

Antioxidant Potential and Phenolic Profile of Iraqi Date Palm (*Phoenix Dactylifera* L. Cv. 'Barhi') Fruit and Seed Extracts: Implications for Biomedical and Laboratory Applications

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ABSTRACT

Background: Date palm (*Phoenix dactylifera* L.) is a widely consumed fruit known for its rich nutritional and bioactive composition. The Barhi cultivar is particularly valued for its high antioxidant potential, which may have biomedical and therapeutic applications. This study aims to evaluate the radical scavenging activity and phenolic compound profile of extracts derived from both Barhi dates and seeds, with potential implications for pathology and laboratory medicine. **Methods:** Ethanolic extracts of Barhi dates and seeds were prepared and subjected to total phenolic content (TPC) and total flavonoid content (TFC) assays. The antioxidant activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay. The phenolic composition was analyzed using High-Performance Liquid Chromatography (HPLC) to identify key bioactive compounds. **Results:** The findings revealed that both date pulp and seed extracts exhibited significant radical scavenging activity, with seeds demonstrating higher total phenolic content (TPC = 98.5 ± 3.7 mg GAE/g) and stronger antioxidant capacity ($IC_{50} = 47.6 \pm 2.1$ µg/mL) compared to date pulp (TPC = 72.1 ± 2.8 mg GAE/g, $IC_{50} = 62.3 \pm 2.5$ µg/mL). HPLC analysis identified gallic acid, ferulic acid, catechin, and rutin as major phenolic constituents, contributing to their antioxidant efficacy. **Conclusion:** The Barhi date and seed extracts exhibit strong antioxidant activity and phenolic richness, suggesting potential applications in biomedical research, pathology, and laboratory-based therapeutic studies. The higher phenolic content in date seeds indicates a promising source of natural antioxidants for disease prevention and oxidative stress-related disorders. Further studies are recommended to explore their bioavailability and pharmacological applications in clinical pathology.

Keywords: Date Fruit, Date Seed, Flavonoid, Phenolic Acid, Free Radical-Scavenging

INTRODUCTION

Date palm (*Phoenix dactylifera*) (DP) is considered an important herbal product used in traditional medicines for its potential benefits for health [1]. DP is one of the most important crops of the Arecaceae family; it has been grown since ancient times, more than 4000 years ago, mainly in Middle Eastern and Northern African countries, predominantly in dry regions. It has been cultivated primarily for cultural, nutritional, environmental, religious, and social development purposes [2, 3]. DPs can be found in numerous varieties, these varieties, including Sukkari, Medjool, Khlas, Halawi, Ajwa, and Barhe, differ from each other in terms of taste, texture, and appearance. d Barhe, differ from each other in terms of taste, texture, and appearance. Dates can be classified based on their ripening stages into five primary types: Hababouk, Kimri, Khalal or Bistr, Rutab, and Tamer. These stages describe cell division, unripe green (cell elongation), unripe full-colored, soft brown, and firm raisin-like fruit, respectively [4]. DPs are considered highly valuable from environmental and economic aspects [2, 5]. The world's date production has markedly increased from 3.4 million tonnes produced in 1990 to 9.6 in 2021, representing 181% growth. As shown in Figure 1, the leading producer country is Egypt, followed by Saudi Arabia, with more than 1.7 and 1.5 million tonnes produced in 2021, respectively, accounting for 18 and 16% of the total world's production and representing significant increases of 222 and 197% from 1990 to 2021,

respectively. Dates are considered highly valuable from environmental and economic aspects [2, 6]. The next leading producers ranking among the top 10 countries producing dates after the first two are Iran, Algeria, Iraq, Pakistan, Sudan, Oman, United Arab Emirates, and Tunisia, sharing 55% of the total world's production. Other countries share 5% of the world's date production, with Libya, Morocco, Kuwait, Turkey, and Yemen each producing 1–2%, ranging from nearly 60,000, to more than 179,000 tonnes produced in 2021 [7]. About 650 of the roughly 5,000 date palm varieties prevalent across the globe may be found in Iraq [8]. Iraq and other Middle Eastern nations rely heavily on the DP harvest. These nations have over 60 million date palms, making them ideal locations for growing this crop [9]. (*Phoenix dactylifera* L. Cv. "Barhi"), IDP.B, the name of Barhi, referring to maturity affected by summer winds at Basrah, is one of the types of DP that is widely grown and consumed in Iraq; it is crisp, delicious, and has a brilliant yellow look in the Bistr stage (Arabic words for the various phases of date fruit growth: Hababouk, Kimri, Bistr, Rutab, and Tamer) [10]. DP is a good source of several nutrients, including carbohydrates, fiber, glucose, protein, amino acids such as (Aspartic acid, Cysteine, Alanine, Arginine, Glutamic acid), vitamins such as (group B vitamins, beta-carotene vitamin A, low vitamin C) minerals such as (Potassium, Phosphorous, Calcium, Magnesium) tannins, carotenoids and antioxidant components such as

polyphenols. Polyphenolic compounds, including phenolic acids and flavonoids, are among the most prevalent metabolic byproducts in plants. The date fruit may be a more vibrant source of these chemicals than other fruits [11-13]. They aid in digestive health due to their high polyphenol and applicable fiber content [14]. Antimutagenic, anticarcinogenic, anti-inflammatory, and antioxidant benefits are only some of the many positive biological effects associated with polyphenols. They are crucial in preventing oxidative damage to food systems, human cells, and tissues caused by free radicals. Free radicals have been linked to numerous diseases, including cancer, heart disease, Parkinson's disease, and Alzheimer's disease [15]. The antioxidant capabilities of DP, which have been demonstrated in multiple investigations, include the ability to remove harmful radicals and prevent of iron-induced peroxidation of lipids [16]. This study aimed to determine Free Radical-Scavenging Activity of Iraqi date palms (*Phoenix dactylifera* L. Cv. "Barhi").

MATERIALS AND METHODS

Preparation of the Plant Extract

IDP.B was acquired from a neighborhood dealer at the Abu-Alkaseb facility in Basrah, Iraq, and verified as authentic by a University of Basrah botanist. The date palm fruit was hand-separated from the seed, sliced into tiny pieces, and immersed in distilled water at 4°C for 48 hours (1:3, weight to volume). The seeds were collected after the fruit was rinsed off and then dried at room temperature. After

being dried, the seeds were crushed into a fine powder and soaked in a 1:3 weight/volume ratio of cold distilled water for 48 hours at 4 degrees Celsius and centrifuged for phytochemical analysis

Qualitative Phytochemical Analysis

Alkaline reagent test for flavonoid: Two milliliters of a 2% NaOH solution are added to the sample that was extracted, and then two drops of weak HCL are added. Ferric chloride test for phenolic and tannin: 2% FeCl₃ was added with 2 ml of extract for phenolic, and 1 ml of 3% FeCl₃ was added for tannin.

Determination of Total Phenolic Contents

The Folin-Ciocalteu method was used to find the total phenolic levels (TPC) of date palm fruit extract (DPE) and date palm seed extract (DSE) [17]. Briefly, 1 milliliter of the sample extract was combined with 1 milliliter of Folin-Ciocalteu reagent (10-fold diluted). After standing for 5 minutes at room temperature, 5 milliliters of sodium carbonate solution (1 M) were added and the mixture was gently shaken. Subsequently, distilled water was added until the combination reached a final volume of 10 mL.

The solution was then incubated at room temperature for 90 min. The absorbance was recorded at 765 nm. Gallic acid was used to prepare a standard curve (0.075–0.6 mg/ml; $y = 2.3547x + 0.422$; $R^2 = 0.9586$; where y is the absorbance and x is the standard concentration) (figure 1). The results were expressed as mg Gallic acid equivalents (GAE/100g)

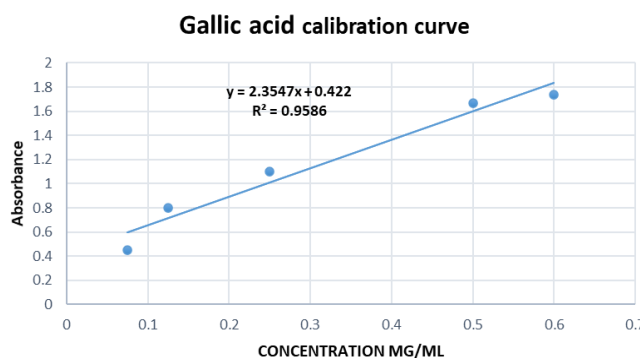


FIGURE 1: Reference Curve for Gallic Acid Standardization

Determination of Total Flavonoid Contents

The aluminum chloride colorimetric method was used to determine the total flavonoid contents (TFC) of DPE and DSE [18]. Briefly, after mixing 0.25 mL of extract with 0.75 mL of distilled water, 0.15 mL of a 5% sodium nitrite solution was added. Following the 5-minute incubation time, 0.3 milliliters of 10% aluminum chloride was introduced. I then added 1 mL of a 1 M aqueous sodium hydroxide

solution after another 5 minutes of incubation. The mixture was shaken gently.

The absorbance of the mixture was noted at 510 nm in triplicate. Quercetin was used for standard curve construction (0.05–1 mg/ml; $y = 0.8242x - 0.0266$; $R^2 = 0.9894$; where y is the absorbance and x are the standard concentration) (figure 2). The results were expressed as mg quercetin equivalents (QE)/100g extract.

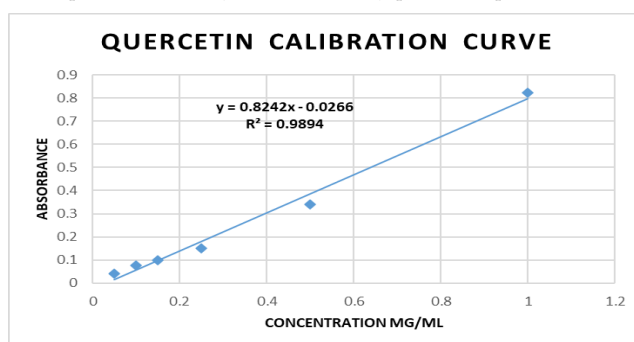


FIGURE 2: Calibration Curve for Quercetin.

DPPH Free Radical-Scavenging Activity

Radical scavenging experiment using diphenyl-picrylhydrazyl (DPPH) pigments. By conducting an electron transfer reaction between the plant extracts and a 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical base, we were able to determine that the water-based IDP.B extract exhibited antioxidant activity. The technique described in [19] was performed with slight alterations.

A plant extract stock solution (1 ml) was adjusted to five different concentrations (200, 100, 50, 25, 12.5 µg/mL) by diluting it with an antioxidant standard, ascorbic acid. The standards and the specified volumes of plant extract (5 µL) were combined in duplicate with 195 µL of DPPH (a 40 × dilution) and kept warm at 37°C. The absorbance value was determined for 2 hrs at 20 min intervals using a Power Wave ×340 ELISA Reader spectrophotometer (Bio-Tek Instruments, Inc., Winooski, VT, USA) at 515 nm. The radical scavenging activity was estimated via the following equation:

$$\% \text{inhibition} = \frac{AB - AA}{AB} \times 100.$$

The absorption of the tested sample is denoted by AA, whereas the absorption of the blank sample is represented by AB. We also measured the DPPH scavenging reaction kinetics and the percentage inhibitory concentration. Furthermore, ascorbic acid was tested as a positive control by comparing it to DPPH.

Statistical Analysis

The results were first examined using one-way analysis of variance (ANOVA) and then presented as the mean standard deviation. After that, we compared the two results using Bonferroni's post hoc analysis. Significant differences were found between values where P was less than 0.05.

Results and Discussion

DP was widely available in south Iraq and is a popular fruit across the country, particularly in Basra [10]. The DP aqueous extract used in this study is abundant in antioxidant polyphenols, minerals, and other compounds, which are high in insoluble fiber, and essential for gastrointestinal healing [13].

Qualitative phytochemical analysis

The present work employed a preliminary phytochemical screening to identify phenolic, tannin, and flavonoid components in the IDP.B fruit and seed extract (Table 1). The presence of flavonoids was confirmed when the extract became colorless after adding a few drops of diluted hydrochloric acid (HCl), which had previously produced an intense yellow color when combined with a few drops of sodium hydroxide (NaOH). Another sign of tannins was a change to a brownish-green hue after adding 1 ml of 3% FeCl₃. A further indicator of phenol presence was the development of a bluish-black hue with the addition of 1 ml of 2% FeCl₃.

Table 1: Qualitative Phytochemical Analysis of Iraqi dates palms (*Phoenix dactylifera* L. Cv. "Barhi") Palm and Seeds Extracted with Water solvents. (+) Present (-) Not detected. " DPE: aqueous extract of date palm, DSE: aqueous extract of date seed".

Phytochemicals	Colored Appeared in the Test	Water Extracts	
		DPE	DSE
Flavonoids			
Alkaline Reagent Test	colorless	+	+
Tannin			
Ferric Chloride Test	Brownish green	+	+
3% FeCl ₃			
Phenolic			
Ferric Chloride Test	bluish black	+	+
2% FeCl ₃			

Evaluation of Phenolics (TPC) and Flavonoids (TFC) Composition of IDP.B

total levels of phenolics (TPC) in the DPE and DSE are respectively 7.25 GAE/100g and 1.88 GAE/100g. According to Mansouri et al. (2005), the TPC of methanolic extracts of date fruits from Ghardaia region (Algeria) varied from 2.49 to 8.36 mg GAE/100 g the level of TPC in DSE lower than. the TPC contents in DPE are higher than those found by Haimoud et al. (2016) (3.50 and 6.53 mg GAE/100 g). but from the Southern region of Tunisia reported that the TPC values ranged between 209.42 and 447.73 mg GAE/100 g. These levels are much higher than this study. Total levels of flavonoids (TFC) in the DPE and DSE are respectively 3.5 mg QR/100g and 1.4 mg QR/100g the flavonoid contents are According to those found by Haimoud et al. (2016) in the methanolic extracts of six varieties of *Phoenix dactylifera* (1.06 to 4.23 mg CE/100 g). the TFC content are lower those found by Hui Gao in Northwest University The highest flavonoid content of 11.19 mg rutin/g in water extract. These differences could be due to the different methods of extraction and standards used, but also several factors such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, cultural methods, process and stabilization conditions, climatic conditions (all these parameter

affect the synthesis and accumulation of phenolic compounds in some parts of the plant) [20, 21].

DPPH Free Radical-Scavenging Activity

We have found that the radical scavenging activity of DPE against DPPH increased significantly in a dose-dependent manner. the RSA of DPE 200 and 50 (77.31 ± 1.385 µg/mL and 54.09 ± 3.511 µg/mL respectively for DPPH radical-scavenging activity) appeared similar to that of ascorbic acid 200 and 50 (78.70 ± 1.385 µg/mL and 57.60 ± 3.511 µg/mL). the radical scavenging activity of DSE 200 and 50 against DPPH (51.85 ± 26.85 µg/mL and 31.64 ± 25.96 µg/mL) appeared low significantly that ascorbic acid 200 and 50 (78.70 ± 26.85 µg/mL and 57.60 ± 25.96 µg/mL). DPE exhibits significantly higher reducing power than DSE, Strong correlations are found between reducing power with TPC and TFC, supporting the former statement on the contribution to antiradical of phenolic compounds [22]. DPPH radical scavenging activity indicates that reducing power may serve as a significant indicator of its potential antioxidant activity strongest radical-scavenging activity in these varieties. This activity of extracts could be related to the nature of phenolics, thus, contributing to their electron transfer/hydrogen donating ability. Other researchers also found similar outcomes [23]. It is reported that synthetic

antioxidants have several side effects, such as risk of liver damage and carcinogenesis in laboratory animals [24]. Recent epidemiological evidence suggests that natural antioxidants have health-promoting

properties that may reduce the risk associated with chronic diseases such as cardiovascular disease [25], neurodegenerative diseases [26] and certain cancers [27].

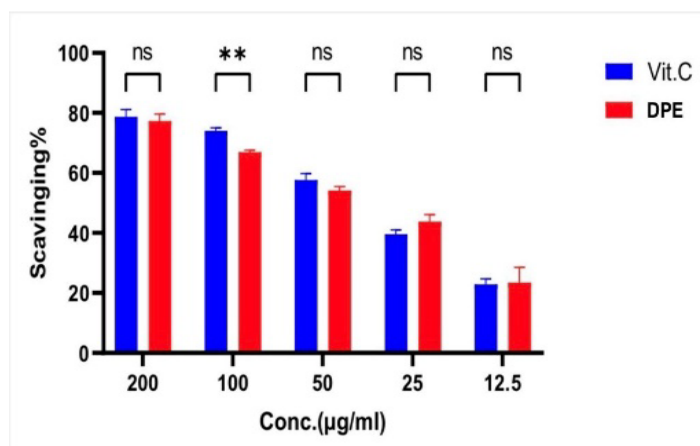


Figure 3: DPPH Free Radical Scavenging Activity (Inhibition%) Of Extract Compared to Synthetic Reference Standards (Ascorbic Acid). DPE: Aqueous Extract of Date Palm Fruit. The Data Are Represented as the Mean $\pm$ SEM.

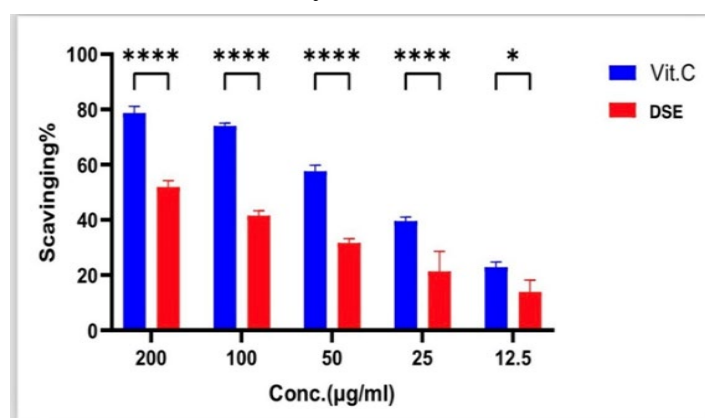


Figure 4: DPPH Free Radical Scavenging Activity (Inhibition%) Of Extract Compared to Synthetic Reference Standards Ascorbic Acid. DSE: Aqueous Extract of Date Seed. The Data are Represented as the Mean $\pm$ SEM.

Conclusion

The present study demonstrates total phenol, flavonoid content in Iraqi Dates Palm (*Phoenix dactylifera* L. Cv. "Barhi") Dates and Seeds extracts. Preliminary phytochemical analysis revealed presence of tannin, flavonoid, and phenolic compounds in date fruit and seed. found that DPE has the highest total phenolic content and the most effective antioxidant compared to DSE. The information points to date palm fruits as a good natural antioxidant source. These results validate

the significance of date consumption and indicate that dates are a valuable source of naturally occurring bioactive compounds that can guard against oxidative stress-related illnesses.

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