

Phytochemical Content and in Vitro Antioxidant, Antibacterial and Antitumor Activities of Phoenix Dactylifera Fruit Extract

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Abstract: Phoenix dactylifera fruits are important sources for carbohydrates, minerals, vitamins, phenols and flavonoids compounds, which are important for human health. The objective of study: To evaluate the chemical composition, minerals, phytochemical, antioxidant activity using [FRAP⁺ and DPPH⁺], and antibacterial properties against some bacterial strains, (*E. coli*, *St. aureus*, *L. monocytogenes* and *B. subtilis*), of Phoenix dactylifera fruits. In addition, cytotoxic study of plant fruit extracts on human cancer cell lines (MCF-7 and HCT-116). The phytochemical screening showed that the fruit extracts contain alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates and except steroids. The Phoenix dactylifera fruits have the highest value of polyphenol and flavonoid content which were 181.27mg GAE/g and 31.09mg QE/g, respectively. The antioxidant activity was evaluated using the ferric reducing antioxidant power (FRAP) radical into 79.17 and 87.05%, at a concentration 16mg/ml of aqueous and methanolic extracts. DPPH assay revealed a good antioxidant activity of methanolic extract which was (IC₅₀ 0.45µg). Moreover, the methanolic extract of Phoenix dactylifera led to the highest growth inhibition (18.75, 20.5 and 17mm), against *E. coli*, *L. monocytogenes* and *B. subtilis* at a concentration 4mg/ml, respectively. While, the aqueous extract provided the medium percentage of growth inhibition (11.5, 9.75 and 12.5mm) against *E. coli*, *St. aureus* and *B. subtilis* at a concentration 4mg/ml, respectively. Moreover, the fruit extracts have a reasonable effect on breast and colon cancer cell lines inhibition when compared with Thymoquinone at a concentration 75µg/ml.

Keywords: Phoenix Dactylifera, Phytochemical, Antioxidant, Antibacterial, Antitumor activity.

INTRODUCTION

Medicinal plants are used and are a source of biologically active chemicals in various parts of the world. Nearly 20% of the plants in the world have been tested in biology and pharmacology fields. New pharmaceutical products have been presented to the markets; a hung amount of such products are derived from either natural or semi-synthetic resources **Al-Daihan *et al.* (2012)**.

Palm dates which scientifically called (*Phoenix dactylifera*) L. Arecaceae) are substantial fruits in many countries. The interest toward studying antioxidant characteristics and the health benefits of the dates have been renewed in the past years. The dates have functions in fighting many disorders such as cancer, cardiovascular, neurological and diabetes. In addition, the date fruit have shown to contain antioxidant, antimutagenic, antiheamolytic, antiviral, antibacterial, antifungal, antitumor activities and

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gastroprotective property. These activities are attributed to the presence of a variety of components such as phenolics, mainly the cinnamic acids and their derivatives, sterols, flavonoids carotenoids, and anthocyanins **El-Arem et al. (2013)**.

The date fruits consists of a high content of carbohydrate, fat, minerals, protein, vitamins and fiber. Antibacterial activities were evaluated of different parts of date plant extract against a variety of gram negative and positive bacteria involving, *Eschereshia coli*, *Listeria monocytogenes*, *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes* **Mainasara et al. (2017)**.

Hence, this current research was planned to extract the active ingredients of the date palm fruit for determining the chemical composition and measuring the best extract accounted for the total polyphenols, flavonoid content, and antioxidant activity. Our investigation was also included the evaluation of the antibacterial characteristics of the date palm fruit extract.

MATERIALS AND METHODS

Plant Material: *Phoenix dactylifera* was kindly obtained from SAER, Mansoura, Egypt. Sample were immersed in water, then washed away to remove the flesh of the dates. The sample was then dried in air, and then was dried again in air oven at 60° C and smashed into a fine powder.



Aqueous extract: Powdered plant fruits (200gm), was extracted using distilled water. The extraction process involved boiling at a temperature ranges from 80 to 100°C to obtain the initial extract. The filtration of the extract was done at room temperature after cooling then lyophilization of the extract and kept at -20°C for the future usage **Kim et al. (2011)**. Methanolic extract: The methanolic extraction was obtained by immersing of 200gm of the fruit powder in methanol (5L) for six times at room temperature. The rotary evaporator was used for obtaining the final methanolic extract by concentrating the extraction to dryness at a temperature of 45 C°, under vacuum and reduced pressure. The methanolic extract was preserved for further use at 4°C **Farid et al. (2016)**.

Chemical Composition of the Fruits

Investigation of moisture content: Moisture, Ash, Fiber, Protein, Lipid and Carbohydrates contents were estimated according to the procedure explained by **(AOAC 2000)**.

Minerals content of investigated fruits: the ached sample was liquefied in 1ml. of concentrated hydrochloric acid and distilled water was used to complete the volume to 100ml. Sodium and potassium were determined using flame photometer **(Hesse 1971)**. Magnesium, copper, calcium, manganese, zinc and, were determined by atomic absorption (Perkin-Elmer 2380) **Cottenie et al. (1982)**. Phosphorous was determined calorimetrically as illustrated by **(Page 1982)**.

Phytochemical estimations of fruits extracts: The estimation was implemented on the methanolic and aqueous extracts to detect the presence of alkaloids, flavonoids, saponins, tannins, phenols, carbohydrates and steroids. Detection was based on the method of **Harborne (1988)**.

Total phenolic contents: Phenolic compounds were extracted from date fruit extract based on the method of **(Lin and Tang, 2007)**. 0.1 ml of the extract was added to distilled water of 2.8 ml, 2 ml of (2% w/v) sodium carbonate and 0.1 ml of 50% (v/v) of Folin-Ciocalteu agent (FCr). The mixed solution was saved at room temperature for 30 min. The absorbance was measured using (Spekol 11 C.Z.J) spectrophotometer) at 750 nm; distilled water was used as the blank. A standard curve was used for the estimation of the phenolic content concentration and it was expressed as milligram gallic acid equivalent (GAE)/g based on dry weight.

Total flavonoid contents: The aluminium chloride colorimetric technique was used for the flavonoids estimation **Chang et al. (2002)**. 0.5 ml of the fruit extract was added to 1.5 ml of 95% ethyl alcohol, 0.1 ml, 0.1 ml, and 2.8 ml of 10% aluminum chloride (AlCl₃), 1M potassium acetate (CH₃COOK) and distilled water, respectively. Incubation of the mixture lasted for 40 min at room temperature. A Spekol 11 (C.Z.J) spectrophotometer, was used to measure the absorbance of the mixture at 415 nm against blank which

was distilled water. The total flavonoids content was obtained from a standardization curve, which was plotted by preparing the quercetin in solutions at concentrations of 12.5 to 100 g/ml in 90 % methanol.

Determination of Reducing properties (FRAP): The antioxidant activity was investigated by (FRAP), based on **Oyaizu (1986)**. An amount of 0-100 mg of the extract from each sample was mixed with 2.5ml potassium ferricyanide (10mg / ml) in a 0.20 mol phosphate buffer at pH 6.6 (2.5ml), the mixture was incubated at 50°C for 20min. Addition of 2.5 ml (100mg/ml) of Trichloroacetic acid (TCA) was done to the mixture, then the mixture was centrifuged for 10 minutes at 650g. 2.5 ml of the supernatant and 2.5 ml of distilled water were mixed with 0.5ml (1mg/ml) ferric chloride solution and the resulting color absorption was measured at 700 nm using a spectrophotometer from (Spekol 11 C.Z.J spectrophotometer). Higher absorbance of the reaction mixture showed greater reduction. The antiradical activity, was calculated using the following equation:

$$\text{Inhibition (\%)} = (A \text{ Test} - A \text{ Blank}) / A \text{ Blank} \times 100$$

Evaluation of (DPPH): The DPPH free radical scavenging activity was measured from blanching of the resulted purple color of (2,2 Diphenyl -1-picryl hydrazyl). This investigation was based on the method of **Coruh et al. (2007)**. 0.1 ml of different concentration of extract was added to 1.4 ml of DPPH 0.1mM and saved for 30 min in dark. The absorbance was measured at 517 nm, using a Spekol 11 (C.Z.J) spectrophotometer and the percentage inhibition was calculated by.

$$\text{Inhibition (\%)} = (A \text{ Blank} - A \text{ Test}) / A \text{ Blank} \times 100$$

Antibacterial activity of fruit extracts: The antibacterial properties of the date fruit extracts were tested using the diffusion disc assay. The bacterial strains including *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Bacillus subtilis*, were pre-cultured at 37°C inside incubator in nutrient broth overnight. The turbidity of the bacterial suspension was set to 0.5 McFarland standards. A sterile cotton swab was used for spreading 100µl of each bacterial strain over the surface of the nutrient agar. In each prepared plate, extracts concentration (1, 2 and 4mg/ml), were poured in all wells using micro-pipette. All plates were then incubated for 24 h at 37°C. After incubation, the inhibition zones' diameters were measured **Samad et al. (2016)**.

Antitumor activity: Breast and Colon carcinoma cell lines (MCF-7 and HCT-116), were obtained from the American Type Culture Collection. They were frozen in liquid nitrogen (-180 °C). The antitumor activity of fruit extracts against these cell lines was measured at the Faculty of Medicine, Zagazig University, Egypt.

Statistical analysis: All data were first analysed by one way ANOVA. Duncan's multiple rang test was used for the determination of the significant differences between treatment means; the p-value <0.05 as the level of the significance.

RESULTS AND DISCUSSION

Chemical composition of date fruits: As shown in table (1) the percentages of moisture, ash, fiber, lipids, protein, total nitrogen and carbohydrate contents of investigated *Phoenix dactylifera* fruits, were 8.75, 1.08, 3.55, 0.90, 3.56, 0.57 and 81.59%, respectively.

Table 1: Chemical composition (%) of investigated fruits

Plant fruits	Moisture Content	Ash Content	Crude Fiber	Crude Lipid	Crude Protein	Total Nitrogen	Carbohydrate Content
<i>Phoenix dactylifera</i>	8.75	1.08	3.55	0.90	3.56	0.57	81.59

Our data were consistent with those obtained by **Djaoudene et al. (2019)**, who evaluated the chemical components of *P. dactylifera* date seeds of the various cultivars and found that the percentage of moisture and ash ranged between 10.8% to 11.4% and 11% to 12%, respectively. The range of carbohydrates were found to be 17.4 to 27.8g/100 g dw, whereas protein was 1.4 to 3.3g BSA/100 g dw. The analysis of proline and FAA contents from diverse cultivars showed large variations, the higher contents were found in the TAG and TAZ cultivars containing 38.7 and 404.3mg/100 g, respectively. **Salomón-Torres et al. (2019)**, mentioned that the average values of protein, lipids, fiber, total sugars, reducing sugars and sucrose, were 3.14, 0.75, 6.34, 75.32, 70.26 and 5.06%, of date pulp, respectively. While the average values of the same chemical composition, were 4.84, 9.94, 66.79, 5.88, 4.40 and 1.46%, of date seeds.

Results were in the agreement with those reported by **Sohail et al. (2018)**, who studied the proximate analysis of Moisture, Ash, Protein, and Fat of date fruit powders and they found that the

percentages were 8.5, 2.2, 1.8 and 1.8% respectively. **Mainasara et al. (2017)**, Carbohydrates were the most dominant component in both the dried and fresh; 92.02% and 95.00, respectively. The moisture content was higher in fresh dates (68.50%) compared to dried dates (5.00%). The crude protein of the dried and fresh dates were 4.3% and 1.14%, respectively. The nitrogen content was higher in dried dates (0.69%) than in fresh date (0.18%). Ash contents of fresh and dried dates were 3.30 and 0.50% respectively. The fiber content of fresh dates (1.20%) was higher than dried dates (0.70%). The lipid content in both date samples was 0.50%. **Oni et al. (2015)**, the moisture, crude protein, and fat contents were 13.40, 2.67 and 0.70 g/100g, respectively. While the dietary fiber, ash, and carbohydrate values were 2.13, 3.29, and 76.95 g/100g respectively.

The mineral contents of the fruits under studying: Minerals have essential role in the nutrition of both plants and humans. Calcium, is necessary for the bones of the skeleton of animals. Iron has critical physiological role in the hemoglobin (Hb) and other elements essential for the activity of certain enzymes and vitamins. In figure (1), showed that the contents of calcium, magnesium, copper, zinc, manganese, iron, sodium, potassium, and phosphorus, were 14.08, 11.66, 9.07, 7.12, 8.78, 12.33, 10.18, 3.57, and 2.44mg/100g, based on dry weight in *Phoenix dactylifera* fruits, respectively.

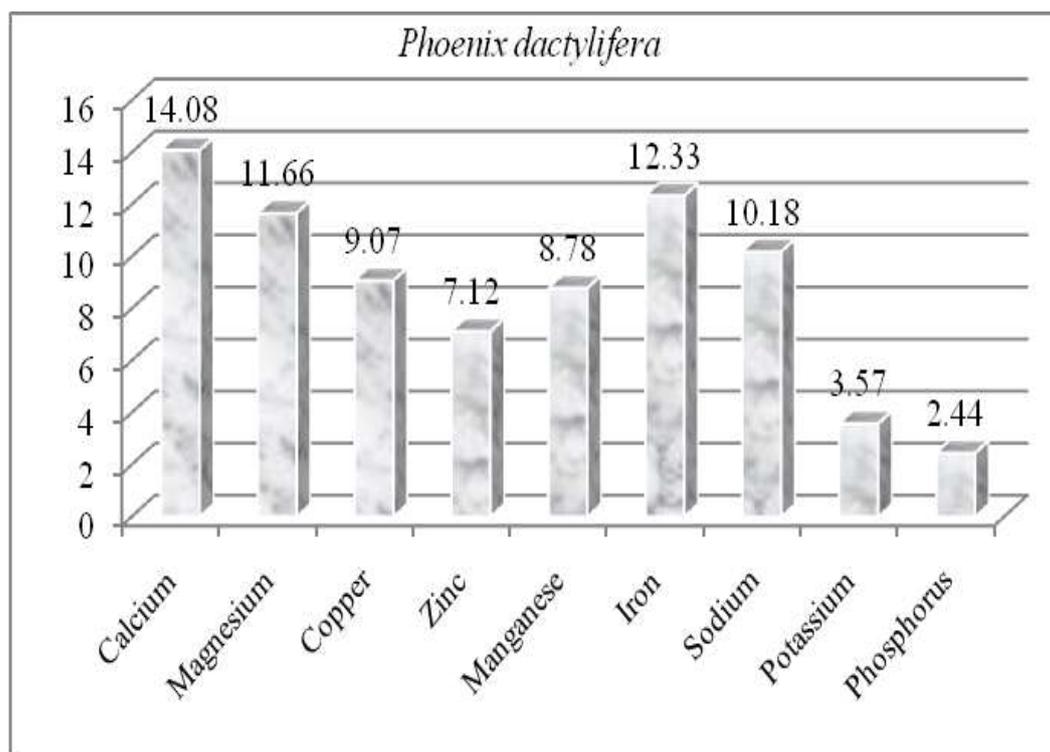


Figure 1: Minerals content of investigated fruits (mg/100g) on air dry weight basis

Our results indicated that the plant contents of minerals were very high. The most prevalent elements of the date pulp, were 851.98, 142.97, and 139.40 mg/100g, of potassium, magnesium, and phosphorus, respectively. Whereas for the seeds, they were potassium 413.36, sulfur 151.36, and phosphorus 92.42mg/100g **Salomón-Torres et al. (2019)**. The results were compatible with those demonstrated by **Mainasara et al. (2017)**, the fresh date have the highest content of sodium (3.5mg/100g) than in dried dates (1.83mg/100g). Magnesium concentration was 0.04 and 0.07mg/100g in fresh and dried dates respectively. Potassium and phosphorus content were higher in fresh date (4.50 and 0.61mg/100g respectively), than in dried dates (3.00 and 0.49mg/100g), respectively. Calcium content in both date samples, was 0.04mg/100g. **Yahaya et al. (2015)**, the mineral analysis of fresh date fruit varieties e.g. calcium, zinc, phosphorus, manganese, and nitrogen ranged from (0.56 to 9.87), (0.46 to 0.81), (4.33 to 7.09), 0.03 to (0.07), and (0.36 to 0.50) mg/g, respectively. The mineral content of *Phoenix dactylifera* fruits was very rich in potassium (360.79mg/100g) and contains calcium and phosphorus of (37.45 and 27.30 g/100g), respectively **Oni et al. (2015)**.

Preliminary phytochemical tests of fruits extracts: Table (2) showed the phytochemical composition of aqueous and methanolic extracts of *Phoenix dactylifera* fruits. The crude aqueous and methanolic extracts of investigating fruits were rich in alkaloids, flavonoids, tannins, saponins, phenols, carbohydrates, and resins within the acceptable limits. All extracts were poor in steroids.

Table 2: Preliminary phytochemical tests of plant fruit extracts

Plant fruits	Extracts	Alkaloids	Flavonoids	Tannins	Saponin	Phenols	Carbohydrates	Steroids	Resins
<i>Phoenix dactylifera</i>	Aqueous	+	++	++	+	++	++	-	+
	Methanolic	++	++	++	++	++	+	-	+

The data about *Phoenix dactylifera* fruits were consistent with those reported by **Jaganathan et al. (2018)**, who studied the phytochemical analysis of methanolic extract of date seed revealed that alkaloids, tannins, flavonoids, proteins, amino acids, terpenoids and phenols were present in the extract. Whereas, cardiac glycoside, saponins, anthraquinones, and steroids were absent. **Sundar et al. (2017)**, the phytochemical screening of *Phoenix dactylifera* fruits demonstrated that the qualitative phytochemical analysis of date seed powder consisted of flavonoids, tannins, saponins, phenol, alkaloids, and sterols and triterpenes while anthraquinone glycosides were not present. The previous results were consistent with those obtained by **Yahaya et al. (2015)**, who detected that the highest amount of anthraquinones, saponins, tannins, reducing sugar, volatile oils, and cardiac glycosides, were in the fresh date fruit. **Oni et al. (2015)**, phytochemical screening of the date fruit consisted of alkaloids, anthraquinones, flavonoids, tannins, saponins, and terpenoids which were 1.59, 0.17, 3.36, 0.69, 1.37, and 1.97g/100g respectively.

Total polyphenols and total flavonoids content: Phenolic compounds can protect the organs and body cells against the injuries caused by hydrogen peroxide. Their action is exerted by neutralizing and scavenging free radicals, as well as damaging lipid peroxides **Sroka et al. (2003)**. Total polyphenolic compounds include several classes are secondary plant metabolites which integral part of human and animal diets. Flavonoids are large group of the phenolic compounds consisting mainly of flavonols, flavanols and anthocyanins. Data in table (3) showed that *Phoenix dactylifera* fruits comprised the highest values of total polyphenol and flavonoid contents which were 181.27mg GAE/g and 31.09mg QE/g, respectively.

Table 3: Total polyphenols and total flavonoids content

Plant fruits	Total polyphenols (mg GAE/g)	Total flavonoids (mg QE/g)
<i>Phoenix dactylifera</i>	181.27	31.09

The results were in harmonious with those mentioned by **Djaoudene et al. (2019)**, who measured the greatest contents of total phenolic, total flavonoids, anthocyanins, flavonols, and proanthocyanidins which were (476mg GAE/g dw), (6.52mg QE/g dw), (1.26mg Q3GE/g dw), (3.36mg Q3GE/g dw), and (85.13mg CE/g dw) respectively. Ascorbic acid were detected in the *Phoenix dactylifera* seeds of the TAG cultivar. The total phenolic and flavonoid content of date fruit powders were 247.47mg GAE/100g and 25.8mg QE/100g **Sohail et al. (2018)**. The dry date contains a considerable level of phenolic content which were 14.80 and 10.31mg GAE/g of aqueous and methanolic extract, respectively **El-Sohaimy et al. (2015)**.

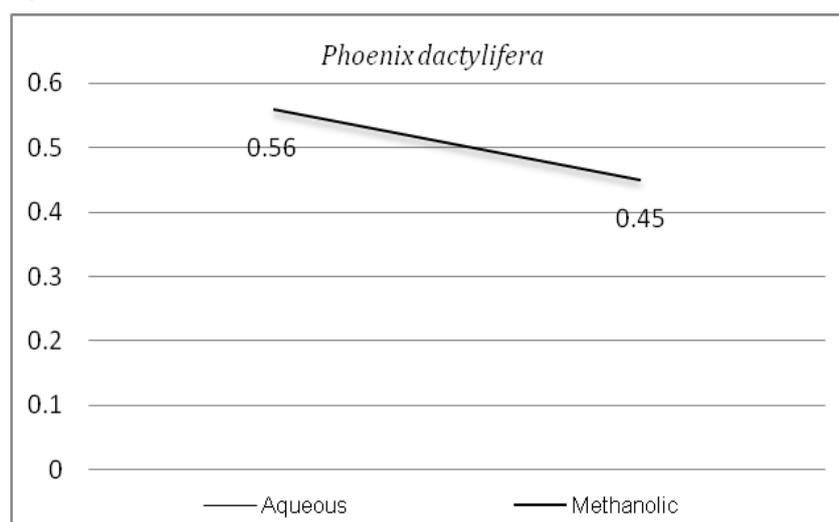
Reducing power of plant fruit extracts: Efficiency of fruit extracts reduce Fe^{+++} to Fe^{++} has been determined by **Sroka et al. (2003)**. The optical density of the solution was measured at 700nm wavelength using a spectrophotometer from (Spekol 11 C.Z.) spectrophotometer). The information acquired in table (4), the absorption reflected the reduction energy for various levels of *Phoenix dactylifera* fruits extracts. Data stated that absorbance at 700nm for producing color as a result and % inhibition for using concentrations (2, 4, 8 and 16mg/ml) of samples. *Phoenix dactylifera* fruits have the largest proportion of reduction energy ranging from 49.01 to 87.05% for methanol extract at concentration 2 and 16mg/ml levels respectively, followed by the aqueous extract of fruits, ranged from 44.03 to 79.17%, at the same concentration. Various studies have indicated that high antioxidant activity is associated with the ability of electronic donation, which represents the reduced strength of biologically active compounds **Siddhuraju et al. (2002)**. The results were in the same line with those demonstrated by **Djaoudene et al. (2019)**, who revealed the antioxidant activity using (FRAP) of seeds of *Phoenix dactylifera* cultivars (Ourous, Tazizaout, Tazarzeit, Tazoughart, Ouaouchet, Oukasaba, Delat and Tamezwertn'telet) which were 2.96, 3.16, 2.59, 2.28, 1.78, 1.67, 1.48, and 1.45 mmol/g, respectively. **Siahpoosh et al. (2016)**, estimated that the antioxidant activity of EC1 of FRAP assay ranged from 0.748 to 2.32 μ g/ml.

Table 4: Reducing power of *Phoenix dactylifera* fruit extracts

Concentration mg/ml of plant fruit	% of Inhibition	
	<i>Phoenix dactylifera</i>	
	<i>Aqueous</i>	<i>Methanolic</i>
2	44.03	49.01
4	55.12	60.00
8	63.09	67.41
16	79.17	87.05

Our results agreed with **El-Arem et al. (2013)**, who stated that the antioxidant activity of different cultivars date fruits using (FRAP) radical, were (0.15, 0.24, 0.10, 0.8, and 0.11EC₅₀, mg/ml), of Beidh Hmam, Degla, Khalt Ahmar, Rtob and Rtob Hodh, respectively.

Antioxidant properties using (DPPH⁺): The antioxidant activity of date fruits aqueous and methanolic extract, reported in figure (2). The concentration of an antioxidant required to reduce the DPPH concentration by 50% (IC₅₀) is widely used to measure the antioxidant activity, **Sanchez et al. (1998)**. The low EC₅₀ refers to the increased activity of the antioxidant. Using (DPPH) radical scavenging activity, antioxidant activity tested on extracts was calculated.

Figure 2: (DPPH⁺) radical of *Phoenix dactylifera* fruit extracts

From revealed that, the scavenging influence (IC₅₀) of methanolic extracts of *Phoenix dactylifera* fruits have the highest effect of inhibition percentage (0.45) followed by aqueous extract of the same plant fruits which were (0.56) respectively. IC₅₀ is the minimum inhibition concentration at 50%. A smaller IC₅₀ means higher antioxidant activity.

Antioxidant activity using (DPPH⁺), of *Phoenix dactylifera* are consistent with those obtained by **Salomón-Torres et al. (2019)**, the antioxidant activities were beta-carotene, 65.50% and 47.75%; DPPH, 0.079 IC₅₀ g/l and 0.0046 IC₅₀ g/L; and ABTS, 13.72 IC₅₀ g/l and 0.238 IC₅₀ g/l respectively. **Djaoudene et al. (2019)**, reported that, the antioxidant activity using (DPPH) of seeds of *Phoenix dactylifera* cultivars (Ourous, Tazizaout, Tazarzeit, Tazoughart, Ouaouchet, Oukasaba, Delat and Tamezwertn'telet) were 3.39, 3.59, 2.74, 2.99, 5.06, 4.52, 3.42 and 3.64mmol/g, respectively. **El-Sohaimy et al. (2015)**, antioxidant capacity using (DPPH), the inhibition values of water extract of date palm fruits, were 19.52, 23.22, 33.18, 68.14, and 79.32%, for concentrations 6.5, 12.5, 25, 50, and 100mg, respectively. While the inhibition values of ethanolic extract, were 10.00, 13.64, 22.70, 49.29, and 66.51%, of the same concentrations respectively. The antioxidant activity of different cultivars of date fruits using (EC₅₀ DPPH) radical, were 1.79, 1.91, 0.93, 1.81 and 1.87µg sample, of Beidh Hmam, Degla, Khalt Ahmar, Rtob and Rtob Hodh, respectively, **El-Arem et al. (2013)**.

Antibacterial activity: The growth inhibition activity of different concentrations (1, 2 and 4 mg/ml) of *Phoenix dactylifera* fruits aqueous and methanolic extracts on *Escherichia coli*, *Staphylococcus aureus*, *Listeria m.cytogenes* and *Bacillus subtilis*, were shown in table (5). It is obvious that the percentage of growth inhibition increased gradually by rising the concentration of extracts under investigation for all microbial strains.

From table (5), it could be noted that the methanolic extract of *Phoenix dactylifera* resulted in the highest inhibition of growth (18.75, 20.5, and 17mm) of *Escherichia coli*, *Listeria monocytogenes*, and

Bacillus subtilis at 4mg/ml, respectively. While the aqueous extract of the same plant led to the medium inhibition for growth (11.5, 9.75, and 12.5mm) of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* at 4mg/ml, respectively.

Table 5: Antibacterial activity of *Phoenix dactylifera* fruits extracts

Fruit extract	Extract	Conc. (mg/ml)	Inhibition zone (mm)			
			<i>Escherichia coli</i>	<i>St.coccus aureus</i>	<i>Listeria m.cytogenes</i>	<i>Bacillus subtilis</i>
<i>Phoenix dactylifera</i>	Aqueous	1	7±0.12	6±0.78	6.5±0.37	8.5±0.39
		2	8.5±0.07	7.5±0.81	7±0.46	10±0.34
		4	11.5±0.31	9.75±0.03	7.05±0.04	12.5±0.71
	Methanolic	1	12.75±0.09	8.75±0.19	14.75±0.59	11.05±0.08
		2	15.5±0.18	9.5±0.77	17.5±1.33	14.5±1.01
		4	18.75±0.44	11±0.01	20.5±1.08	17±1.20

The methanolic extract of *Phoenix dactylifera* caused the highest growth inhibition (18.75, 20.5 and 17mm), against *E. coli*, *L. monocytogenes* and *B. subtilis* at 4mg/ml, respectively. While the aqueous extract of the same plant produced the medium percentage of growth inhibition (11.5, 9.75, and 12.5mm), against *E. coli*, *S. aureus* and *B. subtilis* at 4mg/ml, respectively. While the average effective, were (15.5, 9.5, 17.5, and 14.5mm), of *E. coli*, *S. aureus*, *L. monocytogenes* and *B. subtilis* at a concentration 2mg/ml respectively. When compared with aqueous extract of the same plant fruits, which were 8.5, 7.5, 7, and 10mm, of the same bacteria strains, at a concentration 2mg/ml respectively. This may be accredited to the presence of phenolic compounds having antimicrobial effect as phenolic compounds.

In terms of effects as antibacterial, our results indicated the positive effect of date fruit extracts on some bacteria strains matched with those obtained by **Abdullah et al. (2019)**, the antibacterial activity (diameter of inhibition zone) of hot aqueous *Phoenix dactylifera* fruit extract, were 17.33, 16.33, 17.67, 13.33, and 16.33mm of *E. coli*, *S. typhi*, *S. typhimurium*, *S. flexneri* and *V. cholerae* at a concentration 200mg/ml respectively. While antibacterial activity of methanolic extract, were 17.33, 15.33, 17.33, 24.33, and 17.33mm of the same bacterial strains and same concentration. **Sohail et al. (2018)**, the antimicrobial effectiveness of date fruit powder against *E. coli*, were 11.0, 13.3 and 14.3mm, of Zahidi, Aseel and Muzafati, respectively. The *Phoenix dactylifera* fruit extract of fresh and dried date had higher effect against *S. aureus* with the inhibition zone of 17 and 15mm, respectively. This followed by *B. subtilis* with the inhibition zone of fresh and dried date, which were 16 and 13mm, respectively. Fresh and dried date extract showed efficacy against *Salmonella* spp with zones of inhibition which were 14 and 11mm. The extracts were less effective against *E. coli* with 11 and 9mm for zones of inhibition of fresh and dried date fruit extract, respectively **Mainasara et al. (2017)**. **Sundar et al. (2017)**, demonstrated antibacterial activity for *Phoenix dactylifera* seed acetone extract against *E. coli* and *B. cereus* at various concentrations and showed highest zone of inhibition of 20 and 17mm against *E. coli* and *B. cereus* at 1mg/ml concentration.

Results of several authors were in line with those showed by **El-Sohaimy et al. (2015)**, who studied antibacterial activity and demonstrated that the minimum inhibiting concentration of date palm extract was 50mg/ml for aqueous and ethanol extract. Data presented that the date fruits extract has a strong antibacterial characteristics (for aqueous and ethanol extracts) against *E. coli* (20 and 16mm), *Salmonella enterica* (20 and 14mm) and *Bacillus subtilis* (18 and 15mm) and moderate inhibition against *staphylococcus aureus* (8 and 5mm) and *Enterococcus faecalis* (5 and 2mm). On the other hand, chloramphenicol and ampicillin which were used as positive control, showed toxicity against all five examined pathogenic strains.

The antibacterial properties of the methanolic extracts of date (*Phoenix dactylifera*) cultivars (Beidh Hmam, Degla, Khalt Ahmar, Rtob and Rtob Hodh,) were 9.00, 11.33, 10.33, 9.33, and 10.33%, of *Bacillus cereus* respectively. While the antibacterial activity of the same cultivars dates extract, were 8.33, 13.33, 7.00, 8.66, and 9.66%, of *Staphylococcus aureus*, respectively. Moreover, the antibacterial activity of the same cultivars dates extract, were 13.66, 10.66, 7.33, 9.66, and 0.00% of *Listeria monocytogenes*, respectively. Likewise, the antibacterial activity of the same cultivars dates extract, were 0.00, 7.66, 0.00, 7.66, and 7.33%, of *Escherichia coli*, respectively, **El-Arem et al. (2013)**.

Antitumor activity: Cytotoxicity of aqueous and ethanolic date fruit extracts (*Phoenix dactylifera*) at concentrations of 50 µg/ml on human breast carcinoma cell lines MCF-7 and HCT-116 were detected by measuring the percentage of cell viability using the SRB assay method. Results depicted in table (6)

summarized the cytotoxic effect of different fruit extracts and thymoquinone as a positive control with a concentration (75µg/ml). Methanolic fruit extracts seemed to be highly significant at $P < 0.001$ as they showed the highest cytotoxic activity on the tested cell lines (MCF-7 and HCT-116) which was (33.12 and 30.17%). Followed by aqueous extract of the same cell lines, which was (41.35 and 40.33%), compared with thymoquinone, which was (33.81 and 31.61%), respectively.

Table 6: Antitumor activity of *Phoenix dactylifera* fruit extracts

Concentration µg/ml of extract	Percentage of cell viability (%)	
	MCF-7	HCT-116
75 Aqueous	41.35±0.01	40.33±0.10
75 Methanolic	33.12±0.03	30.17±0.02
75 Thymoquinone	33.81±0.19	31.61±0.26
P-value	< 0.001	

Consistent with many oncology studies and effect of date fruit extracts on cancer cell lines, we found A similar result obtained by **Ibrahim et al. (2017)**, the cytotoxic impact of date palm extracts against human cancer cells (HCT, PC3, MCF7 and HEPG2), were 44.8, 37.1, 53.3, and 55.3%, at a concentration (125µg/ml) respectively. The extract from *Phoenix dactylifera* showed a dose-dependent cytotoxicity across all cell lines of the testes. Cell viability at doses of 250, 500, and 1000µg/ml, was found to be 87, 75 and 48% in HepG2. While for MCF-7 cells, was 95%, 85%, and 78% Followed by A-549, which were 77, 51 and 35%, respectively (**Al-Sheddi 2019**).

CONCLUSION

Phoenix dactylifera fruit contains a high percentage of active biochemical compounds. The effect of bio extracts as antioxidant using (FRAP and DPPH), showed the high inhibition to free radicals in vitro. Moreover, *Phoenix dactylifera* fruit possesses a measurable effect against bacterial strains following: *Escherichia coli*, *St.coccus aureus*, *Listeria monocytogenes* and *Bacillus subtilis*. Moreover, the methanolic extract showed highest tumor inhibition of the breast and colon cell lines.

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REFERENCES

- [1] Abdullah, N., Mohd, N.F., Ishak., & Wan, S.W.S. (2019). In-vitro antibacterial activities of ajwa date fruit (*Phoenix dactylifera* L.) extract against selected gram-negative bacteria causing gastroenteritis. *International Journal of Pharmaceutical Sciences and Research*, 10(6), 2951-2955. <http://doi.org/10.13040/IJPSR.0975-8232>
- [2] Al-Daihan, S., & Bhat, R.S. (2012). Antibacterial activities of extracts of leaf, fruit, seed and bark of *Phoenix dactylifera*. *African Journal Biotechnology*, 11(42), 10021-10025. <http://doi.org/10.5897/AJB11.4309>.
- [3] Al-Sheddi, E.S. (2019). Anticancer potential of seed extract and pure compound from *Phoenix dactylifera* on human cancer cell lines. *Pharmacognosy Magazine*, 15(63), 494-499. http://doi.org/10.4103/pm.pm_623_18.
- [4] AOAC. (2000). *Association of Official Analytical Chemists. Official Methods of Analysis*. 17th edition. The Association, Washington DC. USA.
- [5] Cottenie, A., Verloo, M., Kiekens, L., Velghe, G., & Camerlynck, R. (1982). *Chemical analysis of plant and soil*. Lab Anal and Agro chemistry State Univ. Gent., Belgium.
- [6] Djaoudene, O., López, V., Cásedas, G., Les, F., Schisano, C., Bey, M.B., & Tenore, G.C. (2019). *Phoenix dactylifera* L. seeds: A by-product as a source of bioactive compounds with antioxidant and enzyme inhibitory properties. *Food & function*, 10(8), 4953-4965.
- [7] El Arem, A., Saafi, E.B., Slama, R.B., Zayen, N., Hammami, M., & Achour, L. (2013). Phytochemical composition, antibacterial and antioxidant activities of common date palm (*Phoenix dactylifera* L.) fruit during three maturation stages. *Tunisian Journal of Medicinal Plants and Natural Products*, 10(2), 33-48.
- [8] El-Sohaimy, S.A., Abdelwahab, A.E., Brennan, C.S., & Aboulenein, A.M. (2015). Phenolic content, antioxidant and antimicrobial activities of Egyptian Date Palm (*Phoenix dactylifera* L.) Fruits.

- Australian Journal of Basic and Applied Sciences*, 9(1), 141-147.
- [9] Farid, M., Erian, N.S., Hamed, H., & El-Khateeb, A.Y. (2016). Total polyphenols, flavonoids content and antioxidant activity of crude methanolic and aqueous extracts for some medicinal plant flowers. *The Arab Journal of Sciences & Research Publishing*, 2(2), 53-61.
- [10] Harborne, J.B. (1988). *Phytochemical methods*, 2nd ed. Published in USA by Chapman and Hall 29, west 35th street, New York.
- [11] Hesse, P.R. (1971). *A Text Book of Soil Chemical Analysis* John murry (publishers) Ltd. London. UK, 528.
- [12] Chang, C.C., Yang, M.H., Wen, H.M., & Chern, J.C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of Food and Drug Analysis*, 10(3), 178-182.
- [13] Coruh, N.U.R.S.E.N., Celep, A.S., & Özgökçe, F. (2007). Antioxidant properties of Prangos ferulacea (L.) Lindl., Chaerophyllum macropodum Boiss. and Heracleum persicum Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase. *Food chemistry*, 100(3), 1237-1242. <http://doi.org/10.1016/j.foodchem.2005.12.006>.
- [14] Ibrahim, F.Y., Khalil, M.M., Din, N.E., & Atieya, K.M. (2017). Studies on Biological Effect of some Selected Foods (Un-Pollinated Siwi Date, Date Palm Pollen and Doum Fruit). *Journal of Food and Dairy Sciences*, 8(12), 461-468.
- [15] Jaganathan, V., Shanmugavadivu, M., & Ganesh, S. (2018). Preliminary phytochemical screening and anti-bacterial activity of date seed methanolic extract. *International Journal of Advanced Research in Biological Sciences*, 5(2), 209-215.
- [16] Kim, I.S., Yang, M.R., Lee, O.H., & Kang, S.N. (2011). Antioxidant activities of hot water extracts from various spices. *International journal of molecular sciences*, 12(6), 4120-4131.
- [17] Lin, J.Y., & Tang, C.Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food chemistry*, 101(1), 140-147. <https://doi.org/10.1016/j.foodchem.2006.01.014>
- [18] Mainasara, M.M., Sanusi, S.B., Maishanu, H.M., & Ismail, T. (2017). Antibacterial activity and nutritional content of fresh and dried date fruits (*Phoenix dactylifera*) L. *International Journal of Science and Healthcare Research*, 2(1), 15-20.
- [19] Oni, S.O., Adeosun, A.M., Ladokun, O.A., Ighodaro, O.M., & Oyedele, O.M. (2015). Nutritional and phytochemical profile of niger cultivated date palm (*Phoenix dactylifera* L). *Journal of Food and Nutrition Sciences*, 3(3), 114-118.
- [20] Oyaizu, M. (1986). Studies on products of browning reaction. *The Japanese journal of nutrition and dietetics*, 44(6), 307-315. <http://doi.org/10.5264/eiyogakuzashi.44.307>
- [21] Page, A. (1982). *Method of soil analysis part 2. Chemical and microbiological properties*. 2nd edition. American society of agronomy and soil science society of America, Madison, Wisconsin, USA.
- [22] Salomon-Torres, R., Ortiz-Uribe, N., Valdez-Salas, B., Rosas-González, N., García-González, C., Chávez, D., Córdova-Guerrero, I., Díaz-Rubio, L., Haro-Vázquez, M.P., Mijangos-Montie, J.L., Morales-Maza, A., Mahadevan, P., & Krueger, R. (2019). Nutritional assessment, phytochemical composition and antioxidant analysis of the pulp and seed of medjool date grown in Mexico. *PeerJ* 1-19. <http://doi.org/10.7717/peerj.6821>.
- [23] Samad, M.A., Hashim, S.H., Simarani, K., & Yaacob, J.S. (2016). Antibacterial properties and effects of fruit chilling and extract storage on antioxidant activity, total phenolic and anthocyanin content of four date palm (*Phoenix dactylifera*) cultivars. *Molecules*, 21(4), 1-14. <http://doi.org/10.3390/molecules21040419>.
- [24] Sánchez-Moreno, C., Larrauri, J.A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture*, 76(2), 270-276.
- [25] Siahpoosh, A., Taleb, A.M., & Almasi, H. (2016). In vitro evaluation of antioxidant activity and total phenol contents of some extracts from ripe fruits of *Phoenix dactylifera* Var Berhi. *International Journal of Pharmacognosy and Phytochemical Research*, 8(11), 1855-1862.
- [26] Siddhuraju, P., Mohan, P.S., & Becker, K. (2002). Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food chemistry*, 79(1), 61-67.

- [27] Sohail, A., Rafique, A.A., Abbasi, K.S., & Arif, M. (2018). Comparative impact of drying methods on phytochemical and antimicrobial activities of date fruit powders. *Pakistan Journal of Agricultural Research*, 32(1), 1-7. <http://doi.org/10.17582/journal.pjar/2019/32.1.1.7>
- [28] Sroka, Z., & Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. *Food and Chemical Toxicology*, 41(6), 753-758.
- [29] Sundar, R.D.V., Segaran, G., Shankar, S., Settu, S., & Ravi, L. (2017). Bioactivity of *Phoenix dactylifera* seed and its phytochemical analysis. *International Journal of Green Pharmacy*, 11(2), S292- S297.
- [30] Yahaya, S.A., Omokhudu, C.A., Abdulahi, M.A., & Sanusi, M.K. (2015). Phytochemical screening and mineral evaluation of fresh date fruits (*Phoenix dactylifera* L.) in wet season of Nigeria. *Journal of Agricultural and Crop Research*, 3(3), 47-52.