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Original Article

Phenolic Profile and Antioxidant Activities of Three Date Seeds Varieties (*Phoenix Dactylifera* L.) of Pakistan

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Abstract

Background and objectives: This work aimed to evaluate the phenolic profile and antioxidant capacity of water extracts of three different date seed varieties *i.e.,* Aseel, Karbalaen and Khupro. Date (*Phoenix dactylifera* L.) seeds are available in bulk quantities after manufacturing of pitted dates or syrup and are considered as waste stream.

Methods: Total phenolic content in date seeds was determined with Folin-Ciocalteu's phenol reagent. The phenolic compounds profile was determined by high performance liquid chromatography. Antioxidant capacity of each variety was investigated by 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), oxygen radical absorbance capacity, ferric reducing antioxidant power assay and 2,2-diphenyl-1-picryl-hydrazyl-hydrate assays.

Results: Eight phenolics including gallic acid, caffeic acid, p-coumaric acid, vanillic acid, catechin, epicatechin, chlorogenic acid and sinapic acid were detected in date seeds. The highest content of phenolics was found in Aseel, followed by Karbalaen and Khupro date seeds respectively. Furthermore, the phenolic profile also correlated with the antioxidant capacity of these samples.

Conclusion: Date seeds contain significant level of phenolics and possess high antioxidant activity. Therefore, date seeds could be promising candidates for biomedical applications, functional foods and fortification to increase the shelf life of food products.

Introduction

The date palm (*Phoenix dactylifera* L.) is mainly grown in arid and semi-arid regions of the world. It is popular in countries found in South Asia, America and North Africa. The global production of dates reached up to 8.5 million metric tons in 2016 with approximately 1 million metric tons of date seeds. Pakistan ranks sixth among different date producing countries.[1] The fruit consists of a fleshy pericarp and the seeds comprise 10–15% of total weight of the fruit. The date seeds remain a problem for the date processing industry after manufacturing of pitted date cakes, confectionary and syrups. Ground seeds are sometimes used as base gravel on dirty roads. In Middle and South East countries, seeds are occasionally used in animal feed.[2] Date seed supplemented feeds showed increased weight gain in broilers compared to controls.[3] Other studies also report a positive supplemental effect on animal diet, suggesting the presence of nutritionally important constituents in seeds.[4] Date seeds are a rich source of fiber compared to wheat bran and oats.[5] Phytochemicals with promising health promoting potential, including phenolic compounds, phytosterols, tocopherols and squalene, have also been found in date syrup,[6] grape seeds,[7] blackcurrant seeds,[8] mango pits[9] and citrus seeds.[10,11]

Hence, date seeds could be a rich source of antioxidants. *In vitro* and *in vivo* studies have shown that consumption of date seeds reduces diabetes, dyslipidemia and hypertension, and obesity.[12] These effects might be caused by specific components, including protein, fibers and bioactive compounds. Among all bioactive compounds, phenolic compounds have been reported to have antioxidant, anti-carcinogenic, anti-microbial, anti-mutagenic and anti-inflammatory characteristics.[13,14]

Previous studies have revealed the correlation between total

Keywords: *Phoenix dactylifera* L. seeds; Phenolic compounds; Separation; Identification; Quantification; Antioxidant capacities.

Abbreviations: ABTS, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate; EC_{50}, half-maximal concentration; FRAP, ferric reducing antioxidant power assay; GAE, gallic acid equivalents; HPLC, high performance liquid chromatography; ORAC, oxygen radical absorbance capacity; TE, trolox equivalents; TPC, total phenolic content; TPTZ, 2,4,6-tripyridyl-s-triazine.

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phenolic content (TPC) and antioxidant capacity.[15] Synthetic antioxidants are expensive, possibly toxic, and more immunogenic than naturally occurring phenolics. Omani date seeds showed higher TPC content and antioxidant capacity than other date seed varieties.[16] However, identification and quantification of individual phenolic compounds and the antioxidant capacity of Pakistani date seeds have not been reported. Date seeds may have extractable high value-added nutritional components that could be included in functional foods. The main objectives of this study were to identify and quantity the phenolic components and to investigate antioxidant capacity using different assays.

Experimental

Chemicals

Folin–Ciocalteu's phenol reagent, phenolic standards (caffeic acid, gallic acid, catechin hydrate, sinapic acid, chlorogenic acid, vanillic acid, p-coumaric acid and epicatechin), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,20-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,20-azobis (2-amidinopropane) dihydrochloride were purchased from Fluka (Fisher Scientific, UK). All chemicals and reagents were of analytical grade purchased from Sigma-Aldrich (Dorset, UK) unless otherwise stated.

Plant material

Three types of commonly used ripe date varieties (Karbalaen, Aseel and Khuprowere) were purchased from a local market of Khairpur, Sindh. All samples were carefully checked according to their physical parameters (size, shape and weight) and stored in the refrigerator at 4°C before analysis.

Physical Parameters

A random selection of 20 date fruits from each variety was made. Chosen samples were clean and in good condition. An electrical balance was used to calculate the weight of the dates and seeds. Seed measurements (length (L), width (W), and thickness (T)) were measured with a digital calliper to an accuracy of 0.1 mm. Percent Mass was calculated by using following formula:

$$\text{Mass of date seed (\%)} = \frac{\text{WDS}}{\text{WD}} \times 100$$

where, WDS: weight of date seeds, WD: weight of dates.

Extraction and quantification of phenolic compounds through high performance liquid chromatography

Total phenolics were extracted using a methanol water (4:1) mixture. Briefly, 2 g of ground seeds were transferred to 30 ml of solvent mixture. The extraction was carried in glass tubes, placed in orbital shaker for 4 hours and solution was then centrifuged at 2,000 g for 20 minutes. The supernatant was carefully transferred to brown glass vials to avoid light interference and was kept at −4 °C for further analysis on high performance liquid chromatography (HPLC) and antioxidants activities. Stock standard solutions of each standard (gallic acid, catechin hydrate, chlorogenic acid, epicatechin, caffeic acid, vanillic acid, sinapic acid and p-Coumaric acid) were individually prepared in methanol.

A combined standard solution was also prepared from each standard. Five different concentration standards, from 4 µg/mL to 20 µg/mL, were prepared. Phenolic compound profile was determined using a HPLC-UV (Dionex, Sunnyvale, CA) system. Separation was performed with slight modification from ACE column (250 mm, 4.6 mm, DV10-2950, UK).[17] Injection volume was 20 µL. Detection was monitored over a range of 200–400 nm by a UV Detector (MWD-3000/8018650, UK).

After optimizing the proper conditions for the mobile phase, multi-gradient analysis was selected. Multi-gradient mobile phase flow was set as: 10–15% (A), 90–15% (B) for the first eight minutes, 15–20% (A), 85–80% (B) from 8 to 25 minutes and 20–25% (A), 80–75% (B) from 25 to 60 minutes. Similarly, flow rate was gradually increased from 0.3 mL/min, 0.5 mL/min and 0.6 mL/min, respectively. Data analysis was performed with Chromeleon 6.8 software (Dionex, Sunnyvale, CA).

Total phenolic content

The TPC of date seed extracts was measured with Folin-Ciocalteu's phenol reagent.[18] The absorbance of the reaction mixture was monitored at 725 nm (Jenway 6715, Spectrophotometer, UK). The TPC of each variety of date seeds was expressed in gallic acid equivalents (GAE) per gram.

Antioxidant capacity

DPPH radical scavenging assay

The DPPH assay was performed using 50 µL of date seed extract vortexed with 2 mL of 0.9 mM DPPH in methanolic solution. The mixture was kept in absence of light for an hour then the absorbance was recorded at 517 nm. The half-maximal concentration (EC_{50}) was measured; EC_{50} was defined as the concentration of seed extracts required to scavenge 50% of initial DPPH. This was calculated from results plotted as absorbance versus concentration of seed extracts.[19]

Ferric-reducing antioxidant power assay

Ferric-reducing antioxidant power assay (FRAP) of each date seed extract was measured using reagent prepared with acetate buffer (100 mM, pH 3.5), 2,4,6-tripyridyl-s-triazine (TPTZ) (10 mM in 50 mM HCl) and ferric chloride (25 mM) in a ratio of 10:1:1. 3 mL of this reagent was then added to 75 µL of seed extract and 300 µL of deionized water. The mixture was incubated at 37°C for an hour. The absorbance was recorded at 593 nm for each mixture. The FRAP assay results were expressed in µM Fe^{2+} equivalents per gram.[12]

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) anti-radical assay

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) reagent was prepared as per,[20] this method involves the reaction of ABTS (10 mM) and potassium persulfate (2 mM) in absence of light for 24 h. The prepared 2 mL of ABTS solution and 20 µL of date seed extract were mixed. The absorbance was recorded at 734 nm. Similarly, all samples with Trolox standards were incubated. The results were expressed in Trolox Equivalents (TE) per gram.[21]

Oxygen radical absorbance capacity assay

Oxygen radical absorbance capacity (ORAC) assay was performed for determination of antioxidant capacity. 6-carboxyfluorescein was used as the fluorescence probe, while 2,20-azobis (2-amidinopropane) dihydrochloride as the free radical generator. Fluorescence was measured in a 96 well-plate using a BMGLabtech (Ortenburg, Germany) microplate reader. Data was recorded after every 2 minutes for 2 hours at 37 °C, excitation and emission wavelengths were 485 nm and 520 nm, respectively. Antioxidant capacity of both samples was expressed as TE per gram.[22]

Table 1. Physical parameters of three different date seeds varieties

Variety	Karbalaen	Aseel	Khupro
Length (mm)	92 ± 0.5	90.2 ± 0.24	90.2 ± 2.34
Diameter (mm)	22.35 ± 0.87	20.42 ± 0.21	18.36 ± 0.56
Mass of date seed (%)	7.06 ± 0.65	7.21 ± 0.36	8.05 ± 0.8
Weights of seeds	10.703 ± 0.62	12.32 ± 0.42	13.5 ± 0.21

Data are expressed as the mean ± standard deviation (n = 3).

Statistical analysis

Three replications of each experiment were performed. The results were expressed in mean values ± standard deviations. The antioxidant capacity results were subjected to test the analysis of varience (ANOVA) and least significant difference test using SPSS V25 software. A $p < 0.05$ value was considered significant. The Pearson correlation coefficients among TPC, individual phenolic and antioxidant capacity assays of date seeds of three varieties were calculated using Pearson's test.

Results and discussion

Physical parameters

Date seeds showed physical differences in length, diameter, mass and total weight of the three varieties (Table 1). These characteristics differed depending on the variety, where seed length was highest for Karbalaen, followed by Aseel and Khupro. The diameter for whole date seed changed from 22.35 ± 0.87 mm (Karbalaen), 20.42 ± 0.21 mm (Aseel) and 18.36 ± 0.56 mm (Khupro). The relative percentage weights of Karbalaen, Aseel, and Khupro seeds were 13.5%, 10.7%, and 12.3%, respectively.

Total phenolic content

The TPC of three date seed varieties ranged from 33.6 mg to 44.2 mg GAE/g (Table 2). There was a significant variance ($p < 0.05$) between all three varieties (Aseel, Karbalaen and Khupro). Thouri *et al.* found the TPC of Tunisian date seeds (values) expressed as catechin equivalents per weight of seeds.[24] It should be noted that TPC values of the date varieties presented in our study are two to three-fold higher than those reported for date syrup.[23]

Identification and quantification of phenolic compounds

HPLC chromatogram of Karbalaen and Aseel seed extract depicted eight dominant peaks (1–8) in (Fig. 1). The maximum wavelength, λ_{max}, of compounds (1–8) is presented in Table 3. Aseel seeds showed the highest amount of total phenolics. Individual phenolic compounds detected are also shown in Table 3. Gallic acid quantity in Karbalaen, Aseel and Khupro seeds was 246 mg/kg, 210.42 mg/kg and 517 mg/kg, respectively with retention time (t_R) at 3.4 min. The t_R of caffeic acid was 8.8 min and was present in the lowest quantity 1.62 mg/kg, 4.69 mg/kg and 4.11 mg/kg in Aseel, Karbalaen and Khupro seed respectively. Sinapic acid was found only in Aseel (36.6 mg/kg) and Karbalaen (39.31 mg/kg) varieties with retention times at 15.3 min and 15.8 min, respectively. Sinapic acid was not detected in Khupro. The t_R is shorter in Aseel than that of Karbalaen and its standard suggests that the two varieties might contain different glycosides of sinapic acid. Ethyl gallate and penta-ogalloyl-β-D-glucose could not be detected in any of the three tested varieties. However, presesence of various biofunctional components in date has been reviewed by Wadhwa *et al.*[25] p-Coumeric acid (14.25 mg/kg and 5.33 mg/kg), epicatechin (6.98 mg/kg and 38.68 mg/kg) and vanillic acid (4.64 mg/kg and 11.52 mg/kg) were measured in Aseel and Karbalaen seeds, respectively, but not in Khupro. Among eight phenolics detected from Karbalaen seeds, two are derivatives of hydroxyl benzoic acid (gallic acid and vanillic acid) and four are cinnamic acid derivatives (caffeic acid, p-coumaric acid, synapic acid, and chlorogenic acid).

Catechin and epicatechin are part of the flavan-3-ol family of flavonoids. p-coumeric acid and its glycosides have been found in mango, apple and avocado fruit seeds.[26] The compounds with glycosidic moieties, the use of non-glycodic or even single glycosidic residue can compromise the authentication of obtained results.[27] The two hydroxyl benzoic acid derivatives were present predominantly in the Aseel, Karbalaen and Khupro seed extract, however their quantity was statistically different ($p < 0.05$) from each other. Hydroxycinnamic acid derivatives were the most abundant phenolic compounds in Aseel, Karbalaen and Khupro seed extracts, consistent with the results for lupin seed extracts.[27] In contrast, they found flavones as predominant class of phenolic compounds in *Lupinus angustifolius* L. seeds, where hydroxycinnamic acids constituted only 5% of the total phenolic compounds.[27] El-Rahman *et al.* found a lower amount of total phenolic compounds from *L. albus* seeds compared to the values obtained in this study.[28] The abundance of catechin in date seed varieties is worth emphasizing

Table 2. Total phenolic content and comparative antioxidant capacity of date seeds as determined by the ABTS, ORAC and FRAP assays

Varieties	TPC (mg GAE/g)	ABTS (μM TE/g)	ORAC (μM TE/g)	FRAP (μM Fe^{2+}/g)
Karbalaen	41.3 ±1.3^a	72.4 ± 38^b	21.26 ± 24^a	14.3 ± 57^a
Aseel	44.2 ± 0.7^a	66.71 ± 7^b	23.32 ± 51^c	12.1 ± 34^c
Khupro	33.6 ± 1.1^b	41.9 ± 4^c	12.43 ± 87^c	11.8 ± 62^b
Genotype mean	39.7 ± 5.47	60.3 ± 16.2	19 ± 5.78	12.73 ± 1.36

Data are expressed as the mean ± standard deviation (n = 3). Values in the same column with different letters differ significantly ($p < 0.05$). The different superscript letters, *i.e.*, a, b, and c, represent the statistical significance for TPC, and antioxidant capacity assays of date seeds of three varieties calculated using Pearson's test. ABTS, 2,2′-Azino-bis (3-Ethylbenzothiazoline-6-Sulfonic Acid); FRAP, ferric reducing antioxidant power assay; GAE, gallic acid equivalent; ORAC, oxygen radical absorbance capacity; TE, Trolox equivalents; TPC, total phenolic content.

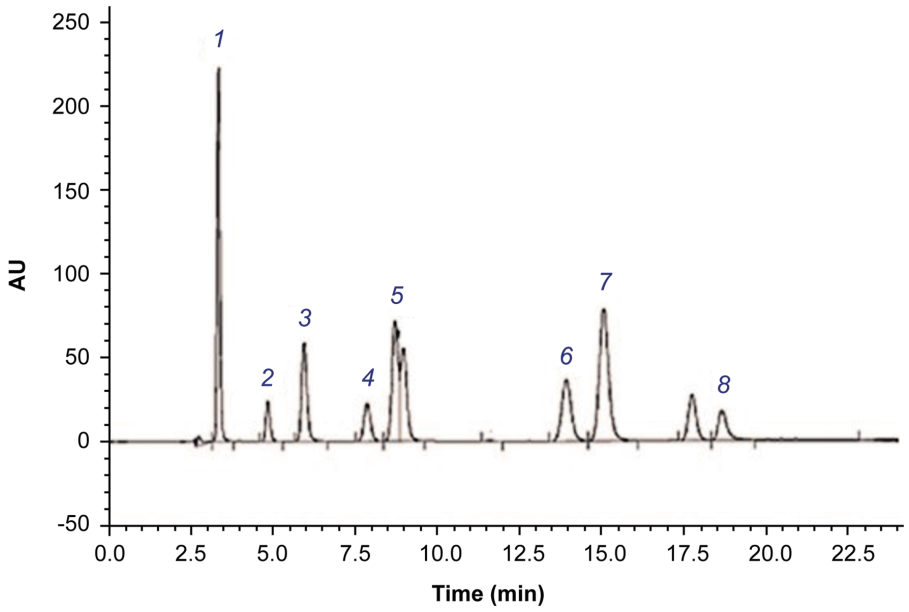

Fig. 1. High performance liquid chromatography chromatogram of phenolic compounds of date seed extracts. Peak 1–8 represent gallic acid, Catechin hydrate, Chlorogenic acid, Epicatechin, Caffeic acid, Vanillic acid, Sinapic acid and p-Coumaric acid, respectively.

as this type of bound phenolic compound is not usually available in most dietary seeds. Many reports have been published that link the health beneficial effects of the aforementioned phenolic compounds to anti-inflammatory, antioxidant, anticancer, hypoglycemic, anti-aging, and cholesterlemic activities.[29]

Antioxidant capacity of date seeds

The free radical scavenging activity of date seed extracts against ABTS (TE/g) is shown in Table 2. Aseel seed extracts showed a lower activity (66 mM TE/g) compared to Karbalaen 72 mM TE/g) and Khupro (41.9 mM TE/g). The results of the present study are much higher than previously reported antioxidant studies in dates.[30,31] The free radical scavenging activity values against ABTS fell within the range reported for different leguminous extracts, including fava bean and black bean extracts (1.5 – 1.75 mM TE/g), and similar values for navy bean extracts (27–45 mM TE/g).[32] Kidney bean and soya bean extract showed comparable antiradical activity against ABTH to date seed extracts.[33]

The DPPH antiradical activity of *P. dactylifera* L. kernel extracts are shown in Figure 2. A significant variation ($p < 0.05$) in the values of three date varieties was observed. Antioxidant capacity decreased in the order of Aseel > Karbalaen > Khupro. The EC_{50} of Aseel was twofold higher than Khupro. The variation in DPPH assay for date syrup of different varieties has previously been reported.[34] The average DPPH antiradical activity of various Omani date species had slightly lower values than Irani date species.[35] The EC_{50} values of date seeds are higher compared to TPC. In turn, mango seed extracts had similar TPC, and showed higher DPPH assay values. This phenomenon could be related to the chemical configuration of phenolic compounds, as bound phenolic might interfere with reaction of Folin-Ciocalteu's reagent and phenols provide inflated TPC values.[36] In addition, in bound phenolic structures, electron donating hydroxyl groups could be blocked and participate in scavenging assay. As a result, bound phenolics usually have lower scavenging potential than free phenolics.

The antioxidant capacity of date seeds extracts to reduce the

Table 3. Identification and quantification of individual phenolic compounds

Peak No.	t_R (min)	Standards (R^2)	$^{\lambda}$max (nm)	Phenolics	Concentration (mg/kg)		
					Aseel	Karbalaen	Khupro
1	3.4	0.994	280	Gallic acid	210.4	246.08	572.46
2	4.9	0.998	280	Catechin hydrate	109.1	178.4	108.48
3	5.8	0.998	280	Chlorogenic acid	16.86	45.09	16.49
4	7.7	0.996	280	Epicatechin	6.98	38.68	39.07
5	8.9	0.997	320	Caffeic acid	1.62	4.69	4.11
6	14.1	0.997	260	Vanillic acid	4.64	11.61	8.98
7	15.3	0.996	320	Sinapic acid	36.65	39.31	ND
8	18.5	0.998	320	p-Coumaric acid	14.25	5.33	5.68

ND, not detected; t_R, retention time.

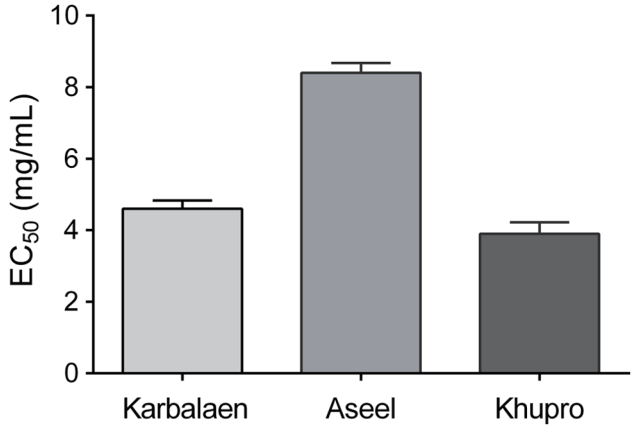

Fig. 2. Antioxidant capacity of the extracts of date seeds against α, α-diphenyl-β-picrylhydrazyl. Data are expressed as the mean ± standard deviation (n = 4).

TPTZ-Fe^{3+} complex to TPTZ-Fe^{2+} is shown in Table 2. The highest FRAP was found for Karbalaen seeds extracts (14.3 µM Fe^{2+}/g), Aseel (12.1 µM Fe^{2+}/g) and Khupro (11.8 µM Fe^{2+}/g). Antioxidant capacity using the ORAC assay measures the total reaction between reactive species and presence of oxidants. Antioxidant capacity for Aseel, Karbalaen and Khupro were 23.26 µM TE/g, 21.32 µM TE/g and 12.43 µM TE/g, respectively. These values are considerably higher than Omani and Irani date seeds extract.[37] However, ORAC values were higher compared to the edible part of dates. Al-Farsi *et al.* reported flesh part values between 1.13–1.75 µM GAE/g^{-1} for Omani dates and these were in agreement with Iranian Kharak dates.[23] Antioxidant capacities of date flesh obtained by Saleh *et al.* were 5.7–6.6 GAE/g,[16] which were higher than those published in.[38] Mansouri *et al.* have reported the positive correlation of the antioxidant capacity and phenolic profile in date fruit from Algeria.[39]

Antioxidant capacity is mainly attributed to the water-soluble component's potential radical scavenging capacity such as cinnamic acid, vinallic acid, and cumeric acid. Many factors such as temperature, soil conditions, maturity, geographic location and post harvesting treatment might be contributing to the differences in phenolic profiles and related antioxidant capacity. Fig, prune and raisin seeds antioxidant capacity values are comparable to date seeds. These values are directly proportional to the amount of phenolics present. High antioxidant capacity for flesh has not been reported with reference to ripening stages of date fruits.

Correlation analysis

The TPC of date seeds demonstrated strong correlation as shown in Table 4 with ABTS (r = 0.903, $p < 0.05$), ORAC (r = 0.996, $p < 0.05$) and FRAP were r = 0.994, $p < 0.05$ (Table 4). Similarly, the correlation coefficient for ABTS and ORAC was 0.937. This is because the reactions of phenolics with ORAC and ABTS and Folin-Ciocalteu's reagent work based on electron transfer principle.[27] The strong linear correlations between ORAC, ABTS and TPC have been reported in previous studies for different leguminous seeds varieties.[40] In DPPH assay, antioxidants scavenge the free radicals by electron transfer yet the EC_{50} measured values were not significantly correlated with FRAP, ABTS and TPC ($p > 0.05$). The above results reveal that date seed extracts contained phenolics, which are more actively participating in FRAP, ABTS and ORAC scavenging, while other phytochemical along with phenolic constituents are also active in DPPH assay. From the dominant phenolic compounds of date seed extract, the gallic acid correlated with TPC (r = 0.937, $p < 0.05$), ABTS (r = 0.996, $p < 0.05$), ORAC (r = 0.964, $p < 0.05$) and FRAP (r = 0.662, $p < 0.05$). Similar relationships were observed for sinapic acid and vanillic acid. The correlations of antioxidant capacity of seed extracts in DPPH assay to the gallic acid, vanillic acid and sinapic acid content were non-significant ($p > 0.05$). Karamać *et al.* have reported that the content of gallic acid in lupin seed extracts was strongly correlated with TPC, however the total scavenging activity was not significant.[27] The less polar p-coumaric acid of date seed extracts likely weakly affected the scavenging potential and TPC, as there was no significant correlations ($p > 0.05$). Previous studies also have shown a strong correlation between TPC and ABTS, ORAC and FRAP assays.[40] Jing *et al.* reported a strong correlation between hydroxyl benzoic acid and Hydroxycinnamic acids derivatives (r = 0.996) and free radical scavenging potentials.[41]

Table 4. Correlation coefficients (r) between the total phenolic content and the antioxidant capacity of date seeds extracts

	TPC	ABTS	ORAC	FRAP
Gallic acid	0.937*	0.996*	0.964*	0.662*
Caffeic acid	0.077^{ns}	0.498^{ns}	0.165^{ns}	0.959*
Catechin hydrate	0.717	0.348^{ns}	0.652*	0.394^{ns}
Sinapic acid	0.979*	0.972*	0.993^{ns}	0.542^{ns}
Chlorogenic acid	0.719*	0.351^{ns}	0.655^{ns}	0.391^{ns}
Vinallic acid	0.127^{ns}	0.312^{ns}	0.038^{ns}	0.881*
p-coumaric acid	0.219^{ns}	0.618^{ns}	0.305^{ns}	0.990*
Epicatechin	0.263^{ns}	0.652^{ns}	0.348^{ns}	0.995*
TPC	1	0.903*	0.996*	0.358^{ns}
ABTS		1	0.937*	0.725*
ORAC			1	0.439
FRAP				1

*Correlation is significant at $P < 0.05$. ^{ns} Correlation is not significant ($P \geq 0.05$). ABTS, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric reducing antioxidant power assay; ORAC, oxygen radical absorbance capacity; TPC, total phenolic content.

However, Hatamnia *et al.* reported that TPC and EC_{50} of DPPH did not exhibit a significant correlation.[42] Positive correlation between phenolic compounds and antioxidant actives have been reported in the different plant extracts. Zakhm-e-hayat rhizomes crude extract showed a similar trend of phenolic and flavonoid content with its antioxidant activity.[43] TPCs and presence of aforementioned free and bound phenolics contribute to the overall antioxidant capacity of date seed extract. Furthermore, this study also suggests that TPCs and a single free radical scavenging assay might not be sufficient to reveal the whole picture of antioxidant capacity and as the best indicator for antioxidant capacities.

Future directions

There is a long history of using plants to cure and manage a variety of diseases. It has been claimed that dates in particular are physiologically active, containing a number of bioactive elements such as phenolic compounds and others. Here three different varieties (Aseel, Karbalaen and Khupro) of date (*Phoenix dactylifera* L.) seeds were explored for their antioxidant potential and quantifica-

tion of phenolic constituents. Phenolics and other phytochemicals affect the bio-functionality of the extracts. The chemical components of these extracts must therefore be further investigated, characterized and isolated through various sophisticated techniques such as high-pressure liquid chromatography. We recommend that these active components be explored for biomedical potential as anti-microbial, anti-inflammatory, and anti-cancer compounds with a focus on the associated molecular mechanisms.

Conclusions

Date seeds are a remarkable source of important phytoconstituents such as phenolic compounds. These chemicals exhibit a variety of activities including antioxidant potential. Date seeds are considered a troublesome waste product, thus it may be advantageous to identify and extract the potential nutritional, and bioactive phytochemicals of these seeds. In this study date (*P. dactylifera* L.) seeds were explored for their phytochemicals (phenolic compounds) and antioxidant potential. Gallic acid (hydroxyl benzoic acid), sinapic acid (Hydroxycinnamic acids) and catechin (Bound phenolic) were the dominant phenolics observed in date seeds. A strong correlation was found between phenolic compounds and antioxidant capacity (ABTS, ORAC and FRAP assays), with no significant variation between the date seeds varieties. The present study indicates that three date seeds varieties from Pakistan can be potential candidates as food ingredients with high phenolic content and antioxidant capacity. However, more studies are suggested for isolation of date seed constituents for their prospective applications in food and biomedical industries.

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Conflict of interest

ARP has been an editorial board member of *Exploratory Research and Hypothesis in Medicine* since December 2018. The authors declare that they have no other conflict of interests.

Author contributions

Contributed to study concept and design (AM and FN), acquisition of the data (AM), assay performance and data analysis (AM), drafting of the manuscript (AM, FN, ARP, and SB), critical revision of the manuscript (AM, ARP and SB), supervision (AM).

Data sharing statement

All data generated and analyzed in the study is available in the manuscript.

References

[1] Qayyum MA, Saleem MA, Saeed S, Wakil W, Ishtiaq M, Ashraf W, *et al*. Integration of entomopathogenic fungi and eco-friendly insecticides for management of red palm weevil, Rhynchophorus ferrugineus (Olivier). Saudi J Biol Sci 2020;27(7):1811–1817. doi:10.1016/j.sjbs.2019.12.018, PMID:32565700.

[2] Sami A, Suliman G, Abudabos A, Abdelrahman M. Effect of feeding different levels of date pits with growth promoters on carcass characteristics and meat quality merits of Naimi lambs. Pak J Agric Sci 2016;53(3):713–717. doi:10.21162/PAKJAS/16.4308.

[3] Alyileili SR, El-Tarabily KA, Belal IEH, Ibrahim WH, Sulaiman M, Hussein AS. Effect of trichoderma reesei degraded date pits on antioxidant enzyme activities and biochemical responses of broiler chickens. Front Vet Sci 2020;7:338. doi:10.3389/fvets.2020.00338, PMID:33015134.

[4] Najafi S, Ghasemi HA, Hajkhodadadi I, Khodaei-Motlagh M. Nutritional value of whole date waste and evaluating its application in ostrich diets. Animal 2021;15(3):100165. doi:10.1016/j.animal.2020.100165, PMID:33500216.

[5] Habib HM, Ibrahim WH. Nutritional quality evaluation of eighteen date pit varieties. Int J Food Sci Nutr 2009;60(Suppl 1):99–111. doi:10.1080/09637480802314639, PMID:18925479.

[6] Nazirah Hamzah N, Ferdosh S, Islam Sarker MZ, Ghafoor K, Yunus K, Jalal Khan Chowdhury A, *et al*. Biological activities and extraction technologies of Pheonix dactylifera: A review. Nat Prod J 2019;9(1):3–13. doi:10.2174/2210315508666180327152800.

[7] Luzio A, Bernardo S, Correia C, Moutinho-Pereira J, Dinis LT. Phytochemical screening and antioxidant activity on berry, skin, pulp and seed from seven red Mediterranean grapevine varieties (Vitis vinifera L.) treated with kaolin foliar sunscreen. Sci Hortic 2021;281:109962. doi:10.1016/j.scienta.2021.109962.

[8] Hui XD, Wu G, Han D, Gong X, Wu XY, Tang SZ, *et al*. The effects of bioactive compounds from blueberry and blackcurrant powder on oat bran pastes: Enhancing *in vitro* antioxidant activity and reducing reactive oxygen species in lipopolysaccharide-stimulated raw264.7 macrophages. Antioxidants (Basel) 2021;10(3):388. doi:10.3390/antiox10030388, PMID:33807689.

[9] Castañeda-Valbuena D, Ayora-Talavera T, Luján-Hidalgo C, Álvarez-Gutiérrez P, Martínez-Galero N, Meza-Gordillo R. Ultrasound extraction conditions effect on antioxidant capacity of mango by-product extracts. Food Bioprod Process 2021;127:212–224. doi:10.1016/j.fbp.2021.03.002.

[10] Rojas-Lema S, Torres-Giner S, Quiles-Carrillo L, Gomez-Caturla J, Garcia-Garcia D, Balart R. On the use of phenolic compounds present in citrus fruits and grapes as natural antioxidants for thermo-compressed bio-based high-density polyethylene films. Antioxidants (Basel) 2020;10(1):14. doi:10.3390/antiox10010014, PMID:33375591.

[11] Campos DA, Gómez-García R, Vilas-Boas AA, Madureira AR, Pintado MM. Management of fruit industrial by-products-a case study on circular economy approach. Molecules 2020;25(2):320. doi:10.3390/molecules25020320, PMID:31941124.

[12] Hasan M, Mohieldein A. *In vivo* evaluation of anti diabetic, hypolipidemic, antioxidative activities of saudi date seed extract on streptozotocin induced diabetic rats. J Clin Diagn Res 2016;10(3):FF06–FF12. doi:10.7860/JCDR/2016/16879.7419, PMID:27134893.

[13] Phull AR, Ali A, Rafiq M, Tahir T, Majid A, Seo SY, *et al*. Antioxidant potential, urease and acetylcholine esterase inhibitory activity and phytochemical analysis of selected medicinal plants from the Republic of Korea. Explor Res Hypothesis Med 2021;6(2):51–59. doi:10.14218/ERHM.2020.00069.

[14] Kazi MA, Sahito R, Abbas Q, Ullah S, Majid A, Phull AR, *et al*. The Inhibitory Effect of polyphenon 60 from green tea on melanin and tyrosinase in zebrafish and A375 human melanoma cells. Evid Based Complement Alternat Med 2022;2022:7739023. doi:10.1155/2022/7739023, PMID:36091602.

[15] Phull AR, Hassan M, Abbas Q, Raza H, Haq IU, Seo SY, *et al*. In vitro, in silico elucidation of antiurease activity, kinetic mechanism and COX-2 inhibitory efficacy of coagulansin A of withania coagulans. Chem Biodivers 2018;15(1):e1700427. doi:10.1002/cbdv.201700427, PMID:29121447.

[16] Saleh EA, Tawfik MS, Abu-Tarboush HM. Phenolic contents and antioxidant activity of various date palm (Phoenix dactylifera L.) fruits from Saudi Arabia. Food Nutr Sci 2011;2:1134–1141. doi:10.4236/fns.2011.210152.

[17] Olennikov DN, Fedorov IA, Kashchenko NI, Chirikova NK, Vennos C.

Khellactone derivatives and other Phenolics of Phlojodicarpus sibiricus (Apiaceae): HPLC-DAD-ESI-QQQ-MS/MS and HPLC-UV profile, and antiobesity potential of dihydrosamidin. Molecules 2019;24(12):2286. doi:10.3390/molecules24122286, PMID:31248222.

[18] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat Protoc 2007;2(4):875–877. doi:10.1038/nprot.2007.102, PMID:17446889.

[19] Alexander B, Browse DJ, Reading SJ, Benjamin IS. A simple and accurate mathematical method for calculation of the EC50. J Pharmacol Toxicol Methods 1999;41(2-3):55–58. doi:10.1016/s1056-8719(98)00038-0, PMID:10598675.

[20] Kumar V, Rani A, Dixit AK, Pratap D, Bhatnagar D. A comparative assessment of total phenolic content, ferric reducing-anti-oxidative power, free radical-scavenging activity, vitamin C and isoflavones content in soybean with varying seed coat colour. Food Re Int 2010;43(1):323–328. doi:10.1016/j.foodres.2009.10.019.

[21] Sridhar K, Charles AL. *In vitro* antioxidant activity of Kyoho grape extracts in DPPH and ABTS assays: Estimation methods for EC_{50} using advanced statistical programs. Food Chem 2019;275:41–49. doi:10.1016/j.foodchem.2018.09.040, PMID:30724215.

[22] Yilmaz Y, Toledo RT. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. J Agric Food Chem 2004;52(2):255–260. doi:10.1021/jf030117h, PMID:14733505.

[23] Al-Farsi M, Alasalvar C, Al-Abid M, Al-Shoaily K, Al-Amry M, Al-Rawahy F. Compositional and functional characteristics of dates, syrups, and their by- products. Food Chem 2007;104(3):943–947. doi:10.1016/j.foodchem.2006.12.051.

[24] Thouri A, Chahdoura H, El Arem A, Omri Hichri A, Ben Hassin R, Achour L. Effect of solvents extraction on phytochemical components and biological activities of Tunisian date seeds (var. Korkobbi and Arechti). BMC Complement Altern Med 2017;17(1):248. doi:10.1186/s12906-017-1751-y, PMID:28472941.

[25] Wadhwa M, Bakshi M, Makkar H. Wastes to worth: Value added products from fruit and vegetable wastes. CAB Reviewes 2015;43:1–25. doi:10.1079/PAVSNNR201510043.

[26] Belviranlı B, Al-Juhaimi F, Özcan MM, Ghafoor K, Babiker EE, Alsawmahi ON. Effect of location on some physico-chemical properties of prickly pear (*Opuntia ficus-indica* L.) fruit and seeds. J Food Process Pres 2019;43(3):e13896. doi:10.1111/jfpp.13896.

[27] Karamać M, Orak HH, Amarowicz R, Orak A, Piekoszewski W. Phenolic contents and antioxidant capacities of wild and cultivated white lupin (*Lupinus albus* L.) seeds. Food Chem 2018;258:1–7. doi:10.1016/j.foodchem.2018.03.041, PMID:29655709.

[28] Abd El-Rahman SS, Mazen MM, Mohamed HI, Mahmoud NM. Induction of defence related enzymes and phenolic compounds in lupin (*Lupinus albus* L.) and their effects on host resistance against Fusarium wilt. Eur J Plant Pathol 2012;134(1):105–116. doi:10.1007/s10658-012-0028-z.

[29] Critselis E, Panagiotakos D. Adherence to the Mediterranean diet and healthy ageing: Current evidence, biological pathways, and future directions. Crit Rev Food Sci Nutr 2020;60(13):2148–2157. doi:10.1080/10408398.2019.1631752, PMID:31272195.

[30] Souli I, Jemni M, Rodríguez-Verástegui LL, Chaira N, Artés F, Ferchichi A. Phenolic composition profiling of Tunisian 10 varieties of common dates (*Phoenix dactylifera* L.) at tamar stage using LC-ESI-MS and antioxidant activity. J Food Biochem 2018;42(6):e12634. doi:10.1111/jfbc.12634.

[31] Al-Yahya M, Raish M, AlSaid MS, Ahmad A, Mothana RA, Al-Sohaibani M, Al-Dosari MS, Parvez MK, Rafatullah S. 'Ajwa' dates (*Phoenix dactylifera* L.) extract ameliorates isoproterenol-induced cardiomyopathy through downregulation of oxidative, inflammatory and apoptotic molecules in rodent model. Phytomedicine 2016;23(11):1240–1248. doi:10.1016/j.phymed.2015.10.019, PMID:26776662.

[32] Amarowicz R, Shahidi F. Antioxidant activity of broad bean seed extract and its phenolic composition. J Funct Food 2017;38:656–662. doi:10.1016/j.jff.2017.04.002.

[33] Amarowicz R, Pegg RB. Natural antioxidants of plant origin. Adv Food Nutr Res 2019;90:1–81. doi:10.1016/bs.afnr.2019.02.011, PMID:31445594.

[34] Makhlouf-Gafsi I, Krichen F, Mansour RB, Mokni A, Sila A, Bougatef A, *et al*. Ultrafiltration and thermal processing effects on Maillard reaction products and biological properties of date palm sap syrups (*Phoenix dactylifera* L.). Food Chem 2018;256:397–404. doi:10.1016/j.foodchem.2018.02.145, PMID:29606465.

[35] Mrabet A, Rodríguez-Arcos R, Guillén-Bejarano R, Chaira N, Ferchichi A, Jiménez-Araujo A. Dietary fiber from Tunisian common date cultivars (*Phoenix dactylifera* L.): Chemical composition, functional properties, and antioxidant capacity. J Agric Food Chem 2012;60(14):3658–3664. doi:10.1021/jf2035934, PMID:22443221.

[36] Goujot D, Cuvelier ME, Soto P, Courtois F. A stoichio-kinetic model for a DPPH-ferulic acid reaction. Talanta 2019;196:284–292. doi:10.1016/j.talanta.2018.12.056, PMID:30683365.

[37] Ghnimi S, Umer S, Karim A, Kamal-Eldin A. Date fruit (*Phoenix dactylifera* L.): An underutilized food seeking industrial valorization. Nutr Food Sci J 2017;6:1–10. doi:10.1016/j.nfs.2016.12.001.

[38] Daoud A, Malika D, Bakari S, Hfaiedh N, Mnafgui K, Kadri A, *et al*. Assessment of polyphenol composition, antioxidant and antimicrobial properties of various extracts of Date Palm Pollen (DPP) from two Tunisian cultivars. Arab J Chem 2019;12(8):3075–3086. doi:10.1016/j.arabjc.2015.07.014.

[39] Mansouri A, Embarek G, Kokkalou E, Kefalas P. Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). Food Chem 2005;89(3):411–420. doi:10.1016/j.foodchem.2004.02.051.

[40] Martín-Sánchez AM, Cherif S, Ben-Abda J, Barber-Vallés X, Pérez-Álvarez JÁ, Sayas-Barberá E. Phytochemicals in date co-products and their antioxidant activity. Food Chem 2014;158:513–520. doi:10.1016/j.foodchem.2014.02.172, PMID:24731377.

[41] Jing P, Zhao SJ, Jian WJ, Qian BJ, Dong Y, Pang J. Quantitative studies on structure-DPPH• scavenging activity relationships of food phenolic acids. Molecules 2012;17(11):12910–12924. doi:10.3390/molecules171112910, PMID:23117431.

[42] Hatamnia AA, Rostamzad A, Malekzadeh P, Darvishzadeh R, Abbaspour N, Hosseini M, *et al*. Antioxidant activity of different parts of Pistacia khinjuk Stocks fruit and its correlation to phenolic composition. Nat Prod Res 2016;30(12):1445–1450. doi:10.1080/14786419.2015.1060593, PMID:26166043.

[43] Ahmed M, Phul AR, Haq IU, Bibi G, Mazhar K, Rehman T, *et al*. Antioxidant, anticancer and antibacterial potential of Zakhm-e-hayat rhizomes crude extract and fractions. Pak J Pharm Sci 2016;29(3):895–902. PMID:27166554.