

PHENOLIC CONTENTS AND ANTIOXIDANT ACTIVITY OF SIX ALGERIAN DATE PALM (*Phoenix dactylifera*L.) CULTIVARS

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Abstract

The present study aimed to estimate total phenols, flavonoids and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity of the methanolic extracts of six date palm (*Phoenix dactylifera* L.) Algerian cultivars. The total phenolic content and total flavonoids were measured using Folin-Ciocalteu's and aluminum chloride colorimetric methods, respectively. The antioxidant capacity were analyzed using DPPH assay. The cultivars of date palm fruit used in this study were dry dates (DD): Abdelazaz, Degla-Beida and Haloua dates (moisture content 10-13%), semi-soft dates (SSD): Deglet-Nour and Hamraya dates (moisture content 22-25%) and soft date, Tinicine cultivar (moisture content 34%). The results revealed the richness of these cultivars in polyphenols (173.71-248.64 mg gallic acid equivalents (GAE)/100 g fresh weight), a mean content of total flavonoids (15.89-40.87 mg quercetin equivalents (QE)/100 g fresh weight) and an interesting antioxidant capacity (51.31-76.88%). With a 2.11 mg/g fresh weight for total phenolic content, Abdelazaz date showed the highest level of total antioxidant capacity (76.88 %) with a BHA (butylated hydroxytoluene), gallic acid, quercetin and vitamin C equivalents antioxidant capacity values of 80.09, 248.27, 191.90 and 722.56 µg/g fw, respectively. High positive significant correlation ($r = 0.827$, $p < 0.01$) was found between total phenols and DPPH radical scavenging capacity, suggesting that phenolics were the major contributor to the antioxidant activity. However no significant correlation was found between flavonoids and DPPH radical scavenging capacity. These results suggest that date palm fruit can act as a chemopreventative agent, providing antioxidant properties and offering effective protection from free radicals.

Keywords: Date palm fruit; Total phenolic content; Total flavonoid content; DPPH; Antioxidant activity

Submitted: 29.12.2014

Reviewed: 14.04.2015

Accepted: 20.05.2015

1. INTRODUCTION

Date fruit of the date palm (*Phoenix dactylifera* L.), very popular in north Africa, especially in the Algerian south, constitutes an essential food for Muslims during all seasons, mainly in the holy month of Ramadan.

Worldwide production of dates in 2010 was 7.626.448 tons. With a production of 710.000 tons, corresponding to 9.3 % of the worldwide production, Algeria is the 5th world producer (FAOSTAT, 2010). This production ensures an important income for the national economy, providing at the same time a significant resource for local consumption.

Date palm fruits is an important diet component in most of the arid and semiarid regions of the world. Typically, date palm fruits

contain carbohydrate (total sugars, 44-88%), fat (0.2-0.5%), protein (2.3-5.6%) dietary fiber (6.4-11.5%), minerals (0.1 to 916 mg/100 g date) and vitamins (such as vitamin C, B1, B2, A, riboflavin and niacin) (Biglari et al., 2008).

Several studies indicate that consumption of fruits and vegetables reduces the risk of several chronic diseases : coronary heart disease, blood pressure, obesity, diabetes and cancers (Wildman, 2009).

The cellular protection offered by fruits and vegetables against oxidative stress in several diseases has been attributed to various antioxidants and vitamins. Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health have only recently been recognized. Flavonoids are

known to possess anti-inflammatory, antioxidant, antiallergic, hepatoprotective, antithrombotic, neuroprotective, and anticarcinogenic activities (Salaet al., 2003). These compounds are secondary metabolites that gather a large set of molecules, divided into fourteen classes (Vermerris & Nicholson, 2006).

These valuable chemicals possess very interesting biological properties, which are used in various fields, such as medicine, cosmetic and nutrition.

The objective of the present study is to determine the levels of total phenolic content, total flavonoids and to evaluate the antioxidant activity of methanolic extract from six Algerian date cultivars : Abdelazaz, Degla-Beida, Deglet-Nour, Haloua, Hamraya and Tinicine.

2. MATERIAL AND METHODS

2.1. Plant material

Fresh ripe date samples used in this study consisted of three cultivars of dry dates (DD) locally known as Abdelazaz, Degla-Beida and Haloua dates, two cultivars of semi-soft dates (SSD) locally known as Deglet-Nour and Hamraya dates and soft date (SD) locally known as Tinicine date. These cultivars were harvested in ripening stage (tamr) from the agricultural experimental station of the National Institute of Agronomic Research in Touggourt, Algeria (33°04'N; 06°06'E; altitude: 85 m). The samples were selected identically in terms of size, colour, ripening stage, without damage and calamity, and were stored in paper bags at 4 °C until use.

2.2. Chemicals and standards

Aluminum chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), BHA (butylated hydroxytoluene), Folin-Ciocalteu's reagent, gallic acid, methanol, quercetin, sodium carbonate and vitamin C were purchased from Fluka Chemie (Switzerland), Merck (Germany) and Sigma-Aldrich (USA). All chemicals and reagents used in the experiments were of analytical grade.

2.3. Moisture content

Moisture was determined according to standard AOAC method 920.151 (AOAC, 1998).

2.4. Extraction of the phenolic compounds

One gram of cleaned pitted fruits was extracted with 40 ml of methanol at room temperature for 24 hours with continued agitation. After centrifugation and filtration, the extracts were concentrated under reduced pressure at 40 °C in a rotary evaporator. The extracts were then redissolved in 10 mL of the same solvent. These concentrated extracts were used to determine total phenolics, flavonoids content, and antioxidant activity of date palm fruits.

2.5. Total phenolic content

Total phenolics were estimated using Folin-Ciocalteu's reagent as described by Cai et al. (2004) with a little modification. Briefly, 0.5 ml of each sample was mixed with 5 ml of distilled water and 0.5 ml of Folin-Ciocalteu's reagent, after 3 min, 0.5 ml of 7.5 % sodium carbonate (Na_2CO_3) was added. The final mixture was shaken and then incubated for 1 h in the dark at room temperature. The absorbance was measured at 760 nm using Beckman 34UV-Vis spectrophotometer.

2.6. Total flavonoids

Total flavonoids content were estimated using the colorimetric assay according to Gursoy et al. (2009). One ml of 2 % aluminium methanolic trichloride solution (AlCl_3) was mixed with 1 ml of the methanolic extracts. Test tubes were incubated at room temperature for 10 min and the absorbance was determined at 415 nm.

2.7. Free radical scavenging by using DPPH radical

The DPPH radical scavenging capacity was determined using the method described by Mansouri et al. (2005). Twenty five μl of sample were added to 975 μl methanolic solution of DPPH (6×10^{-5} M) and vortexed, the mixture was left in the dark for 30 min and the absorbance measured at 515 nm.

DPPH radical scavenging capacity was estimated according to the following equation (Lu et al., 2011) :

$$\text{DPPH radical scavenging capacity (\%)} = \frac{[(\text{Abs}_{515} \text{ DPPH} - \text{Abs}_{515} \text{ Sample}) / \text{Abs}_{515} \text{ DPPH}] \times 100}{}$$

Where $\text{Abs}_{515} \text{ DPPH}$ is the absorbance of the control solution (containing only DPPH), and $\text{Abs}_{515} \text{ Sample}$ is the absorbance in the presence of the date extracts.

The scavenging activity was calculated from the calibration curve. BHA (0-200 $\mu\text{g/mL}$), gallic acid (0-80 $\mu\text{g/mL}$), quercetin (0-200 $\mu\text{g/mL}$) and vitamin C (0-500 $\mu\text{g/mL}$) were used as a references to produce a standard curves. All the measurements were taken in triplicate and the mean values were calculated. The same antioxidant capacity of the methanolic extracts for all date cultivars was compared to those of the standards and all results were expressed as a microgram standard equivalent antioxidant capacity per gram of fresh weight ($\mu\text{g SEAC/g fw}$).

2.8. Statistical analysis

Duncan's multiple range method and Pearson's correlation were carried out for analyzing the data obtained from different types of dates, and to study the relationship between DPPH radical scavenging capacity, Total phenolics and Total flavonoids. Data were reported as means \pm standard deviation of triplicate experiments. p values < 0.05 were regarded as significant and p values < 0.01 very significant. Data were analyzed using SPSS statistical software (Version 20.0. Armonk, NY: IBM Corp.).

3. RESULTS AND DISCUSSION

The six cultivars of Algerian date palm fruit used in this study included the dry dates (DD) namely Abdelazaz, Degla-Beida and Haloua dates (moisture content 10-13%), semi-soft dates (SSD) namely Deglet-Nour and Hamraya dates (moisture content 22-25%) and soft date which was Tinicine date (moisture content 34%).

3.1. Total phenolic content

With exceptions of some studied cultivars, Degla-Beida and Tinicine, significant differences ($p < 0.05$) in total phenolic contents were observed (Table 1). The total phenolic contents of the six cultivars ranged between 173.71 to 248.64 mg GAE/100 g fw. Results of the total phenolic content showed that maximum phenolic content was found in Hamraya date (249 mg GAE/100 g of fw) and the minimum phenolic content was found in Tinicine date (174 mg GAE/100 g of fw) and ranked as: Hamraya > Haloua > Deglet-Nour > Abdelazaz > Degla-Beida > Tinicine. It is evident that date fruits are rich in phenolics.

Results of the total phenolic content agreed with those found in some Omani varieties which ranged between 172-246 mg GAE/100 g fw (Al-Farsi et al. 2007). However, Mansouri et al. (2005) reported much lower content of total phenolics of several Algerian dates in the range of 2.49 - 8.36 mg/100 g fw. Furthermore, while total phenolic content of the Algerian date cultivars reported here are higher than those reported for some Iranian cultivars (Biglari et al., 2008), the TPC of Medjool cultivar reported by Wu et al. (2004), was the much higher.

In the present study, it was found that Deglet Nour variety contained 219.59 mg/100 g fw of total phenolics content. However, Wu et al. (2004) reported much higher content of total phenolics in the same date variety than that found by Mansouri et al. (2005), which were 661 and 6.73 mg of GAE/100 g fw, respectively. Various factors such as growing condition, geographic origin, fertilizer, soil type, storage conditions, and received sunlight amount, might be responsible for the observed differences.

3.2. Total flavonoids

The results showed that total flavonoid content of the six studied cultivars varied considerably from 15.89 to 40.78 mg QE/100 g of fw. The order of total flavonoid content of the studied varieties was: Abdelazaz < Tinicine < Deglet-Nour < Hamraya < Degla-Beida < Haloua. With some exceptions (Degla-Beida and Hamraya),

significant differences ($p < 0.05$) in total flavonoid content values were observed among date cultivars (Table 1).

The results agreed with those found by Biglari et al. (2008), who gave values ranging between 1.62 and 81.79 mg/100 g dw for Iranian varieties. They are similar with those obtained by Chibane et al. (2007): for Degla-Beida 27.43 mg/100 g, Frezza 22.61 mg/100 g, but lower than Mech-Degla variety with a content of 69.28 mg/100 g fw.

3.3. DPPH scavenging capacity of date extracts

Activity is measured as the relative decrease in absorbance of DPPH as it reacts with the antioxidant (Rumbaoa et al., 2009). Substances which are able to perform this reaction could be considered as antioxidants and therefore radical scavengers (Hinneburg et al., 2006). This method is widely used to evaluate antioxidant activity in foods.

Results of radical scavenging capacities which determined by DPPH assays are shown in Table 1. Antioxidant capacity of the studied date cultivars were in the range of 51-77% and listed in decreasing order: Abdelazaz > Hamraya > Haloua > Deglet-Nour > Degla-Beida > Tinicine.

These results agree with those found by Chibane et al. (2007), who obtained antioxidant capacity by the inhibition of the linoleic acid's oxidation, show that the three date varieties Mech-Degla, Degla-Beida and Frezza present an inhibitive activity of 61.82, 61.56 and 61.60 %, respectively. Singh et al. (2012) reported a comparable DPPH free radical scavenging activity of some Omani varieties: Fardh (72.7%), Khalas (72.1%) and Khasab (69.5%).

The results of the present study showed that Abdelazaz cultivar, had higher antioxidant capacity than Deglet-Nour, Haloua and Hamraya dates. However, the total phenolic content of Deglet-Nour, Haloua and Hamraya dates was higher than Abdelazaz date. This suggests that the antioxidant activity depends probably on the quality of the existing

phenolics or there are some other active components in Abdelazaz date, other than phenolic compounds, which may contribute to its antioxidant capacity.

Results of comparison between the same radical scavenging capacities of the methanolic extracts for all date cultivars and those of the standards are shown in Table 2. With a 2.11 mg/g fw for total phenolic content, Abdelazaz date showed the highest level of total antioxidant capacity (76.88 %) with a BHA, gallic acid, quercetin and vitamin C equivalents antioxidant capacity values of 80.09, 248.27, 191.90 and 722.56 $\mu\text{g/g}$ fw, respectively and with a 1.74 mg/g fw for total phenolic content, Tinicine date exhibited the lowest level of antioxidant capacity (51.31 %) with a BHA, gallic acid, quercetin and vitamin C equivalents antioxidant capacity values of 53.74, 169.02, 121.52 and 497.54 $\mu\text{g/g}$ fw, respectively. Total antioxidant activity was also found to increase in a dose dependent manner.

The antioxidant activity of dates was reported by several authors (Allaith 2008; Al-Humaid et al. 2010; Awad et al. 2011; Anjum et al. 2012; Singh et al. 2012; Mohamed Lemine et al. 2014). Although different assays have been used in the assessment of their antioxidant activity such as DPPH, β -carotene bleaching, ferric-reducing antioxidant potential (FRAP), 2,2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), they all concluded to the powerful antioxidant activity of date fruit. For instance, Mohamed Lemine et al. (2014) using the DPPH assay reported antioxidant activity values that ranged from 91.2 to 99.3 μmol trolox equivalents/100 g dm for the Tamr stage in six Mauritanian date palm cultivars. Mansouri et al. (2005), using the same assay to estimate the antioxidant activity expressed as the mass ratio (μg sample/ μg DPPH) in seven different ripe date palm fruits from Algeria, including Deglet Nour, found an antiradical efficiency that ranged from 0.08 to 0.22.

Table 1. Total phenolic contents, total flavonoids and DPPH scavenging capacity of six Algerian date cultivars

Cultivar name	Total phenolic contents (mg/100 g fw)	Total flavonoids (mg/100 g fw)	DPPH scavenging capacity (%)
Abdelazaz	211,58 ± 6,21 ^b	15,89 ± 0,72 ^a	76,88 ± 3,38 ^d
Degla-Beida	176,92 ± 2,07 ^a	27,67 ± 0,55 ^d	62,17 ± 0,41 ^b
Deglet-Nour	219,59 ± 1,95 ^{bc}	21,96 ± 0,89 ^c	72,01 ± 0,46 ^c
Haloua	228,95 ± 12,07 ^c	40,78 ± 1,78 ^c	74,93 ± 0,98 ^{cd}
Hamraya	248,64 ± 4,14 ^d	27,26 ± 1,07 ^d	75,57 ± 0,32 ^d
Tinicine	173,71 ± 1,37 ^a	19,54 ± 1,75 ^b	51,31 ± 2,57 ^a

Values are means ($n = 3$) ± SD. Values within columns with the same superscript letter are not statistically different at the 5% level. fw: Fresh weight

Table 2. Standards equivalent antioxidant capacity per gram of fresh weight of date fruits (µg SEAC/g fw).

Cultivar	TPC (mg/g fw)	TF (mg/g fw)	DPPHSC (%)	BHAEAC (µg /g fw)	GAEAC (µg /g fw)	QEAC (µg /g fw)	VitCEAC (µg /g fw)
Abdelazaz	2,11 ± 6,21 ^b	0,16 ± 0,72 ^a	76,88 ± 3,38 ^d	80,09 ± 1	248,27 ± 4,08	191,90 ± 2,23	722,56 ± 22,83
Degla-Beida	1,77 ± 2,07 ^a	0,28 ± 0,55 ^d	62,17 ± 0,41 ^b	64,93 ± 1,07	202,68 ± 3,83	151,41 ± 1,97	593,11 ± 20,16
Deglet-Nour	2,20 ± 1,95 ^{bc}	0,22 ± 0,89 ^c	72,01 ± 0,46 ^c	75,07 ± 1,02	233,17 ± 4	178,49 ± 2,15	679,70 ± 21,94
Haloua	2,29 ± 12,07 ^c	0,41 ± 1,78 ^c	74,93 ± 0,98 ^{cd}	78,08 ± 1	242,22 ± 4,04	186,53 ± 2,2	705,40 ± 22,47
Hamraya	2,49 ± 4,14 ^d	0,27 ± 1,07 ^d	75,57 ± 0,32 ^d	78,74 ± 1	244,21 ± 4,05	188,29 ± 2,21	711,03 ± 22,59
Tinicine	1,74 ± 1,37 ^a	0,19 ± 1,75 ^b	51,31 ± 2,57 ^a	53,74 ± 1,14	169,02 ± 3,67	121,52 ± 1,79	497,54 ± 18,2

TPC: Total phenolic contents; TF: Total flavonoids; DPPHSC: DPPH scavenging capacity;

BHAEAC: BHA equivalent antioxidant capacity; GAEAC: Gallic acid equivalent antioxidant capacity;

QEAC: Quercetin equivalent antioxidant capacity; VitCEAC: Vitamin C equivalent antioxidant capacity

Table 3. Correlation matrix between DPPH scavenging capacity and antioxidants

	DPPH SC	TPC	FLA
DPPH SC	1		
TPC	0,827**	1	
FLA	0,240	0,342	1

Abbreviations: DPPH SC : DPPH scavenging capacity ; TPC : total phenolic contents; FLA : flavonoids; ** $p < 0.01$,

3.4. Correlation

Pearson's correlation (Table3) shows a high positive significant relationship between DPPH scavenging capacity and phenol content ($r = 0.827$, $p < 0.01$). These results were in agreement with those reported by Mansouri et al. (2005), Biglari et al. (2008) and Vayalil (2012). The relationship between flavonoids and DPPH scavenging capacity ($r = 0.240$, $p > 0.05$), flavonoids and phenol content ($r = 0.342$, $p > 0.05$) in methanolic extracts is not significant. The DPPH scavenging capacity of phenolic constituents could be attributed to the presence of hydroxyl groups which can donate the electron and neutralize the existing free radical in the reaction mixture.

4. CONCLUSION

In this work, the antioxidant capacity of six selected Algerian date cultivars were studied

by DPPH essay. The total phenolic content and total flavonoid content of the date palm fruit were measured. The methanolic extracts of six date palm cultivars revealed an interesting DPPH scavenging capacity (51.31-76.88 %). However, the antioxidant capacity of Abdelazaz was the highest among studied date cultivars.

The antioxidant activity of dates due mainly to the presence of polar-soluble compounds with potent free radical-scavenging effects, such as phenolic compounds (mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones). Various factors such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage conditions and amount of received sunlight, might be responsible for the observed differences (Biglari et al., 2008).

Overall, it could be concluded that fruit of date palm is an excellent dietary source of natural

antioxidants and may be considered as food with remarkable benefits for human health.

5. ACKNOWLEDGMENTS

The authors thank Mr. Acourene Said and Mr. Tama Mouhammed of the National Institute of Agronomic Research (INRA) in Touggourt, Ouargla Algeria, for supplying the date palm cultivars used in this study.

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