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Phenolic Content, Antioxidant and Antimicrobial activities of Egyptian Date Palm *(Phoenix dactylifera L.)* Fruits.

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ABSTRACT

Date palm fruits are one of the most popular fruits packed with an impressive list of essential nutrients, vitamins, and minerals that are required for normal growth, development and overall well-being. They contain health benefiting polyphenolic antioxidants. This work is aimed to determine the phenolic profile and phenolic content of date palm fruits (Tamr stage) and evaluate their functional properties like antioxidant and antimicrobial activities to confirm the date palm health benefits. Water extract of Tamr stage showed a higher content (14.80 mg GAE/g sample) of phenolic compounds than ethanol extract (10.31mg GAE/g sample). HPLC analysis showed the extracts contain the high concentration in esculetin (15.11 and 17.30mg/100g) and tannic acid (2.85 and 1.79 mg/100g). On the other hand, protocatechuic acid, catechol, Pyrogallol and cinnamic acid didn't detect in both extracts. Moderate concentrations of gallic acid (7.51 and 5.28 mg/100g), itaconic acid (6.40 and 5.91 mg/100g) and traces of ferulic acid (0.15 and 0.22 mg/100g) were detected. DPPH assay revealed a good antioxidant capacity of water extract, which was higher than of ethanol extract. Antimicrobial data exhibited an impressive antibacterial activity for date extract. Date extract showed a strong antibacterial activity (for water and ethanol extracts) against *E. coli (*20 ±0.57 *and* 16 ±0.57 *mm), Salmonella enterica (*20 ±0.54 *and* 14 ±0.52 *mm) and Bacillus sabtilis (*18±0.32, 15±0.23 mm) *and* moderate inhibition against *staphylococcus areues (*8 ±0.48 *and* 5 ±0.52 *mm)* and *Enterococcus faecalis (*5 ±0.36 *and* 2 ±0.57*mm).* These findings may enhance our knowledge for the value and importance of using the dates in our daily diet; and can be used as natural antioxidants and antimicrobial agents for various food products

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INTRODUCTION

The date palm (*Phoenix dactylifera L.*), a tropical and subtropical tree, belonging to the family Palmae (Arecaceae) is one of mankind's oldest cultivated plants. It has played an important role in the day-to-day life of the people in the Arabian Peninsula for the last 7000 years (Ahmed *et al*., 1995). Also, date palm is one of the most important crops in Egypt. At present, Egypt is recorded as one of the top ten in date palm producing countries. The fruit is considered a vital component of the Bedouin daily diet in Egypt and other Arabic Sahara. The potential health benefits of date fruits have been attributed to their polyphenols content, in particular flavonoids that have received much attention in the literature for its biological effects (Hertog *et al*., 1993; Hertog *et al*., 1995). Nowadays, the consumption of fruits and vegetables is regarded as important and good for health (Mansouri *et al.,* 2005). Date palm fruits are a good source of vitamins minerals simple carbohydrate and dietary fibers (El Sohaimy and Hafez, 2010). Many researchers reported the nutritional and biochemical aspects of date fruits (El Sohaimy & Hafez, 2010; Saleh *et al*., 2011; Al-Farsi 2005; Sourial *et al*., 1986). Pulps of dates hold easily digestible sugars (70%), mostly glucose, sucrose and fructose, dietary fibers and enclose less proteins and fats (Al Farsi & Lee, 2008). They also contain vitamins like riboflavin, biotin, thiamine, ascorbic acid and folic acid that are essential in the human body. Also, it is rich in calcium, iron, copper, cobalt,

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magnesium, fluorine, manganese, phosphorus, potassium, copper, sodium, boron, sulfur, zinc and selenium (El Sohaimy & Hafez, 2010; Alfarsi & Lee, 2008; Mohammed and Khamis, 2004; Elias, 2008). In addition, date palm fruit is a good source of energy and a mixture of antioxidants including ascorbic acid, carotinoids, flavonoids and polyphenols. It is essential to mention that various factors such as cultivars, seasons, and pre-and post-harvest conditions may affect on the chemical composition of plan food (Fakqir *et al*., 2012). The date fruit pulp is rich in phytochemicals like phenolics, sterols, carotenoids, anthocyanins, procyanins and flavonoids (Manjeshwar *et al*., 2011). The ratio and concentrations of these constituents depend on the type of the fruit, stage of the fruit picking, location and soil conditions, these phytochemicals also contribute to the nutritional and organoleptic properties of the fruits (Abdelhak *et al*., 2005; Abdul & Allaith, 2008). There was also found a good correlation between the total phenolic content and antioxidant activities of the nonvolatile extracts (Faqir *et al*., 2012). Beside the nutritional value, date fruits are rich in phenolic compounds possessing antioxidant activity (Saleh *et al*., 2011). They are a good source of antioxidants, mainly carotenoids and phenolics (Alfarsi & Lee 2008) that possess antioxidant and antimutagenic properties *in vitro* (Vayalil, 2002; Mansouri *et al*., 2005). In this concern, the authors mentioned that date fruits can be considered a rich source of hydrophilic antioxidants and this reducing property is generally associated with the presence of polyphenols specifically flavones. The PHLC-DAD-MS analysis showed that the major phenolic compounds of date fruits are cinnamic acid, ferulic, sinapic and coumaric acids and their derivatives (Mansouri *et al*., 2005). Ayachi *et al.*, (2009) reported that, the date fruits have low antimicrobial activity against *E. coli* ATCC 25922 and *Salmonella typhimurium*. Also, Madiha (2012) reported moderate antibacterial activities for date palm extract compared to control of antibiotic against some pathogenic bacterial strains. However, Simin & Jochen, (2010) found antimicrobial and antifungal activity of date syrup under various conditions and they referred the antimicrobial activity to the presence of specific compounds naturally present in date fruits. The aim of the present study is to analyze the phenolic profile of date palm fruits (Tamr-dry date) and to evaluate their functional properties like antioxidant and antimicrobial activities to confirm their nutritional benefits for human uses.

MATERIALS AND METHODS

Date palm *(Phoenix dactylifera L.)* fruits (Tamr stage) were collected from Alexandria markets, Alexandria Governorate, Egypt. Standards of phenolic compounds for HPLC analysis (purity> 99.0%) including gallic acid, Itaconic acid, protocathechuic acid, catechin, esculetin, catechol, tannic acid, ferulic acid, pyrogallol and cinnmaic acid were purchased from Sigma-Aldrich. The used solvents of methanol, acetic acid, acetonitrile, DPPH (2,2-diphenyl-1-picryhydrazyl), gallic acid and Folin-Ciocalteu were obtained also from Sigma Company.

Preparation of Date Fruit Extract:

The dry date palm fruits (Tamr stage, 10g) were dried, ground and soaked in water (1:20 w/v) then stirred for 60 min at room temperature (25°C). The mixture was centrifuged at 4000 xg for 20 min. to discard the precipitate and the supernatant was lyophilized and stored at -20°C for further analysis. The same procedure was repeated to prepare ethanol extract using 95% ethanol instead of water.

Total Phenolic Content:

Total phenolic content of the date palm fruits was determined in extracts using Folin-Ciocalteu assay (El Sohaimy, 2013). A 0.5 ml extract was transferred to test tube containing 2.5 ml of Folin-Ciocalteu reagent followed by addition of 2 ml sodium carbonate (Na_2CO_3) (75 g/l). The contents were incubated for 5 min at 50°C then the absorbance was measured at 760 nm against different concentrations of Gallic acid as standard. The phenolic content was expressed as mg gallic acid equivalents per gram of extract (mg GAE/g dry weight sample).

HPLC Analysis:

Phenolic compounds in the date palm extracts were analyzed by high performance liquid chromatography (HPLC) (Agilent-1100 Series) as described by (Öztürk *et a*l., 2007). Reversed phase Zorbax Eclipse XDB-C18 column (4.6 x 150 mm, 5 µm) was used using a gradient program with two solvent systems (A: 0.5 % acetic acid in 50:50 acetonitrile: water (1:1); B: 2 % acetic acid in water at a constant solvent flow rate of 1.2 ml/min and injection volume was 20 µl. The signals were detected at 280 nm by UV-VIS detector. Ten phenolic compounds were analyzed as standard (gallic acid, Itaconic acid, protocathechuic acid, catechin, esculetin, catechol, tannic acid, ferulic acid, pyrogallol and cinnmaic acid).

Antioxidant Activity:

The free radical scavenging activity in sample extracts was measured according to the method of El Sohaimy, (2013) and Brand-Williams *et al*., (1995). A serial concentrations of lyophilized extract (6.5-100mg)

were dissolved in 1 ml methanol and then 1 ml of α,α-diphenyl-β-picrylhydrazyl (DPPH) solution was added and incubated for 15 min at room temperature (25°C). The absorbance was then measured at 515 nm using SHIMADZU UVmini-1240 UV-VIS Spectrophotometer. The antioxidant activity was expressed as percentage of reduction of initial DPPH absorption by test sample as follows:

DPPH scavenging effect %= [(A_0 -A_t)/ A0] X 100

A_0 is the absorbance of control at zero time, A_t is the absorbance of the antioxidant at 15 min. The IC_{50} is defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration by 50%. The IC_{50} of the sample was derived from the % Scavenging activity against concentration plot and is expressed as mg/ml.

Antimicrobial Activity:

The antibacterial activity of the date palm fruit extracts was tested against five pathogenic bacterial strains (*Escherichia coli 0-143, Salmonella enterica ATCC 13076, Bacillus subtilis ATC6633, Staphylococcus aureus ATCC 25923, Enterococcus faecalis ATCC 19433*), which obtained from Cairo-MIRCEN (Microbiological Resource Center) Faculty of Agriculture, Ain Shams University. The dried extract was dissolved in DMSO (75%) for antimicrobial activity experiment. The minimal inhibitory concentration (MIC) was achieved by an adaptation of the agar streak dilution method based on radial diffusion (Ferreira *et al*., 2004). The MIC was considered to be the lowest concentration of the tested sample able to inhibit the growth of bacteria after 24 hr (Barbary et al., 2010). DEMSO solution was used and a negative control; Ampicillin and Chloramphenicol were used as a positive control.

Statistical Analysis:

Each value is the mean of triplicates and statistical analysis was carried out using Tukey HSD Test for Post-ANOVA Pair-Wise Comparisons in a One-Way ANOVA

RESULTS AND DISCUSSION

Total Phenolic Content:

The total phenolic content of date extract (Tamer stage) was carried out and the obtained results evident that the tamer stage (dry date) contains a considerable level of phenolic content (table 1). Water extract had 14.80 mg GAE/g sample but methanol extract had 10.31 mg GAE/g sample. From the above-mentioned results; it is clear that water extract has higher phenolic content than ethanol one ($p<0.05$). This declared that, the date extract (Tame stage) contains higher aliphatic (hydrophilic) phenolic compounds than aromatic (hydrophobic) compounds. These results agreed with that of Myhara *et al*., (2000) and Zagoskina *et a*l., (2013) whom reported that the phenolic contents in water extract were higher than that in ethanol extract for dates and lichens respectively. The same trend was appeared in the study of Saleh *et al*., (2011). This refer to the date palm may contain water-soluble phenolic compounds

Table 1: Total phenolic content of date palm extract (Tamer stage),

Sample	Total phenolic content (mg GAE/ g sample)
Water extract	14.80±0.61
Ethanol Extract	10.31±0.29

The values is mean of triplicates ±SD, ($p<0.05$)

HPLC analysis:

Data obtained from HPLC chromatogram of water and ethanol date palm extracts are given in (Table 2). The results in the table showed the detected phenolic compounds and their concentrations. Esculetin gave the highest concentration in both water and ethanol extracts (15.11 and 17.30 μg/g). Also Gallic acid (7.51 and 5.28 μg/g), itaconic acid (6.40 and 5.91μg/g), tannic acid (2.85 and 1.79 μg/g) and ferulic acid (0.15 and 0.22 μg/g) were detected in both extracts respectively. However, Protochatechuic acid, Catechol, Pyrogallol and Cinnamic acid were not detected in neither water nor ethanol extract. In the same time the Cathechin was detected in water extract and showed low content in ethanol extract (1.10 μg/g). These results revealed that the concentrations of phenolic compounds in water extract were higher than that in ethanol extract. This means that the dry date (Tamr stage) contains hydrophilic phenolic compounds greater than hydrophobic compounds. Similar findings were obtained by Saleh *et al*., (2001), who analyzed the phenolic compounds in date palm fruits and confirmed that date fruit can be considered a rich source of hydrophilic antioxidant.

Table 2: HPLC -Phenolic compounds in date palm fruits extracts

Phenolic standards (μg/g)	Water extract (μg/g)	Ethanol extract (μg/g)	Molecular structure
Gallic acid	7.51± 0.123	5.28±0. 167	3,4,5-Trihydroxybenzoic acid $C_7H_8O_5$
Itaconic acid	6.40±0.113	5.91±0.137	2-Methylidenebutanedioic acid $C_5H_6O_4$
Protocatechuic acid	-----	-----	3,4-Dihydroxybenzoic acid $(HO)_2C_6H_3CO_2H$
Cathechin	-----	1.10±0.213	2*R*,3*S*)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2*H*-chromene-3,5,7-triol $C_{15}H_{14}O_6$
Esculetin	15.11±0.213	17.30±0.165	6,7-Dihydroxycoumarin; Cichorigenin $C_9H_6O_4$
Catechol	-----	-----	1,2-dihydroxybenzene $C_6H_4(OH)_2$
Tannic acid	2.85±0.097	1.79±0.110	2,3-dihydroxy-5-({[(2R,3R,4S,5R,6R)-3,4,5,6-tetrakis({3,4-dihydroxy-5-[(3,4,5 trihydroxyphenyl)carbonyloxy]phenyl}carbonyloxy) oxan-2-yl]methoxy}carbonyl)phenyl 3,4,5-trihydroxybenzoate $C_{76}H_{52}O_{46}$
Ferulic acid	0.15±0.194	0.22±0.215	(E)-3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid $C_{10}H_{10}O_4$
Pyrogallol	-----	-----	Benzene-1,2,3-triol $C_6H_6O_3$
Cinnamic acid	-----	-----	(E)3-phenylprop-2-enoic acid $C_9H_8O_2$

Antioxidant Activity:

The antioxidant capacity of date palm fruits was determined by DPPH and ascorbic acid was used as standard and the obtained results are given in table (3). Data from table revealed that both water and ethanol extract have high antioxidant capacity and free radical scavenging activity and the effect was concentration dependent which has the same trend with L-ascorbic acid. As mentioned the extracts exhibited concentration dependence across the range tested but ascorbic acid was slightly more efficient than date palm extract. The percentage % 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 100 mg respectively. The percentage inhibition of ethanol extract was 10.00, 13.64, 22.70, 49.29 and 66.51 for concentrations 6.5, 12.5, 25, 50 and 100 mg respectively (Table 3). It is observed from results in the table that the required concentration for 50% inhibition was significant ($P < 0.05$) and exhibited strong free radical scavenging activity against the stable free radical DPPH. Water extract of date palm fruits showed higher antioxidant capacity than ethanol extract at the same concentrations ($P < 0.05$). These findings revealed that the date palm fruits have a significant antioxidant potential in both water and ethanol extract but the antioxidant capacity of water extract higher than of ethanol extract. The present findings revealed a positive relation between the antioxidant activity and phenolic compounds. The findings agreed with Saleh *et al*. (2011) who reported that date fruits can be considered a rich of hydrophilic antioxidants and this reducing property is generally associated with the presence of polyphenols. Also, Faqir *et al*., (2012), and Foroogh *et al.*,

(2008) showed that there was a linear relationship between antioxidant activity and total phenolic content in dates. The present study showed that; Egyptian date fruits contained a high level of phenolic compounds and antioxidants potential, which encourage its uses as a functional food.

Table 3: Antioxidant capacity (% inhibition) of date palm extract.

Concentration (mg)	Water Extract	Ethanol Extract	Ascorbic acid
100	79.32 ±0.32	66.51 ±0.26	85.32 ±0.27
50	68.14 ±0.30	49.29 ±0.26	65.56 ±0.31
25	33.81 ±0.26	22.70 ±0.28	44.76 ±0.31
12.5	23.22 ±0.31	13.64 ±0.28	32.86 ±0.32
6.5	19.52 ±0.32	10.00 ±0.18	27.64 ±0.31

The values expressed as mean of triplicates± SD ($p<0.05$).

Antimicrobial Activity:

The antimicrobial activity of date palm extract was carried out against five pathogenic bacterial strains (*Escherichia coli 0-143*, *Salmonella enterica ATCC 13076*, *Bacillus subtilis ATC6633*, *Staphylococcus aureus ATCC-25923*, *Enterococcus faecalis ATCC 19433*) and both Ampicillin and Chloramphenicol were used as antibiotic control. The antibacterial study revealed that the minimum inhibitor concentration (MIC) of date palm extract was 50 mg/ml for water and ethanol extract as well (Table 4). Data in the table showed that the date palm fruits extract has a strong antibacterial activity (for water and ethanol extracts) against *E. coli (*20 ±0.57 *and* 16 ±0.57 *mm), Salmonella enterica (*20 ±0.54 *and* 14 ±0.52 *mm) and Bacillus sabtilis (*18±0.32, 15±0.23 mm) *and* moderate inhibition against *staphylococcus areues (*8 ±0.48 *and* 5 ±0.52 *mm)* and *Enterococcus faecalis (*5 ±0.36 *and* 2 ±0.57*mm).* On the other hand, ampicillin and chloramphenicol as positive control, showed toxicity against all five examined pathogenic strains. Water extract showed higher toxicity than ethanol extract and chloramphenicol against *E. coli*, *Salmonella enterica and Bacillus sabtilis* ($p<0.05$). The activity of date palm extracts as antibacterial may be due to the ability of phenolic compounds to bind with the bacterial cell wall and therefore inhibiting the bacterial growth (Barbary *et al.*, 2010). Polyphenols played an important role in protein precipitation and enzyme inhibition of microorganisms (Naz *et al*., 2007). The results showed a good correlation between the concentrations of phenolic compounds and antimicrobial activity. The obtained results demonstrated the potentiality of date palm fruits as antibacterial source for application as functional food.

Table 4: Antibacterial activity of date palm extract

Strains		IZD (mm)		IZD (mm)		IZD (mm)		IZD (mm)		IZD (mm)
E. coli 0-143	Water Extract	20 ±0.57	Ethanol Extract	16 ±0.57	Ampicillin	29 ±0.33	Chloramphenicol	19 ±0.33	*DEMSO*	-
Sal. enterica ATCC 13076		20 ±0.54		14 ±0.52		25 ±0.36		15 ±0.52		-
B. subtilis ATC6633		18±0.32		15±0.23		23±0.41		17±0.35		-
Staph. aureus ATCC 25923		8 ±0.48		5 ±0.52		19±0.25		18±0.12		-
Enter.faecalis ATCC 19433		5 ±0.36		2 ±0.57		20±0.23		16±0.22		-

The values expressed as mean of triplicates ± ($p<0.05$), IZD= Inhibition Zone Diameter, (-)= No Inhibition zone detected,

Conclusion:

The results of this study concluded that the date palm fruits consider a good source of natural polyphenolic compounds and have high antioxidant and antibacterial potentials. These fruits can be used as natural antioxidants and antimicrobial agents for various food and food products. These findings may enhance our knowledge for the value of using the date palm fruits in our daily diet as a functional food. We can recommend it as valuable and healthy food and providing a wide range of essential nutrients and potential health benefits. The fruits can be taken directly or after immersing in water for human use as healthy juice.

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