing the 70% flesh extract to both the 70% and 100% seed extract groups, as well as to the negative control ($p=0.002$). Furthermore, the Mann-Whitney test revealed a significant disparity between the two concentrations of seed extract ($p=0.010$). A defined and significant difference was also noted between the combined flesh/seed extract groups and the positive control ($p=0.004$).

The non-significant result for the flesh extract concentrations aligns with their respective mean inhibition zones of 8.82 ± 1.02 mm (70%) and 10.51 ± 1.28 mm (100%). This finding is consistent with the research of Bachtiar et al. (2023), which reported that 100% ajwa date flesh extract demonstrated greater efficacy against *Porphyromonas gingivalis* than lower concentrations. As noted by Mawaddah and Mayasari (2024), antibacterial efficacy is governed by multiple factors, including extract concentration, bioactive compound profile, diffusion properties, and the target bacterial species.

The inhibition zone diameters for ajwa date seed extract exhibited a statistically significant concentration-dependent response ($p=0.010$), with the 100% concentration (16.84 ± 0.57 mm) outperforming the 70% concentration (15.61 ± 0.72 mm). This pattern reflects a well-documented pharmacological relationship where higher extract concentrations typically yield larger inhibition zones, a principle supported by Leing and Rini (2025). Comparable concentration-dependent effects have been observed in related research; for instance, Amiruddin et al. (2025) reported that 50% date seed extract demonstrated superior inhibition of *Streptococcus mutans* compared to lower concentrations.

Comparative analysis revealed that ajwa date seed extract demonstrates significantly greater inhibitory activity against *A. actinomycetemcomitans* than the flesh extract ($p=0.004$).

This finding is consistent with existing literature that characterizes date seed extract as a strong antibacterial agent, producing inhibition zones typically between 10-20 mm. The differential efficacy likely stems from variations in bioactive composition. Date flesh contains a diverse array of phytochemicals including flavonoids, tannins, and sterols, whereas the seed is notably richer in proteins and lipids (Fikayuniar et al., 2022). These compositional differences may explain the observed variance in antimicrobial potency between the two extract types.

Identical phytochemical profiles were observed in both ajwa date flesh and seed extracts, comprising flavonoids, saponins, triterpenoids, alkaloids, and tannins. This compositional uniformity supports their documented effectiveness against oral bacteria, as noted by Amiruddin and Rusyd (2025). Each compound class contributes uniquely to the overall antibacterial effect: Flavonoids operate through triple mechanisms suppressing nucleic acid formation, disrupting membrane function, and halting metabolic energy pathways (Rodriguez et al., 2023). Saponins compromise cellular integrity, provoking leakage of intracellular components (Fikayuniar et al., 2022). Triterpenoids bind to lipid membranes, inducing structural damage and inhibiting nutrient absorption (Ardelia et al., 2025). Tannins sequester vital metal ions, depriving bacteria of essential cofactors. Alkaloids target peptidoglycan biosynthesis, leading to defective cell wall synthesis and bacterial death (Fikayuniar et al., 2022).

A post-hoc Mann-Whitney analysis revealed a significant disparity in efficacy between ajwa date (*Phoenix dactylifera L.*) extracts (both flesh and seed) and the 0.2% chlorhexidine positive control ($p=0.002$). The chlorhexidine group demonstrated a mean inhibition zone of 19.65 ± 0.38 mm, surpassing that of the natural extracts. This indicates that, while bioactive, ajwa date extracts do not yet match the potent, broad-spectrum antimicrobial action of chlorhexidine against *Aggregatibacter actinomycetemcomitans*. This finding aligns with research by Bachtiar et al. (2023), which reported chlorhexidine's superior efficacy over various concentrations of ajwa date extract against *Porphyromonas gingivalis*.

Chlorhexidine's established mechanism involves its cationic molecules binding to negatively charged microbial cell surfaces, disrupting membrane integrity, increasing permeability, and inducing cytoplasmic coagulation, ultimately leading to cell death (Tjiptoningsih et al., 2023). Given its robust performance, future studies could explore chlorhexidine as a benchmark when evaluating other natural extracts against *A. actinomycetemcomitans* or test ajwa date extracts against different bacterial species to further delineate its antimicrobial spectrum.

Antibacterial activity was assessed via the disc diffusion method, with inhibition zones interpreted as evidence of growth suppression (Tjiptoningsih & Trisanto, 2022). However, this approach has recognized limitations, as zone size can be affected by technical factors like incubation settings, inoculum load, pre-diffusion and pre-incubation protocols, and the thickness of the seeded agar (Goetie et al., 2022).

Key limitations of this study include the omission of dilution-based assays to quantify the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *A. actinomycetemcomitans*. Furthermore, the potential toxicity of the ajwa date flesh and seed extracts was not evaluated, leaving a gap in the safety assessment of these materials.

CONCLUSIONS

The findings of this study confirm that both Ajwa date flesh and seed extracts possess antibacterial properties against *Aggregatibacter actinomycetemcomitans*. However, the seed extract demonstrated superior inhibitory activity at both 70% and 100% concentrations compared to the flesh extract, though neither matched the efficacy of the 0.2% chlorhexidine positive control. Statistical analysis revealed significant differences in antibacterial effectiveness between the flesh and seed extracts at each concentration, as well as between the two concentrations of seed extract. In contrast, no significant difference was observed between the two concentrations of flesh extract. In summary, Ajwa date seed extract exhibits greater antibacterial potential than the flesh extract, yet remains less effective than the standard antiseptic chlorhexidine against *A. actinomycetemcomitans*.

To build upon these findings, follow-up research employing dilution assays is advised to establish the MIC and MBC values of Ajwa date extracts. This would refine the understanding of their antimicrobial potential. Concurrently, toxicity testing must be conducted to ensure biocompatibility for clinical use. The development of practical formulations such as oral rinses or topical gels could facilitate real-world application. Additional studies exploring the extract's activity against a broader range of periodontal bacteria, as well as *in vivo* evaluation, are also recommended to substantiate its viability as an adjunct in periodontal treatment.

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