

DENTINO
JURNAL KEDOKTERAN GIGI
VOL. XI. No 1. MARCH 2026

**IDENTIFICATION OF SECONDARY METABOLITES IN AJWA DATE SEED
EXTRACT (*PHOENIX DACTYLIFERA L.*) USING PHYTOCHEMICAL SCREENING,
UV-VIS, FTIR, AND GC-MS METHODS**

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ABSTRACT: Previous studies concluded that Ajwa date (*Phoenix Dactylifera L.*) seed extract significantly reduced the roughness of acrylic resin plates used as denture bases, and has the potential to be used as a raw material for denture cleaning tablets. Several previous studies also showed that Ajwa date seeds contain various secondary metabolites, such as phenolic compounds and flavonoids, which have the potential as antioxidants and other bioactive agents. However, most of these studies were still limited to the use of specific analytical methods separately, such as phytochemical screening or chromatographic analysis, without a comprehensive integrated approach. **Objective:** To identify the levels of active secondary metabolites in ethanol extract of Ajwa date seeds using a comprehensive method with phytochemical screening, UV-Vis, FTIR, and GC-MS. **Results:** Ajwa date seeds were found to contain alkaloids, flavonoids, phenolics, tannins, and saponins. UV-Vis analysis showed consistent levels of polyphenols, tannins, and flavonoids. FTIR analysis showed that Ajwa date seeds are a rich natural source of polysaccharides, lipids, and phenolic compounds, and GC-MS results revealed various secondary metabolites detected at various retention times, rich in essential fatty acids, esters, and phenolic derivatives. All test methods were repeated three times. **Conclusion:** The combination of the four analytical methods complemented each other in identifying and confirming the presence of secondary metabolites in Ajwa date seed extract, thus providing a comprehensive picture of its chemical composition and potential bioactivity.

Keywords: Ajwa date seeds, Phytochemical screening, UV-Vis, FTIR, GC-MS

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INTRODUCTION

Ajwa date seeds (*Phoenix Dactylifera L.*) contain bioactive components such as phenolic compounds, polyphenols, flavonoids, pectin, and fiber in large quantities, which have health-protective properties.¹ The isolated phenolic and flavonoid compounds exhibit antifungal and antibacterial properties, and terpenoid compounds can also function as antibacterial agents. Date seeds contain oil rich in secondary metabolites, particularly phenolic and steroidal compounds.² This makes this natural plant product an alternative ingredient for solutions that are expected to prevent bacterial resistance, a growing problem in society.³ Flavonoid and phenolic compounds have been reported to damage the cell membranes of microorganisms and inhibit biofilm formation, while tannins can precipitate proteins and interfere with the activity of microbial enzymes.⁴ Furthermore, saponins act as natural surfactants that can increase cleaning effectiveness by reducing surface tension.⁵ These findings align with previous research showing that date seed extract has antibacterial activity against oral microorganisms, including plaque-forming bacteria and biofilms on dentures.⁶ The main problem in denture use is related to the accumulation of microbial biofilms such as *Candida albicans* which can cause denture stomatitis.⁷ Therefore, the presence of these bioactive compounds indicates the potential of Ajwa date seed extract as a natural ingredient in denture cleaning formulations. Thus, the results of phytochemical analysis not only provide information on chemical composition, but also serve as a scientific basis in the development of functional applications as an effective and safe cleaning agent. Amiruddin et al⁸ stated that a concentration of Ajwa date seed extract of 3.25% inhibited the growth of *Streptococcus mutans* and *Candida albicans*, which are the dominant microorganisms in the oral cavity of denture wearers. Meanwhile, another study found that a 3.25% extract of Ajwa date flesh and seeds inhibited the growth of *Candida albicans*.¹ Qualitative and quantitative tests

used to analyze secondary metabolites include Ultraviolet-Visible (UV-Vis) spectroscopy, phytochemical screening, Fourier Transform Infrared Spectroscopy (FTIR), and Gas Chromatography-Mass Spectrometry (GC-MS). UV-Vis is an analytical technique that utilizes the interaction of ultraviolet and visible light with molecules, enabling the identification, quantification, and structural characterization of compounds.⁹ Phytochemical screening is used to detect bioactive compounds in plants, such as alkaloids, flavonoids, and saponins, for qualitative identification.¹⁰ FTIR works by detecting molecular bond vibrations through the infrared spectrum.¹¹ GC-MS is a combination of two techniques: the separation of volatile compounds through gas chromatography and molecular identification; this method is known to be highly sensitive and accurate in the qualitative identification of compounds.^{12,13} Several previous studies have shown that ajwa date seeds contain various secondary metabolites, such as phenolic compounds and flavonoids, which have potential as antioxidants and other bioactive agents.¹⁴ However, most of these studies are still limited to the use of specific analytical methods separately, such as phytochemical screening or chromatographic analysis, without a comprehensive integrated approach.¹⁵ Some other studies have utilized advanced techniques such as LC-MS or metabolomics to identify compound profiles, but have not integrated basic spectroscopic methods such as UV-Vis and FTIR in a single analysis suite.¹⁶ Furthermore, there are also studies that only use FTIR to identify functional groups without combining it with other more specific techniques such as GC-MS. On the other hand, most studies also focus more on the date fruit than the seeds, so the study of ajwa date seeds is still relatively limited.¹⁷ Therefore, research is needed that combines phytochemical screening methods, UV-Vis, FTIR, and GC-MS in an integrated manner to obtain more comprehensive information regarding the secondary metabolite profile in ajwa date seed extract.

MATERIALS AND METHODS

This study used a descriptive design structured within a step-by-step analytical framework, encompassing sample preparation, extraction, qualitative identification, quantitative analysis, and compound characterization and confirmation. The study began with ethical approval obtained through Ethics Approval Recommendation No. 835/A.1/KEP-UMI/X/2025 from the Research Ethics Committee of the Muslim University of Indonesia. The extraction process was carried out at the Fisheries Laboratory of Hasanuddin University, while sample testing was carried out at the Chemistry Laboratory of the Ujung Pandang State Polytechnic, Makassar. Ajwa date (*Phoenix dactylifera* L.) samples were separated into flesh and seeds, then the date seeds were extracted using the maceration method with 96% ethanol as the main solvent with a material to solvent ratio of 1:10 (w/v). The simplicia was soaked in 96% ethanol in a closed container and left for 72 hours at room temperature ($\pm 25\text{--}27^\circ\text{C}$) with occasional stirring every 24 hours to optimize the extraction of active compounds. After the maceration process was complete, the mixture was filtered using filter paper to separate the filtrate and residue. The filtrate was then evaporated using a rotary evaporator at 60°C with a pressure of $\pm 150\text{--}200$ mbar until a thick extract was obtained, then concentrated to remove residual solvent. Thus, the extraction process was carried out consistently using 96% ethanol without adding water during the maceration stage so that the solvent system remained stable and optimal in extracting secondary metabolite compounds. The qualitative analysis stage was carried out through phytochemical screening to identify the presence of secondary metabolites, including an alkaloid test using Dragendorff's reagent which was characterized by the formation of an orange-brown precipitate, a flavonoid test using 10% AlCl_3 which produced a yellow-orange color, and a phenolic and tannin test using 1–5% FeCl_3 which produced a dark blue or greenish color, as well as a saponin test through the formation of stable foam ≥ 1 cm for ± 10 minutes. The quantitative analysis stage was carried out using Ultraviolet-Visible (UV-Vis) spectrophotometry with a wavelength range of 200–800 nm using a quartz cuvette (path length 1 cm) and 96% ethanol as a blank. Determination of flavonoid levels was carried out at a maximum wavelength of ± 415 nm using a quercetin standard curve (0–100 ppm) with an incubation time of 30 minutes, while total polyphenols were analyzed at ± 765 nm using a gallic acid standard with an incubation time of 30–60 minutes in the dark. Tannin levels were measured at a wavelength of $\pm 725\text{--}760$ nm using a tannic acid standard with an incubation time of ± 30 minutes. The calibration curve was prepared using a minimum of five concentration points with a coefficient of determination (R^2) value of ≥ 0.99 . The calculation of the content of each compound was carried out by determining the linear regression equation ($y = ax + b$), then the sample absorbance value was entered into the equation to obtain the concentration (C), which was then calculated using the formula $\text{Content (mg/g)} = (C \times V \times \text{FP}) / m$. The results were expressed as mg QE/g for flavonoids, mg GAE/g for polyphenols, and mg TAE/g for tannins. All measurements were carried out three times (triplicate) to assess data variability, which was evaluated through the average value, standard deviation, and Relative Standard Deviation (RSD), with good precision criteria if $\text{RSD} \leq 2\%$. The characterization and confirmation stages of the compounds were carried

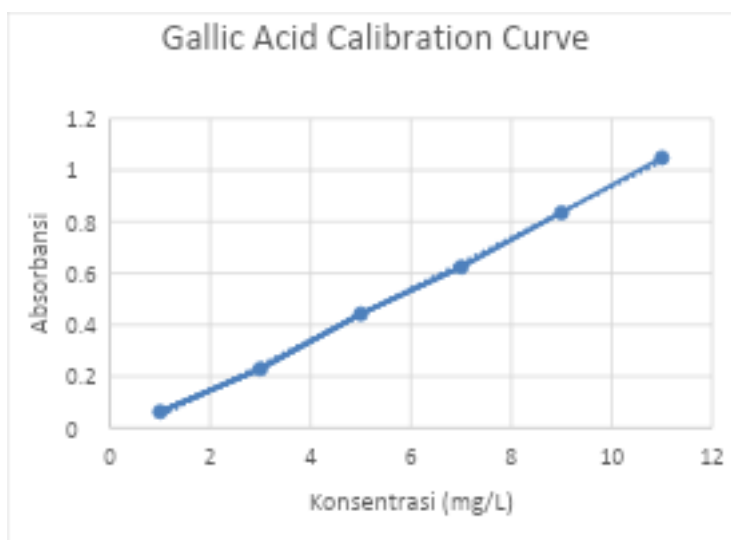
out using Fourier Transform Infrared Spectroscopy (FTIR) for functional group analysis in the wavenumber range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} and a scan count of 16–32 times, as well as Gas Chromatography–Mass Spectrometry (GC–MS) for identification of volatile and semi-volatile compound components. GC–MS analysis was carried out using a Shimadzu QP2010S instrument with a 30 m long, 0.25 mm diameter capillary column (DB-5MS), helium carrier gas ($\pm 1 \text{ mL/min}$), split injection mode ($\pm 1:10$), injector temperature of 250°C, and an oven temperature program from 60°C to 280°C. Detection was carried out using 70 eV Electron Impact (EI) ionization with an m/z range of 40–600, and compound identification was based on the conformity of the mass spectrum to the NIST data library ($\geq 90\%$). Method validation was conducted through linearity tests ($R^2 \geq 0.99$), accuracy (recovery 80–120%), and precision ($\text{RSD} \leq 2\%$). Instrument calibration was conducted periodically, including UV–Vis using a standard potassium dichromate solution, FTIR using polystyrene, and GC–MS through a tuning process using standard compounds such as perfluorotributylamine (PFTBA). The equipment used in this study included drop plates, spatulas, beakers, measuring cylinders, test tubes, stoves, gauze, dropper pipettes, and UV–Vis, FTIR, and GC–MS spectrophotometer instruments (QP2010S, Shimadzu, Japan).

RESULTS

UV-Vis Spectroscopy

Polyphenols were calculated as Gallic Acid Equivalent per gram of sample

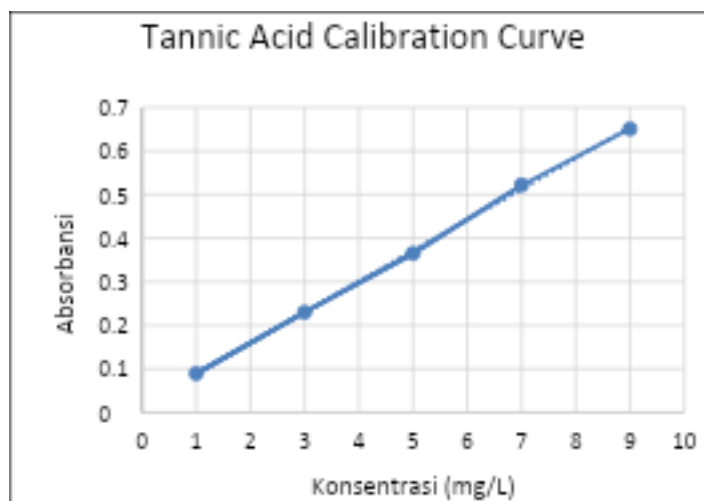
Sample Code	Absorbance	C (mg/L)	vol/1000	fp	g sample	mg GAE/g sample
Simple	0.709	7.708797	0.0002	50	0.01	7.708797
Duplo	0.705	7.668352	0.0002	50	0.01	7.668352
Tripla	0.7	7.617796	0.0002	50	0.01	7.617796



UV-Vis spectrophotometric analysis using a gallic acid standard curve with three replicates showed that Ajwa date seeds have consistent polyphenol content. The absorbance values of the samples ranged from 0.700 to 0.709, which after being converted yielded polyphenol content in units of mg gallic acid equivalents (GAE)/g sample. The consistency between replicates confirmed the reliability of the UV-Vis method as a technique for measuring polyphenols. The average total polyphenol content was 7.664982 mg GAE/g sample, indicating that Ajwa date seed extract has a relatively stable polyphenol content between replicates. The variation between replicates was very small, indicating that the analytical method has good precision. The values obtained showed a very slight decreasing trend from single to triple replicates, but were still within a very close range. This suggests that the differences may originate from small variations in the pipetting process, solution homogeneity, or spectrophotometer absorbance readings. Therefore, the data can be considered reliable due to the low variation between replicates.

Tannins were calculated as Tannic Acid Equivalent per gram of sample

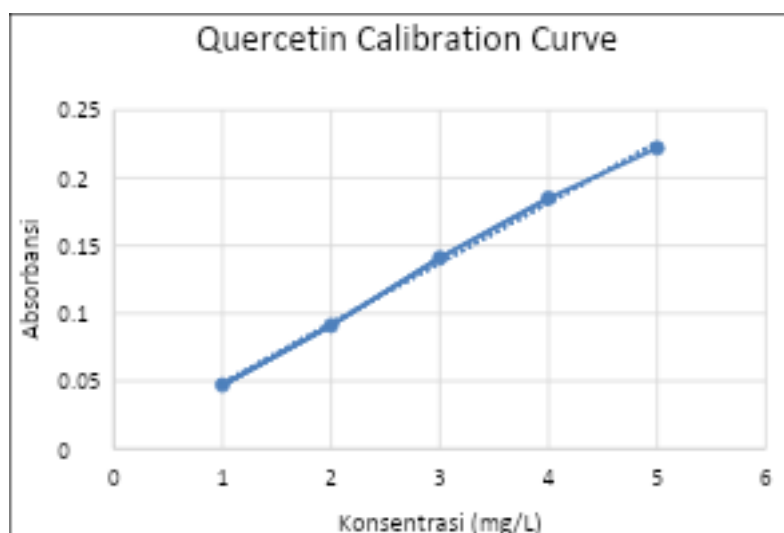
Sample Code	Absorbance	C (mg/L)	vol/1000	fp	g sampel	mg TAE/g sampel
Simplo	0.588	8.059322	0.0002	50	0.01	8.059322034
Duplo	0.58	7.946328	0.0002	50	0.01	7.946327684
Triplo	0.598	8.200565	0.0002	50	0.01	8.200564972



The results of tannin content measurements in ajwa date seed extract showed relatively consistent absorbance values in three replications (triples), namely 0.588 (simplo), 0.580 (duplo), and 0.598 (triplo). Based on the tannic acid standard curve, the concentrations (C) were 8.059322 mg/L; 7.946328 mg/L; and 8.200565 mg/L, respectively. The differences in values between replications showed very small variations. This indicates that the analytical method has good precision, with possible variations arising from technical factors such as pipetting accuracy, solution homogeneity, or fluctuations in absorbance readings. Overall, the data show good consistency between replicates.

Flavonoids were calculated as Quercetin Equivalent/gram of sample

Sample Code	Absorbance	C (mg/L)	vol/1000	fp	g sampel	mg QE/g sampel
Simplo	0.071	0.757062	0.002	5	0.01	0.757062147
Duplo	0.066	0.686441	0.002	5	0.01	0.686440678
Triplo	0.072	0.771186	0.002	5	0.01	0.771186441



The results of flavonoid content measurements in ajwa date seed extract showed relatively consistent absorbance values in three replications (triples), namely 0.071 (simplo), 0.066 (duplo), and 0.072 (triplo). Based on the quercetin standard curve, the concentrations (C) were 0.757062 mg/L; 0.686441 mg/L; and 0.771186 mg/L, respectively. The average flavonoid content was 0.738230 mg QE/g sample, indicating that ajwa date seed extract contains low-moderate but stable flavonoids between replications. Variations between replications showed very small differences, indicating that the analytical method has good precision. Differences in values can be caused by minor variations in the pipetting process, solution homogeneity, and the stability of the $AlCl_3$ color complex during measurement. In general, the data showed good consistency with low variation between replications.

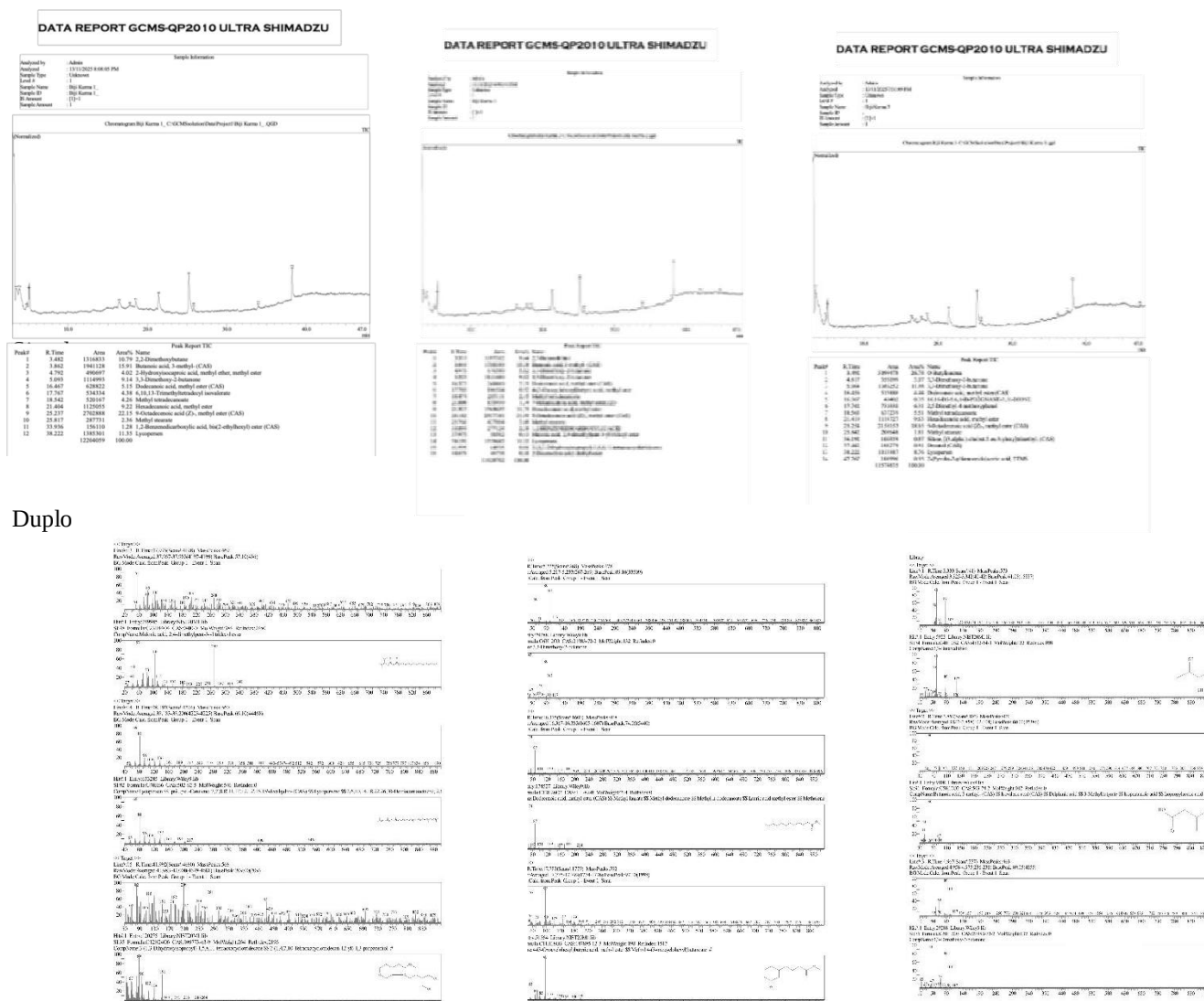
Phytochemical screening of Ajwa date seed ethanol extracts showed positive results for the following groups of bioactive compounds:

Alkaloids	Flavonoids	Phenolics	Tannins	Saponins
+	+	+	+	+

- **Alkaloids**, characterized by a dark brown color change after the addition of Dragendorff's reagent.
- **Flavonoids**, producing a yellow-orange color after the addition of $AlCl_3$.
- **Phenolics**, showing a blackish-blue color with $FeCl_3$ reagent.
- **Tannins**, producing a brownish-green color with gelatin reagent.
- **Saponins**, characterized by the formation of stable foam after shaking with water.

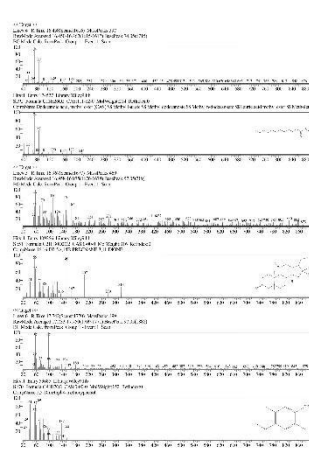
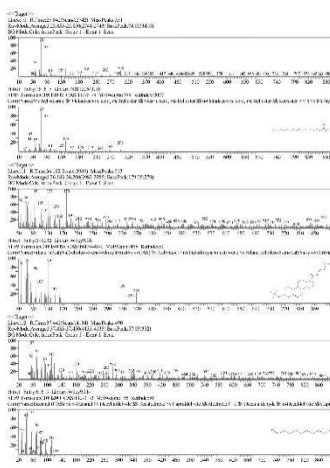
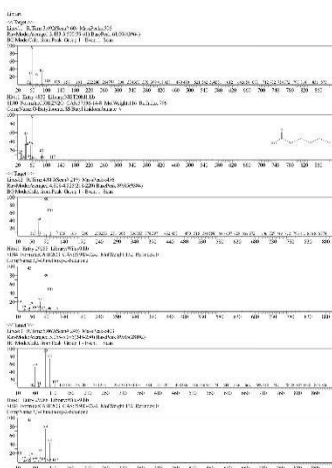
Ajwa date seed extract exhibits a comprehensive phytochemical profile, with phenolics and flavonoids playing a dominant role as antioxidants, tannins and alkaloids as antimicrobial agents, and saponins as cleansing enhancers. This combination provides a strong scientific basis for the development of natural product-based functional applications

GC-MS CHROMATOGRAM



Duplo

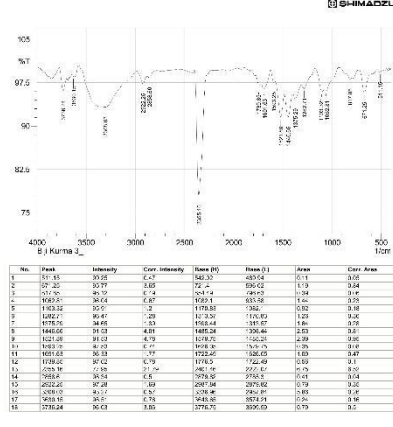
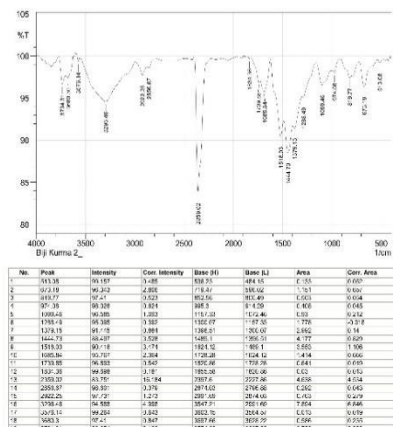
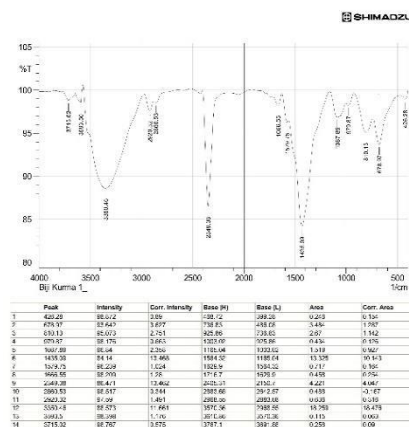
Triplo



Analysis of the GC-MS chromatogram of Ajwa date seed extract shows a diversity of secondary metabolites detected at various retention times. The dominant compounds identified include:

- 9-Octadecenoic acid (Z)-, methyl ester (oleic acid methyl ester) with an area of 22.15%,
 - Butanoic acid, 3-methyl- (15.91%),
- Hexadecanoic acid, methyl ester (palmitic acid methyl ester) (9.22%),
 - Lycopersene (11.35%)

FTIR



This spectrum indicates that the date Ajwa seed sample contains:
 Cellulose/hemicellulose (O-H, C-O, C-H)/ (C=O, C-O, C-H) : 1087, 1435, 3350 cm^{-1}
 Lignin/aromatic compounds (aromatic C=C, aromatic C-H) : 1579, 810 cm^{-1}
 High hydroxyl groups (-OH) indicative of lignocellulosic material

Alkanes (hydrocarbon chains) (C–H stretching) : 2860–2920 cm^{-1}
Possible carbonyls (C=O) : 1666 cm^{-1}

DISCUSSION

UV-Vis spectrophotometric analysis was performed to identify the presence of chromophore compounds in date palm seed extract related to phenolic and flavonoid content. Based on the results of absorbance measurements converted into Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) values, the phenolic content was ± 7.67 mg GAE/g and flavonoids were ± 8.07 mg TAE/g and ± 0.74 mg QE/g. These values indicate that the extract contains quite significant phenolic compounds. Phenolic and flavonoid compounds are known to have a conjugated electron system so that they can absorb radiation in the UV region and act as antioxidants through the mechanism of electron donation or free hydrogen.¹⁸ The results of chemical characterization using FTIR showed strong absorption bands in the –OH (3200–3700 cm^{-1}), C=O (~ 1700 cm^{-1}), and C–O (~ 1000 – 1100 cm^{-1}) regions, which indicate the presence of hydroxyl, carbonyl, and ether bond groups that are typical of phenolic compounds, flavonoids, and lignocellulosic polysaccharides. The presence of large amounts of –OH groups correlates with antioxidant activity because it acts as a proton producer in neutralizing free radicals.¹⁹ This finding is consistent with phytochemical tests that show positive results for alkaloids, flavonoids, phenolics, tannins, and saponins as biologically active secondary metabolites. Further GC-MS analysis identified the main compounds as fatty acids such as palmitic acid, oleic acid, and stearic acid, as well as other compounds such as esters, aldehydes, ketones, and long-chain hydrocarbons. Unsaturated fatty acids such as oleic and linoleic acids are reported to have antibacterial activity through the mechanism of disrupting cell membrane integrity and inhibiting microbial metabolism.²⁰ In addition, saturated fatty acids such as palmitic and stearic acids also contribute to antimicrobial activity, although with lower potency.²¹ The presence of these bioactive compounds plays an important role in the development of date seed extract as a denture cleanser. Biofilms on dentures are complex communities of microorganisms dominated by *Candida albicans* and *Streptococcus mutans*, which play a role in the occurrence of denture stomatitis and oral cavity infections.²² Therefore, denture cleaning agents must have the ability to inhibit the growth of microorganisms and damage the biofilm structure. In this case, the identified fatty acid compounds play an important role in antibiofilm activity. Studies have shown that unsaturated fatty acids are able to disrupt the biofilm matrix and cause the biofilm to detach from the acrylic resin surface through penetration into the extracellular matrix and disruption of microorganism adhesion.²³ This mechanism is very relevant in the denture cleaning process because biofilms attached to the acrylic resin surface are a major factor causing infection. In addition, phenolic and flavonoid compounds detected through UV-Vis and FTIR also contribute to antimicrobial and antioxidant activities. These compounds can disrupt the permeability of microbial cell membranes, inhibit enzyme activity, and induce oxidative damage to microorganism cells.²⁴ Tannins play a role in precipitating proteins and damaging microbial cell walls, while saponins have surfactant properties that can reduce surface tension and inhibit the adhesion of microorganisms to the denture surface.²⁵ These findings are in line with research by Amiruddin et al who reported that date seed extract has antibacterial activity against *Streptococcus mutans* and antifungal activity against *Candida albicans*, thus having potential as a natural antimicrobial agent in dentistry.²⁶ The use of natural ingredients as denture cleansers is growing due to their advantages of high biocompatibility, broad biological activity, and minimal side effects compared to synthetic chemicals. The combination of compounds in date palm seed extract has shown a synergistic effect between antimicrobial, antibiofilm, and antioxidant activities, thus offering potential for development as an active ingredient in denture cleanser formulations.

However, the effectiveness of these bioactive compounds is highly influenced by concentration and formulation. The antibiofilm activity of fatty acids is reportedly dose-dependent, requiring concentration optimization to achieve maximum effectiveness without causing cytotoxic effects on oral tissues.²⁷ Therefore, further research is needed to evaluate the stability, safety, and effectiveness of the extract under in vitro and in vivo conditions before clinical application.

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