Research Article

Variation in bioactive composition, antioxidant attributes and fatty acids profile of *Phoenix dactylifera* L. fruits in relation to different extraction solvents

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Abstract

The present study was planned to determine the antioxidant and fatty acids profile of different varieties of *Phoenix dactylifera* L. The sample of Ajwa date, Ajwa paste, Moti jhari and Basrah were taken to evaluate the antioxidant potential and fatty acids of their pulp and seeds, because unsaturated fatty acids are useful for heart diseases. The extract yields from these samples ranged from 32.68 to 55.0 g/100g dry weight (DW). Maximum yield was obtained by 80% ethanol. All extracts contained appreciable amount of total flavonoid and total phenolic contents (20.6-43.5 CE mg/g DW), (110.6-490.9 GAE mg/100g DW), respectively. All date varieties exhibited good reducing power and DPPH radical scavenging activity with contribution of EC₅₀ 2.56 to 6.2 mg/mL and IC₅₀ 2.5-7.6 mg/mL of extract, respectively. Maximum amount of total flavonoids and total phenolics was obtained by 50% methanol and 100% methanol extract, respectively, whereas DPPH radical scavenging activity and reducing potential was higher for 50% ethanol and 100% ethanol extract. Extracts of ajwa date pulp and seed exhibited maximum value of total phenolic contents and extracts of ajwa paste exhibited maximum value of total flavonoid contents. Ajwa date exhibited greater DPPH scavenging activity and reducing potential. All samples contained a wide range of phenolic acids among which Gallic acid is the most diversified phenolic acid. Ajwa date contained maximum value of total phenolic acids (1005.03mg/kg). All samples contain appreciable amount of saturated and unsaturated fatty acids among which percentage of oleic acid (21%) was higher in Ajwa dates. The present results indicated that pulp and seeds of *Phoenix dactylifera* L. have good antioxidant potential, considerable contents of phenolics and appreciable amount of fatty acids, so can be used for prevention of heart diseases, in food industry, oil industry and pharmaceuticals as a natural source of antioxidants and preservatives.

Keywords: Dates; Antioxidant potential; Reducing powers; Fatty acids; Phenolics; GC and HPLC

Introduction

Role of nutritional factors on health status has long been identified. But recent clinical and epidemiological studies demonstrated

the clear insight on physiochemical and chemical mechanisms of effects of bioactive compounds present in food on human health [1]. Bioactive compounds are the

phytochemicals which are produced naturally, being nonnutritive are synthesized by the plants as safeguards for attack by pathogenic microorganisms and external stress [2]. Naturally occurring bioactive compounds such as, fatty acids, terpenoids, phenolics, coumarins, flavonoids, xanthonenes, curcuminoids and tannins are found in plant products such as peels, leaves, oils, seeds and fruits, several of these are proved as efficient as synthetic antioxidant in model systems [3, 4]. Bioactive compounds are reported to have diverse biological effects for instance antimutagenic, antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory activities [5]. Current studies have declared that date fruit has been shown to have tremendous antioxidant activity among the twenty-eight fruits commonly consumed in China [6]. Phenolic acid content and antioxidant activity of dates demonstrated a linear relationship in some recent studies [7]. *Phoenix dactylifera* commonly called dates are grown in over 30 countries. Nutrient-rich dates are enjoyed for their sweet, exotic and succulent flavor. In recent years, dates have found acceptance among consumers in European Countries and North America. Date fruit is also processed into a broad variety of value-added products beside fresh consumption [8, 9]. They serve as good source of natural antioxidants, so, considered as functional food [10, 11].

Pakistan being one of the top ten *dates* producing countries of the world and Pakistan has become the fourth largest country in the world that is exporting dates to the rest of the world. However, very little is known about fatty acids, phenolics composition, antioxidant activity and bioactive compounds of different varieties of locally available dates. So, it would be worthwhile to evaluate the fatty acid profile and antioxidant attributes of date fruit by different solvents such as chloroform,

methanol and ethyl acetate. The nature of extracting solvent have a central role in the resulting extracts yields for their antioxidant activities and other important bioactive compounds of date's fruit leading to exploring their potential prospects in pharmaceuticals and food industries as natural preservatives and antioxidants. With this in mind, TPC, TFC, fatty acids and antioxidant properties along with individual phenolic acids composition of the dates from three important and widely cultivated dates were studied in the current work

Materials and methods

Sample preparation and extraction

All samples (Ajwa date, Moti, jhari and Basrah) were cut manually into small pieces and the seeds were separated and were air dried and ground to fine powder. The ground material was stored in polythene bags. The Ajwa paste purchased from market to check the difference between commercial and Ajwa dates available in market. The ground material that passed through 80-mesh sieve was used for the extraction of antioxidant components and other bioactives compounds. All other chemicals (analytical grade) used in different experiments were purchased from Sigma-Aldrich Chemical Corporation, Germany. Extraction of all samples for antioxidant and other bioactive compounds was done by orbital shaker, using different solvents such as 100 % methanol, 100% ethanol, 80% methanol, 80% ethanol, 50% methanol and 50% ethanol.

Estimation of antioxidant activity

TPC determination

Folin-Ciocalteu assay was used for determination of total phenolic contents (TPC) as described by Kalpna *et al.*, [12]. Briefly, 0.5 mL (2mg/1mL) of extract solutions were taken in test tube and 0.1 mL of Folin-Ciocalteu (0.5 N) reagent was added in each solution. Then they were incubated at room temperature for 15 min.

Following that 2.5 mL of saturated solution (7% W/V) of sodium carbonate was added. The mixtures were again incubated for 30 minutes at room temperature. The absorbance was done at 700 nm using spectrometer (CECIL CE 7200). Amount of TP was calculated using Gallic acid as standard.

TFC determination

Total flavonoid contents (TFC) were calculated by following the experiment reported by Zhishen *et al.*, [13] 1 mL of aqueous solution of extracts (10mg/mL) was placed in 10 mL volumetric flasks. Distilled water was added to make the total volume of every flask up to 5 mL. Then 0.3 mL NaNO₂ (1:20) was added in each mixture. For 5 min mixtures were kept at room temperature. Following the addition of 0.3 mL AlCl₃, the mixture was kept again at room temperature for 6 minutes, followed by 2 mL 1 mol litre⁻¹ NaOH. Total volume was made up to 10 mL by adding distilled water. The solutions were mixed again and absorbance was taken at 510 nm against blank. The results were expressed as catechin equivalent (10-500 ppm) ($R^2 = 0.9919$) CE mg/g of dry weight.

DPPH scavenging activity assay

The antioxidant activity of *Phoenix dactylifera* L. varieties was assessed by 1, 1 diphenyl-2-picrylhydrazyl radical (DPPH[•]) scavenging assay. The DPPH assay was performed by using the method followed by Zhuang *et al.*, [14] According to the assay, 1 mL of extracts solutions were taken with 0.25 mg-2mg/mL concentration. 2 mL of 0.1 mM DPPH solution in methanol was added to each mixture. Methanol was added to make the solutions up to 4 mL. The extracts were kept in dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. BHA was used as a positive control. IC₅₀, the concentration of extract which scavenged 50 % of DPPH free radicals, was calculated.

% scavenging activity = $(A_{\text{control}} - A_{\text{extract}}) / A_{\text{control}} \times 100$
The % scavenging activity at 50 % is IC₅₀

Determination of reducing power

The reducing power of *Phoenix dactylifera* L. extracts were determined by using method following by Zhuang *et al.*, [14] with minute alteration. According to which different extracts concentrations (2-10) mg were formed by mixing the extracts with 3.5 mL phosphate buffer (0.2 M, pH 6.6), then 2 mL of 1 % potassium ferricyanide in all test tubes was added. The mixtures were incubated for 20 minutes at room temperature. After that 2.5 mL of 10 % trichloroacetic acid was added to the mixtures. For 10 minutes mixtures were centrifuged at 3000 rpm. 2.5 mL of supernatant fluid was collected and its volume made up to 5mL by adding 2.5 mL of distilled water. After that 0.5 mL of 0.1 % ferric chloride was added. The absorbance of mixtures was taken at 700 nm.

Calculation of phenolic acids profile by HPLC

Individual phenolic acids were determined by HPLC. Separation of phenolic acids was done by Varian HPLC. Acidified acetonitrile (99.5%) was used as mobile phase. By using a microsyringe, the column was injected with 20-μL sample. It was detected at 280 nm. By matching the relative retention times with standards of targeted compounds, phenolic compounds were identified. Peak area measurement gives the contents of individual compounds.

Extraction of oil for fatty acids profile

In this method the sample was dried, grind into fine particles and placed in porous cellulose thimble which was placed in an extraction chamber. About 500ml of n-hexane was placed in round bottom flask. The flask was heated on heating mantle, solvent evaporates and drop into the extraction chamber containing the sample. The process was repeated thrice. After that the solvent was evaporated and mass of the remaining oil was measured.

Formation of methyl esters for fatty acids profile determination of *Phoenix dactylifera* L.

One drop of oil was taken in round bottom flask having 100ml capacity. Then one pellet of KOH was added in round bottom flask having one drop of oil. Then 30ml of pure methanol was added. After that a condenser was attached to this flask with proper circulation of water. Then the round bottom flask with attached condenser was placed in heating mantle for about 30 min only to condense methanol. After 30 min heating was stopped and the round bottom flask was cooled in water immediately. Whole mixture in round bottom flask was transferred into separating funnel (250-500ml), then only 10 ml of n-hexane were added in this separating funnel, shaken it gently and allowed to stand for the formation of 2 layers. Lower layer was discarded. Then small amount of distilled water was added in separating funnel, shaken it slowly and allowed it to stay for 5 min. After the formation of two layers again, lower layer was again discarded. Repeat the addition of water two times to wash all residues of methanol in mixture. Then FAMES were poured into beaker, small amount of sodium sulphate were added in it to absorb moisture then filtered it.

Fatty acids profile by GC

The dates oil methyl esters were analyzed on a SHIMADZU gas chromatograph, model 17-A, coupled with flame ionization detector (FID). Separation was made on a methyl-lignocerate coated polar capillary column SP-2330(30 m*0.32mm*0.20µm; Supelco, Inc, Bellefonte, PA., USA). Nitrogen was used as a carrier gas at a flow rate of 3.0 mL/min, temperature column was programmed from 180 to 220°C at the linear rate of 5°C/min. Initial and final temperatures was set for 2 and 10 min, respectively. Injector and detector were set at 230 and 250°C, respectively. A sample

volume of 1.0 µL was injected using split mode (split ratio of 1:75). By comparing their relative and absolute retention times to those of authentic standards, FAMES were identified. The internal standard used was heptadecanoic acid (C17:0).

Statistical analysis

Data was expressed as mean values. Two way ANOVA procedures were used to carry out analysis of variance. Significant difference for values relative to means of solvents was determined. $P > 0.05$ makes difference that is not significant. Analysis was done by using SPSS software [15].

Results and discussions

The extraction yield for antioxidant components of *Phoenix dactylifera* L. with methanol (100%, 80% and 50%) and ethanol (100%, 80% and 50%) is shown in Table 1. The extract yield of *Phoenix dactylifera* L. varied over range of 32.68% to 55.04% (g/100g). Maximum yield (55.04%) was obtained with 80% ethanol while minimum (32.68%) by 50% methanol for *Phoenix dactylifera* L. In the present study by seeing results, the order of extraction value of different solvents was seen to be: 80% ethanol > 100% ethanol > 50% ethanol > 100% methanol > 80% methanol > 50% methanol. The higher yield of *Phoenix dactylifera* L. with 80% ethanol revealed that 80% ethanol has greater effectiveness to extract antioxidant components from *Phoenix dactylifera* L. A significant difference ($p < 0.05$) has been observed in the extracted yield relative to the solvent system and *Phoenix dactylifera* L. used.

Some previous study [16] also reported higher extraction yield by ethanol but its value was lower than the present work yield. Kachau *et al.*, [17] reported higher % extract yield by methanol. Such differences may be attributed to different agro climatic factors and nature of extracted material and extraction solvent. Present study exposed ethanol more effective solvent for extraction

of antioxidant compounds as compared to other solvents from *Phoenix dactylifera* L. According to studies, ethanol is preferred solvent for extraction of organic compounds

[18] due to its lower toxicity while methanol and ethanol are also effective solvents for extraction [19].

Table 1. Extract yields (g/100 g) from different extracts of *Phoenix dactylifera*

Samples	Percent yield (g/100g)					
	100% Ethanol	80% Ethanol	50% Ethanol	100% Methanol	80% Methanol	50% Methanol
Ajwa Date	50.45±0.49 ^a _A	55.04±1.26 ^a _A	45.65±1.6 ^b _A	40.86±1.35 ^a _A	45.04±1.76 ^a _A	31.66±0.63 ^b _A
Ajwa Paste	49.50±0.35 ^a _B	51.06±1.54 ^a _B	40.23±1.34 ^b _B	44.24±0.45 ^a _B	38.16±1.77 ^a _B	37.56±1.27 ^b _B
Basrah	40.68±1.32 ^a _C	39.54±0.15 ^a _C	38.56±0.35 ^b _C	46.50±1.26 ^a _C	44.89±0.18 ^a _C	32.68±0.40 ^b _C
Moti jhari	39.34±1.65 ^a _D	40.28±0.43 ^a _D	41.34±0.86 ^b _D	43.77±1.72 ^a _D	46.19±0.61 ^a _D	43.31±0.29 ^b _D

Values are means ± SD (n=3) of three separate experiments. Different caps letter in subscript within the same column show significant differences of means among *Phoenix dactylifera* L. varieties. Different superscript letters within the same row show significant differences of means among extraction solvent.

Total phenolic contents

Total phenolic contents of different date varieties (Ajwa Date, Basrah and Moti Jhari) by different solvents (100% methanol, 80% methanol, 50% methanol, 100% ethanol, 80% ethanol, 50% ethanol) ranged from 220.9 GAE (mg/100g) to 290.56 GAE (mg/100g) of dry weight (DW) as shown in Table 2. The lowest value was obtained for basrah (110.6 GAE mg/100g) from 50% methanol, while highest for ajwa date (490.9 GAE mg/100g). TPC values (GEA mg/100g) of DW for extracts of different species were 243.45-490.9 for ajwa date, 246.76-440 for ajwa paste, 224.3-340.9 for moti jhari and 110.6-240.8 for basrah.

The order of extraction of solvents was: methanol 50% > methanol 80% > methanol

100% > ethanol 50% > ethanol 80% > ethanol 100%. The present trends for effect of solvent system is similar to that observed by Saleh *et al.*, [20]. There are many factors which affect the recovery of phenolic contents in different samples such as polarity of solvent solubility of the compounds in solvent used for extraction. TPC for different date varieties were found to be in the order of: ajwa date > ajwa paste > moti jhari > basrah showing significant ($p < 0.05$) variations. The present results are in line with the values of TPC investigated by Saleh *et al.*, [19] for ajwa date (455.88 mg/100g). Present TPC values are also higher than those determined by Saleh [20].

Table 2. Total phenolic contents (GAE mg/100g (DW) of *Phoenix dactylifera*

Samples	TPC (GAE mg/100g DW)					
	100% Methanol	80% Methanol	50% Methanol	100% Ethanol	80% Ethanol	50% Ethanol
Ajwa Date	451.9 ± 6.7 ^a _A	475.6 ± 4.5 ^a _B	490.9 ± 6.7 ^a _C	243.45 ± 4.5 ^a _A	260.45 ± 2.5 ^a _B	290.56 ± 5.8 ^a _C
Ajwa Paste	439.6 ± 6.5 ^a _B	440 ± 6.6 ^b _B	430.5 ± 5.7 ^b _C	246.76 ± 3.5 ^a _B	270.63 ± 3.5 ^b _B	250.6 ± 6.7 ^b _C
Moti Jhari	300 ± 6.0 ^c _A	315.2 ± 5.4 ^c _B	340.9 ± 7.2 ^c _C	224.3 ± 5.3 ^c _A	230.6 ± 2.3 ^c _B	240.7 ± 4.5 ^c _C
Basrah	225.7 ± 8 ^d _A	240.8 ± 6.8 ^d _B	220.9 ± 5.9 ^d _C	110.6 ± 7.3 ^d _A	115.6 ± 4.6 ^d _B	120.5 ± 3.4 ^d _C

Total flavonoid contents (TFC)

Total flavonoid contents (TFC) in different *Phoenix dactylifera* L. species as extracted by different solvents are given in Table 3. Values of TFC for different *Phoenix dactylifera* L. species pulp and seeds ranged from 20.6-43.5 CE mg/g of dry weight (DW). The maximum value was observed for 100% methanol ajwa paste (43.5 CE mg/g) DW extract while minimum for 100% ethanol moti jhari (20.6 CE mg/g) of DW. The TFC values for different *Phoenix dactylifera* L. were 29.8-39.2 for ajwa date, 38.4-43.5 for ajwa paste, 20.6-32.4 for moti jhari and 22.2-29.8 for basrah. Among solvents the order of ability to extract TFC

is: methanol 100% > ethanol 100% > methanol 80% > ethanol 80% > methanol 50% > ethanol 50% showing significant ($p < 0.05$) effects. The order of TFC values of peels *Phoenix dactylifera* L. on basis is ajwa paste > ajwa date > basrah > moti jhari. Similarly, the values of TFC of peels varied significantly among the *Phoenix dactylifera* L. analyzed. The present values of TFC are comparable to the values studied by ochratoxin, who worked on dates and applied his results on rats. It is concluded from this study that dates contain the high number of flavonoids. So the dates can be used in pharmaceutical industries for the cure of many diseases.

Table 3: Total flavonoid contents (CE mg/g (DW) of the extracts from *Phoenix dactylifera*

Samples	TFC (CE mg/g DW)					
	100% Methanol	80% Methanol	50% Methanol	100% Ethanol	80% Ethanol	50% Ethanol
Ajwa Date	29.8 ± 0.9 ^A ^a	32.9 ± 0.8 ^A ^b	30.6 ± 0.3 ^A ^c	35.6 ± 0.9 ^A ^a	39.2 ± 0.5 ^A ^b	30.8 ± 0.4 ^A ^c
Ajwa Paste	43.5 ± 0.3 ^B ^a	39.7 ± 0.4 ^B ^b	40.8 ± 0.1 ^B ^c	40.6 ± 0.5 ^B ^a	38.4 ± 0.9 ^B ^b	41.6 ± 0.7 ^B ^c
Moti Jhari	24.5 ± 0.5 ^C ^a	29.5 ± 0.8 ^C ^b	32.4 ± 0.2 ^C ^c	31.5 ± 0.3 ^C ^a	20.6 ± 0.6 ^C ^b	23.5 ± 0.4 ^C ^c
Basrah	22.2 ± 0.10 ^D ^a	29.8 ± 0.04 ^D ^b	26.5 ± 0.5 ^D ^c	23.9 ± 0.2 ^D ^a	26.8 ± 0.7 ^D ^b	24.5 ± 0.6 ^D ^c

DPPH scavenging activity assay

It is observed from graphical representation in Figure 1 A & B that the required concentration for reducing 50% of lipid peroxidation was significant ($P < 0.05$). The IC₅₀ data for different date varieties. The IC₅₀ for the *Phoenix dactylifera* species peels ranged from 2.5-8.2 mg/mL. The highest value was noted for 100% ethanolic extract of basrah (8.2 mg/mL) while lowest for 50% ethanolic extract of ajwa date (2.5 mg/mL). IC₅₀ (mg/mL) values for different species peels were as: 2.5-4.25 for ajwa date, 2.9-4.3 for ajwa paste, 2.9-4.5 for moti jhari and 5.2-8.2 for basrah. The ability to scavenge free radicals of the *Phoenix*

dactylifera extracts varied significant ($p < 0.05$) in relation to extraction solvents. 50% ethanol extract exhibited highest antioxidant activity among others. Overall, effect of extraction solvents on radical scavenging ability is significant ($p < 0.05$). Ajwa date has highest radical scavenging activity showing significant ($p < 0.05$) variations among species. Ajwa shows strongest antioxidant capacity more willingly than other varieties, particularly in aqueous extract, so, it may have some potent polyphenolic antioxidant such as proanthocyanidins. These bio components [21, 22] perhaps largely contribute to the antioxidant activity.

Figure. 1A and B. Graphical representation of DPPH by using different concentration of solvents

Reducing power assay

Antioxidant activity and reducing capacity assessment are directly related to each other. So, reducing capacity may serve as significant indicator to determine the antioxidant activity. This assay is basically based on the capability of antioxidants to reduce ferric III ions to ferrous II ions. EC_{50} is the concentration at which 0.5 absorbance is obtained. Greater is the value of EC_{50} , smaller is the reducing potential [23].

The EC_{50} for different date varieties from different solvents is given in table 5.5. EC_{50} value ranged from 2.56 to 6.2 mg/mL of extract. The lowest value is for moti jhari (2.56 mg/mL) from 100% ethanol as solvent while the highest was for ajwa paste (6.2 mg/mL) using 50% ethanol. The EC_{50} values for *Phoenix dactylifera* are 3.86-4.9 for ajwa date, 4.56-6.2 for ajwa paste, 2.56-4.5 for moti jhari and 2.65-4.4 for basrah.

The ability of extraction solvent towards recovery of reducing agents from *Phoenix*

dactylifera is in the order of: 100% methanol > 80% methanol > 50% ethanol > 80% ethanol > 50% methanol > 100% ethanol as shown in Figure 2 A & B While 50% ethanol extract exhibited higher reducing potential among others indicating a significant ($P < 0.05$) among solvents. The reducing potential for is in *Phoenix dactylifera* the order of ajwa paste > ajwa date > moti jhari > basrah and thus indicates significant variations among dates variety. These readings confirmed Al-Farsi *et al.*, [12] suggested that mostly antioxidants in dates are hydrophilic. However, the majority of antioxidants in date fruit are water soluble which is the major contribution to act as antioxidant in lipids membranes system. This can be, to some extent, due to the property of flavanols which have the ability to react in both side of water and lipid layers of cell membranes [22], and thus protecting from oxidative degradation.

Figure. 2A and B. Graphical representation of Reducing Power assay by using different concentration of solvents

Phenolic profile

Estimation of phenolic acids was done by HPLC. Phenolic acids of *Phoenix dactylifera* species were compared with standard phenolic acids. Ten phenolic acids including Gallic acid, Caffeic acid, ferulic acid, Protocatechuic acid, 3-hydroxybenzoic acid, rutin, Hydroxy cinnamic acid, *p* Coumaric acid, 3,5-dihydroxybenzoic acid and Vanillic acid. Gallic acid was found to be present in almost every sample. The range of gallic acid is 10.50-91.06 mg/L. The highest value for gallic acid was found in basrah

(9.06mg/L) from 100% methanol while lowest was from 80% ethanol for ajwa date (10.50mg/L). Highest amount of phenolic acids was found in 100% methanolic extract of ajwa paste which is 1005.03mg/L of extract. The range for protocatechuic acid is 144.39-55.52 mg/L of extract as shown in Table 4. These phenolic acids have antioxidant and medicinal properties. The most abundant phenolic acid is gallic acid. Another phenolic acid is *p*-coumaric acid that is supposed to have ability to reduce the stomach cancer risk [24].

Table 4. Individual phenolic acids (mg/kg of extract) profile of extract of different dates

Sample	Extract	Phenolic Acids (mg/Kg)										Total amount of phenolic acids (mg/Kg)
		Gallic acid	Caff-ic acid	Feru-lic acid	Proto Catechuic acid	3-hydroxy benzoic acid	Rutin	Hydroxy cinnamic acid	p-coumaric acid	3,5 dihydroxylbenzoic acid	Vanillic acid	
Ajwa date (Ethanol)	100%	90.01	81.22	-	144.39	-	-	-	-	-	-	315.62
	80%	10.50	82.72	8.91	-	-	-	-	-	-	-	102.13
	50%	12.30	-	-	-	-	15.62	-	-	10.22	-	38.14
Ajwa date (Methanol)	100%	13.02	758.22	-	233.79	-	-	-	-	-	-	1005.03
	80%	61.43	32.67	-	-	-	-	-	-	9.20	-	103.3
	50%	21.52	-	-	244.18	-	129.66	-	80.31	-	-	475.67
Ajwa paste (Ethanol)	100%	66.34	-	-	575.50	72.71	-	-	-	-	-	714.55
	80%	86.79	-	109.23	-	424.97	-	-	-	73.61	-	694.42
	50%	22.31	-	-	-	-	-	-	-	-	-	22.31
Ajwa paste (Methanol)	100%	66.88	-	-	-	-	-	-	-	172.65	264.98	504.43
	80%	12.66	-	105.16	-	-	142.63	-	-	-	-	260.45
	50%	21.05	-	-	-	-	-	-	-	-	-	21.05
Basrah (Ethanol)	100%	91.06	82.32	-	140.24	-	-	-	-	-	-	313.62
	80%	19.53	79.61	10.81	-	-	-	-	-	-	-	109.95
	50%	13.32	-	-	-	-	-	-	-	-	-	13.32
Basrah (Methanol)	100%	12.02	732.20	-	221.73	-	-	-	-	-	-	965.95
	80%	44.31	36.62	-	-	-	-	-	-	-	-	80.93
	50%	22.61	-	-	244.19	-	133.54	-	-	-	-	400.34

Fatty acids profile

All varieties of date seeds contain a number of saturated and unsaturated fatty acids. Oleic acid was found to be primary fatty acid in date seeds of all three varieties with concentrations of 21, 12, and 17% of total fatty acids, respectively. It was followed by lauric (19, 25 and 13%), myristic (13, 7.63 and 9%), stearic (12, 20 and 15%), palmitic (10, 10 and 8%), linoleic acid (8, 15 and 15.78%) and trace amounts of the other fatty acids like undecylic, capric, trideclic and caprylic acids. Results indicated that there is no significant relationship found between fatty acids levels in three date seed varieties, although there is difference in the amount of each fatty acid in all three varieties. Fatty acid values are shown in Table 5.

The three varieties were similar in the type of fatty acids present in their seed oil but

caprilic acid and capric acid were not found in moti jhari Besbes [25] also worked on some quality characteristics of date seed oil from varieties of date palm and reported unsaturated and saturated fatty acids.

We conclude that oil from dates would serve as useful source of these fatty acids and replace the other vegetable oils. On the other hand, for safety purpose it should be evaluated before use for human feed. They are renewable resources. Depending on the local industry, present data recommend the possible application of date seed oil in cosmetics and pharmaceuticals and may be the other more applications. Hence, it would be very significant to do more research on date seed oil in future to explore its potential effects.

Table 5. Fatty acid profile of oil extract from *Phoenix dactylifera*. L

Relative percentage of fatty acids in different date pulp and seed oils				
Fatty acids	Type of Fatty acids	Ajwa Date%	Moti Jhari %	Basrah%
Caprilic acid	Saturated	0.06	0	0.05
Capric acid	Saturated	0.59	0	0.1
Undecylic acid	Saturated	0.45	1.50	2
Lauric acid	Saturated	13	7.63	9
Tridecyllic acid	Saturated	2	0.96	3
Myristic acid	Saturated	19	25	13
Dendritic acid	Unsaturated	0.39	0.02	0.06
Palmitic acid	Saturated	10	10	9
Margaric acid	Saturated	0.0	2	0.01
Stearic acid	Saturated	2.51	20	16.62
Oleic acid	Unsaturated	21	12	17
Linoleic acid	Unsaturated	18	15	15.78
α -linolenic acid	Unsaturated	9	3	9.38
Arachidic acid	Saturated	4	2.89	5

Conclusion

All results indicated that pulp and seeds of *Phoenix dactylifera* L. have good antioxidant potential so can be used in food and pharmaceutical industry as a natural antioxidant and as a preservative. Maximum value of TPC was found in extract of ajwa

date and TFC was in ajwa paste extract while values of DPPH and reducing power were higher in extract of ajwa date. Phenolic acids have antioxidant and medicinal properties. The most abundant phenolic acid in dates was gallic acid. So, present findings conclude that seeds from dates would serve

as valuable source of fatty acids and substitute the other vegetable oils. Depending on the regional industry, present data recommend the possible application of date seed oil in cosmetics and pharmaceuticals and may be the some other more applications. Our findings supported the use of dates as a potential source of valuable natural antioxidants as well as for pharmaceutical, for development of nutraceutical, in value addition and food industries.

Authors' contributions

Conceived and designed the experiments: T Mehmood, Performed the experiments: ZA Khan & Abdul Karim, Analyzed the data: T Mehmood, M Akram, A Afzal & F Siddique, Contributed reagents/ materials/ analysis tools: MA Shaheen, Wrote the paper: T Mehmood & MA Shaheen.

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