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Assessment of Phenolic Contents and Antioxidant and Antibacterial Activities of Extracts from Four Varieties of Iranian Date Palm (*Phoenix dactylifera* L.) Seeds

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Abstract

Background and objective: Every year, large quantities of date palm seeds are produced as byproducts in date processing and packaging industries, which is discarded or used as low-value materials for animal feeds and composts. However, these bioresources may include potentials to produce high-value added products in food industries. The major aim of the current study was to assess phenolic profiles and contents and antioxidant and antibacterial activities of four Iranian date palm seed extracts, namely Zahedi, Kabkab, Mazafati and Rabbi.

Material and methods: Total phenolic contents, phenolic compounds profile and antioxidant and antibacterial activities of extracts from four Iranian date palm seeds were assessed using Folin-Ciocalteu, reversed-phase high-performance liquid chromatography, 2, 2-diphenyl-1-picrylhydrazyl radical scavenging, agar disc diffusion and broth microdilution methods, respectively.

Results and conclusion: Total phenolic contents varied 1480-3380 mg GAE 100 g^{-1} dw. cinnamic, chlorogenic, caffeic and 3, 5-dihydroxybenzoic acid included the primary phenolic compounds, respectively. Of the varieties, Kabkab and Mazafati seed extracts with IC_{50} values of 16.56 and 22.6 µg ml^{-1} demonstrated the highest and lowest radical scavenging activity, respectively. Results obtained from disc diffusion method revealed that all extracts included inhibitory effects against *Staphylococcus aureus*, but not against *Escherichia coli*. Minimum inhibitory concentration and minimum bactericidal concentration of the extracts ranged 1.56-3.125 and 3.125-12.5 mg ml^{-1} for *Staphylococcus aureus*, respectively. Based on the findings, Iranian date seeds are good sources of extractable phenolic compounds with notable antioxidant activities, which can be used as natural additives in formulations of various products such as functional foods and dietary supplements. Furthermore, these seeds can be converted to value added products through biotechnological processes.

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1. Introduction

It is suggested that the date palm (*Phoenix dactylifera L.*) is one of the earliest planted trees in world [1,2]. It is cultivated in most tropical and subtropical regions worldwide, including Middle East, North Africa, the United States and Southern Europe [3]. One of these regional countries, Iran, produces more than 1 million tons of dates annually; ranked highest after Egypt, Algeria, Saudi Arabia and Iraq [4]. The edible part of date fruit is pericarp surrounding the seed. Date seed, as one of the major byproducts produced during processing operations, constitutes approximately 10-15% of the total fruit mass [5]. Currently, date seeds are discarded or used in limited scales

in animal feeds and natural fertilizers. However, there are possibilities of producing high-value added products from these byproducts [6]. Date palm seeds (mainly in the form of hydrolysates) have been used as solid substrates for the production of various fermented products, including antibiotics (such as nisin and oxytetracycline), single cell proteins, citric acids and biofuels (from seed lipids) [7-11]. Most importantly, it is possible to produce valuable functional foods from date seed extracts [12]. These products include environmental and economic advantages for the farmers and food industries. Based on the phytochemical analysis, date seeds contain natural compounds such as carbohydrates, oils, dietary fibers, minerals and vitamins [13-14]. Moreover, biologically active compounds such as phenolic acids, anthocyanins, carotenoids, sterols and flavonoids are other bioactive components of the date seeds, which offer health improvement properties such as anti-carcinogenic [15], antioxidant [16], anti-mutagenicity [17], antibacterial and anti-inflammatory effects [18-19]. Use of natural bioactive compounds extracted from plant sources is rising in food products, mainly due to the public concerns on adverse effects of artificial additives and preservatives on human health [20]. Although studies have been carried out on date palm seed properties, there are no recently published studies on compositions and characteristics of the Iranian date seeds. The major aim of this study was to assess and compare total phenolic contents and antioxidant and antibacterial activities of four Iranian date seed varieties, namely Zahedi, Kabkab, Mazafati and Rabbi.

2. Materials and methods

2.1. Materials and bacterial strains

Folin-Ciocalteau phenol reagent, 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), gallic acid, cinnamic acid, caffeic acid, ferulic acid, vanillic acid, dihydroxybenzoic acids and chlorogenic acid were purchased from Sigma-Aldrich (Deisenhofer, Germany). Methanol, dimethyl sulfoxide (DMSO), hexane and butylhydroxytoluene (BHT) and culture media (including Mueller-Hinton agar, tryptic soy broth and tryptic soy agar) were purchased from Merck (Germany). *Staphylococcus* (*S.*) *aureus (*ATCC 29213*)* and *Escherichia* (*E.*) *coli* O157:H7 (ATCC 35218) were provided by the Iranian Research Organization for Science and Technology (Iran).

2.2. Date seeds

Four Iranian date varieties (Kabkab, Rabbi, Zahedi and Mazafati) were provided by the Iranian Date Palm and Tropical Fruits Research Center, Ahwaz, Iran. Date seeds were removed from the fruits and washed to remove impurities. Pits were dried in oven at 40°C for 24 h and then completely grounded to make fine powders using laboratory scale mortar grinder (Pulverisette, Fritsch, Germany) [21]. These powders were stored at -40°C in deep freezer (Media, WHS-109 FW1, USA) until use [22].

2.3. Preparation of the date palm pit extracts

Extracts of date seeds were prepared based on a previously described method by Al-Farsi and Lee with modifications [23]. Briefly, 1 g of each date pit powder was soaked in 20 ml of hexane. After rinsing, these were extracted at 40°C for 2 h using 60 ml of 80% ethanol and stirring rate of 120 rpm. During the extraction process, conical flasks were covered with aluminum foils to prevent solvent evaporation and light-induced changes. Mixtures were centrifuged at 3913 ×g for 20 min at room temperature. Supernatants were then filtered through Whatman No. 1 filter papers. Filtered extracts were concentrated to dryness at 40°C and reduced pressure using rotary evaporator (HS 2005s Hahnshin Scientific, South Korea). The remaining extracts were stored at -20°C until use.

2.4. Assessment of total phenolic content

Phenolic contents of the date seed extracts were assessed based on the method of Al-Farsi and Alasalvar [24]. Briefly, 200 µl of the seed extracts were mixed with 1.5 ml of Folin-Ciocalteau reagent (10-fold diluted). After incubation of the mixtures at 22°C for 5 min, 1.5 ml of sodium bicarbonate solution (60 g l^{-1}) were added to the mixture. After 90 min incubation at 22°C, absorbance of the mixtures was measured at 725 nm using UV-Vis Absorption 1601 Spectrophotometer (Shimadzu, Japan). Total phenolic contents were assessed using calibration curve (R^2 = 0.99) of gallic acid as standard (0.001-0.006 mg ml^{-1}). Total phenolic contents of the extracts were expressed as mg of gallic acid equivalent per 100 g of dry weight of the date seeds (mg GAE 100 g^{-1} dw).

2.5. Assessment of antioxidant activity

Antioxidant activity of the seed extracts was assessed using DPPH free radicals scavenging method based on a method described by Bakhtiyari et al. [25]. Various concentrations of each extract and BHT (as reference compound) were prepared in DMSO. After adding 50 µl of the prepared solutions to microplates, 200 µl of DPPH in methanol (100 µM) were added to microplates. Reaction mixtures were stored at darkness for 30 min at room temperature. Absorbance of the mixtures was read immediately at 517 nm using BioTek XS2 PowerWave Microplate Reader (BioTek Instruments, USA). Control included 50 µl of DMSO and DPPH solutions and blank included pure methanol. The percentage of inhibition for each extract was calculated based on the following equation 1:

$$\text{Inhibition (\%)} = [1 - (A_{extraxt} - A_{blank}) / A_{control}] \times 100 \quad \textbf{Eq. 1}$$

Each extract was prepared in three replicates and the mean values of data were used for the calculation of IC_{50}. The IC_{50} values of antioxidants were calculated from the calibration curve [26].

2.6. Analysis of phenolics by HPLC-RP

Phenolic compounds were analyzed using HPLC-RP system with UV-photodiode array detector (Smartline 2800, Knauer, Germany). Compounds were separated on a C-18 reverse phase column (Waters Spherisorb S5 ODS2, 250 × 4.6 mm, particle size of 5 µm). The HPLC profiles of phenolic compounds were monitored at 254, 275, 305 and 320 nm. Injection volume of the extract included 20 µl. The mobile phase included methanol (99.5% v v^{-1}) with 0.05% of trifluoroacetic acid (TFA) as Eluent A and H_2O with 0.05% of TFA as Eluent B. The flow rate included 0.5 ml min^{-1} and the gradient conditions were programmed as 0–10 min, 5% A; 10-20 min, 5–25% A; 20–30 min, 25–35% A; 30-48 min, 35-45% A; 48-50 min, 45-90% A; 50-52 min, 90-95% A; 52-70 min; 95-5% A; and 70-80 min, 5-100% A. Total time for the analysis of samples included 80 min [27]. Method for the quantification of phenolic compounds included external standard procedure. Used standards included gallic, cinnamic, 3,4- dihydroxybenzoic (DHB), vanillic, chlorogenic, caffeic, and 2,5-dihydroxybenzoic acid. Six various concentrations of the standard stock solutions were prepared and injected to HPLC and then peak areas of chromatograms were plotted against concentrations of the stock solutions to prepare calibration curves. These curves were used to calculate concentrations of polyphenols in the samples. For each calibration curve, the best fit line was determined using linear regression analysis. Contents of the identified polyphenols were calculated from the regression equations of the respective curves. The correlation of coefficient (r) for the regression equations included 0.993-0.997.

2.7. Assessment of antibacterial activity

2.7.1. Bacterial strains

Antibacterial activity of the date seed extracts was assessed against *S. aureus* and *E. coli* O157:H7. Bacterial suspensions from overnight culture at 37°C were prepared in tryptic soy broth . Turbidity of the bacterial cultures was adjusted to 0.5 McFarland standard (1.0×10^8 CFU ml^{-1}) [28].

2.7.2. Disc diffusion method

Antibacterial activity of the extracts against *S. aureus* and *E. coli* O157:H7 was assessed using disc diffusion method [28,29]. All extracts were prepared in sterile conditions and dissolved in distilled water to prepare concentrations of 200 mg ml^{-1}. These were sterilized through Millipore membrane filters with pore sizes of 0.45 µm. Then, 10 µl of the prepared extracts were dropped onto sterile paper discs (6 mm in diameter). The solvent control (water) did not show any antimicrobial activity. Commercial discs of ampicillin (10 µg) were used as positive control. Then, 100 µl of the overnight incubated bacterial cultures (10^8 CFU ml^{-1}) were spread over the Muller Hinton agar plates. Discs were placed on the inoculated plates and incubated at 37°C for 24 h. Zones of inhibition were measured and expressed in millimeters.

2.7.3 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

Based on the results from disk diffusion method, MIC of the seed extracts against *S. aureus* was assessed using broth microdilution technique described by Hammer et al. and Mahmoudzadeh et al. [30,31]. Stock solutions of the extracts (200 mg ml^{-1}) were prepared in 5% of DMSO and sterilized through 0.45 µm filter papers. From these stock solutions, double serial dilutions in tryptic soy broth (100-0.2 mg ml^{-1}) were prepared using microtiter trays with 96 U-bottom wells. Bacteria cultures at a final concentration of 10^8 CFU ml^{-1} were inoculated into each well containing 400 µl of the dilutions. Wells containing bacteria in media and without extracts were considered as positive and those containing 5% of DMSO as negative controls. Bacterial growth was assessed by measuring turbidity at 600 nm using Bioscreen C FB-1100 (MBR, Helsinki, Finland) after incubation at 37°C for 24 h. For MBC, 10 µl of broth from each well with no visible bacterial growth were streaked on Mueller-Hinton agar plates. After incubation at 37°C for 24 h, the lowest concentration of the extracts that completely inhibited the bacterial growth was considered as MBC.

2.8. Statistical analysis

All analyses were carried out in three replicates and expressed as means ±SD (standard deviation). One way of analysis of variance was used to compare the groups and significance of differences was reported using Duncans post hoc test at $P \leq 0.05$. All the analyses were carried out using SPSS Software v.10.1 (SPSS, Chicago, IL).

3. Results and discussion

3.1. Assessment of total phenolic content

Phenolic contents of the date seed extracts were assessed using Folin-Ciocalteu method. This method has been chosen because of its low-cost, simplicity, repeatability and rapidity [32]. Calculated phenolic contents for the date pit extracts ranged 1483-3377 mg GAE 100 g^{-1} dw (Fig. 1). The highest phenolic content was observed in Kabkab variety seeds (3377 ±0.45 mg GAE 100 g^{-1} dw) followed by Zahedi (3310 ±0.27 mg GAE 100 g^{-1} dw), Rabbi (2423 ±0.50 mg GAE 100 g^{-1} dw) and Mazafati (1483 ±0.60 mg GAE 100 g^{-1} dw). Based on Marshall et al. findings [33], total phenolic contents of three date seed varieties supplied in the UK market (namely Deglet Nour, Khouat Allig and Zahedi) ranged 2058-2983 mg GAE 100 g^{-1} w. In another study, Al Farsi et al. [13] studied the phenolic contents of syrups and

byproducts (press cakes and seeds) of three Omanian date varieties. Results showed that the total phenolic content of seeds (3102-4430 mg GAE 100 g^{-1} w) was higher than those found in date syrups and byproducts (press cakes). However, the phenolic content reported in the current study was higher than those reported in Mansouri et al. [21] study (661 and 572 mg GAE 100 g^{-1} dw for Deglet Noor and Medjool varieties, respectively) and Juhaimi and Ghafoor [34] (1.98 and 4.65 mg GAE 100 g^{-1} dw for Barhi and Soughi varieties, respectively). Variations in results are mainly linked to date varieties, extraction methods, physiological conditions of fruits (e.g. stage of ripening) and growing conditions (e.g. growing seasons, soil fertilization and cultivation place) [13,35]. It can be suggested that date seeds are good sources of phenolic compounds including phenolic acids and their derivatives.

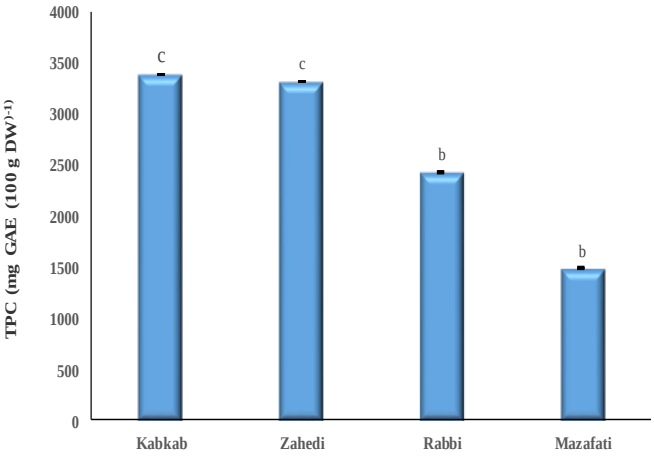


Figure 1. Total phenolic contents of four Iranian date palm seed extracts. Values are expressed as the means of three replicates and error bars represent standard deviation (SD) (n = 3). Means with a different superscript letter in each column are significantly different at P≤0.05

3.2. Phenolic profiles of the extracts

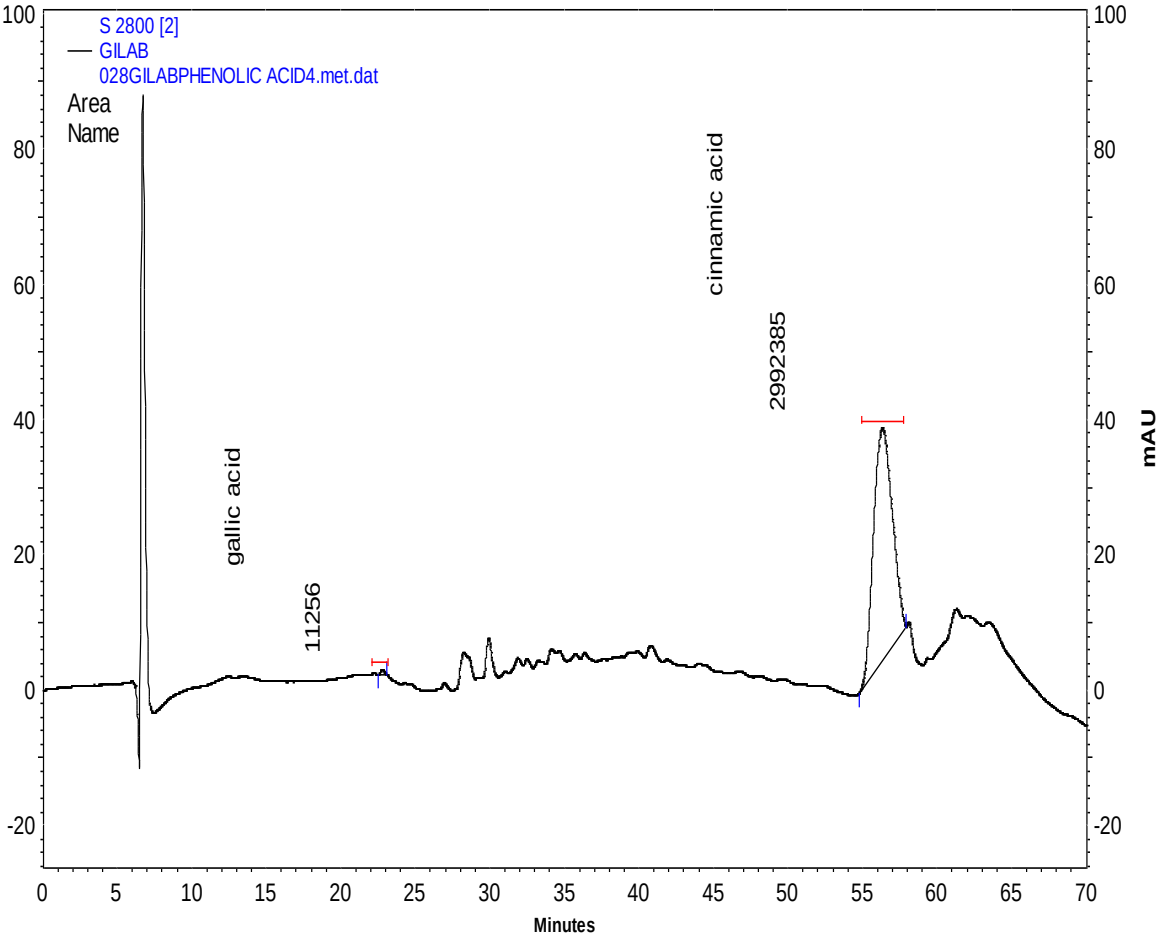
Seven phenols of chlorogenic acid, caffeic acid, vanillic acid, gallic acid, cinnamic acid, 3,5-DHB and 2,5-DHB were identified and quantified in four varieties of date seeds as listed in Table 1. Results of HPLC analysis demonstrated all phenolic compounds in the extracts. Fig. 2. Shows selected chromatograms of cinnamic acids; the most abundant phenolic compound found in date seed extracts. Concentrations of the phenolic compounds in the extracts varied from one variety to another one. Cinnamic acid was the most abundant phenolic compound found in date seed extracts with the total concentration of 1.54-3.53 mg g^{-1} dw; respectively followed by chlorogenic acid (0.17–0.28 mg g^{-1} dw), caffeic acid (0.17-0.26 mg g^{-1} dw), 3,5-DHB (0.15–0.27 mg g^{-1} dw), vanillic acid (0.11-0.2 mg g^{-1} dw) and gallic acid (0.11-0.18 mg g^{-1} dw). The least phenol quantity belonged to 2,5-DHB with concentrations ranged 0.1-0.17 mg g^{-1} dw. The highest content of phenols belonged to Zahedi seed extract; followed by Kabkab seed extract. In contrast, Mazafati and Rabbi seed extracts included the lowest quantities of phenols with total contents of 2.4 and 2.8 mg g^{-1} dw, respectively. Mansouri et al. [21] reported a similar phenolic profile from seven Algerian date pits. They found that cinnamic acid and its derivatives (di-hydrocinnamic, coumaric and sinapic acids) were the major simple phenols presented in all varieties. Al Juhaimi and Ozcan [36] in a study reported six phenolic compounds including caffeic acid, protocatechuic acid, gallic acid, rutin, catechin and syringic acid in seeds of 12 date varieties from five countries. Similarly, Al-Farsi and Lee [23] found nine phenolic compounds in seeds of Omanian dates. In general, p-hydroxybenzoic, protocatechuic and m-coumaric acids were three major compounds, respectively. Contrary to the findings of previous studies, detected hydroxybenzoic compounds in the current study included the least abundant phenolic acids (Table 1).

Table 1. Phenolic profiles of the four Iranian date seed extracts

	Concentration(mg g^{-1} dw)							
	Gallic acid	Vanillic acid	3,4 DHB[a]	2,5 DHB[b]	Cinnamic acid	Caffeic acid	Chlorogenic acid	Sum of polyphenols
Rabbi	0.12 ±0.08^{c}	0.13 ±0.09^{c}	0.15±0.01^{b}	0.10 ±0.01^{c}	1.54 ±0.09^{b}	0.17 ±0.05^{b}	0.20 ±0.01^{c}	2.41^{c}
Zahedi	0.18 ±0.13^{a}	0.20 ±0.01^{a}	0.27 ±0.02^{a}	0.17 ±0.09^{a}	3.36 ±0.04^{d}	0.26 ±0.06^{d}	0.28 ±0.01^{a}	4.72^{a}
Kabkab	0.16 ±0.01^{b}	0.15 ±0.02^{b}	0.26 ±0.07^{a}	0.14 ±0.05^{b}	3.53 ±0.05^{c}	0.19 ±0.08^{c}	0.17 ±0.09^{b}	4.6^{b}
Mazafati	0.11 ±0.01^{d}	0.11 ±0.03^{d}	0.15 ±0.01^{b}	0.10 ±0.04^{c}	1.90 ±0.03	0.22 ±0.05^{a}	0.21 ±0.01^{c}	2.8^{d}

Results are expressed as mean ±SD (standard deviation) (n = 3). Means with a different lowercase letter in each row are significantly different at P≤0.05
[a]3,4-dihydroxybenzoic acid
[b]2,5-dihydroxybenzoic acid

A



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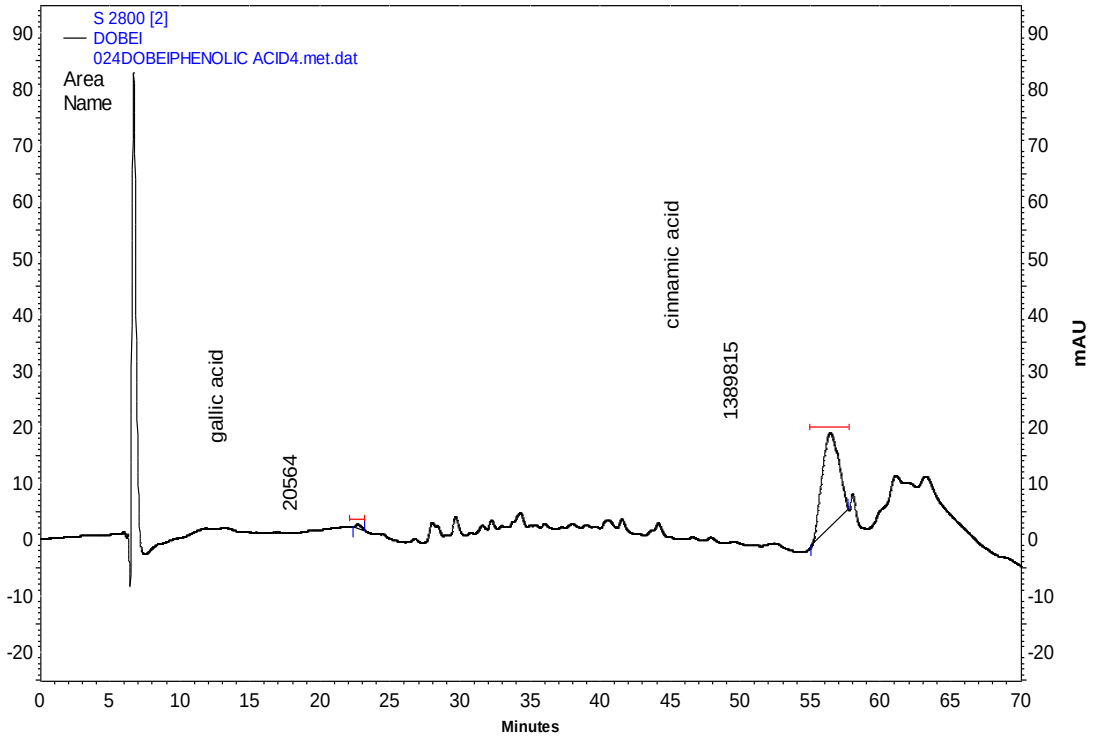

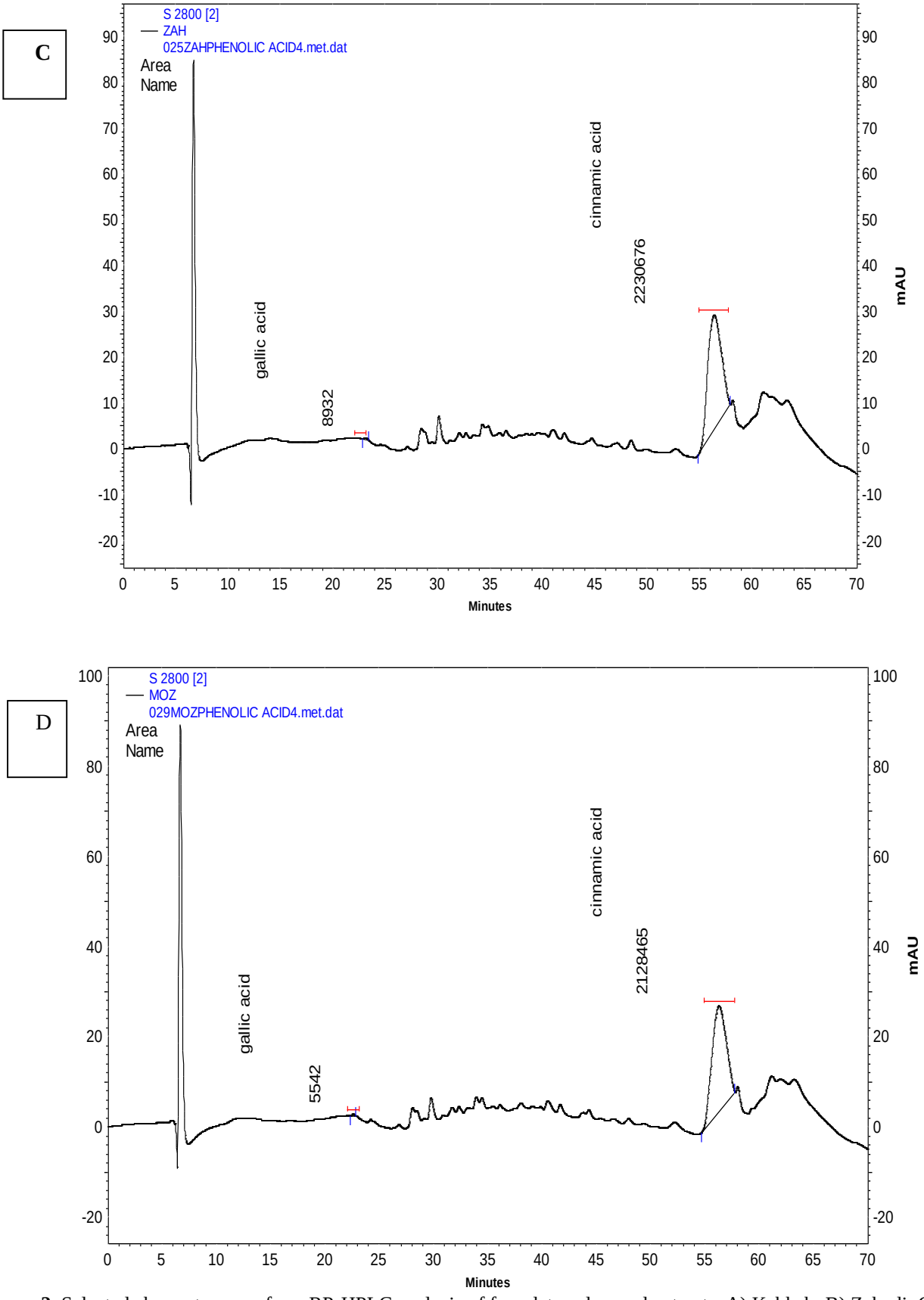

Figure 2. Selected chromatograms from RP-HPLC analysis of four date palm seed extracts. A) Kabkab; B) Zahedi; C) rabbi and D) Mazafati

3.3. Assessment of antioxidant activity

Antioxidant activity of the date seed extracts and BHT are presented in Fig. 3. The IC_{50} values of the seed extracts varied 16.55-21.58 μg ml^{-1}. Kabkab seed extracts included the highest antioxidant activity against DPPH radicals; followed by Rabbi (20.4 μg ml^{-1}) and Zahedi (20.8 μg ml^{-1}) seed extracts. Mazafati variety with a high IC_{50} of 21.58 μg ml^{-1} included the least antioxidant activity. However, the radical scavenging effect of all extracts was less than that of BHT, but the IC_{50} value of Kabkab variety was close to that of BHT as standard reference. It is noteworthy that pure BHT was more active than crude BHT [37]. To assess the contribution of total phenolic contents to antioxidant capacity of the date seeds, Pearson's correlation coefficient was calculated. The analysis of correlation ($P < 0.05$) showed a significant negative correlation between the IC_{50} and the total phenolic content of the date seed extracts ($r = -0.75$). Based on previously published studies, correlations have been reported between the antioxidant activity and the total phenolic content of dates [35,38].

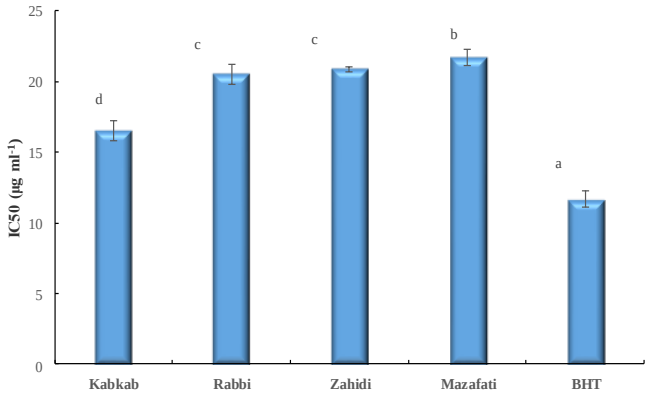

Figure 3. DPPH radical scavenging activities (IC_{50} μg ml^{-1}) of four Iranian date palm seed extracts. Values are expressed as the means of three replicates and error bars represent standard deviation (SD) (n = 3). Means with a different superscript letter in each column are significantly different at $P \leq 0.05$.

Studies have reported that seeds from various fruits including mango, basil-seed, wild pear, pomegranate and red grape include antioxidant activities [39-43]. Antioxidant activity of the date seed extracts is mainly due to the presence of phenolic compounds. Results of several studies have demonstrated that phenolic compounds such as cinnamic acid and its derivatives (caffeic acid, p-coumaric acid, ferulic acid, chlorogenic acid and sinapic acid) offer a higher antioxidative capacity, compared to other polyphenols such as hydroxybenzoic acid derivatives (gallic acid, vanillic acid, dihydroxybenzoic acids and protocatechuic acid). Considering that date seeds include considerable quantities of these compounds (especially cinnamic acid derivatives), it can be concluded that date seeds are rich sources of antioxidant compounds [44-46].

3.4. Assessment of antibacterial activity

In the current study, antibacterial activity of the ethanolic extracts of date seeds was assessed against *E. coli.* O157:H7 as Gram-negative and *S. aureus* as Gram-positive bacteria (Table 2). Findings from the disc diffusion method revealed that all evaluated seed extracts included varying antibacterial effects against *S. aureus* but not against *E. coli*. Differences in susceptibilities of the two bacteria against date seed extracts could be attributed to the differences in structure of the bacterial cell walls. Cytoplasm of the Gram-positive bacteria is surrounded by a thick peptidoglycan layer, while in Gram-negative bacteria an additional outer membrane mainly containing lipopolysaccharides is present [47,48]. Antimicrobial strength of the phenolic compounds against Gram-positive bacteria is associated with their ability to interact directly with the peptidoglycan layer and thereby to decrease cell integrity and to increase bacterial sensitivity to osmotic pressure and ionic strength. However, the lipopolysaccharide layer in outer membrane of Gram-negative bacteria, which functions as a tight permeability barrier, hinders polyphenols to have direct connections with the peptidoglycan layer [49].

Regarding antibacterial effects of the highlighted extracts against *S. aureus*, the maximum effect was observed for Kabkab variety (12.2 mm), followed by Zahedi (11.5 mm), Rabbi (10 mm) and Mazafati (0.9 mm) varieties. Differences in antibacterial activities of the extracts could be attributed to the differences in their qualitative compositions. As previously shown in analysis of phenolic compounds, the highlighted seed extracts included the highest quantity of cinnamic acid and its derivatives (including chlorogenic acids). These compounds in comparison with other phenolics such as hydroxyl benzoic acids include notable antibacterial activity, especially against Gram-positive bacteria such as S. *aureus* [48]. The antibacterial potency of these extracts against *S. aureus* was subsequently assessed using MIC and MBC methods (Table 3). Kabkab and Zahedi date extracts included the highest inhibition activity or lowest MIC against *S. aureus* (1.56 mg ml^{-1}) followed by Rabbi and Mazafati dates (3.125 mg ml^{-1}). Furthermore, MBC method showed that all investigated extracts included antibacterial activity. Despite the fact that a similar inhibitory effect against *S. aureus* was reported for Kabkab and Zahedi dates, the bactericidal effect of Kabkab date was more than that of Zahedi date (the lowest MBC of 3.125 mg ml^{-1}). In contrast to Kabkab date, Mazafati and Rabbi date seed extracts demonstrated the weakest antibacterial activity (MBC of 12.50 mg ml^{-1}).

Table 2. Antibacterial activities of the four date seed extracts using disc diffusion method

	Zahedi	Kabkab	Rabbi	Mazafati	Ampicillin
	Inhibition zone (mm)[a]				
Staphylococcus aureus	11.5 ±0.06[b]	12.2 ±0.05[b]	10.0 ±0.2[a]	0.9 ±0.05[a]	18 ±0.25[c]
Escherichia coli	ND	ND	ND	ND	21 ±0.5

Results are expressed as mean ±SD (standard deviation) (n = 3); ND, not detected; means with a different lowercase letter in each row are significantly different at P≤0.05
[a]Inhibition zone diameter (mm)

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the four date seed extracts against *Staphylococcus aureus* (mg m^{-1})

	Zahedi		Kabkab		Rabbi		Mazafati	
	MIC (mg m^{-1})	MBC (mg m^{-1})	MIC (mg m^{-1})	MBC (mg m^{-1})	MIC (mg m^{-1})	MBC (mg m^{-1})	MIC (mg m^{-1})	MBC (mg m^{-1})
Staphylococcus aureus	1.56[b]	6.25[C]	1.56[b]	3.125[B]	3.125[a]	12.5[A]	3.125[a]	12.5[A]

Results are expressed as mean ±SD (standard deviation) (n = 3)
Different lowercase and capital letters respectively indicate significant differences between MIC and MBC of the four date seed extracts (P≤0.05)
MIC=Minimum inhibitory concentration
MBC= minimum bactericidal concentration

According to Qadoos et al. [50], date fruit and leave extracts show inhibitory effects against Gram-positive bacteria (*S. aureus* and *B. subtilis)*; however, no effects are seen for Gram-negative bacteria (*E. coli* and *Pseudomonas aeruginosa*). Samad et al. [51] studied effects of methanolic extracts of various date fruits against Gram-positive and Gram-negative bacteria (*Serratia marcescens* and *E. coli*) and reported that the significant differences against studied bacteria were associated to various concentrations of methanolic extracts (100-500 mg ml^{-1}) from date varieties. Of these varieties, Ajwa dates with the highest quantities of phenolic compounds showed antimicrobial activities against all four bacteria at higher concentrations; however, no inhibitory activities were observed against Gram-negative bacteria in Mabroom and Mariami study. Based on the results from the current study, increased phenolic contents can result in increased antimicrobial activity of the extracts with a correlation factor of 0.99. Several studies have shown that antimicrobial activity of the extracts is directly linked to their polyphenolic compounds [52-55]. The mechanism of action of these compounds in inhibiting the bacterial growth can be summarized as interaction with the bacterial cell walls [54,56], precipitation of proteins and disruption of enzyme activities [52,53,55,57,58].

4. Conclusion

This study demonstrated that all the assessed extracts included significant phenolic contents, mainly cinnamic acid and its derivatives, as well as antioxidant activities. The best results were observed for Kabkab date seed extracts. Moreover, the study revealed that all palm date seeds included antibacterial activities against *S. aureus* but not against *E. coli*. In general, palm date seeds (as byproducts of the processing industries) are rich sources of phenolic compounds, which can be used as complementary biotechnological substrates or as nutraceuticals for the production of novel, healthy innovative foods. However, further human clinical studies seem necessary.

5. Acknowledgements

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6. Conflict of Interest

The authors declare no conflict of interest.

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بررسی محتویات فنولی، فعالیت ضداکسایشی و ضد باکتریایی عصاره های هسته چهار رقم خرمای ایرانی (*Phoenix dactylifera* L.)

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چکیده

سابقه و هدف: هر ساله، در صنایع فرآوری و بسته بندی خرما مقادیر زیادی هسته خرما تولید می شوند که یا دور ریخته می شوند یا به عنوان مواد کم ارزش به مصرف خوراک دام و تولید کمپوست می‌رسند. در حالی‌که، اینها منابع زیستی[۱] می باشند، که امکان تولید مواد با ارزش افزوده بالا در صنایع غذایی از آنها وجود دارد. هدف اصلی تحقیق حاضر، تعیین پروفایل و محتوای فنولی، فعالیت ضد باکتریایی و ضد اکسایشی عصاره‌های هسته چهار رقم خرمای ایرانی مضافتی، ربی، کبکاب و زاهدی بوده است.

مواد و روش ها: محتوی فنولی کل، پروفایل ترکیبات فنولی، فعالیت ضداکسایشی و فعالیت ضد باکتریایی عصاره هسته چهار رقم خرمای ایرانی، به ترتیب با استفاده از روش‌های فولین- سیوکالتو، کروماتوگرافی مایع با کارایی بالا با فاز معکوس، گیرنده ۲، ۲- دی فنیل -۱- پیکریل هیدرازیل، و روش‌های انتشار دیسک در آگار، تعیین گردیدند.

یافته‌ها و نتیجه‌گیری: محتوای فنولی کل بین ۱۴۸۰ تا ۳۳۸۰ میلی گرم بر ۱۰۰ گرم وزن خشک بر حسب اسید گالیک و شامل اسیدهای سینامیک، کلروژنیک، کافئیک و ۳ و ۵- هیدروکسی بنزوئیک به ترتیب عمده‌ترین-ترکیبات فنولی شناسایی شده بودند. در میان ارقام مورد آزمایش، عصاره های هسته ارقام کبکاب و مضافتی با داشتن IC_{50} به ترتیب معادل ۱۶/۵۶ $\mu g\ ml^{-1}$ و ۲۲/۶ کمترین و بیشترین فعالیت گیرندگی رادیکال را به خود اختصاص دادند. نهایتاً، یافته های حاصل از آزمون انتشار دیسک نشان داد که کلیه عصاره‌های هسته خرما روی باکتری *استافیلوکوکوس اورئوس* اثر بازدارندگی داشتند، اما روی باکتری اشرشیا کلی تاثیری نداشتند. حداقل غلظت مهاری[۲] و حداقل غلظت کشندگی[۳] عصاره هسته های خرما برای باکتری *استافیلوکوکوس اورئوس* به ترتیب بین ۱/۵۶ تا ۳/۱۲۵ و ۳/۱۲۵ تا ۱۲/۵ میلی گرم بر میلی لیتر تعیین شد. بر اساس یافته های این تحقیق هسته خرماهای ایرانی منبع خوبی برای ترکیبات فنولی قابل استخراج با فعالیت‌های قابل توجه ضداکسایشی می باشند و از این رو می توانند به عنوان افزودنی‌های طبیعی در فرمولاسیون فرآورده‌های گوناگون مانند غذاهای فراسودمند و مکمل‌های رژیمی مورد استفاده قرار گیرند. علاوه بر این، این هسته‌ها می توانند از طریق فرایندهای زیست فناوری به فرآورده‌هایی با ارزش افزوده تبدیل شوند.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

تاریخچه مقاله

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واژگان کلیدی

- خواص ضد باکتریایی
- فعالیت ضداکسایشی
- هسته خرما
- پروفایل فنولی

***نویسنده مسئول**

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