Phytochemical Screening And Biological Activities Of Tamr Dry Date Variety Cultivated In Upper Egypt

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ABSTRACT

Egypt is considered one of the main producers of date fruits in the world. Tamr dry date is widely cultivated in Upper Egypt, however, investigating the chemical components and biological activities of such common variety has not been performed yet. In this study, the chemical components of the Tamr variety and its biological activities were investigated. Results showed that the percentages of sugar, dietary fibers, metal, protein were 61.0\%, 13.7\%, 2.1\%, and 2.6\%, respectively. Total phenolic and flavonoids were 17.6 and 2.3 mg/100 g, respectively. 17 amino acids, 8 metals, 15 fatty acids, 30 terpenes, vitamins (A, B1, B2, B3), and 5 flavonoids were identified. The presence of phenolics and flavonoids in ethyl acetate extract resulted in high biological activity than chloroform and methanolic extracts. The biological activity of chloroform extract could be ascribed to the presence of polyunsaturated fatty acids and terpenes. These results are in good agreement with the chemical-biological relationships.

INTRODUCTION

The date palm, Phoenix Dactylifera, is a well-known crop in north Africa and the Middle East (Al-Alawi et al., 2017). The flush of the date fruits has high nutritional values. It is considered one of the richest sources of glucose and fructose (Al-Alawi et al., 2017; Al-Farsi and Lee, 2008; Al-Shahib and Marshall, 2003; Awad et al., 2011; El-Mergawi et al., 2019; Vayalil, 2012). Also, metals, dietary fibers, proteins, fatty acids, phenolics, volatile compounds, and vitamins exist in the date fruits (Al-Farsi and Lee, 2008; Baliga et al., 2011; El Arem et al., 2011, 2012; Elleuch et al., 2008; Siddeeg et al., 2019; Vayalil, 2012). Volatile compounds such as alkanes, alkenes, esters, terpenoids, aldehydes, ketones, and esters were determined in different date varieties (Mezroua et al., 2017; El Arem et al., 2011, 2012; Khalil et al., 2017). Total phenolics and flavonoids were in the range of 2.9 ~ 945.6 mg gallic acid equivalents (GAE) and 1.6 ~ 299.7 mg quercetin equivalents (QUE) in 100 g of date flush, respectively (Al-Najada and Mohamed, 2014; Barakat et al., 2020; Benmeddour et al., 2013; Biglari et al., 2008; R. M. A. Mohamed et al., 2014; S. A., 2016; Siddeeg et al., 2019). Besides the high nutritional values of date fruits, they exhibited cytotoxicity, antimicrobial, antifungal, hemolytic, and antioxidant activities (Al-Alawi et al., 2017; Assadi et al., 2019; Baliga et al., 2011; El Abed et al., 2018; Vayalil, 2012).

According to FAOSTAT, Egypt is considered one of the main producers of date fruits (Al-Alawi et al., 2017). The chemical components and biological activities of wet and semidry date varieties cultivated in
Egypt have been investigated (Abdallah et al., 2018; El Sohaimy et al., 2015; Khalil et al., 2017). Tamr dry date variety is commonly cultivated in Upper Egypt, however, no detailed studies about the chemical components and biological activities have been performed. Thus, the objectives of this study are to screen the chemical components of Tamr with illustrating the biological impacts of its different extracts. Total amounts of sugars, protein, metals, dietary fiber, phenolics, and flavonoids were estimated to highlight its nutrition values. Also, different extraction, separation methods and modern instruments were applied for chemical analysis. Antimicrobial, antioxidant, and cytotoxicity activities of the different extracts were investigated. Knowing the chemical constituents and biological activities could highlight the nutrition and economic values of this variety, which would call attention to this sector of national income.

**Experimental**

**Plant Material**

The date sample was collected from Kharga city, New Valley Governorate, Egypt. The harvesting was in 2010, and the variety was authenticated at Department of Pomology, Faculty of Agriculture, Assiut University, Egypt. Dates with similar size, color, appearance and without obvious defects were selected and stored at < 10°C.

**Protein and Amino Acids**

To estimate the quantity of protein, the Kjeldahl method was used (Assirey, 2015; Bouhlali et al., 2017; Elleuch et al., 2008). The identification and quantification of amino acids were performed using LC3000 amino acid analyzer (Eppendorf- Biotronik, Germany) of HCl-digested sample versus authentic amino acids. Citrate buffers at pH values of 2.2, 2.8, 3.3, and 3.7 were used as eluent, and the flow rate was 0.2 ml/min at pressure 0–50 bar and 123°C.

**Sugars and Dietary Fibers**

The sugar content was estimated by the anthrone-sulfuric acid method (Castro-Enríquez et al., 2020). To quantify the dietary fibers, 1 g was ground and placed in a pre-weighed filter bag. Fats were removed, and the residue was digested using concentrated NaOH, and concentrated sulfuric acid, respectively. The residue was washed, dried at 102°C, and weighted.

**Metals**

A 100 g was calcined at 300°C in a muffle furnace, and the metallic residues were dissolved in nitric acid. The concentration of every metal was determined using an atomic absorption spectroscopy instrument (Buck model 210 VGP).

**Fatty Acids and Volatile Compounds**

Fatty acids were extracted from 50 g of fruit sample in CH₃OH: CHCl₃ (1:2) for 2 h. This step was repeated three times, and the filtrate was concentrated using a rotary evaporator at 35°C. Afterthought, KOH (6.0 N) was added to saponify fatty acids. Saponified and non-saponified compounds were fractionated between water and petroleum ether (40–60°C). Saponified fatty acids that existed in the aqueous layer were liberated by neutralization using HCl (6.0 N). Free fatty acids were extracted using petroleum ether (40–60°C) and esterified using ethanol/ sulfuric acid mixture. Using GC/MS analyzer (Agilent 6890 gas chromatograph equipped with an Agilent mass spectrometric detector), the esters were separated and identified. Non-saponified components and volatile compounds were also identified by GC/MS.
**Vitamins (A and B)**

To extract B vitamins, a mixture (50 mL) of glacial acetic acid, acetonitrile, water (1:5:94) was stirred with 10 g of the fruits for 24 h at 70°C. For vitamin A, the fruit flush (5 g) was blended with 40 mL of 95% ethanol and 10 mL of 100% (w/v) potassium hydroxide for 3 min. Then, the mixture was refluxed for 30 min, and vitamins were extracted using n-hexane for three times. Vitamins were identified and quantified using the HPLC versus authentic samples.

**Phenolics and Flavonoids**

Total phenolic and flavonoid contents were quantified by Folin–Ciocalteu method and AlCl₃ method, respectively (Velioglu et al., 1998; Zhishen et al., 1999). The phenolic and flavonoid contents were expressed as GAE and QUE, respectively. Besides, we examined the presence of quercetin; 3-mono-hydroxyflavone; 5-monohydroxyflavone; 6-monohydroxyflavone; and 7-monohydroxyflavone in the ethyl acetate extract using the HPLC apparatus versus the authentic samples.

**Biological Importance**

The biological activities of methanol (70%), CHCl₃, and ethyl acetate were investigated as below.

**Antimicrobial Activities**

The antimicrobial activity of the extracts was determined using the agar diffusion assay (El Sohaimy et al., 2015). Different concentrations of these extracts were prepared in DMSO. The extracts were impregnated and placed on filter paper discs (5 mm). The strains of bacteria and fungi were inoculated using nutrient agar and Sabouraud’s dextrose media. The plates of bacteria and fungi were incubated at 37 °C for 24 h and 25 °C for 6 days, respectively. Inhibition zones were measured in units of mm and recorded. The activity of the extracts was referenced to chloramphenicol.

**Antioxidants Activities**

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) assay (Allaith, 2008; Bouhlali et al., 2017; El Abed et al., 2018; Siddeeg et al., 2019), where 25 μL of the extract (100 μg/mL) was mixed with 975 μL DPPH solution (6 × 10⁻⁵ M). After 30 min, the absorbance was determined at 515 nm.

**Cytotoxicity**

Cytotoxicity of the extracts was examined on two cancer cell lines (Hela and HEK293T cells) by MTT assay (El Abed et al., 2018). After 24 h, 3-(4,5-dimethylthiazol- 2-yl)-2,5- diphenyl tetrazolium bromide was added. A blue precipitate was formed, dissolved in DMSO, and the absorbance was measured at 540 nm.

**Results and Discussion**

**Nutrition Value**

Amounts of sugar, protein, dietary fibers and metals in Tamr were found to be 61.0%, 2.6%, 13.7%, and 2.1%, respectively. Comparing the estimated amount of sugar with the reported amounts (44.0 ~ 88.6%) indicates that the Tamr variety could be considered as a good source of carbohydrates. Percentages of protein and dietary fibers were comparable with the reported values of 1.7 ~ 3.0, and 3.0 ~ 18, respectively (Al-Shahib and Marshall, 2003; Assirey, 2015; R. M. A. Mohamed et al., 2014; Siddeeg et al., 2019). Tamr fruit can be considered as a good source of different metals, as their quantities are comparable with the previously reported amounts in different date varieties (1.3 ~ 3.9%), as will be discussed later (Al-Farsi and Lee, 2008; Al-Shahib and Marshall, 2003;
Assirey, 2015; R. M. A. Mohamed et al., 2014; Siddeeg et al., 2019). Fortunately, Aflatoxins (G1, G2, B2, B1), and 400 pesticides were not detectable in the investigated Tamr fruit.

**Protein and Amino Acids**

Nitrogen content in the HCl-digested sample equaled 0.34 g/100 g as calculated from the Kjeldahl method. No free nitrogen was determined, which means that the source of nitrogen was mainly proteins. Calculated amounts of proteins equaled to 2.6 g/100 g, and the reported amounts in the other varieties are in the range of 1.1–6.5 g/100 g (Assirey, 2015; Baliga et al., 2011; Bouhlali et al., 2017; El Arem et al., 2011; R. M. A. Mohamed et al., 2014). Table 1 illustrates the amounts of amino acids in Tamr compared with the highest reported amounts in different varieties. Tamr contained high amounts of alanine, phenylalanine, arginine, proline, leucine, histidine, valine, and lysine as compared with the highest reported amounts (Al-Farsi and Lee, 2008; Assirey, 2015; Baliga et al., 2011).

**Sugar and Dietary Fibers**

The sugar content in Tamr (61.0%) was represented as glucose and fructose in equal amounts, and disaccharides were not detectable. The high sugar content indicates that Tamr can be considered a rich source of energy. As previously reported, date fruits can provide energy up to 314 kcal/100 g, which means that 100 g of dates can provide 12 ~ 15% of total energy requirements for adults (Al-Farsi and Lee, 2008; Baliga et al., 2011). Consuming 100 g of Tamr can provide the body up to 265 kcal, which means that the intake of 100 g of Tamr can cover 10 ~ 13% of the total energy for adults. Intake date fruit would support the body with high amounts of glucose, which would be rapidly digested resulting in elevating the blood sugar.

On the other hand, the dietary fibers in Tamr variety were ~ 13.7%, which means that intake of 100 g of Tamr can provide more than 40% of the recommended daily intake of total dietary fibers (25 ~ 30 g/day). The content of total dietary fibers in the other date fruits ranges 2.4–18.4% depending on the variety and climate (Al-Farsi and Lee, 2008; Al-Shahib and Marshall, 2003; Elleuch et al., 2008; Maqsood et al., 2020; R. M. A. Mohamed et al., 2014; Siddeeg et al., 2019).

| Amino acids     | Tamr | HRA  | DRIA |
|-----------------|------|------|------|
| Aspartic acid   | 0.240| 0.309| ......|
| Serine          | 0.110| 0.128| ......|
| Glutamic acid   | 0.330| 0.382| ......|
| Glycine         | 0.080| 0.268| ......|
| Alanine         | 0.180| 0.133| ......|
| Tyrosine        | 0.050| 0.156| ......|
| Arginine        | 0.160| 0.148| ......|
| Proline         | 0.240| 0.148| ......|
| Phenylalanine   | 0.140| 0.067| 1.750|
| Valine          | 0.130| ......| 1.330|
| Methionine      | 0.010| 0.062| 1.050|
| Isoleucine      | 0.090| 0.055| 1.050|
| Leucine         | 0.190| 0.242| 2.380|
| Histidine       | 0.100| 0.046| 0.770|
| Lysine          | 0.190| 0.154| 2.170|
| Threonine       | 0.090| 0.095| 1.120|
Metals

Surveying the quantities of elements in Tamr, highly reported amounts, and safe daily intake amounts are illustrated in Table 2 (Al-Farsi and Lee, 2008; Assirey, 2015; Baliga et al., 2011; R. M. A. Mohamed et al., 2014). Potassium, sodium, and magnesium were estimated in high amounts in Tamr. Also, Fe, Zn, and Mn were determined. Hence, Tamr is considered a rich source of minerals especially K. Consumption of 100 g of date fruits provides > 15% of the daily recommended amounts of K. High K and low Na in dates are desirable for people suffering from hypertension (Bouhlali et al., 2017; R. M. A. Mohamed et al., 2014; Vayalil, 2012).

Fatty Acids

Fatty acids were identified as ethyl esters as illustrated in Table S1 (Supporting Information), where the retention time and fragments are listed. According to Table 3 and Table S1, the percentages of saturated, monounsaturated, and polyunsaturated fatty acids were 15.0%, 16.2%, and 68.8% of the total fatty acids, respectively. Linoleic acid was the major fatty acid (68.6%), which is considered the highest reported ratio of polyunsaturated fatty acids compared to the total amounts of fatty acids in any date fruits (El Arem et al., 2011, 2012). Oleic, heptadecanoic, and stearic acids existed in high percentages of 14.7%, 4.5%, and 4.5%, respectively. The percentage of pentadecanoic acid was 2.5%. Decanoic, dodecanoic, tetradecanoic, palmitic acid, palmitoleic acid, and linolenic acids were the minor fatty acids presented in Tamr fruits. Heptadecanoic acid presented in large amounts (4.5%), which is unusually quantified in date fruits (El Arem et al., 2011, 2012).

As illustrated in Table S2 (Supporting Information), Tamr contained 23 monoterpenoids, which were classified into 6 aromatics, 5 alkenes, 6 aliphatic alcohols, 6 aliphatic carbonyl compounds. In

| Metal | Tamr | HRM | Safe intake |
|-------|------|-----|-------------|
| K     | 1566.7 | 1287.0 | 4700 |
| Na    | 166.7  | 261.0 | 2300 |
| Mg    | 14.9   | 150.0 | 450 |
| Mn    | 0.30   | 0.4   | 11 |
| Fe    | 3.60   | 1.5   | 45 |
| Zn    | 0.15   | 0.6   | 40 |
| Cu    | 0.80   | 0.8   | 10 |
| Co    | 0.10   | ...... | 0.1 |

Table 2. Elements detected in Tamr compared with the HRA and safe daily intake amounts.

| Fatty acids         | Tamr (%) | HRA (%) |
|---------------------|----------|---------|
| Decanoic acid       | 0.5      | 0.8     |
| Dodecanoic acid     | 0.7      | 24.1    |
| Tetradecanoic acid  | 0.8      | 14.4    |
| Pentadecanoic acid  | 2.5      | 2.2     |
| Hexadecanoic acid   | 1.5      | 15.0    |
| Hexadec-9-eino acid | 1.5      | ......   |
| Heptadecanoic acid  | 4.5      | ......   |
| Oleic acid          | 14.7     | 55.2    |
| Linoleic acid       | 68.6     | 21.0    |
| Stearic acid        | 4.5      | 27.0    |
| Linolenic acid      | 0.2      | 27.0    |

Table 3. The percentage of fatty acids in Tamr compared with the HRA.
Tunisian date fruits, volatile compounds were classified into terpenoids, hydrocarbons, esters, aldehydes, and ketones. El Arem et al. identified different terpenoids in different maturation stages (El Arem et al., 2011, 2012). Khalil et al. screened 89 volatile compounds that existed in 11 Egyptian date varieties, and Tamr variety was not among the examined varieties (Khalil et al., 2017).

**Vitamins**

Vitamins A and B1 are present in small amounts in Tamr as shown in Table S3 (Supporting Information). Vitamins B2 and B3 in Tamr had the highest reported amounts in all date varieties (Al-Shahib and Marshall, 2003; Baliga et al., 2011; Biglari et al., 2008; Bouhlali et al., 2017). Comparing these amounts with the estimated amounts in Tamr fruits illustrated that the daily intake of date fruits supports the body with vitamins without exceeding the allowed amounts as illustrated in Table S3 (Al-Shahib and Marshall, 2003).

**Phenolics and Flavonoids**

Total phenolic and flavonoid contents in 100 g of Tamr fruit were 17.6 mg GAE and 2.3 mg QUE, respectively. In 100 g of different date varieties, the reported total phenolics and flavonoids amounts are 2.9–945.6 mg GAE and 1.6–299.7 mg QUE, respectively (Benmeddour et al., 2013; Biglari et al., 2008; El Sohaimy et al., 2015; R. M. A. Mohamed et al., 2014; Siddeeg et al., 2019). Amounts of 3-monohydroxyflavone, 6-monohydroxyflavone, 7-monohydroxyflavone, and quercetin in 100 g Tamr were 0.05 mg, 0.11 mg, 0.19 mg, and 0.096 mg, respectively. The amount of 5-monohydroxyflavone was < 0.01 mg in Tamr.

**Biological Importance**

**Antimicrobial Activities**

The inhibition zones on the examined bacterial strains using ethyl acetate extract were larger than that of CHCl₃ extract as illustrated in Table 4. Ethyl acetate extract had the lowest minimal inhibition concentration (MIC) compared with methanol (70%) and CHCl₃. The MIC of ethyl acetate extract that influenced on staphylococcus aureus (+ve) and bacillus cereus (+ve) with inhibition zones of 8 mm and 10 mm was 6.25 µg/mL, respectively. The effective MIC of ethyl acetate extract on micrococcus luteus (+ve), escherichia coli (+ve), pseudomonas aeruginosa (-ve) was 100 µg/mL, and the inhibition zones were 22, 9, and 20 mm, respectively. The high influence of ethyl acetate extract was attributed to the presence of phenolics and flavonoids, which might bind with the cell wall of bacteria resulting in inhibiting bacterial growth (El Sohaimy et al., 2015). Such compounds could precipitate the proteins and inhibit enzymes of these microorganisms (El Sohaimy et al., 2015). CHCl₃ extract did not influence micrococcus luteus (+ve). The MIC of CHCl₃ extract that inhabited an 8 mm zone of Staphylococcus aureus (+ve), bacillus cereus (+ve), and escherichia coli (+ve) was 25 µg/mL. The MIC of CHCl₃ extract that inhabited 10 mm of Serratia marcescens (-ve) and pseudomonas

| Table 4. Antibacterial activity of extracts by illustrating the inhibition zone in mm and MIC in µg/mL |
|----------------|-----------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| Bacterial strains | Inhibition zone in mm (MIC in µg/mL) | CH₂OH | CHCl₃ | AcOEt | Chloramphenicol |
|----------------|-----------------------------|---------------------------|-----------------------------|-----------------------------|-----------------------------|
| Staphylococcus aureus (+ve) | 0 | 8 (25.0) | 8 (6.3) | 12 (50.0) |
| Micrococcus luteus (+ve) | 0 | 0 | 22 (100.0) | 13 (25.0) |
| Bacillus cereus (+ve) | 0 | 8 (25.0) | 10 (6.3) | 13 (50.0) |
| Escherichia coli (+ve) | 0 | 8 (25.0) | 9 (100.0) | 12 (25.0) |
| Serratia marcescens (-ve) | 0 | 10 (50.0) | 11 (25.0) | 10 (0.8) |
| Pseudomonas aeruginosa (-ve) | 0 | 10 (25.0) | 20 (100.0) | 12 (6.3) |
**Table 5.** Antifungal activity of extracts by illustrating the inhibition zone in mm and MIC in μg/mL.

| Extracts          | Inhibition zone in mm (MIC in μg/mL) |
|-------------------|--------------------------------------|
| Fungal strains    | Ethyl acetate                        |
|                   | Chloramphenicol                      |
| Candida albicans  | 8 (50.0)                             | 12 (1.6)                   |
| Geotrichum candidum | 14 (100.0)                           | 12 (0.4)                   |
| Trichophyton rubrum | 22 (100.0)                           | 10 (0.1)                   |
| Fasarium oxysporum | 14 (100.0)                           | 15 (0.1)                   |
| Aspergillus flavus | 13 (100.0)                           | 24 (1.6)                   |
| Scopulariopsis brevicaulis | 8 (50.0) | 18 (0.1)                   |

aeruginosa (-ve) were 50 and 25 μg/mL, respectively. The presence of polyunsaturated fatty acids especially linoleic acid and terpenes in CHCl₃ extract might be the reason for the antibacterial activity (Bentrad et al., 2017; Gallucci et al., 2009). The inhibition zones of 100 μg/mL of ethyl acetate extract on geotrichum candidum, trichophyton rubrum, fasarium oxysporum, and aspergillus flavus were 14, 22, 14, and 13, respectively. The MIC of ethyl acetate extract on candida albicans and scopulariopsis brevicaulis was 8 μg/mL, and the inhibition zone was 8 mm. The presence of phenolics and flavonoids could be also for the fungal activity. The CH₃OH and CHCl₃ extracts did not affect the examined fungal strains as illustrated in Table 5. In methanol extract, the presence of sugar and proteins could inhibit the antimicrobial activities.

**Antioxidant Activities**

As illustrated in Figure 1A, the inhibition percentages of methanol, CHCl₃, ethyl acetate, vitamin C and rutine were < 3.0%, 23.5%, 41.6%, 75.6% and 84.5%, respectively. The presence of phenolics and flavonoids in ethyl acetate extract was the reason for the inhibition percentage. Phenolics and flavonoids are working as radical scavengers, and they can quenched DPPH radicals (Shahdadi et al., 2015). The antioxidant efficiency of chloroform extract could be ascribed to the presence of polyunsaturated fatty acids and volatile compounds, especially aromatic terpenes (Wang et al., 2019).

**Cytotoxicity**

The concentration of extracts was 0.0025 mg dissolved in 1 mL of DMSO. According to Figure 1B, adding this concentration of ethyl acetate extract to HeLa and to HEK293T cells decreased the cancer
cells to 12.3% and 16.7%, respectively. The percentages of cell viability after treating HeLa and HEK293T by CHCl₃ extract were 26.3% and 33.3%, respectively. The cytotoxicity of ethyl acetate extract might be attributed to the presence of phenolics and flavonoids (Al-Alawi et al., 2017; El Abed et al., 2018), while the presence of polyunsaturated fatty acids and volatile oils caused the cytotoxicity of CHCl₃ extract (Bratton et al., 2019). The presence of sugars in methanolic extracts would inhibit cancer cell death (Takatani-Nakase et al., 2014).

Conclusions
According to this study, Tamr fruits contained 61.0% sugar, 13.7% dietary fibers, 2.6% protein, 2.1 metals, 17.6 mg/100 g phenolics, and 2.3 mg/100 g flavonoids. Also, 17 amino acids were identified. K, Na, and Mg existed in adequate amounts, while the amounts of Mn, Fe, Zn, Cu, and Co were small. 15 fatty acids, 30 terpenes were identified. The biological activities of extracts can be ordered as ethyl acetate > CHCl₃ > methanol extracts. The presence of phenolics and flavonoids in the ethyl acetate extract and polyunsaturated fatty acids and terpenes in the CHCl₃ extract could explain the chemical-biological relationship of these extracts. This report summarizes the nutrition values and therapeutic applications of one of the most abundant date varieties in upper Egypt.

Disclosure statement
The authors declare that there is no conflict of interest.

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