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Original Research Article

Date Seeds (*Phoenix dactylifera*.L,) in Morocco: Phenolic Profiling and *In vitro* Antioxidant Potency

Hamza Ourradi[1]*, Said Ennahli[2], Lahcen Hssaini[3], Marto-Viuda Martos[4], Fracisca Hernadez[5], Hafida Hanine[1]

[1]*Laboratory of Bioprocessing and Bio-interfaces. Faculty of Science and Technics, BO 523 Beni-Mellal, Morocco*
[2]*National School of Agriculture. BO S/40 Meknes, Morocco*
[3]*National Institute of Agricultural Research (INRA) Meknes, Morocco*
[4]*Dpto. TecnologíaAgroalimentaria, IPOA. Escuela Politécnica Superior de Orihuela.(Universidad Miguel Hernández), CtraBeniel, km 3.2, E-03312 Orihuela (Alicante), Spain*
[5]*Dpto. Producción Vegetal y Microbiología, Grupo de Investigación de Producción Vegetal yTecnología, cuelaPolitécnica Superior de Orihuela (Universidad Miguel Hernández deElche), Ctra. de Beniel, km 3,2, E- 03312 Orihuela, Alicante, Spain*

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ABSTRACT

Date seeds are thus far the least studied part of the *Phoenix dactylifera* L. species, as they are usually discarded as waste despite their richness in functional and bioactive compounds. The purpose of this study was to assess the phytochemical compositions and *in vitro* antioxidant capacities of crude acetone extract from date seed powder in order to designate the most promising valorisation modes. Quantitative analysis using colorimetric and HPLC-DAD methods was conducted to determine the phytochemical profile of date seeds, while *in vitro* antioxidant activity of extracts was evaluated using DPPH (2,2-diphényl 1-picrylhydrazyle), ABTS (2,2′-Azinobis-(3-Ethylbenzthiazolin-6-Sulfonic Acid)), FRAP (Ferric ion reducing antioxidant power) and *β*-carotene methods. The phytochemical analysis showed that date seeds are a valuable resource for phenolic compounds such as tannins, flavonoids, anthocyanins and flavonols. The analysis of individual phenolic compounds using the HPLC-DAD method identified ten phenolic acids. The most abundant among them were *p*-coumaric acid, protocatechuic acid, rutin, caffeic acid and quercetin. Moreover, *in vitro* antioxidant activity showed that the acetone extracts exhibited a reducing antioxidant power, as well as an inhibition of lipid peroxidation using FRAP and *β*-carotene methods, respectively. In addition, acetone extracts also showed an efficient ability to scavenge free radicals. Rutin, *p*-coumaric acid and kaempferol were the compounds mostly involved in the inhibition of free radical scavenging activities. Furthermore, rutin showed a high inhibition of lipid peroxidation. These results strengthen the evidence that date seeds are rich in phytoconstituents with excellent antioxidant potency, which can be used for nutraceutical and medicinal applications.

Keywords: Date seeds, (*Phoenix dactylifera* L.), HPLC-DAD, phenolic acids, antioxidant activity.

Introduction

Plant-based by-products have become a key source of bioactive compounds with numerous health benefits.[1] In fact, plant extracts and their bioactive compounds are used at the industry level to produce functional foods.[2] For this reason, those by-products rich in bioactive compounds are sought out by industry and consumers. Therefore, nutraceutical profiles can be used to promote the consumption of by-products as natural functional ingredients. Date palm trees (*Phoenix dactylifera* L.) are an essential crop of the oasis ecosystem worldwide. Their cultivation extends from North Africa to Southwest Asia.[3]Also, their fruits have been recognized as an excellent source of energy and nutrition.[3] They are well known for their higher content in anthocyanins, phenolics, sterols, carotenoids, procyanidins and flavonoids.[4,5]

*Corresponding author. E mail: hamza.ourradi@usms.ma
Tel: +212650806034

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These natural bioactive compounds work as free radical scavengers and antioxidants as well as anti-inflammatory, antibacterial and cardioprotective agents.[6,7] Date seeds, generated by many different industries, are considered waste products that are usually discarded and rarely used in animal feed. Previous reports suggest that the seeds do not induce toxic effects.[6] In addition, compared to date flesh, they are an excellent source of polyphenols, natural antioxidants and dietary fibres.[8-10]For that reason, they are used both in the production of coffee,[2] and to improve the texture and sensory properties of wheat bread.[11] Furthermore, previous studies reported that date seeds are rich in macro-elements, such as potassium, magnesium, calcium, phosphorus, iron, dietary fibres and edible oils.[7,9,12] Phenolic compounds are bioactive substances and are considered important constituents of daily human diet, as they are rolled as protective against different diseases because of their multiple health-beneficial components.[13] Date palm seed extracts were reported to contain phenolics, tocopherols, and sterols, with higher antioxidant, antimicrobial, and anticancerproperties.[6,7,10] They are rich in phenolics (0.73 – 4.76 g GAE/100 g DW), flavonoids (0.65 – 1.84 g RE/100 g DW), anthocyanins (126 mg Q3GE/100 g), flavonols (336 mg Q3GE /100 g), proanthocyanidins (851.30 mg Q3GE/100 g), and antioxidants (274 – 506 mmol Trolox equivalent/100g).[7,10] Unfortunately, studies on the antioxidant activity of date seeds have been sparsely reported. Date seeds have not gained attention as sources of antioxidants, most likely because they lack popularity and have few practical commercial

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applications. However, these derived by-products have gained attention as fruit-processing methods yield higher ratios of these natural ingredients.[2] Therefore, the main purpose of this study was to assess the phytochemical compositions (total phenolic, condensed tannins, total flavonoids, anthocyanins and flavonols), the phenolic compounds profile and *in vitro* antioxidant capacities of crude acetone extract from date seeds (*Phoenix dactylifera* L) belonging to eight cultivars, most of which are grown in the southern region of Morocco. In order to designate the most promising valorisation modes.

Materials and Methods

Plant materials

Ten kilograms of seeds from each of the eight cultivars (*Boufgous, Bouslikhan, Bousthami, Khalt a, khalt z, Lassian, Majhoul and Tadmamt*) were collected from the Arfoud region in southern Morocco (31°27'18"N 4°15'02"W) in November 2020. Seeds were pitted, washed in distilled water, air-dried at room temperature and subsequently oven-dried.[10] Dried seeds were powdered and stored at 4°C for further analysis.

Preparation of crude extracts

The crude extracts were prepared according to the method described by El-Mergawi et al., (2016).8 Briefly, 1 g of powdered date seeds was shaken with 250 mL of acetone-water (70:30, v/v) at room temperature for 12 hours. Afterward, solids were separated by centrifugation for 20 min at 5000×*g*. Then, the extracts were dried at 45°C under vacuum using a rotary evaporator. The yield of extraction was determined for each date seed cultivar (Table 1).

Biochemical assessment

Total phenolic

The total phenolic content of seeds powder was carried out using Folin-Ciocalteu method according to the protocol described by Al-Farsi and Lee (2008).[14] Briefly, 1 mL of each extract was mixed with 1 mL of Folin-Ciocalteu reagent and incubated at room temperature for 5 min. Then, 10 mL of sodium carbonate solution 7% (w/v) were added. The final volume was adjusted to 25 mL by distilled water. After 1h of incubation in the dark at room temperature, the absorbance was measured at 750 nm using a spectrophotometer (JASCO V-630), and the total phenolic content was expressed as mg Gallic Acid Equivalent per 100 g Dry weight.

Total Flavonoids

Total flavonoids content was measured based on the method described by Brighente *et al*. (2007).[15] Briefly, 1 mL of each extract was mixed with 1 mL of the $AlCl_3$ solution (2%) prepared in methanol (w/v), and after 40 minutes of incubation in the dark at room temperature, absorbance was determined at 430 nm, and the total flavonoids content was expressed as mg Rutin equivalent per 100g DW.

Total tannins

The dosage of condensed tannins was carried out according to the method described by Bajaj and Devsharma (1997).[16] Briefly, 1 mL of extract was mixed with 75 mL of distilled water. Then 5 mL of Folin-Denis and 10 mL of saturated sodium carbonate solution were added, and the mixture was stirred and incubated for 30 min in the dark. Absorbance was measured at 760 nm. Condensed tannins content was expressed as mg Tannic Acid Equivalent per 100g DW.

Total anthocyanin and flavonols

Total anthocyanins and flavonols contents were measured according to the procedure described by Yoshitama *et al*. (1992).[17] One gram of seed powder was mixed with 10 mL of acidified methanol (methanol/0.1N HCl) and stirred constantly. A 900 µL of the supernatant was mixed with 900 µL methanol/0.1 N HCl. The concentration of anthocyanins and flavonols were measured at 530 nm and at 360 nm, respectively. Anthocyanins and flavonols were expressed as mg Querctin-3-Glucoside Equivalent per 100g DW.

In vitro antioxidant activities

DPPH radical-scavenging assay

The antioxidant activity was carried out according to the protocol described by Bondet *et al*. (1997).[18] The stock solution DPPH (0.1 mM) was prepared in methanol, and then stoked at 4°C in darkness until further analysis. 0.5 mL of diluted extracts or standards was mixed with 3.5 mL of DPHH solution. The sample absorbance was measured at λ = 515 nm against a blank after 30 min of storage in the darkness. The antioxidant activity value, which represents the percentage of scavenging ability, was estimated according to the following formula:

$$\text{Percentage Inhibition (\%)} = \frac{\text{Ab}_{515\text{ nm control}}\text{ nm} - \text{Ab}_{515\text{ nm sample}}\text{ nm}}{\text{Ab}_{515\text{ nm control}}\text{ nm}} \times 100$$

Where,

$Ab_{515\ control}$: control absorbance.

$Ab_{515\ sample}$: control sample absorbance.

IC_{50} is the concentration of extract responsible for 50% of the inhibition of DPPH radicals. It was determined from the plot of inhibition percentage against extract concentration.

ABTS radical-scavenging assay

ABTS radical scavenging activity was evaluated according to the method described by *Re et al*. (1999).[19] The stock solution was prepared by mixing equal volumes of ABTS salt (7 mM) and potassium persulfate (2.45 mM) and allowing them to react for 12h in the darkness at room temperature. Then, the solution was diluted with methanol to obtain an absorbance 0.7 ± 0.02 at 734 nm. An aliquot of 25µL of each extract was added to 2000 µL of ABTS solution. The absorbance of the sample was measured at λ = 734 nm after 5 min of storage in the darkness. The antioxidant activity value was estimated according to the following equation:

$$\text{Percentage Inhibition (\%)} = \frac{\text{Ab}_{734\text{ nm control}} - \text{Ab}_{734\text{ nm sample}}}{\text{Ab}_{734\text{ nm control}}} \times 100$$

Where,

$Ab_{734\ control}$: control absorbance.

$Ab_{734\ sample}$: control sample absorbance

IC_{50} is defined as above-described.

FRAP reducing antioxidant power assay

FRAP was determined according to Benzie and Strain (1996).[20] Briefly, 100 µL of extract were added to 3000 µL of FRAP solution by mixing 25 mL sodium acetate buffer solution (300 mM, pH=3.6), 2,5 mL of TPTZ solution (400 mM prepared in HCL 40 mM) and 2,5 mL $FeCl_3$ solution (20 mM), then incubated 30 min at 37°C. The absorbance was measured at 593 nm after 30 min incubation in the darkness at room temperature.

The antioxidant activity value was estimated according to the following equation

$$\text{Percentage Inhibition (\%)} = \frac{\text{Ab}_{593\text{ nm sample}} - \text{Ab}_{593\text{ nm control}}}{\text{Ab}_{593\text{ nm sample}}} \times 100$$

Where,

Ab_{593nm} sample: sample absorbance.

Ab_{593nm} control: control sample absorbance.

Bleaching or β-carotene discolouration test (β-carotene/Linoleic Acid system)

β-carotene assay was carried out according to Boros *et al*. (2010).[21] Firstly, 2.5 mL of emulsion composed of 0.5mg of β- carotene, 1 mL chloroform, 25 µL linoleic acid, 200 mg Tween 20 and 100 mL distilled water was prepared, then the mixture was added to 0.5mL plant extract then the whole mixture was incubated for 2 hours at 50°C in a water bath. The absorbance was read at 490 nm.

The percentage of lipid peroxidation inhibition (LPI %) was calculated according to the following formula

$$\text{lipid peroxidation inhibition (\%)} = \frac{\text{Ab}_{490\text{ nm after 2 h}}}{\text{Ab}_{490\text{ nm initial}}} \times 100$$

Where,

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Ab_{490nm} initial: initial absorbance.
$Ab_{490nm\ after\ 2\ h}$: absorbance after 2 hours.

Quantitative analysis by HPLC–DAD method

Reversed phase high performance liquid chromatography with tandem mass spectrometry was used to determine the major phenolic compounds present in the palm date seeds. A slightly modified version of the method outlined by Zheng and Wang (2001).[22] was used. Briefly, the samples were prepared by dissolving a 0.02 g extract in 2.0 mL of 50% HPLC-grade methanol and passed through a 0.45-µm filter before injection into a reverse phase C18 column (250 mm length, 4.6 mm i.d., 5 µm particle size, Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada) with a guard column. The mobile phase was acetonitrile (A) and acidified water containing 2.5% formic acid (B). The gradient was as follows: 0 min, 5% A; 10 min, 15% A; 30 min, 25% A; 35 min, 30% A; 50 min, 55% A; 55 min, 90% A; 57 min, 100% A and then held for 10 min before returning to the initial conditions. The flow rate was 1.0 mL/min and the wavelengths of detection were 280, 350, and 380 nm. An external standard method using authentic compounds was used to confirm the identified compounds. Compounds were identified on the basis of comparing their retention times, UV-vis spectra. Phenolic compounds were identified according to peak retention time and UV/Vis spectra by comparing them with those of the standards. The quantities of the different phenolic compounds were based on peak areas, and expressed as µg/g dw and in comparison, with those of standards (caffeic acid, protocatechuic acid, rutin, gallic acid, hydroxytyrosol, oleuropein, tyrosol, chlorogenic acid, caffeic acid, ferulic acid, p coumaric acid, vanillic acid, rutin, kaempherol, and quercetin. Three samples per cultivar were analysed and all the samples were run in triplicate. The Empower 2 software (Waters, Spain) was used for data processing.

Statistical analysis

The statistical analysis was performed using SPSS software version 19, the Duncan post-hoc test ($p < 0.05$) was performed to indicate signification differences and Pearson's correlation coefficient (r) was used to assess the association between the variables. Differences at $p < 0.05$ were considered significant among studied samples and the results were reported as mean ± SD (standard deviation).

Results and Discussion

Phenolic compounds

Date seeds are considered an excellent source of phenolics and flavonoid compounds.[10] As shown in Table 1, total phenolic and flavonoid contents were significantly different among cultivars ($p < 0.05$); they ranged from0.72 ± 0.08 to 3.59 ± 0.19 g GAE /100g DW. The highest total phenolic levels were observed in *Lassian*, whereas the lowest amounts were recorded in*Tadmamt* seeds. Flavonoid levels ranged from 0.10 ± 0.02 to 1.11 ± 0.01 g RE/100g DW; the highest values were observed in *Bouslikhan*, while *Bousthami* exhibited the lowest flavonoid levels.

Date seeds are a valuable source of phytochemical compounds, which display high biological activities beneficial for human health.This has been documented in previous studies, particularly those conducted by Bouhlali *et al*. (2017)[9] on seeds from Moroccan date palm cultivars and by El-Rahman *et al*. (2017)[7] and El-Mergawi *et al*. (2016)[8] on Saudi Arabian cultivars. In our study, total phenolic and flavonoid levels were lower than those reported by Bouhlali *et al*. (2017)[9], who reported a range of total phenolic and flavonoid levels of5.34 to 1.84 g GAE/100g DW and 1.22 to 1.84 RE/100g DW, respectively. The phenol content was higher than that reported by El-Rahman *et al*. (2017)[7] (0.73 g GAE/100g). However, flavonoid contents were similar to those reported by Djaoudene *et al*. (2019)[10] (0.65 mg QE/100g) except for *Bousthami*, *Boufgous* and *Bouslikhan* cultivars. These differences could be attributed to the cultivar effect [9] or culture conditions[23] as well as extraction methods.[24]Also, phenolic compounds of date seeds were higher than those reported for sesame seeds (*sesamum indicum*) (1.57g GAE/100g),[25] olive stones (*Olea europaea*) (0.51 – 0.72 g GAE/100g), [26]and avocado seeds (*Persea americana*) (0.61-1.65 g GAE /100g).[27] Comparable amounts of tannins were exhibited in date seeds and fruit flesh.[7] In addition, tannins represent the most abundant phenolic forms in date seeds.[8]The tannin contents of date seeds are summarized in Table 1. Significant differences ($p < 0.05$) were observed between all date seed cultivars. The tannin levels ranged between 0.28 ± 0.03 to 0.96 ± 0.04 g TAE/100g DW. Furthermore, the highest value was in *Lassian*,while *Tadmamt* had the lowest content. Higher amounts of tannins in date seeds were reported in previous studies; [8] recorded the highest total tannin levels (3.05 - 5.21 g/100g), while El-Rahman *et al*. (2017)[7] reported an amount (1.57 g/100 g) that approximated our data. This variation can be linked to the extraction method[24]or to culture conditions.[23] Furthermore, compared to data previously reported on grape seeds (0.54 g/ 100g), tea (3.70 g/100g) and green beans (6.60 g/100g).[29] It was concluded that Moroccan date seeds are an excellent source of tannins. Therefore, owing to this richness, they can be used to develop products of high nutritional value. Anthocyanin and flavonol pigments are members of the flavonoid group. They are well known for theirability to protect against cardiovascular diseases and reduce proliferation of cancer cells.[30]Date seeds are a rich source of these pigments.[10] The current (Table 1) show asignificant cultivar effect($p < 0.05$). The highest levels of anthocyanins (39.77 ± 0.12 mg Q-3-GE/100g DW) and flavonols(150.18 ± 1.11 mg Q-3-GE/100g DW)were observed in *Bouslikhan* seeds, whereas*Bousthami*seeds recorded the lowest levelsof both anthocyanins (5.37 ± 0.08 mg Q-3-GE/100g DW) and flavonols (34.27 ± 1.01 mg Q-3-GE/100g DW). However, these values are lower than those reported by Djaoudene *et al*. (2019),[10] who found a range of anthocyanin levels between 64 and 126 mg Q-3-GE/100 g DW, and a range of flavonols between 211 and 336 mg Q-3-GE/100g DW. These differences could be due to the cultivar effect[9] or to culture conditions.[23]

Table 1: Extraction yield and phytochemical composition of date seeds (*Phoenix dactylifera* L.) cultivars

Variety		TP	TF	TCT	TA	TFL
	Yield (%)	g GAE /100g DW	g RE /100g DW	g TAE/100g DW	mg Q-3-GE/100gDW	
Boufgous	14.20 ± 0.42^{b}	1.59 ± 0.14^{b}	0.29 ± 0.04^{b}	0.66 ± 0.03^{b}	16.10 ± 0.13^{b}	64.16 ± 1.24^{b}
Bouslikhan	22.90 ± 0.57^{e}	2.40 ± 0.23^{c}	1.11 ± 0.01^{f}	0.90 ± 0.01^{g}	39.77 ± 0.12^{g}	150.18 ± 1.11^{g}
Bousthami	16.60 ± 0.71^{c}	2.22 ± 0.14^{c}	0.10 ± 0.02^{a}	0.69 ± 0.04^{c}	5.37 ± 0.08^{c}	34.27 ± 1.01^{a}
Khalt a	15.25 ± 0.21^{b}	1.59 ± 0.11^{b}	0.47 ± 0.05^{c}	0.75 ± 0.05^{d}	24.27 ± 0.07^{c}	70.71 ± 2.31^{d}
khalt z	23.90 ± 0.28^{e}	2.80 ± 0.10^{d}	0.79 ± 0.06^{e}	0.84 ± 0.02^{f}	29.62 ± 0.10^{d}	69.67 ± 0.91^{cd}
Lassian	27.75 ± 0.35^{f}	3.59 ± 0.19^{e}	0.66 ± 0.04^{d}	0.96 ± 0.04^{h}	31.66 ± 0.08^{e}	109.82 ± 0.35^{f}
Majhoul	19.80 ± 0.42^{d}	1.65 ± 0.17^{b}	0.85 ± 0.12^{e}	0.80 ± 0.05^{e}	35.23 ± 0.28^{f}	90.42 ± 2.13^{e}
Tadmamt	11.95 ± 0.49^{a}	0.72 ± 0.08^{a}	0.43 ± 0.04^{c}	0.28 ± 0.03^{a}	16.21 ± 0.24^{a}	67.68 ± 0.64^{c}

Value in average ± SD. Values marked by the same letter, are not significantly different ($p < 0.05$) using Duncan post hoc test ($p < 0.05$). DW: dry weight, TP: total phenolics, TF: total flavonoids, TCT: total condensed tannins, TA: total anthocyanins, TFL: total flavonols, GAE: gallic acid equivalent, RE: rutin equivalent, TAE: tannic acid equivalent, mg Q-3-GE: mg querctin-3-glucoside equivalent.

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In vitro antioxidant activity

The antioxidant activities of the different date seed cultivars were evaluated using four in-vitro antioxidant assays: DPPH, ABTS, FRAP, and β-carotene. As shown in Tables 2 and 3, all date seed cultivars exhibited a higher antioxidant capacity.

DPPH and ABTS radical-scavenging assay

The free radical scavenging ability of date seed extracts was analysed using DPPH, which is a stable free radical with high free radical scavenging capacity, commonly used to screen for antioxidants.[18] All date seeds, regardless of cultivar, showed efficient antioxidant ability using the DPPH method (Table 2). Varietal effect was significant ($p < 0.05$). The IC_{50} values ranged between 179.66 ± 0.33 to 235.72 ± 1.44 µg/mL.*khalt z* recorded the highest capacity to inhibit 50% of DPPH radicals, while *Tadmamt* exhibited the lowest one. The remaining cultivars fell between these extremes in this order, from higher to lower:*Lassian, Bouslikhan*and*Majhoul.* The present values are lower than those reported by Djaoudene *et al*. (2019)[10] (41.72 - 68.90 µg/mL), but close to those reported by Bouhlali *et al*. (2017)[9] (122 - 166 µg/mL). These differences are probably related to the cultivar effect[9] or extraction methods.[24]

The results of antioxidant activity using the ABTS method are summarized in Table 2. A statistically significant difference ($p < 0.05$) was observed among all date seed cultivars based on IC_{50} values. The IC_{50} values varied between 48.38 ± 0.54 to 68.21 ± 1.41 µg/mL. The highest inhibition capacity was exhibited by *khalt z*, followed, in order, by *Lassian* (52.417 ± 1.271g µg/mL), *Bouslikhan*(52.69 ± 1.06 µg/mL), *Majhoul* (53.48 ± 0.65) *Bousthami* (55.35 ± 1.33 µg/mL), *Khalta* (56.56 ± 1.08 µg/mL), *Boufgous* (60.70 ± 0.12 µg/mL) and *Tadmamt* seeds. these values were lower than those reported by Djaoudene *et al*. (2019),[10] who noted an IC_{50} range between13.80 and 32.31 µg/mL. This variation can be attributed to the cultivar effect[9] and extraction methods.[24] In this investigation, the existing differences that were recorded between DPPH and ABTS radical scavenging tests could be due to the mechanism of action for each method.[31]

FRAP reducing antioxidant power assay

Antioxidant activity of date seed extracts was also evaluated according to its ability to scavenge ferric radicals according to the FRAP assay.The results of the FRAP assay are summarized in Table 3. Significant differences between cultivars were recorded. The antioxidant activity ranged between 31.01 ± 1.27 and 117.39 ± 1.65. According to the results, *Lassian* seeds displayed the highest reducing power, while *Tadmamt* exhibited the lowest. The other cultivars in between followed in this order, from higher to lower: *khalt z, Bouslikhan* and *Bousthami.* The current findings are lower than those reported by Djaoudene *et al*. (2019)[10] (145 – 316 mmol Fe^{2+} equivalent /100g DW). This difference may be due to the cultivar effect[9] and analysis conditions.[24] Therefore, date seeds can be considered among the most remarkable sources of bioactive molecules that display an efficient antioxidant power.

Table 2: Antioxidant activity of date seeds (*Phoenix dactylifera* L.) cultivars were estimated following DPPH and ABTS assays.

Variety	IC_{50} (µg/mL)		Antioxidants mmolTrolox equivalent/100g dw	
	DPPH	ABTS	DPPH	ABTS
Boufgous	193.45 ± 1.76^{e}	60.69 ± 0.07^{d}	6.26 ± 0.07^{b}	14.08 ± 0.24^{b}
Bouslikhan	182.29 ± 0.05^{bc}	52.69 ± 1.48^{b}	10.38±0.17^{f}	27.49 ± 0.12^{f}
Bousthami	189.47 ± 1.54^{d}	55.35 ± 1.84^{c}	8.12 ± 0.13^{d}	16.94 ± 0.21^{d}
Khalt a	189.65 ± 0.81^{d}	56.56 ± 1.41^{c}	6.91 ± 0.08^{c}	15.46 ± 0.15^{c}
khalt z	179.66 ± 0.33^{a}	48.55 ± 0.64^{a}	12.02 ± 0.12^{h}	33.26 ± 0.31^{h}
Lassian	180.48 ± 1.07^{ab}	52.55 ± 1.77^{b}	11.30 ± 0.18^{g}	28.12 ± 0.25^{g}
Majhoul	183.37 ± 1.42^{c}	53.48 ± 0.92^{b}	10.00 ± 0.14^{e}	19.95 ± 0.31^{e}
Tadmamt	235.72 ± 1.44^{f}	68.21 ± 1.98^{e}	5.25 ± 0.03^{a}	14.88 ± 0.16^{a}

Value in average ± SD. Values marked by the same letter, are not significantly different ($p < 0.05$) using Duncan post hoc test ($p < 0.05$). Dry weight, IC_{50}: Concentration of extract responsible for 50% of inhibition of DPPH and ABTS radical

Table 3: Antioxidant activity of date seeds (*Phoenix dactylifera* L.) measured following FRAP and β-carotene bleaching method.

Variety	Antioxidants mmol Fe^{2+} equivalent /100g dw	Percentage of lipid peroxidation inhibition (%)
Boufgous	57.21 ± 2.23^{b}	44.63 ± 2.18^{b}
Bouslikhan	88.21 ± 0.60^{f}	67.88 ± 1.86f
Bousthami	83.18 ± 2.67^{a}	48.42 ± 2.82^{c}
Khalt a	64.41 ± 0.66^{c}	54.53 ± 2.17^{d}
khalt z	88.86 ± 1.65^{f}	64.06 ± 2.09^{e}
Lassian	117.39 ± 1.36^{g}	54.95 ± 1.66^{d}
Majhoul	79.78 ± 1.32^{d}	64.76 ± 1.79^{e}
Tadmamt	31.01 ± 1.27^{e}	36.13 ± 1.45^{a}

Value in average ± SD. Values marked by the same letter, are not significantly different ($p < 0.05$) using Duncan's post hoc test ($p < 0.05$).

β-carotene bleaching test (β-carotene/Linoleic Acid system)

The β-carotene bleaching method is currently used to evaluate the antioxidant ability of the extract to inhibit the formation of conjugated hydroperoxides resulting from the oxidation of linoleic acid. These hydroperoxides are known to react with β-carotene, which is responsible for its bleaching and the disappearance of its yellow colour.[32] As summarized in Table 3, date seed samples exhibited an efficient lipid peroxidation inhibition (LPI%) with significant differences ($p < 0.05$) among sampled cultivars. The LPI% varied from 36.13 ± 1.45 to 67.88 ± 1.86%. *Bouslikhan* seeds exhibited the highest value followed by *Majhoul* and *khalt z*, while *Tadmamt* recorded the lowest value. Therefore, the antioxidant ability of these seed extracts can be explained by their richness in bioactive substances.

Quantitative composition of phenolic compounds

The major phenolic compounds in date seed samples were identified on the basis of their retention times and absorbance spectra. Figure 1 shows the identified peaks in the HPLC-DAD and ESI-MS chromatogram. Profiles of the phenolic acids of each date seed cultivar is presented in Table 4. The total phenolic acid amount in the date seed cultivars varied from 456.97 ± 5.26 to 585.83 ± 5.29 µg/g DW (Figure 2). The highest amount was detected in *Lassian* seeds,

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which differed significantly from the other cultivars. The lowest content was in the*Tadmamt* seed cultivar. For the date seeds, *p*-coumaric acid was the major compound (228.96 ± 1.28 µg/g DW), followed by protocatechuic acid, rutin, caffeic acid, quercetin and gallic acid. However, kaempferol and vanillic, chlorogenic and ferulic acids were the minor phenolic compounds; vanillic acid was not detectable in *Khalt a* seeds. *p*-coumaric was the most abundant phenolic compound in all seed samples across all cultivars. The major phenolic compounds herein identified were consistent with those found in similar studies on date seeds.[7,8,14] Comparing the data reported for other date palm cultivars, chlorogenic acid contents were generally lower, which is likely attributable to the cultivar effect. Also, there was no catechin, syringic acid or luteolin in date seed powder or in the seeds of other cultivars.[33] Other compounds detected in the date seed samples in our study were rutin.

The major phenolic compounds are identified in the table regarding date palm seeds (Table 4). The seed powder contained chlorogenic acid (4.13±0.07 to 12.56±0.04µg/g) and vanillic acid (4.19±0.04 to 9.74±0.07µg/g). Three common phenolic compounds are identified in date seeds: *p*-coumaric acid, quercetin and ferulic acid. However, the phenolic compound levels of date seeds cultivars were lower than those reported by [33]for date seeds. Moreover, these compound levels are substantially impacted by environmental factors, soil composition and maturation stage and are less than those observed in other fruits and vegetables.[34,35] These results are consistent with those reported by[33]. They describe the abundant phenols, such as flavonols, flavones, flavan-3-ols (monomers, dimers, and trimers), proanthocyanin oligomers and catechin. These data are the most complete and comprehensive description of phenolic compound profiles and their quantification in date seeds.

Figure 1: HPLC chromatogram of *Lassian* date seeds cultivar.

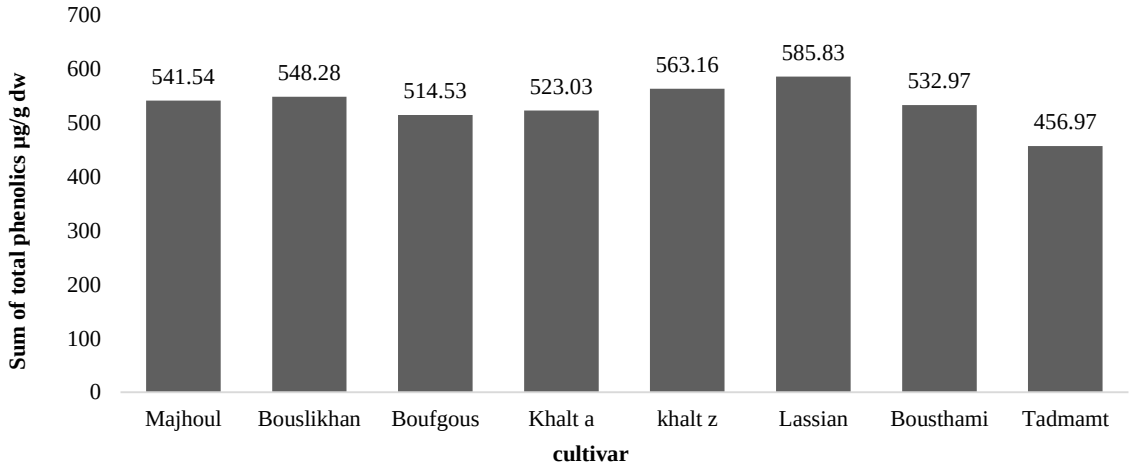

Figure 2: Sum of total phenolic in date seeds

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Table 4: Polyphenolic profiles of palm date seeds cultivars.

Sample	Gallic acid	Caffeic acid	Chlorogenic acid	Protocatechuic acid	Ferulic acid	*p*-coumaric acid	Vanillic acid	Rutin	Kaempferol	Quercetin
Majhoul	15.69 ± 0.25^{c}	63.36 ± 0.52^{b}	8.32 ± 0.09^{b}	148.96 ± 1.59^{d}	4.12 ± 0.04^{e}	185.95 ± 2.98^{c}	6.58 ± 0.03^{e}	82.56 ± 0.15^{b}	6.96 ± 0.09^{e}	19.04 ± 0.17^{g}
Bouslikhan	16.89 ± 0.17^{b}	58.45 ± 0.87^{d}	4.13 ± 0.07^{f}	155.69 ± 1.63^{b}	4.18 ± 0.05^{e}	174.21 ± 1.18^{d}	6.99 ± 0.08^{d}	94.25 ± 0.25^{a}	8.48 ± 0.12^{d}	25.01 ± 0.09^{f}
Boufgous	14.56 ± 0.14^{e}	69.36 ± 0.41^{a}	7.96 ± 0.12^{c}	151.22 ± 2.45^{c}	5.21 ± 0.04^{b}	155.83 ± 1.32^{f}	7.18 ± 0.07^{d}	63.41 ± 0.09^{f}	9.28 ± 0.10^{c}	30.52 ± 0.15^{e}
Khalt a	18.63 ± 0.08^{a}	41.48 ± 0.56^{f}	5.61 ± 0.08^{e}	126.14 ± 1.36^{g}	4.89 ± 0.08^{c}	228.96 ± 1.28^{a}	N.D.	71.27 ± 0.17^{d}	4.58 ± 0.18^{g}	21.47 ± 0.21^{h}
khalt z	12.32 ± 0.17^{g}	53.17 ± 0.15^{e}	6.87 ± 0.12^{d}	135.96 ± 0.98^{e}	3.14 ± 0.11^{f}	210.99 ± 1.04^{b}	7.96 ± 0.06^{c}	82.14 ± 0.24^{b}	13.74 ± 0.14^{a}	36.87 ± 0.08^{d}
Lassian	15.17 ± 0.21^{cd}	76.96 ± 0.32^{a}	12.56 ± 0.04^{a}	164.52 ± 0.97^{a}	6.05 ± 0.06^{a}	189.12 ± 3.28^{c}	4.13 ± 0.04^{g}	75.24 ± 0.18^{c}	11.56 ± 0.06^{b}	30.52 ± 0.13^{e}
Bousthami	16.98 ± 0.18^{b}	57.23 ± 0.84^{d}	8.62 ± 0.13^{b}	149.15 ± 1.39^{d}	5.29 ± 0.14^{b}	163.25 ± 1.12^{e}	9.74 ± 0.07^{a}	70.12 ± 0.15^{e}	5.03 ± 0.17^{g}	47.56 ± 0.17^{a}
Tadmamt	13.69 ± 0.09^{f}	61.14 ± 0.66^{c}	6.66 ± 0.14^{d}	134.51 ± 1.34^{f}	4.79 ± 0.09^{cd}	123.89 ± 2.44^{g}	5.32 ± 0.04^{f}	55.69 ± 0.17^{g}	6.07 ± 0.08^{f}	45.21 ± 0.21^{b}

Values expressed as µg/g, Value in average ± SD. Values marked by the same letter, are not significantly different ($p < 0.05$) using Duncan post hoc test ($p < 0.05$).

Table 5: Pearson correlation coefficients of total phenolics, condensed tannins, total flavonoids, anthocyanines, flavonols content and antioxidant activities

	Tn	PT	Flv	Anth	Flvols	DPPH	DPPH IC_{50}	ABTS	ABTS IC_{50}	FRAP	β-Carotene	Ga	Ca	Cha	Pra	Fa	p-Ca	Va	Rut	Kae	Qer
Tn	1																				
PT	0.834*	1																			
Flv	0.530	0.324	1																		
Anth	0.626	0.387	0.962**	1																	
Flvols	0.516	0.362	0.865**	0.868**	1																
DPPH	0.831*	0.841**	0.672	0.674	0.487	1															
DPPH	-0.961**	-0.747*	-0.391	-0.478	-0.311	-0.766*	1														
C_{50}																					
ABTS	0.704	0.810*	0.693	0.657	0.526	0.934**	-0.607	1													
ABTS	-0.916**	-0.801*	-0.521	-0.550	-0.326	-0.916**	0.928**	-0.804*	1												
C_{50}																					
FRAP	0.670	0.659	0.079	0.041	0.006	0.634	-0.745*	0.461	-0.772*	1											
-	0.910**	0.956**	0.400	0.476	0.418	0.877**	-0.836**	0.751*	-0.864**	-	1										
Carotene										0.876**											
Ga	0.240	-0.064	-0.087	-0.009	0.128	-0.192	-0.246	-0.364	-0.063	-0.063	0.152	1									
Ca	0.110	0.326	0.016	0.079	0.245	0.167	-0.006	0.132	0.082	0.082	-0.172	-0.390	1								

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Cha	0.227	0.505	-0.288	-0.138	-0.167	0.249	-0.182	0.112	-0.132	-0.132	-0.189	-0.209	0.718*	1							
ra	0.507	0.590	0.215	0.253	0.477	0.435	-0.408	0.331	-0.295	-0.295	0.219	-0.010	0.827*	0.559	1						
a	-0.069	0.115	-0.550	-0.403	-0.111	-0.358	0.146	-0.428	0.355	0.355	-0.548	0.361	0.487	0.615	0.430	1					
-Ca	0.693	0.463	0.318	0.446	0.126	0.524	-0.720*	0.438	-0.733*	-0.733*	0.656	0.312	-0.484	-0.041	-0.239	-0.267	1				
Va	0.028	-0.082	-0.320	-0.445	-0.503	-0.025	-0.258	-0.095	-0.246	-0.172	0.123	0.231	-0.704	-0.378	-0.284	-0.347	0.120	1			
Rut	0.809*	0.555	0.826*	0.787*	0.694	0.812*	-0.761*	0.731*	-0.830*	-0.830*	0.970**	0.190	-0.107	-0.232	0.339	-0.485	0.511	0.139	1		
Kae	0.480	0.673	0.427	0.437	0.301	0.693	-0.418	0.824*	-0.532	-0.532	0.376	-0.701	0.410	0.304	0.345	-0.287	0.220	-0.243	0.346	1	
Qer	-0.556	-0.082	-0.585	-0.736*	-0.573	-0.264	0.515	-0.129	0.362	0.362	-0.622	-0.403	0.080	0.152	-0.090	0.140	-0.573	0.323	-0.532	-0.020	1

PT: Total phenolics, Tn: condensed tannins, Flv: Total flavonoids, Anth: Anthocyanines, Flvol: Flavonols, Caro: carotenoids IC_{50} DPPH: IC_{50} values using DPPH method, IC_{50} ABTS: IC_{50} values using ABTS method, β-Car: β-Carotene. Ga: Gallic acid, Ca: Caffeic acid, Cha: Chlorogenic acid, Pra: protocatechuic acid, Fa; Ferulic acid, p-Ca: p-Coumaric acid, Va: Vanillic acid, Rut: Rutin, Kae; Kaempferol, Qer; Quercetin

*Correlation is significant at the 0.05 level (Two-tailled) ** Correlation is significant at the 0.01 level (Two-tailled)

Correlation among variable

To better assess the relationship between bioactive compounds that were studied and antioxidant properties of sampled date seeds from all cultivars, a bivariate correlation analysis using the Pearson coefficient was conducted. The significant correlations at both 0.05 and 0.01 levels are summarized in Table 5; total phenolics strongly correlated with antioxidant activities assessed via all four methods. Negative correlations between total phenolics and the free radical scavenging assessed by DPPH and ABTS assays were observed with correlation coefficients (*r*) of -0.747* and -0.801**, respectively. Whereas this association was positive in the case of β-carotene blanching assays (r = 0.956**). Furthermore, a strong correlation was established between IC_{50} DPPH and condensed tannins (r = -0.961**). The results were similar between IC_{50} ABTS and condensed tannins (r = -0.916**). The latter also displayed a strong correlation with β-carotene (r = 0.910**). In addition, antioxidant activity showed significant correlation with phenolic acids and individual flavonoids, such as rutin, kaempferol and *p*-coumaric acid. This signifies that these phenolic components were the main contributors to the free radical scavenging activity based on DPPH and ABTS assays as well as the lipid peroxidation inhibition. It is noteworthy that among these compounds, rutin seemed to record the highest correlation levels with values of *r* that were 0.812*, -0.830* and 0.970** for DPPH, ABTS and β-carotene assays, respectively. Furthermore, a significant positive correlation was established between kaempferol and ABTS assays (r=0.824*). Similarly, *p*-coumaric acid exhibited a significant negative correlation with IC_{50} using a DPPH assay. Overall, our results, which were similar to those reported by Bouhlali *et al*. (2017), who reported a higher correlation between total phenolic and antioxidant activity; the corresponding values for *r* were 0.998, 0.915 and -0.856 using ABTS, FRAP and DPPH assays, respectively. In particular, phenolic acids and individual flavonoids can be considered the main antioxidants. In addition, phenolic compounds comprise one (phenolic acids) or more (polyphenols) aromatics rings with attached hydroxyl groups in their structures, which induces efficient radical scavenging.[32,36] In the radical scavenging mechanism, polyphenols reduce ROS/RNS, such as $^{\bullet}OH$, $O_2^{\bullet -}$, $NO^{\bullet}$ or $OONO^-$ after their generation, thus preventing damage to biomolecules or the formation of more reactive ROS.[37] Furthermore, they protect lipid peroxidation by blocking the Fenton reaction.[38]

The results described above are very promising given that they may attract the attention of local or export-oriented industries. They could possibly be guided to appreciate the economic value of date palm seeds, which are usually discarded, in various industrial applications – primarily as ingredients and nutraceuticals.

Conclusion

In summary, the date seeds (*Phoenix dactylifera* L) have high levels of polyphenolic compounds. Condensed tannins, flavonoids, anthocyanins and flavonols) and phenolic acids (*p*-coumaric, protocatechuic, caffeic, and gallic acids were found to be major contributors to the antioxidant activity of date seeds, as confirmed through the strong correlations that were revealed between these compounds and antioxidant methods. Rutin, *p*-coumaric acid and kaempferol were the major contributors to the antioxidant properties. The richness of date seeds in valuable molecules can potentially be used in the recovery of phenolic compounds with various applications in the food, cosmetic and pharmaceutical industries.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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