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. subtillis), 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. subtillis 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. subtillis 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. Areceaceae 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
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 agiants a variety of gram negative and positive bacteria involving, Escheresia 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: Moister, Ashe, 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–Ciocalteure 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:

\[
\text{Inhibition} \% = \left( \frac{A_{\text{Blank}} - A_{\text{Test}}}{A_{\text{Blank}}} \right) \times 100
\]

Evaluation of (DPPH): The DPPH free radical scavenging activity was measured from blanching of the resulted purple color of (2.2 Diphenyl -1-picyl 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.

\[
\text{Inhibition} \% = \left( \frac{A_{\text{Blank}} - A_{\text{Test}}}{A_{\text{Blank}}} \right) \times 100
\]

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 range 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>
<thead>
<tr>
<th>Plant fruits</th>
<th>Moisture Content</th>
<th>Ash Content</th>
<th>Crude Fiber</th>
<th>Crude Lipid</th>
<th>Crude Protein</th>
<th>Total Nitrogen</th>
<th>Carbohydrate Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phoenix dactylifera</td>
<td>8.75</td>
<td>1.08</td>
<td>3.55</td>
<td>0.90</td>
<td>3.56</td>
<td>0.57</td>
<td>81.59</td>
</tr>
</tbody>
</table>

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% to11.4% 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. Mainsara 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.

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

<table>
<thead>
<tr>
<th>Plant fruits</th>
<th>Extracts</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Saponin</th>
<th>Phenols</th>
<th>Carbohydrates</th>
<th>Steroids</th>
<th>Resins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phoenix dactylifera</td>
<td>Aqueous</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Methanolic</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

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, anthraquiones, 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. Thier 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, flavonols 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

<table>
<thead>
<tr>
<th>Plant fruits</th>
<th>Total polyphenols (mg GAE/g)</th>
<th>Total flavonoids (mg QE/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phoenix dactylifera</td>
<td>181.27</td>
<td>31.09</td>
</tr>
</tbody>
</table>

The results were in harmonious with those mentioned by Djaoudene et al. (2019), who measured the greatest contents of total polyphenol, total flavonoids, anthocyanins, flavonoids, 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/g and 25.8mg QEQ/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, Taziaouat, Tazarzeit, Tazoughart, Ouaouchet, Ouksasba, Delat and Tamezwen'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 2.32μg/ml.
Table 4: Reducing power of *Phoenix dactylifera* fruit extracts

<table>
<thead>
<tr>
<th>Concentration mg/ml of plant fruit</th>
<th>% of Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phoenix dactylifera</td>
</tr>
<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

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 0.11EC$_{50}$ 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 reduce DPPH concentration by 50% (IC$_{50}$) is widely used to measure the antioxidant activity, Sanchez et al. (1998). The low EC$_{50}$ refers to the increased activity of the antioxidant. Using (DPPH) radicals scavenging activity, antioxidant activity tested on extracts was calculated.

From revealed that, the scavenging influence (IC$_{50}$) 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. IC$_{50}$ is the minimum inhibition concentration at 50%. A smaller IC$_{50}$ 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 IC$_{50}$ g/l and 0.0046 IC$_{50}$ g/L; and ABTS, 13.72 IC$_{50}$ g/l and 0.238 IC$_{50}$ g/l respectively. Djoudene et al. (2019), reported that, the antioxidant activity using (DPPH) of seeds of *Phoenix dactylifera* cultivars (Ourous, Tazizaout, Tazarzeit, Tazoughart, Ouaouchet, Ouakasaba, Delat and Tamezewntet) 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 (EC$_{50}$ 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 monocytogenes* 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

<table>
<thead>
<tr>
<th>Fruit extract</th>
<th>Extract</th>
<th>Conc. (mg/ml)</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phoenix dactylifera</td>
<td>Aqueous</td>
<td>1</td>
<td>7±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>8.5±0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>11.5±0.31</td>
</tr>
<tr>
<td></td>
<td>Methanolic</td>
<td>1</td>
<td>12.75±0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>15.5±0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>18.75±0.44</td>
</tr>
</tbody>
</table>

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 Muzaafati, 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 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 aqueousand 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 (Beidi Hmam, Degla, Khalt Ahmar, Rtof and Rtof 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 (33.61 and 31.61%), respectively.

Table 6: Antitumor activity of Phoenix dactylifera fruit extracts

<table>
<thead>
<tr>
<th>Concentration μg/ml of extract</th>
<th>Percentage of cell viability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF-7</td>
<td>HCT-116</td>
</tr>
<tr>
<td>75 Aqueous</td>
<td>41.35±0.01</td>
</tr>
<tr>
<td>75 Methanolic</td>
<td>33.12±0.03</td>
</tr>
<tr>
<td>75 Thymoquinone</td>
<td>33.81±0.19</td>
</tr>
<tr>
<td>P-value</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

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 tests. 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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