

The Antibacterial Comparative Analysis of Ajwa Dates With and Without Tahnik on *Staphylococcus aureus*

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DATA OF ARTICLE:

Received:

Reviewed:

Revised:

Accepted: ...

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DOI:

.....

TYPE OF ARTICLE:

Research

Abstract: Neonatal infections are a leading cause of infant mortality in Indonesia, especially in low birth weight infants. *Staphylococcus aureus* is one of the most common pathogens with high antibiotic resistance. Ajwa dates contain bioactive compounds such as flavonoids, alkaloids, tannins/polyphenols, and saponin. Tahnik, a traditional practice of chewing dates and applying them to an infant's palate, is believed to offer both antimicrobial and immunomodulatory benefits. This study aims to analyze the phytochemical content and evaluate the antibacterial effects of dates with and without tahnik against *Staphylococcus aureus*. A quasi-experimental in vitro design was used, involving six treatment groups: dates without tahnik, tahnik dates chewed for 15, 30, and 60 seconds, a positive control (ampicillin), and a negative control (aquadest). The phytochemical test showed that all dates contained alkaloids, tannins/polyphenols, and saponins. All groups of dates demonstrated antibacterial activity. The highest inhibition zone was observed in without tahnik group (6.84±0.87 mm). The Kruskal-Wallis analysis showed significant differences between groups ($p<0.05$), but post hoc analysis showed that only the positive and negative control groups differed significantly. No statistically significant difference was found between dates with and without tahnik groups. Further research is needed to explore other potential biological effects of tahnik.

Keywords: Antibacterial Activity; Phytochemistry; *Staphylococcus aureus*; Tahnik Dates

INTRODUCTION

Neonatal infections are still the leading cause of infant mortality in Indonesia, especially babies born with low birth weight (LBW). These infections occur during the prenatal period or in the first four weeks of life. According to the Indonesian Ministry of Health (2022), LBW was the highest cause of neonatal mortality in 2021, at 34.5% of cases, followed by asphyxia at 27.8%.¹ Among the most frequently identified bacterial pathogens is *Staphylococcus aureus*, a Gram-positive bacterium known for its high resistance to antibiotics. Reports indicate that this bacterium was the most prevalent pathogen in neonatal infections, with a prevalence of 26.9%, surpassing other common pathogens such as *Streptococcus pyogenes* and *Klebsiella pneumoniae*.² The increasing threat of antibiotic resistance highlights the need to explore alternative antibacterial agents derived from natural products.

One of the potential natural ingredients is dates (*Phoenix dactylifera*), especially the Ajwa variety. Ajwa dates are widely known in Indonesia, not only because of their religious value in Islamic tradition, but also because of their bioactive compounds such as alkaloids, flavonoids, and tannins.^{3,4} These compounds are known to have antibacterial activity by damaging bacterial cell membranes, disrupting metabolic processes, and inhibiting protein synthesis.⁵ Previous studies have shown that date extract can inhibit the growth of *Staphylococcus aureus*, with inhibition zones ranging from 6 to 20 mm depending on the variety and concentration of the extract.⁶

In Islamic teachings, the practice of tahnik is a sunnah recommended by the Prophet Muhammad, it's made by chewing dates and then rubbing them on the roof of the mouth of a newborn baby while praying for him. This practice is believed to provide health benefits such as improving the immune system and supporting digestive tract health.⁷ In addition to the active content of dates, human saliva also plays an important role through antimicrobial components such as lysozyme, lactoferrin, and secretory immunoglobulin A (sIgA).⁶ Lysozyme damage bacterial cell walls, leading to cell lysis.⁸

Although the cultural and religious significance of tahnik is well recognized, the effect of chewing duration on its antibacterial activity has not yet been scientifically studied. Observational findings suggest that the practice is often carried out with an odd number of chews, such as three, five, or seven, without any standardized duration.⁹ However, no previous studies have specifically investigated whether the duration of chewing impacts the antibacterial potential of tahnik preparations.

This study aims to evaluate the phytochemical content and antibacterial activity of Ajwa dates with and without tahnik, using varied chewing durations (15, 30, and 60 seconds), against the growth of *Staphylococcus aureus*. A quasi-experimental in vitro design was employed, and the outcomes are expected to provide scientific insight into the antibacterial potential of tahnik, while preserving and supporting traditional Islamic health practices.

MATERIALS AND METHOD

This study employed an in vitro quasi-experimental design with a non-equivalent control group, conducted at the Preparation and Research Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Prof. DR. HAMKA between February and April 2025. The dates used in this study were Ajwa variety dates, Castle Farm brand, imported from Medina and packaged by PT Hasta Niaga Berkah Bekasi. Six treatment groups were examined: Ajwa date extract without tahnik, tahnik date extract chewed for 15, 30, and 60 seconds, a positive control (ampicillin), and a negative control (distilled water). Each group underwent three replications. Date and tahnik extracts were prepared by homogenizing 8 g of dates in 40 mL of distilled water, followed by filtration, centrifugation at 5000 rpm for 10 minutes at 5°C, and sterilization using a 0.45 µm membrane filter.¹⁰ The tahnik date extract was prepared in the same way, but the dates were first chewed for specific durations before processing. To minimize the potential inhibitory effect of the oral microbial flora from the chewer, all date and tahnik extracts were prepared on the same day.

Phytochemical screening was performed to detect the presence of alkaloids, flavonoids, tannins/polyphenols, saponins, triterpenoids, and steroids using standard colorimetric qualitative tests.^{11–13} Identification of *Staphylococcus aureus* involved culturing on Mannitol Salt Agar and Gram staining.¹⁴ Antibacterial testing was performed using the disk diffusion method. A bacterial suspension standardized to 0.5 McFarland was spread on MHA plates. Sterile paper disks were soaked in the test solutions and placed on the agar surface. Plates were incubated at 37°C for 18–24 hours. Inhibition zones were measured in millimeters and categorized based on Greenwood's classification.^{10,15,16}

The antibacterial activity data were analyzed statistically. Data normality was assessed using the Shapiro-Wilk test and homogeneity with Levene's test. If data were normally distributed and homogeneous, one-way ANOVA followed by LSD Post Hoc test was applied. Otherwise, the Kruskal-Wallis and Dunn's tests were used. Statistical analysis was performed using GraphPad Prism software version 10.4.1. This study received ethical approval from the Health Research Ethics Committee of Universitas Muhammadiyah Prof. DR. HAMKA (No. KEPKK/FK/009/03/2025).

RESULT

Phytochemical screening was conducted to identify bioactive compounds in the tahnik and non-tahnik date extracts. The results showed that all samples contained alkaloids, tannins/polyphenols, and saponins. However, no flavonoids, triterpenoids, or steroids were detected. Detailed results of the tests and reagents used are presented in Table 1.

Table 1. Phytochemical Screening Results

Phytochemicals	Reagent	Reference	Result			
			Dates without tahnik	Tahnik dates 15 seconds	Tahnik dates 30 seconds	Tahnik dates 60 seconds
Alkaloid	Meyer	White precipitate	+	+	+	+
	Wagner	Reddish-brown precipitate	+	+	+	+
	Dragendorff	Reddish-brown precipitate	+	+	+	+
Flavonoid	Wilstater	Red, orange, and yellow	-	-	-	-
Tannin/ Polyphenols	FeCl ₃	Dark bluish-green	+	+	+	+
Saponin	Concentrated HCL	Stable foam	+	+	+	+
Triterpenoids	Liebermann-Burchard	Red to brown color	-	-	-	-
Steroid	Liebermann-Burchard	Bluish-green ring	-	-	-	-

Description :

(+) : Positive, indicating the presence of compounds based on the test performed

(-) : Negative, indicating no detectable compounds based on the test performed

Bacterial identification was carried out using Mannitol Salt Agar (MSA) medium, which showed the growth of golden-yellow colonies. Gram staining confirmed the presence of purple-stained, round-shaped cocci arranged in clusters, indicating that the bacteria were *Staphylococcus aureus*. The colonies were further cultured on Nutrient Agar (NA), producing milky-white, round colonies with smooth surfaces and convex elevation. The visualization of the bacterial identification process is shown in Figure 1.

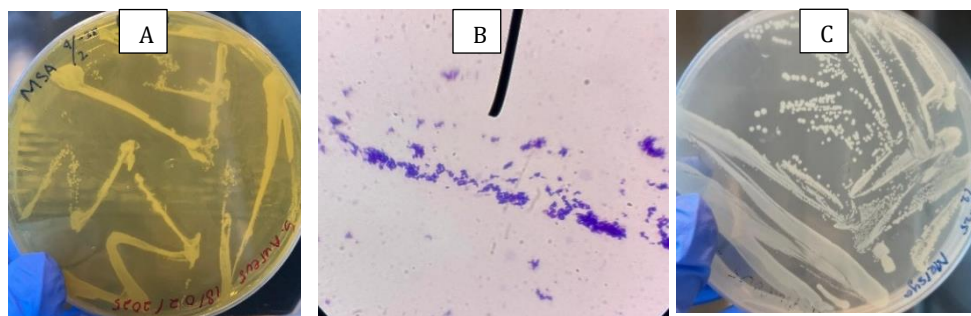


Figure 1. (A) *Staphylococcus aureus* on MSA, (B) Gram Staining, (C) *Staphylococcus aureus* on NA

Antibacterial activity was evaluated using the disk diffusion method, performed in four replications for each treatment. Inhibition zones were observed in all date extract treatments, except for the negative control (distilled water). The positive control (ampicillin) produced the largest inhibition zone, followed by the non-tahnik date extract with an average zone of 6.84 ± 0.87 mm. The next highest inhibition zones were observed in the 30-second, 15-second, and 60-second tahnik extracts, respectively. The inhibition zones are visualized in Figure 2, and the measurement results are detailed in Figure 3.

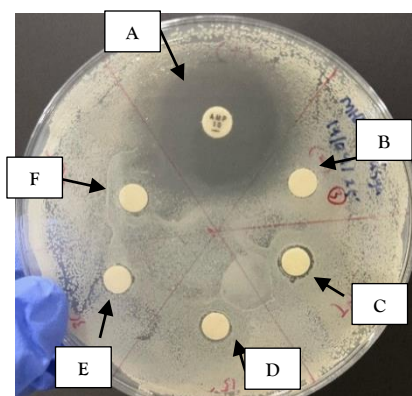


Figure 2. Visualization of Antibacterial Test Using Disc Diffusion Method: (A) Positive Control, (B) Negative Control, (C) Dates without tahnik, (D) Tahnik dates 15 seconds, (E) Tahnik dates 30 seconds, (F) Tahnik dates 60 seconds. Arrow sign (↑) = Inhibition zone.

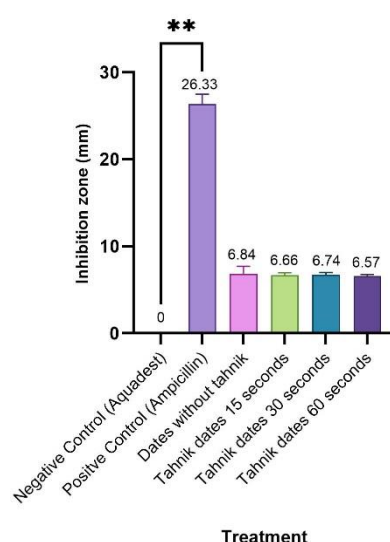


Figure 3. Antibacterial Effects of Ajwa Dates with and without Tahnik on *Staphylococcus aureus*. Mean inhibition zones (mm) \pm SD (n = 3), Kruskal-Wallis test, $p = 0.038$; only the positive and negative controls showed significant difference, (**) = $P < 0,01$

DISCUSSION

The results of phytochemical tests showed the presence of secondary metabolite compounds, namely alkaloids, polyphenols or tannins and saponin in dates with and without tahnik.^{4,6,11,17} Alkaloids are known to have various biological activities, including as analgesics, antimicrobials, and anti-inflammatories.⁴ Phenolic components, namely polyphenols and tannins have antioxidant and antimicrobial properties.⁶ Meanwhile, Saponin has benefits as an antimicrobial, antiviral, antioxidant, and can increase the activity of the body's immune system.¹⁷⁻¹⁹ This finding is different from several previous studies that generally reported the presence of flavonoids as one of the dominant compounds in dates.^{4,6,17,20} This can be caused by various factors such as the location of the growth, the use of inappropriate solvents or suboptimal extraction methods.²¹ Another study reported that flavonoid compounds are sensitive to temperature and light exposure. Careless processes in sample preparation can result in the loss of these compounds and cause negative test results.²² The phytochemical tests that have been carried out provide information on the content of metabolite compounds in untreated and treated dates.

Bacterial identification was carried out to ensure that the test bacteria were *Staphylococcus aureus*. Mannitol Salt Agar (MSA) media showed a picture of a round colony, golden yellow in color and changed the color of the surrounding medium to yellow due to fermenting mannitol.²³ Gram staining showed the morphology of the bacteria was purple, round (coccus), and arranged in groups resembling a series of

grapes.²⁴ Subculture to Nutrient Agar produced round, milky white colonies with a smooth surface and convex elevation.²⁵ The results of the antibacterial test showed that all dates, both with and without tahnik, had antibacterial effects against *Staphylococcus aureus*. The largest average inhibition zone was found in dates without tahnik, followed by dates with tahnik for 30 seconds, 15 seconds, and finally 60 seconds. All treatments were categorized as moderate antibacterial (5–10 mm) according to the classification of Greenwood.¹⁶ These results are in line with previous findings, which reported that the aqueous extract of Ajwa date fruit showed an average inhibition zone diameter of 5.87 mm against *Staphylococcus aureus*, and was also included in the moderate category.²⁶ Although there was a difference in the mean, no significant difference was found between groups, indicating that their antibacterial activity was relatively comparable.

The antibacterial potential of dates is believed to come from bioactive compounds such as polyphenols, alkaloids, and saponins.^{3,4} These compounds can interact with the cell wall or DNA (Deoxyribonucleic Acid) of bacteria, disrupt metabolism, increase membrane permeability, and ultimately cause cell lysis.²⁷ In this study, the antibacterial activity of tahnik dates appeared lower than that of without dates. This is thought to be related to the mixing of dates with saliva during the tahnik process, which can affect the concentration of active compounds and provide a nutrient substrate for bacteria. This finding is in accordance with previous studies, which stated that Tahnik Dates themselves are not effective in inhibiting *E. coli* bacteria, but become more effective when combined with LAB (Lactic Acid Bacteria) from breast milk.²⁸ This indicates that Tahnik Dates can act as a modulator or supporter of the effectiveness of Lactic Acid Bacteria, but are not effective alone against pathogenic bacteria. Of the tahnik group, a chewing duration of 30 seconds showed the highest antibacterial activity. This duration is thought to be the optimal point between the release of active compounds and sufficient saliva volume for their distribution. Chewing increases saliva secretion and breaks down food structures, making active compounds more easily dissolved and distributed.^{29,30} A duration of 15 seconds may not be enough, while chewing for 60 seconds risks increasing soluble sugar levels that support bacterial growth. Ripe dates are known to contain glucose (34.5%), fructose (25.6%), and sucrose (0.5%).^{31,32}, which are energy sources for *Staphylococcus aureus*.³³

Human saliva contains various components with antimicrobial activity, including lysozyme, sialoperoxidase, and lactoferrin that play a role in maintaining the balance of the oral microbiota.⁸ Lysozyme is particularly effective against Gram-positive bacteria such as *Staphylococcus aureus* by breaking down the bacterial cell wall.³⁴ However, the most abundant salivary protein is α -amylase which only works on starch which is not abundant in ripe dates so its possible role in the antibacterial effect is very small.^{32,35} The content of lysozyme itself also varies greatly and is difficult to measure consistently.³⁶ Therefore, it is not certain whether a longer chewing duration will increase the concentration of antibacterial enzymes or digestive enzymes. Overall, the results of this study indicate that the antibacterial effectiveness of tahnik dates is influenced by many factors, namely, the content of active compounds from dates, but also influenced by additional factors such as chewing duration, volume and composition of saliva, and the amount of simple sugars dissolved and can be a substrate for bacterial growth. Although there was a difference in the average antibacterial effect between tahnik dates with varying chewing duration and without tahnik, no statistically significant difference was found. However, this study can be the basis for a hypothesis for further research, especially related to the release of active compounds and their interactions with saliva components.

Several limitations of this study are: first, phytochemical analysis used in this study was only qualitative, so it did not provide information on the relative levels of each secondary metabolite compound that might contribute to the antibacterial effect. Second, this study did not include a comprehensive evaluation of saliva characteristics such as volume, pH, soluble sugar content, and antimicrobial compound content.

The findings of this study still provide an important contribution that the potential of tahnik is not limited to antibacterial activity. In the Islamic tradition, tahnik is seen as a natural immunization for newborns. Although the biological mechanism of this effect is still not fully understood, there is a hypothesis that microRNAs (miRNAs) in adult saliva used during the tahnik process play a role in enhancing the infant's immune response through regulating gene expression and its influence on the immune system.⁹ In addition, tahnik dates have also been shown to prevent neonatal hypoglycemia more effectively and safely than oral dextrose gel because they have a low glycemic index.³⁷ Therefore, the biological potential of tahnik deserves further study from various aspects, not limited to antibacterial effects, but also from the immunological and metabolic aspects for newborns.

CONCLUSION

This study concluded that Ajwa dates with and without tahnik (chewing duration of 15, 30, and 60 seconds) have similar bioactive compounds, namely alkaloids, tannins or polyphenols, and saponins, and did not show the presence of flavonoids, triterpenoids, or steroids. All groups showed antibacterial activity against *Staphylococcus aureus*, with the highest average inhibition zone in the group without tahnik. Although chewing duration influenced the inhibition zone, the differences among the tahnik groups were not statistically significant.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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