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In vitro inhibitory effects on α-glucosidase and α-amylase level and antioxidant potential of seeds of *Phoenix dactylifera* L.

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ABSTRACT

Objective: To evaluate and compare the antioxidant activity, total phenolic contents (TPCs) and *in vitro* antidiabetic activity of various pits extracts obtained from five Omani date cultivars.

Methods: Sun-dried mature fruits of five Omani date varieties, namely, Fardh, Naghal, Khalas, Khinazi and Khasab were purchased from the local market in Muscat, Oman in the month of September 2014. Four seed extracts *viz.* water, ethanol, methanol and acetone were prepared for each date variety and their antioxidant activities were investigated by 1,1-diphenyl-2-picrylhydrazyl, hydrogen peroxide scavenging method and reducing power assay method, respectively. *In vitro* antidiabetic activity of the date pit extracts was evaluated by measuring their inhibitory effect on α-glucosidase and α-amylase level. TPCs were also quantified colorimetrically.

Results: The results indicated that TPC of date seeds was solvent dependent. Acetone, ethanol and methanol were found to be significantly better solvents than water in extracting phenolic compounds from the date seeds. Pit extracts exhibited moderate to good *in vitro* antioxidant activity and increased reducing power. Among all date pit extracts, water extract exhibited significant *in vitro* antidiabetic activity in comparison to standard drug, acarbose.

Conclusions: The present study confirms that disposed waste of Omani dates is a rich source of dietary antioxidant because of its high TPC. The pits due to their inhibitory effects on α-glucosidase and α-amylase level could be used as a monotherapy along with an appropriate diabetic diet and exercise or might be in conjunction with antidiabetic therapy to manage and prevent progression of diabetes.

1. Introduction

Plants have played an important role in drug development [1]. They have always been a common source of medications, either in the form of traditional preparations or as pure active principles [2]. Plants known for their curative powers are used for a wide spectrum of diseases, from the common cold and fever to paralysis and diabetes [3].

Nature has bestowed Oman with an enormous wealth of medicinal plants. One common plant is the date palm [*Phoenix dactylifera* (*P. dactylifera*)] with almost 10 million growing along the Northern Batinah coastal strip [4]. The fruit of these palms "dates" are considered as the most traditional and popular food especially for breaking the Ramadan fast. Eating dates after fasting helps to maintain blood sugar levels and is an excellent source of dietary fiber, potassium, magnesium, and complex sugars [5]. The beneficial effects of dates are not limited to the date fruit only but date seeds or pits, which are left after consuming date fruits, considered as disposed waste are equi-important. These pits, seeds or disposed waste of date food have been used traditionally as

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animal feed, a source of oil, a coffee substitute in "Arabic Qahwa", a raw material for activated carbon and an adsorbent for dye-containing waters throughout gulf region [6]. The date seeds contain a high percentage of carbohydrate (81.0%–83.1%), protein (5.17%–5.56%), oil (10.19%–12.67%), ash (1.12%–1.15%) and oleic acid (41.3%–47.7%). Date seed oil is also reported to contain various polyphenolic compounds, namely, hydroxytyrosol, protocatechuic acid, tyrosol, gallic acid, caffeic acid, *p*-coumaric acid and oleuropein [7]. Several reports have indicated that there may be a correlation between phenolic content and antioxidant capacity of plant extracts [8]. Hence, date seeds might be considered as a potential source of natural edible oil, antioxidants, and pharmaceuticals [9].

Preclinical studies have shown that the date fruits as well as seeds possess free radical scavenging, antioxidant, anti-mutagenic, antimicrobial, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, anticancer and immune–stimulant activities [10]. Crude methanol, acetone and water extracts obtained from pits of three varieties of *P. dactylifera* grown in Saudi Arabia were found to be more effective than the leaves extracts of the plant against the selected Gram positive and Gram negative pathogenic bacteria [11]. The study concluded that the antimicrobial activity of date pits might result from the selective or synergistic action of various chemicals present in date palm. It has also been reported that treatment with date seed extract could protect against cerebral ischemic damages in male rats most probably due to its antioxidant properties [12]. Ardekani *et al.*, screened seed extracts of fourteen date varieties grown in Iran for their antioxidant activities and polyphenol contents. They observed a significant correlation between the total phenolic content (TPC) and antioxidant activity [13]. Seeds of five varieties of date palm grown in Qassim region, Saudi Arabia, were analyzed by Ammar and Habiba for their proximate composition, TPC and antioxidant activity by using different solvents for extraction *viz.* water, acetone, methanol and ethanol [14]. They reported that ethanol was the best solvent for extracting phenolic compounds, followed by acetone and methanol. All seed extracts also exhibited significant antioxidant activity determined as Trolox equivalent. However, results of another similar study conducted on Egyptian date seeds revealed that both non polar and polar fractions of the date seeds possess potent antioxidant and estrogen-like activity [15]. Many studies conducted elsewhere have shown a direct relationship between antioxidant, TPC and antidiabetic activity [16,17].

Because there have been no studies assessing the *in vitro* antidiabetic effect of seeds of *P. dactylifera* dates grown in Oman, this study was carried out to evaluate the *in vitro* anti-oxidant potential and the inhibitory effect of date pits on α-glucosidase and α-amylase level as an indication of antidiabetic activity.

2. Materials and methods

2.1. Drugs and chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), caffeic acid, α-glucosidase, α-amylase and acarbose were purchased from Sigma–Aldrich (USA). Folin–Ciocalteu reagent and ascorbic acid were obtained from Merck, Germany. All other chemicals and solvents used in the study were of analytical grade procured locally.

2.2. Collection of plant material

Mature fruits of five locally grown Omani date varieties, namely, Fardh, Naghal, Khalas, Khinaizi and Khasab which are listed among top 10 date palm cultivar produce of Oman [18] and are commonly consumed, were purchased from the local market of Muscat, Oman in the month of September 2014. The dates were authenticated by the botanist and sample vouchers (DS/PH/1–5) were also deposited in the pharmacy lab of Oman Medical College for future reference.

2.3. Evaluation of physical properties and preparation of date seed extracts

Five different cultivars of mature date fruits having uniform size, free of physical damage, injury from insects and fungal infections were picked up by hand and used for preparation of extract. Samples were washed with tap water and the pits were removed manually for preparation of extract of varying polarities.

2.3.1. Evaluation of physical characteristics of date fruits

Twenty dates from each variety were randomly selected and individually weighed using an analytical balance. The date pit was removed manually and it was re-weighed again. The average weight of date fruit, fruit pulp, date pit, fruit flesh percentage, total number of fruits/kg and pulp/pit ratio *etc.* were calculated.

2.3.2. Preparation of date seed extracts

Pits obtained from the date fruit were thoroughly washed under running tap water and then dried at 45 °C for 24 h. Date pits were coarsely powdered using heavy duty grinder and finally passed through 1 mm sieve to obtain fine powder of uniform particle size. Approximately 0.5 g of powdered date seeds was suspended in 10 mL of four different solvents *viz.* water, ethanol, methanol and acetone separately and kept overnight in a tight closed container. Each mixture was stirred briefly (5–10 min) at 6 h interval to speed up the extraction and finally centrifuged to obtain clear supernatant liquid extracts of varying polarities.

2.3.3. Qualitative phytochemical screening

The freshly prepared date pit extracts were also subjected to qualitative chemical tests to identify various classes of bioactive chemical constituents present in the date pits using standard procedures [19].

2.4. Estimation of TPCs

Total phenolics in date pit extracts were determined colorimetrically by using Folin–Ciocalteu reagent as per the reported method of Ardekani *et al.* [13]. Caffeic acid was used as a reference standard for constructing the external calibration curve in the concentration range of 4–64 μg/mL. The linear regression equation from the standard plot of caffeic acid was used to calculate TPC in various extracts and TPC results

were expressed as mg/g caffeic acid equivalent (CAE) of dry powder.

The TPCs were calculated using the following linear regression equation obtained from the external calibration curve of caffeic acid:

$$y = 0.0133x - 0.0133; R^2 = 0.9925$$

where y is absorbance and x is amount of caffeic acid in μg.

2.5. Determination of antioxidant activity

2.5.1. DPPH free radical-scavenging assay method

The antioxidant activity of various date pit extracts (0.1 mL) was evaluated by using DPPH (1 mmol/L in methanol) free radicals as per the previously reported method [20]. Ascorbic acid (5–100 μg/mL) was used as a positive control for comparison purpose.

2.5.2. Hydrogen peroxide (H_2O_2) scavenging assay method

The ability of the date seed extracts (0.1 mL) to scavenge H_2O_2 was determined according to the UV spectrophotometric method of Vadnere *et al.* [21]. Ascorbic acid (5–200 μg/mL) was used as a reference material for comparison of antioxidant activity.

2.5.3. Ferric reducing power assay method

Total reducing power was determined by colorimetric method as previously described by Nizam and Mushfiq [22]. Ascorbic served as a positive control. Increased absorbance at 700 nm by the reaction mixture of standard or extracts indicated increased reducing power *i.e.* antioxidant action. Percentage increase in reducing power was also calculated by the following formula:

$$\%\ \text{Increase in reducing power} = [(A_T/A_B) - 1] \times 100$$

where, A_B is absorbance of blank (containing all reagents except the test material) and A_T is absorbance of test solution.

2.6. In vitro antidiabetic activity

2.6.1. α-Amylase inhibition

The α-amylase (bovine pancreatic α-amylase; EC 3.2.1.1, Sigma) inhibition by the standard drug acarbose (12.5–200 μg/mL) and pit extracts (0.1 mL) was performed by using the chromogenic method adapted from Ali *et al.* [23]. Potato starch (0.5%, w/v) in 20 mmol/L phosphate buffer (pH 6.9) was used as a substrate solution while a mixture of sodium potassium tartrate and 3,5-dinitrosalicylic acid was used as colorimetric reagent. Inhibition of α-amylase activity by extract or standard was determined by measuring the absorbance at 540 nm which was due to the reduction of 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid.

2.6.2. Inhibition of α-glucosidase enzyme

α-Glucosidase inhibitory activity of pit extracts (0.1 mL) was measured at 540 nm using a reported method [24]. Briefly, 2% w/v sucrose and acarbose (12.5–200 μg/mL) served as a substrate and positive control, respectively.

2.7. Data analysis

All samples were analyzed in triplicate and the results were expressed as mean ± SD. The obtained data were statistically analyzed by SPSS (version 19) using one way of ANOVA and significant difference between means of tested parameters was determined by using Turkey *post hoc* multi-comparison test. A P-value less than 0.05 was considered statistically significant.

3. Results

3.1. Physical characteristics of date fruits

The physical characteristic data of five commonly consumed Omani date varieties were presented in Table 1. It could be seen from the results that a significant difference ($P < 0.05$) existed between varieties for almost all the physical parameters studied. Average number of fruits/kg in date varieties ranged from 125 to 239. Khalas and Fardh were found to contain the highest and lowest number of fruits/kg, respectively. Therefore, mean weights of fruit and flesh were also the highest (8.33 g and 8.01 g) for Fardh date. Though, Khalas date had the highest number of date fruits/kg *i.e.* smaller in size, the lowest fruit and flesh weights were observed for Khinazi variety (4.28 g and 3.65 g). However, there was no significant difference between the Khalas and Khinazi varieties. Average pit weights of all five cultivars ranged from 0.24 to 0.92 g. It was expected that Khalas being lighter and smaller in size (239 fruits/kg) would have the lowest pit weight (0.46 g) but contrary to this Fardh had the lowest pit weight (0.24 g). Naghal had the heaviest pit followed by Khinazi (0.63 g) and Khasab (0.54 g). A significant difference was observed in mean pit weights of all date varieties. The percentage of fruit flesh in date varieties ranged from 82.84% to 96.21% and Naghal date had the lowest edible portion (82.84%) with respect to other four varieties. The highest fruit flesh was found in Fardh (96.21%). On the other hand, the percentage of disposed waste *i.e.* seed or pit and flesh/pit ratio varied greatly from 3.79% to 17.16% and 4.83% to 33.36%, respectively (Table 1).

3.2. Phytochemical screening

Preliminary phytochemical testing showed that pits of all date varieties contained bioactive macromolecules such as tannins, carbohydrates, proteins and amino acids in all date varieties, however, steroidal compound and alkaloids were found to be absent in the date pit extracts.

3.3. TPCs

The results of TPC of the various date pits extracted by using four different solvents indicated that acetone, ethanol and methanol were significantly better solvents than water in extracting phenolic compounds from the date seeds (Table 2). Among three organic solvents, acetone appeared to be the best solvent for the extraction of maximum phenolic compounds [(1.41 ± 0.16)–(1.58 ± 0.06) mg/g CAE]. TPC of ethanol [(1.32 ± 0.21)–(1.51 ± 0.01) mg/g] and methanol [(1.40 ± 0.03)–(1.46 ± 0.04) mg/g] solvents showed little variation but were better than the content of phenolic compounds in water [(0.85 ± 0.03)–(1.29 ± 0.04) mg/g]. For Fardh and Khinazi, the order of solvents according to extraction of phenolic compounds

Table 1
Physical characteristic of date fruits.

Date variety	No. of fruits/kg	Average weight in g (mean ± SD)			Flesh/pit ratio	Fruit/pit ratio	% Flesh	% Pit
		Fruit	Flesh	Pit				
Naghal	189[a]	5.36 ± 0.32[a]	4.44 ± 0.26[a]	0.92 ± 0.07[a]	4.83	5.84	82.84	17.16
Khinazi	235[b]	4.28 ± 0.20[b]	3.65 ± 0.19[b]	0.63 ± 0.03[b]	5.79	6.79	85.28	14.72
Khalas	239[b]	4.33 ± 0.17[b]	3.87 ± 0.16[b]	0.46 ± 0.02[c]	8.41	9.41	89.38	10.62
Khasab	155[c]	6.57 ± 0.25[c]	6.03 ± 0.26[c]	0.54 ± 0.03[d]	11.17	12.17	91.78	8.28
Fardh	125[d]	8.33 ± 0.19[d]	8.01 ± 0.19[d]	0.24 ± 0.03[e]	33.36	34.69	96.21	3.79

Means within a column with no common letter differ significantly ($P < 0.05$).

was observed to be acetone > methanol > ethanol > water while for Khasab, it was in the order of ethanol > methanol > acetone > water. Fardh date pits showed the highest TPC in acetone [(1.58 ± 0.06) mg/g] and methanol [(1.46 ± 0.04) mg/g]. Further, no significant statistical difference was observed in TPC of date cultivars extracted by using acetone and methanol (Table 2).

Table 2
TPC as mg/g of CAE in date seed using different solvents.

Date variety	mg/g CAE of total phenol			
	Acetone	Methanol	Ethanol	Water
Fardh	1.58 ± 0.06[a]	1.46 ± 0.04[a]	1.32 ± 0.21[a]	1.24 ± 0.01[a*]
Khalas	1.50 ± 0.07[a]	1.44 ± 0.03[a]	1.45 ± 0.03[b]	1.08 ± 0.01[b*]
Khinazi	1.45 ± 0.01[a]	1.43 ± 0.01[a]	1.33 ± 0.21[a]	0.85 ± 0.03[c*]
Khasab	1.41 ± 0.16[a]	1.44 ± 0.05[a]	1.51 ± 0.01[b]	1.16 ± 0.18[b**]
Naghal	1.48 ± 0.06[a]	1.40 ± 0.03[a]	1.44 ± 0.04[b]	1.29 ± 0.04[a*]

Values are mean ± SD ($n = 3$). Means within a column with no common letter differ significantly ($P < 0.05$); *: Within a horizontal row differ significantly ($P < 0.05$) from other extracts; **: Within a horizontal row differ significantly ($P < 0.05$) from ethanol extract.

3.4. In vitro antioxidant activity

The antioxidant activity of four crude extracts of date pits was investigated by ferric reducing power assay method and by two other commonly used radical scavenging methods such as DPPH and H_2O_2. The scavenging effect of pit extracts on the DPPH and peroxide free radicals were expressed as % inhibition and were compared with standard antioxidant, ascorbic acid.

Ascorbic acid showed a dose dependent scavenging activity of DPPH radical, inhibiting 93.83% of free DPPH radicals at a concentration of 100 μg/mL. The IC_{50} value of ascorbic acid was found to be 13.68 μg/mL. The results of antioxidant activity of date pits presented in Table 3 indicated that the % inhibition of DPPH varied with the polarity of solvent. All the solvents exhibited moderate to good antioxidant activity by DPPH method. For Fardh, Khasab and Khinazi date pits, acetone extract exhibited the highest antioxidant activity (78.25 ± 2.38, 73.50 ± 8.37 and 70.92 ± 10.16, respectively) while surprisingly, it was the least active extract for Naghal variety (17.21 ± 11.62). Methanol extract displayed better activity than the ethanol and water extracts. In fact, antioxidant activity of aqueous extract for all date varieties except Fardh was noted to be better than the ethanol extract. In general, Fardh and Khasab pits were observed to possess significant antioxidant activity by DPPH scavenging method. Acetone and ethanol extract of Naghal variety appeared to be the weakest antioxidant but methanol and water extract showed promising activity. The highest inhibition of DPPH for each date variety in a particular solvent were observed as follows: Fardh in acetone (78.25), Khalas in water (66.27), Khinazi in acetone (70.92), Khasab in acetone (73.50) and Naghal in methanol (69.44). A significant statistical difference in activity of each extract was observed.

No significant difference in % inhibition of H_2O_2 by ascorbic acid was noted at a concentration of 25 μg/mL (55.76%) and 100 μg/mL (58.68%) or higher concentration of 200 μg/mL (59.33%). The H_2O_2 scavenging ability of prepared date pit extracts was shown in Table 4. The antioxidant activity of all extracts at a concentration of 5 mg/mL was found to be between 24.49 and 40.75 *i.e.* their activity was comparable to ascorbic acid at a concentration of 5–10 μg/mL (30.90%–46.15%). In other words, the percent inhibition produced by ascorbic acid at concentration of 25 μg/mL was much greater than the scavenging activity of all date pit extracts at a concentration of 5 mg/mL. Acetone and water extracts exhibited better inhibition as compared to alcoholic extracts. In terms of H_2O_2 scavenging activity of Khinazi, Khasab and Naghal date pits, acetone appeared to be the best solvent while methanol and water seemed to be ideal for Fardh and Khalas, respectively. The highest antioxidant activity was exhibited by aqueous extract of

Table 3
Antioxidant activity of date seed extracts at 5 mg/mL concentration by DPPH method.

Date variety	% Inhibition of DPPH			
	Acetone	Ethanol	Methanol	Water
Fardh	78.25 ± 2.38[a**φ]	63.63 ± 4.75[a ψφγ]	77.95 ± 5.36[a**φ]	50.15 ± 10.53[a**ψγ]
Khalas	56.83 ± 5.10[b**ψφ]	40.56 ± 7.74[bψφγ]	65.51 ± 7.03[b**γ]	66.27 ± 6.93[b**γ]
Khinazi	70.92 ± 10.16[c**φ]	44.20 ± 6.72[bψφγ]	70.77 ± 1.72[c**φ]	56.47 ± 6.64[c**ψγ]
Khasab	73.50 ± 8.37[d**]	50.48 ± 6.74[cψφγ]	69.36 ± 2.78[c**]	69.54 ± 2.64[d**]
Naghal	17.21 ± 11.62[e**ψφ]	32.12 ± 7.08[dψφγ]	69.44 ± 2.04[c**φγ]	55.58 ± 5.01[c**ψγ]

Values are mean ± SD ($n = 3$). Means within a column with no common letter differ significantly ($P < 0.05$); **: Within a horizontal row differ significantly ($P < 0.05$) from ethanol; ψ: Within a horizontal row differ significantly ($P < 0.05$) from methanol; φ: Within a horizontal row differ significantly ($P < 0.05$) from water; γ: Within a horizontal row differ significantly ($P < 0.05$) from acetone.

Table 4
Antioxidant activity of date seed extracts at 5 mg/mL concentration by H_2O_2 scavenging assay method.

Date variety	% Inhibition of H_2O_2			
	Acetone	Ethanol	Methanol	Water
Fardh	28.62 ± 3.90^{a}	27.12 ± 1.50^{a}	33.82 ± 1.34^{a}	31.96 ± 0.60^{a}
Khalas	$28.82 \pm 1.18^{a\varphi}$	$26.35 \pm 1.50^{a\varphi}$	$30.49 \pm 0.54^{b\varphi}$	$40.75 \pm 1.43^{b**\psi\gamma}$
Khinazi	$32.11 \pm 2.14^{b**}$	$24.49 \pm 1.41^{a\gamma}$	28.36 ± 3.10^{b}	30.46 ± 0.83^{a}
Khasab	32.93 ± 1.08^{b}	27.98 ± 1.06^{a}	29.51 ± 2.96^{b}	31.35 ± 1.14^{a}
Naghal	$35.89 \pm 7.71^{b**}$	$26.49 \pm 2.40^{a\gamma}$	29.56 ± 1.47^{b}	31.74 ± 3.03^{a}

Values are mean ± SD (n = 3). Means within a column with no common letter differ significantly ($P < 0.05$); **: Within a horizontal row differ significantly ($P < 0.05$) from ethanol; $^{\psi}$: Within a horizontal row differ significantly ($P < 0.05$) from methanol; $^{\varphi}$: Within a horizontal row differ significantly ($P < 0.05$) from water; $^{\gamma}$: Within a horizontal row differ significantly ($P < 0.05$) from acetone.

Khalas (40.75 ± 1.43) and lowest activity by ethanol extract of Khinazi pits.

The reducing power of the date pit extracts at a concentration of 5 mg/mL was determined using the potassium ferricyanide reduction method and the results were given in Table 5. Ascorbic acid was used a positive control and a standard curve was plotted to show the increase in ferric reducing power at different concentration (Figure 1). A linear relationship was obtained in the concentration ranging from 10 to 100 μg/mL with a regression coefficient (r^2) of 0.979 4. The highest % increase in reducing power of Khinazi in acetone (81.80%) and Khasab in ethanol (80.88%) was found to be comparable to ascorbic acid (85.36%) obtained at 100 μg/mL. In general, it was observed that the reducing power of organic extracts for all date pits were better than the aqueous extract. The activity of acetone, methanol and ethanol extracts at tested concentration was very much similar to the ascorbic acid at concentration of 75 μg/mL. It was interesting to note that reducing power of aqueous extract of Fardh pits (67.39%) was significantly different from aqueous extract of other date varieties (31.57%–41.65%).

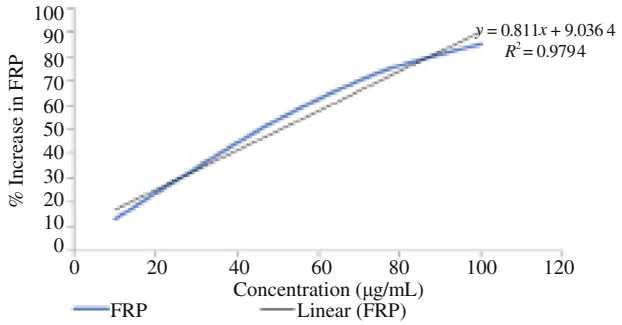

Figure 1. Plot showing increase in ferric reducing power of standard ascorbic acid solution at different concentration.
FRP: Ferric reducing power.

3.5. In vitro antidiabetic activity

In vitro antidiabetic activity of the date pits was investigated by studying the inhibitory effects on the level of α-amylase and α-glucosidase. Acarbose was used as a standard drug to compare the inhibitory effects.

The standard curve of the inhibitory effects of acarbose (12.5–200 μg/mL) against intestinal α-glucosidase showed the dose dependent activity (r^2 = 0.952 9). The IC_{50} value of acarbose was found to be 137.64 μg/mL. At concentration of 5 mg/mL, pits extract of five date varieties exhibited moderate to good inhibitory activity, ranging from 5.91% to 51.71% (Table 6). It was quite evident from the results that solvent had pronounced effect on the inhibitory activity of α-glucosidase. Water extract by far exhibited superior % inhibition (34.46 ± 2.33 to 51.71 ± 8.20) in comparison to the organic extracts (5.91 ± 0.65 to 42.40 ± 6.43). It was observed to be the best solvent for all date varieties in exhibiting potent *in vitro* antidiabetic activity by α-glucosidase inhibition method followed by methanol and ethanol. It could be concluded that the inhibitory effect of water extract at 5 mg/mL was approximately equivalent to IC_{50} value of acarbose *i.e.*, 137.64 μg/mL. Interestingly, acetone extract exhibited the weakest activity (5.91%–24.78%) among all pit extracts with Fardh and Naghal showing the highest and the lowest activity. For Khalas, Khinazi and Naghal, the order of solvents according to activity from the highest to lowest was as follows: water > methanol > ethanol > acetone; for Fardh it was, water > acetone > ethanol > methanol and for Khasab it was water > methanol > acetone > ethanol.

The IC_{50} value calculated from the standard curve of acarbose (12.5–200 μg/mL) on the inhibitory effects on the level of α-amylase was found to be 95.37 μg/mL. The results presented

Table 5
Ferric reducing power of date seed extracts at 5 mg/mL concentration.

Date variety	% Increase in reducing power			
	Acetone	Ethanol	Methanol	Water
Fardh	$68.39 \pm 1.00^{a,b}$	$73.84 \pm 0.98^{a,b}$	67.40 ± 0.18^{a}	67.39 ± 3.49^{a}
Khalas	$76.37 \pm 3.75^{a\varphi}$	$75.82 \pm 2.30^{a\varphi}$	$72.15 \pm 2.44^{a\varphi}$	$34.11 \pm 1.94^{b**\psi\gamma}$
Khinazi	$81.80 \pm 0.83^{a,c\varphi}$	$79.71 \pm 3.05^{a\varphi}$	$79.05 \pm 0.15^{b\varphi}$	$41.65 \pm 0.61^{b**\psi\gamma}$
Khasab	$70.26 \pm 7.53^{a**\varphi}$	$80.88 \pm 2.21^{a,c\gamma\varphi}$	$74.26 \pm 10.28^{a\gamma\varphi}$	$31.57 \pm 15.82^{b**\psi\gamma}$
Naghal	$69.76 \pm 5.75^{a\varphi}$	$77.03 \pm 2.03^{a\varphi}$	$74.83 \pm 5.82^{a\varphi}$	$34.07 \pm 7.14^{b**\psi\gamma}$

Values are mean ± SD (n = 3). Means within a column with no common letter differ significantly ($P < 0.05$); **: Within a horizontal row differ significantly ($P < 0.05$) from ethanol; $^{\psi}$: Within a horizontal row differ significantly ($P < 0.05$) from methanol; $^{\varphi}$: Within a horizontal row differ significantly ($P < 0.05$) from water; $^{\gamma}$: Within a horizontal row differ significantly ($P < 0.05$) from acetone.

Table 6

α-Glucosidase inhibitory activity of date seed extracts at 5 mg/mL concentration.

Date variety	% Inhibition of α-glucosidase			
	Acetone	Ethanol	Methanol	Water
Fardh	$24.78 \pm 3.45^{a\psi\varphi}$	$21.21 \pm 2.43^{a\psi\varphi}$	$10.91 \pm 1.98^{a^{**}\gamma\varphi}$	$34.46 \pm 2.33^{a^{**}\psi\gamma}$
Khalas	$10.91 \pm 1.32^{a^{**}\psi\varphi}$	$18.56 \pm 1.54^{a\psi\varphi\gamma}$	$38.26 \pm 3.50^{b^{**}\gamma}$	$46.90 \pm 6.33^{b^{**}\gamma}$
Khinazi	$8.26 \pm 0.98^{a,b^{**}\psi\varphi}$	$33.87 \pm 2.65^{a\gamma\varphi}$	$39.09 \pm 5.20^{b\gamma}$	$47.51 \pm 4.98^{b^{**}\psi}$
Khasab	$16.97 \pm 1.45^{a\psi\varphi}$	$10.92 \pm 0.32^{a,b\psi\varphi}$	$42.40 \pm 6.43^{b,c^{**}\gamma\varphi}$	$51.71 \pm 8.20^{b,c^{**}\psi\gamma}$
Naghal	$5.91 \pm 0.65^{a,c\psi\varphi}$	$9.93 \pm \pm 0.55^{a,c\psi\varphi}$	$31.12 \pm 4.31^{b,d^{**}\gamma}$	$36.59 \pm 2.69^{a^{**}\gamma}$

Values are mean ± SD (n = 3). Means within a column with no common letter differ significantly ($P < 0.05$); **: Within a horizontal row differ significantly ($P < 0.05$) from ethanol; $^{\psi}$: Within a horizontal row differ significantly ($P < 0.05$) from methanol; $^{\varphi}$: Within a horizontal row differ significantly ($P < 0.05$) from water; $^{\gamma}$: Within a horizontal row differ significantly ($P < 0.05$) from acetone.

in Table 7 indicated the percentage inhibition shown by water extract (13.71%–51.45%) at concentration of 5 mg/mL was greater than all other extracts (1.44%–26.23%). Further, it could be seen that water was the best solvent for displaying antidiabetic activity by inhibition of α-amylase. It exhibited approximately 2, 2.24 and 8.21 folds greater activity for Fardh pits than methanol, ethanol and acetone extracts, respectively. The inhibitory activities for water, methanol, ethanol and acetone were found in the range of 13.71%–51.45%, 8.55%–26.23%, 4.96%–22.99% and 1.44%–6.26%, respectively. Thus, it could be inferred that water was almost 2–3 folds better than ethanol and methanol and approximately 7–10 folds better than acetone solvent. The inhibitory effects on the level of α-amylase by water and acetone extracts of date varieties from highest to lowest was observed to be in the following order: Fardh > Khasab > Khinazi > Khalas > Naghal; for methanol it was, Fardh > Khinazi > Khalas > Khasab > Naghal; for ethanol it was, Fardh > Khalas > Khinazi > Khasab > Naghal. Thus, Fardh was the most potent and Naghal was the least potent varieties in all solvent extracts (Table 7).

Table 7

α-Amylase inhibitory activity of date seed extracts at 5 mg/mL concentration.

Date variety	% Inhibition of α-amylase			
	Acetone	Ethanol	Methanol	Water
Fardh	$6.26 \pm 2.33^{a^{**}\psi\varphi}$	$22.99 \pm 6.20^{a\varphi\gamma}$	$26.23 \pm 8.49^{a\gamma\varphi}$	$51.45 \pm 5.29^{a^{**}\psi\gamma}$
Khalas	$3.37 \pm 1.43^{a^{**}\psi\varphi}$	$15.33 \pm 1.30^{a\varphi\gamma}$	$15.39 \pm 7.01^{a\gamma\varphi}$	$33.31 \pm 1.39^{b^{**}\psi\gamma}$
Khinazi	$4.91 \pm 3.38^{a^{**}\psi\varphi}$	$14.36 \pm 6.68^{a\varphi\gamma}$	$19.98 \pm 6.14^{a\gamma\varphi}$	$41.69 \pm 2.08^{b,c^{**}\psi\gamma}$
Khasab	$7.66 \pm 4.60^{a\varphi}$	$12.92 \pm 2.66^{a\varphi}$	$13.40 \pm 1.35^{a\varphi}$	$48.93 \pm 1.30^{a^{**}\psi\gamma}$
Naghal	$1.44 \pm 0.43^{a\psi\varphi}$	$4.96 \pm 2.11^{a,b\varphi}$	$8.55 \pm 0.84^{a,b\gamma}$	$13.71 \pm 4.23^{d^{**}\gamma}$

Values are mean ± SD (n = 3). Means within a column with no common letter differ significantly ($P < 0.05$); **: Within a horizontal row differ significantly ($P < 0.05$) from ethanol; $^{\psi}$: Within a horizontal row differ significantly ($P < 0.05$) from methanol; $^{\varphi}$: Within a horizontal row differ significantly ($P < 0.05$) from water; $^{\gamma}$: Within a horizontal row differ significantly ($P < 0.05$) from acetone.

4. Discussion

P. dactylifera or date palm is one of the oldest and most popular fruit trees in the hot arid regions of the world, particularly in the gulf countries of the Middle East [25]. Date fruits are popular worldwide due to their taste, health benefits and nutritional values owing to the presence of good amount of essential nutrients such as sugars, proteins, fibers, trace elements, *etc.*, thus forming an important part of the daily diet, especially in Oman [26]. A high degree of biodiversity has been reported in size, shape, color, texture, quality and chemical composition among numerous date fruits varieties grown in different parts of the Sultanate which probably could be due to difference in cultivation time, harvesting time, geographical conditions and other environmental factors, *etc* [4]. Thus, it could be hypothesized that this variation might also affect the chemical composition and other physical characteristics of disposed waste or pits of dates. Therefore, the present study was undertaken to investigate the beneficial actions of pits of five date (*P. dactylifera*) varieties widely grown and consumed in Oman by assessing their physical characteristics, *in vitro* antioxidant activity, TPC and inhibitory effects against α-amylase and α-glucosidase enzymes.

The selected date cultivars showed variation in the physical characteristics such as average weight of date fruit, fruit pulp, date pit, fruit flesh percentage, total number of fruits/kg and pulp/pit ratio which is mainly due to genetic variations, different environmental and growth conditions. A good quality date is generally characterized by a high flesh percent and fruit/pit ratio. Though Fardh is commonly used for industrial purposes and is a moderate quality date, it showed the highest pulp and flesh/pit ratio, even much higher than the Khalas, a premium quality date. Similarly, the other two moderate quality dates, Naghal and Khinazi had the greatest pit weights as expected.

The preliminary phytochemical screening was also done to confirm the presence of various classes of biologically active plant metabolites in date pit extracts which revealed the presence of poly phenols (tannins), carbohydrates, proteins and amino acids. Numerous *in vitro* studies have suggested that antioxidant potential and other useful biological actions of medicinal plants could be attributed to their high phenolic contents [27]. The phenolic compounds by virtue of their reducing properties can absorb and neutralize free radicals, quench singlet and triplet oxygen, or decompose peroxides to act as antioxidant [28]. Therefore, the TPC of date pits and effect of solvents of varying polarity in extraction of TPC was determined by using a colorimetric reagent Folin–Ciocalteu, which reacts non specifically with phenolic compounds and forms a complex to be measured at 765 nm [29].

The TPC determined in different solvent extracts of date pits is expressed as CAE and ranges from 0.85 to 1.58 mg/g of dry date pit powder. Al Harthy *et al.* determined the TPC of four date fruits native to Oman and it ranged from 32.24 to 35.84 mg/100 g of CAE. Therefore, it can be deduced that phenolic content of date pits is lower than the date fruits [20]. It was observed that organic solvents, namely, acetone, ethanol and methanol are significantly better than water in extracting phenolic compounds from the date seeds. This difference is mainly due to their polarity and good solubility for phenolic components from plant materials [30]. Acetone extract showed the highest content of total phenol followed by ethanol and methanol. A similar study conducted on different varieties of date pits also reported that organic solvents extract phenolic compounds better than water; however, they observed ethanol to be the best solvent followed by acetone and methanol [14].

A number of studies have ascribed the antioxidant activity of medicinal plants to the presence of polyphenolic compounds. Also natural antioxidants are known to play an important role in the prevention of many age-related diseases and promotion of health. Due to the complexity of the antioxidant mechanism, it is always preferred to adopt the multi-method approach to test the antioxidant activity [31]. The antioxidant activity of date seeds was therefore, evaluated by reducing power assay method and against DPPH and H_2O_2 anion radicals by *in vitro* experiment. Positive control, ascorbic acid significantly scavenged the DPPH and H_2O_2 radicals and also showed increased reducing power in a concentration dependent manner. Though all the tested extracts exhibited moderate to good antioxidant activity, a significant difference ($P < 0.05$) in the activity of different solvents was observed by One way ANOVA. The acetone extract showed better antioxidant activity as compared to other extracts which could be due to the fact that extraction of antioxidant compounds is solvent dependent or in other words it depends on the TPC. Therefore, this difference in antioxidant activity could be attributed to variation in their TPC [22]. Several other studies have also shown a good correlation between TPC and antioxidant activity [32]. In contrary to this, water extract of Khalas and Khasab in DPPH assay method, Khalas against H_2O_2 and Fardh in ferric reducing power assay also showed potent antioxidant activity suggesting that phenols are not the only phytochemicals responsible for antioxidant activity, and there might be some other water soluble non phenolic phytoconstituents which contribute to the antioxidant activity. Nevertheless, acetone seems to be the best choice for the extraction of phenolic compounds irrespective of the date variety, vis-a-vis it exhibited high antioxidant activity. Thus, it could be concluded that date seeds can serve as a natural source of natural antioxidant due to their phenolic content.

Diabetes mellitus is a chronic metabolic disorder affecting millions of people worldwide. Its prevalence is on rise globally at an alarming rate which makes it as one of the major growing health problem [33]. Oral hypoglycemic agents and insulin are the main components of antidiabetic therapy in addition to life style modification. One of the therapeutic strategies to control postprandial hyperglycemia in diabetics is to retard the hydrolysis of carbohydrates by the inhibition of α-glucosidase and α-amylase enzymes in order to slow down the intestinal absorption of glucose [34]. These two enzymes, α-glucosidase and α-amylase, in the digestive tract are responsible for the hydrolysis of starch and disaccharides to glucose and breakdown of long chain carbohydrates, respectively. The inhibitors of these enzymes are considered as the potential targets in the management of diabetes mellitus.

Many natural products of phenolic nature have been shown to inhibit the activity of α-glucosidase and α-amylase. Moreover, a positive relationship between the phenolic content and inhibitory activity has been reported [16,17]. In the present study, aqueous extract of almost all date pits exhibited significant α-glucosidase and α-amylase inhibition activity by indicating the presence of natural α-glucosidase and α-amylase inhibitors in date pits. However, unlike a previous study, no direct correlation between the content of total phenols or antioxidant activity and inhibitory activity against α-glucosidase and α-amylase could be observed [35]. Contrary to this, water extract which extracted the lowest amount of phenolic components exhibited the best inhibitory activities. Thus, it could be proposed that date pits contain some water soluble non phenolic bioactive compounds in addition to phenolic acids and flavonoids, which have the ability to act as α-glucosidase and α-amylase inhibitors. Thus, it can be concluded that date pits might prove promising and beneficial in lowering the blood glucose in diabetics by inhibiting the digestion and suppressing the hydrolysis of dietary carbohydrates. Further, detailed phytochemical investigation of date pits can provide a lead compound that can be used effectively in the prevention and management of diabetes and its complications.

The results of the present study confirmed that disposed waste of Omani dates is a rich source of dietary antioxidant because of its high TPC. Acetone was found to be a better solvent than alcohol or water for extraction of phenolic compounds from the date pits and for exhibiting antioxidant activity. Pits of all date varieties possess significant *in vitro* antidiabetic activity. However, no direct correlation was observed in the antidiabetic activity and the antioxidant activity. Water extract displayed the maximum inhibition of α-glucosidase and α-amylase suggesting presence of some non phenolic water soluble compounds as the potential inhibitor of these enzymes. The pits due to their inhibitory effects on α-glucosidase and α-amylase level could be used as a monotherapy along with an appropriate diabetic diet and exercise or might be in conjunction with antidiabetic therapy to manage and prevent progression of diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

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