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**EFFECTIVENESS OF AJWA DATE SEED EXTRACT EFFERVESCENT TABLETS
 (*Phoenix dactylifera* L.) AGAINST MICROORGANISMS ON ACRYLIC RESIN**

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ABSTRACT

Background: Suboptimal denture cleaning methods present a problem for denture users, leading to the buildup of bacteria and fungi on the denture base. *Streptococcus mutans* is a bacterium involved in plaque formation, while *Candida albicans* is the most abundant fungus in the oral cavity. Ajwa date seeds, with a concentration of 3.25%, have been shown to strongly inhibit the growth of both bacteria and fungi. This study utilizes effervescent tablets containing Ajwa date seed extract.

Purpose: The aim of this study is to evaluate the effect of immersing effervescent tablets containing Ajwa date seed extract on the growth of *Streptococcus mutans* and *Candida albicans* on acrylic resin. **Methods:** The research employed a True Experimental design with a Post Test Only Control Group. A spectrophotometer was used to measure bacterial and fungal growth, and various concentrations of the extract in effervescent tablet preparations were tested. Alkaline peroxide was used as a positive control, while distilled water served as a negative control. **Results:** The One-Way ANOVA test showed that the smallest bacterial count was observed in the alkaline peroxide group (0.008) and the 13% extract concentration group (0.011). The largest bacterial count was observed in the distilled water group (0.065). The smallest fungal count was also observed in the alkaline peroxide group (0.022), while the largest fungal count was recorded in the distilled water group (0.210). **Conclusion:** The One-Way ANOVA test revealed significant differences in the number of bacteria and fungi based on the treatment groups.

Keywords: Ajwa Date Seed, *Candida Albicans*, Denture Cleaning, Effervescent Tablet, *Streptococcus Mutans*

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INTRODUCTION

Suboptimal denture cleaning and maintenance methods pose a significant issue for denture users.¹ This leads to plaque accumulation containing bacteria and fungi on the denture base, which can result in swelling and pain in the oral mucosa (Denture stomatitis). Scully et al. (2013) defined denture stomatitis as a condition in denture users characterized by chronic inflammation with erythema in the mucosal tissue that contacts the denture base. This condition is often asymptomatic but may cause bleeding in the inflamed mucosal areas, a burning sensation, halitosis, discomfort, and xerostomia. *Streptococcus mutans* is a bacterium involved in plaque formation in the oral cavity, while *Candida albicans* is the most common fungus found in the oral cavity of denture users.²

The biofilm formation process begins with the formation of a salivary pellicle, followed by physicochemical, hydrophobic, and electrostatic interactions. This is followed by stronger molecular and cellular interactions, resulting in the attraction, absorption, and attachment of bacteria to the surface of acrylic denture resin. *Candida albicans* is known to respond to environmental stimuli and can penetrate the surface of the denture.³

Chemical cleaning methods for dentures are more effective than mechanical methods.⁴ One such cleaning agent is the effervescent tablet. The advantage of effervescent tablets over other forms of cleaning agents is their practicality, rapid dissolution in water without the need for stirring, sparkling effect, and longer shelf life.⁵ The common base material for dentures is polymethyl methacrylate (PMMA) resin, polymerized by heat. The benefits of acrylic include good aesthetics, biocompatibility, non-toxicity, and stable color resembling gingiva. However, it has drawbacks, such as being prone to fracture and liquid absorption.⁶

Prophetic Medicine, based on the teachings of the Prophet Muhammad (PBUH), includes principles for disease prevention. One herbal plant mentioned in these teachings is the date palm.⁷ Ajwa dates are considered the best type of dates in terms of composition, with superior nutritional content and texture compared to ordinary dates with thin skins. The seeds of Ajwa dates contain alkaloids, which act as antibacterial agents, and flavonoids, which function as antioxidants. Compared to the flesh of the fruit, the seeds have higher antioxidant content, yet the seeds are often underutilized, as people generally consume only the flesh of the date.⁸

Research by Fikayuniar et al. (2022) indicated that ethanol extract from Ajwa date seeds has strong antibacterial properties due to the presence of active compounds such as flavonoids, alkaloids, and phenols, which are known for their antibacterial, antiviral, and antifungal effects.⁹ Another study by Amiruddin et al. (2024) found that extracts from both the flesh and seeds of Ajwa dates at a concentration of 3.25% could significantly inhibit the growth of *Streptococcus mutans* and *Candida albicans*.¹⁰

The novelty of this research lies in utilizing effervescent tablet preparations for denture cleaning, incorporating Ajwa date seed waste as an alternative ingredient to inhibit the growth of *Streptococcus mutans* and *Candida albicans*.

METHODS

This research was conducted using a True Experimental design with a Post Test Only Control Group Design. The study began with ethical approval recommendation No: 586/A.1/KEP-UMI/XI/2024 issued by the Research Ethics Committee of the University of Muslim Indonesia. The extraction process was carried out in the Pharmacognosy Laboratory of the Faculty of Pharmacy, University of Muslim Indonesia, while the preparation of effervescent tablets took place in the Technology Laboratory of the Faculty of Fisheries and Marine Science, Hasanuddin University, Makassar. Ajwa dates (*Phoenix Dactylifera* L.), originating from Madinah, were separated into pulp and seeds, and the extract was prepared using the maceration method with 96% ethanol as a solvent. The Ajwa date seed extract was then mixed with citric acid, tartaric acid, sodium bicarbonate, PVP K30, and lactose. The effervescent granules were prepared with extract concentrations of 1.6%, 3.25%, 6.5%, and 13%, which were subsequently processed into effervescent tablets.

The acrylic resin was fabricated by molding it into the specified dimensions using wax, followed by immersion in a cuvette of boiling water, finishing, and polishing the acrylic resin. The acrylic resin, conforming to ISO 1567 standards with dimensions of 65x10x3.3 mm, was polymerized and contaminated with *Streptococcus mutans* and *Candida albicans*. Bacterial growth was measured using BHIB liquid medium, while fungal growth was assessed using SDA medium. The samples were collected using simple random sampling techniques. The immersion groups consisted of six groups: Ajwa date seed extract effervescent tablets with concentrations of 1.6%, 3.25%, 6.5%, 13%, alkaline peroxide tablets as a positive control, and distilled water as a negative control. The immersion process lasted for 15 minutes and was repeated four times, resulting in a total of 24 samples.

Bacterial and fungal growth was measured using a Thermo UV-Vis Spectrophotometer. The measurement process involved selecting a wavelength of 560 nm, inserting the blank solution (distilled water), and setting the meter to 100%. The blank solution was then replaced with a McFarland solution, and the absorbance of the McFarland solution and the bacterial/fungal media was measured at the same wavelength.

Data analysis was performed using the Shapiro-Wilk test and Levene's Test to assess the mean absorbance values of *Streptococcus mutans* and *Candida albicans* in each treatment group after immersion. One-Way ANOVA and multiple comparisons were conducted to evaluate the differences in bacterial and fungal growth between the various treatment groups.



Figure 1. Immersion of Acrylic Plate Using Ajwa Date Seed Extract Effervescent Tablet

RESULTS

Table 1. Average Absorbance Values of *Streptococcus mutans* Bacteria After 15-Minute Immersion.

Group	N	Mean
C+	4	0.008
C-	4	0.065
1.6%	4	0.042
3.25%	4	0.031
6.5%	4	0.021
13%	4	0.011

Table 1 shows results of Absorbance Measurements: The smallest absorbance value in the positive control group showed an average value of 0.008. In the 13% extract group, the value was 0.011, in the 6.5% extract group it was 0.021, in the 3.25% extract group it was 0.031, in the 1.6% extract group it was 0.042, and the largest absorbance value was measured in the negative control group with a value of 0.065.

Table 2. Average Absorbance Values of *Candida albicans* Fungi After 15-Minute Immersion.

Group	N	Mean
C+	4	0.022
C-	4	0.210
1.6%	4	0.157
3.25%	4	0.127
6.5%	4	0.101
13%	4	0.076

Table 2 shows results of Absorbance Measurements: The smallest absorbance value in the positive control group showed an average value of 0.022. In the 13% extract group, the value was 0.076, in the 6.5% extract group it was 0.101, in the 3.25% extract group it was 0.127, in the 1.6% extract group it was 0.157, and the largest absorbance value was measured in the negative control group with a value of 0.210.

Table 3. Comparison of Bacterial Counts Based on Treatment Groups of Ajwa Date Seed Extract Effervescent Tablets (*Phoenix dactylifera* L.).

Group	Mean	p-value
C+	0.008	
C-	0.065	
1.6%	0.042	0.000*
3.25%	0.031	
6.5%	0.021	
13%	0.011	

Note: One way ANOVA test, *significance (p<0.05)

Table 3 depicts the comparison test using One-way ANOVA showed a p-value of 0.000, which is smaller than 0.05 (p-value < 0.05). This comparison test indicates a significant difference in the number of *Streptococcus mutans* bacteria.

Table 4. Comparison of Fungal Counts Based on Treatment Groups of Ajwa Date Seed Extract Effervescent Tablets (*Phoenix dactylifera* L.).

Group	Mean	p-value
C+	0.022	
C-	0.210	
1.6%	0.157	0.000*
3.25%	0.127	
6.5%	0.101	
13%	0.076	

Note: One way ANOVA test, *significance (p<0.05)

Table 4 displays the results of the One-Way ANOVA test for *Candida albicans* fungi. The comparison test yielded a p-value of 0.000, which is smaller than 0.05 (p-value < 0.05), suggesting a significant difference in the number of *Candida albicans* fungi across the treatment groups.

Table 5. Comparison of *Streptococcus mutans* Bacterial Counts.

Group	Mean	C+	C-	1.60%	3.25%	6.50%	13%
C+	0.008		0.014*	0.000*	0.002*	0.010*	0.031*
C-	0.065			0.149*	0.054*	0.026*	0.017*
1.6%	0.042				0.020*	0.001*	0.000*
3.25%	0.031					0.030*	0.003*
6.50%	0.021						0.023*
13%	0.011						

Note: Multiple comparisons test, *significance (p<0.05)

Table 5 presents the results of the comparison for each extract treatment. It was concluded that the 13% extract treatment was more effective in killing *Streptococcus mutans* bacteria compared to the 1.6%, 3.25%, and 6.5% extract treatments. The comparison between the positive control group and the negative control group showed a difference of -0.057, indicating that the positive control group was more effective in eliminating *Streptococcus mutans* bacteria than the negative control group. Additionally, the comparison between the positive control group and the extract concentration groups yielded a p-value smaller than 0.05 (p-value < 0.05), confirming that the positive control group was more effective in killing *Streptococcus mutans* bacteria than the extract treatment groups.

Table 6. Comparison of *Candida albicans* Fungal Counts.

Group	Mean	C+	C-	1.60%	3.25%	6.50%	13%
C+	0.022		0.000*	0.000*	0.000*	0.000*	0.000*
C-	0.210			0.015*	0.006*	0.003*	0.001*
1.6%	0.157				0.039*	0.005*	0.001*
3.25%	0.127					0.001*	0.000*
6.50%	0.101						0.001*
13%	0.076						

Note: Multiple comparisons test, *significance (p<0.05)

Table 6 presents the comparison results for each extract treatment. It was concluded that the 13% extract treatment was more effective in killing *Candida albicans* fungi compared to the 1.6%, 3.25%, and 6.5% extract treatments. The comparison between the positive control group and the negative control group showed a difference of -0.188, indicating that the positive control group was more effective in killing *Candida albicans* fungi than the negative control group. Furthermore, the comparison between the positive control group and the concentration groups yielded a p-value smaller than 0.05 (p-value < 0.05), confirming that the positive control group was more effective in eliminating *Candida albicans* fungi compared to the extract treatment groups.

DISCUSSION

The results of the study show that the data are homogeneous and normally distributed (p>0.05). The lowest average absorbance value for *Streptococcus mutans* was found in the positive control group, which used alkaline peroxide, with a value of 0.008, followed by the 13% extract group with a value of 0.011. The group with the highest average absorbance value for *Streptococcus mutans* was the negative control group (aquades) with a value of 0.065.

For *Candida albicans*, the lowest absorbance value was found in the positive control group (alkaline peroxide tablet) with a value of 0.022, followed by the 13% Ajwa date seed extract group with a value of 0.076, and the highest value was found in the negative control group with a value of 0.210. The lower the average absorbance value, the more effective the treatment is in inhibiting *Streptococcus mutans* and *Candida albicans*. This occurs because both the alkaline peroxide tablets and the extract tablets contain effervescent ingredients, such as sodium bicarbonate and citric acid. When dissolved in water, these ingredients create a chemical cleaning effect, generating hydrogen peroxide and releasing oxygen.¹¹

The released oxygen has a mechanical cleaning effect, which differs from the negative control group that used distilled water. Effectively, both the alkaline peroxide and extract groups can remove organic deposits and kill microorganisms. The antimicrobial mechanism of alkaline peroxide can inhibit the growth of *Candida albicans*.¹²

The comparison results between the positive control group, negative control group, and the concentration groups yielded a p-value ($p\text{-value} < 0.05$) for the 1.6%, 3.25%, 6.5%, and 13% groups. It was concluded that the positive control group, which used alkaline peroxide, was more effective than the Ajwa date seed extract groups at various concentrations. Additionally, the Ajwa date seed extract groups at all concentrations were more effective than the negative control group (aquades). This study aligns with research conducted by Nurfadillah A et al. (2022), which used herbal materials, such as algae extract, known to contain antibacterial compounds like flavonoids, to examine their bacteriostatic properties against *Streptococcus mutans*.¹³ Similarly, the research by Hartomo TB (2018), using herbal materials such as dragon fruit extract, which also contains flavonoids like those in dates, showed that it could inhibit the growth of *Streptococcus mutans*.¹⁴

Sekeon NH (2018) used herbal materials, specifically *Gedi* leaf extract, to assess the minimum inhibitory concentration (MIC) for *Streptococcus mutans*. The study showed that *Gedi* leaf extract could inhibit the growth of *Streptococcus mutans* with a minimum inhibitory concentration of 6.5%. Alkaline peroxide is a chemical cleaning solution effective in reducing microorganisms in the oral cavity by immersing dentures for 15 minutes due to its bactericidal and fungicidal properties.¹⁵

Herbal plants are commonly used as natural therapy materials. Ajwa date seeds contain alkaloids that serve as antibacterial and antifungal agents. Additionally, Ajwa date seeds contain tannins and flavonoids. Tannins are compounds that act as antibacterial agents found in fruits, while flavonoids in Ajwa date seeds function as antioxidants. The advantage of date seeds over the fruit pulp is that the seeds have a higher antioxidant content, which helps prevent cell damage from free radicals. A study by Amiruddin M et al. (2024) found that both the pulp and seed extract of Ajwa dates at a concentration of 3.25% effectively inhibited the growth of *Streptococcus mutans* and *Candida albicans*.¹⁰

The use of herbal materials tends to have fewer side effects compared to conventional medicines and is often regarded as safer because they are natural substances. However, the continuous and large-scale use of chemicals may affect the roughness of acrylic resin surfaces, which can influence bacterial and fungal growth in the oral cavity of denture wearers. This is consistent with the research by Pertiwisari A et al. (2022), which showed significant differences in the surface roughness of acrylic resin before and after immersion in alkaline peroxide solution. A combination of natural substances and effervescent tablet technology could provide a more environmentally friendly and safer alternative for daily use among denture users.¹⁶ Based on the research, it can be concluded that Ajwa date seed extract has an effect on killing *Streptococcus mutans* and *Candida albicans*. The positive control group, alkaline peroxide, was the most effective in killing both bacteria and fungi. In contrast, the negative control group, aquades, had no effect on killing the bacteria.

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