American Journal of Food Science and Health
Vol. 1, No. 3, 2015, pp. 63-68
http://www.aiscience.org/journal/ajfsh

Total Phenolic, Flavonoid Contents and *in Vitro* Antioxidant Activity of Algerian Date Palm Varieties: A Comparative Study

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Abstract

The objective of this study was to determine the content of bioactive substances (total phenolic compounds and flavonoids) and the antioxidant activity of aqueous extracts of date varieties grown in Algeria (*Ourrous, Deglet Nour, Beid Lahmam, Outkbala, Taneslit, Tamjouherte, Tazerzeite* and *Takermoust*) using spectrophotometric methods. Phenolic compounds were determined by the Folin-Ciocalteu reagent. The total phenolic content ranged from 169.18 to 381.76 mg of gallic acid equivalents per 100 g dry matter, while the flavonoid content varied between 28.68 and 95.22 mg quercetin equivalents per 100 g dry matter. The antioxidant capacity was determined *in vitro* using scavenging effect of diphenylpicryl-hydrazyl radical (DPPH) and phosphomolybdenum assay. The results indicated that cultivar had pronounced effects on both phenolic compound levels and antioxidant potential. Among the studied samples, *Ourrous* variety contained the highest amounts of bioactive compounds and exhibited the best antioxidant activity. The evaluation of antioxidant activity of the eight date varieties revealed highly significant correlation ($p<0.001$) between anti-radical ability and total phenolic ($r = 0.77$), and flavonoid contents ($r = 0.88$).

Keywords

Phoenix Dactylifera, Varieties, Phenolic Content, Antioxidant Activity

Received: April 10, 2015 / Accepted: May 3, 2015 / Published online: June 8, 2015

1. Introduction

The date palm (*Phoenix dactylifera L.*) is a subtropical fruit tree, native to Iraq and other countries of the Middle East. It is the only *Phoenix* species grown for its edible fruits (Serrano *et al*., 2001). Fruits of the date palm are very commonly consumed in the world and are a vital component of the diet in most of the Arabian countries, due to their high-energy value and high levels in simple sugars and minerals. With over 789 350 tons per year, Algeria is one of the main producer of dates. It occupies the fourth position with 11.24% of the world production (FAO, 2012), but from a qualitative point of view, it ranks first with a dominant production of the most appreciated worldwide variety "*Deglet Nour*" (Chibane *et al*., 2007).

The date fruits are highly nutritious and may have great potential as a medicinal food for a number of diseases inflicting human beings (Vayalil, 2012; Assirey, 2015). Several studies from different countries including Algeria (Benmeddour *et al*., 2013), Bahrain (Allaith, 2008), Iran (Biglari *et al*., 2008), Oman (Al- Farsi *et al*., 2005) and Kuwait (Vayalil, 2002) have demonstrated that dates are a good source of antioxidant compounds. Phytochemical investigations have revealed that the date fruits contain phenolics, flavonoids, procyanidins and anthocyanins, compounds known to possess multiple beneficial effects.

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Free radicals play a crucial role in the pathophysiology of human diseases such as cancer and neurodegenerative diseases. Consequently, considerable attention has been focused on functional foods that are able to decrease the level of free radicals and to protect against these diseases (Lee *et al*., 2009).

In our previous study (Benmeddour *et al*., 2013), we have characterized the antioxidant activity of ten date palm varieties (*Mech Degla, Deglet Ziane, Deglet Nour, Thouri, Sebt Mira, Ghazi, Degla Beida, Arechti, Halwa* and *Itima*) cultivated in Algeria. The objective of the current work was to investigate and compare phenolic and flavonoid contents and antioxidant potential and free radical scavenging capacity of other varieties (*Ourrous, Beid Lahmam, Outkbala, Tamjouherte, Taneslit, Tazerzeite* and *Takermoust)* grown in Algeria.

2. Material and Methods

2.1. Chemicals

Folin-Ciocalteu reagent was from Biochem, Chemopharma (Montreal, Quebec); 1,1-diphenyl-2-picrylhydrazyl (DPPH), ammonium molybdate and quercetin were from Sigma-Aldrich (Sternheim, Germany); gallic acid was from BDH, Prolabo (CE); aluminium chloride and sodium carbonate were from Biochem, Chemopharma (Georgia, USA).

2.2. Plant Material

The samples of date varieties (*Ourrous, Deglet Nour, Beid Lahmam, Outkbala, Taneslit, Tamjouherte, Tazerzeite* and *Takermoust*) were harvested from the region of Ghardaïa (Algeria), at full maturity (Tamr stage) in autumn 2013. Fruits with uniform size, free of physical damage, insect injury and fungal infection, were selected and used.

2.3. Preparation of the Extracts

Two grams of crushed dates were mixed with 50 ml of distilled water. The mixture was stirred for 20 min and then centrifuged at 5000 rpm for 10 min. The supernatant was collected and filtered (paper filter) then, stored at 4 °C.

2.4. Total Phenolic Content

The total phenolic compound contents were determined according to Al-Farsi *et al.* (2005). Date extract (200 µl) was mixed with 1.5 ml Folin-Ciocalteu reagent (diluted 10 folds). The mixture was kept at room temperature for 5 minutes, then, 1.5 ml of sodium carbonate (6%) was added. After 90 minutes, the absorbance was measured at 725 nm (Uvline 9400, Secomam, Alès, France). The results were expressed as mg of gallic acid equivalent per 100 g dry matter (mg GAE/ 100g DM).

2.5. Total Flavonoids Content

The total flavonoid contents of the date extracts were measured according to the colorimetric method of Kim *et al.* (2003); 500 µl of the extract were added to 150 µl of the sodium nitrite solution (5%) and 300 µl of aluminum chloride (10%). The tubes were incubated at room temperature for 5 minutes; 1 ml of sodium hydroxide (1 M) was added. The absorbance of the mixture was measured at 510 nm and the results were expressed as mg of quercetin equivalent per 100 g dry matter (mg QE/100 g DM).

2.6. Antioxidant Activity

2.6.1. Total Antioxidant Capacity (Reduction of Molybdate)

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to Kumaran and Karunakaran (2007). This method is based on the reduction of Mo (VI) to Mo (V) by the extracts, leading to the formation of green complex of phosphate/Mo (V) at acid pH. Extract (300 µl) was combined with 3 ml of the phosphomolybdic solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). After incubation at 95°C for 90 minutes, the absorbance was measured at 695 nm. The antioxidant capacity was expressed as mg of gallic acid equivalent per 100 g dry matter (mg GAE/100 g DM).

2.6.2. DPPH Radical Scavenging Capacity

The antiradical activity of date samples was estimated according to Hatano *et al*. (1988). Extract (500µl) was mixed with 1.5 ml of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) solution (60 µM). The mixture was stirred and left for 60 minutes in the dark. The reduction of DPPH radical was determined by measuring the absorbance at 517 nm. The DPPH scavenging capacity of extracts was calculated using the following equation:

$$\text{DPPH radical scavenging (\%)} = [(A_C - A_e)/A_C] \times 100\ \%,$$

where A_e is the absorbance of the extract; A_C is the absorbance of the control.

2.7. Statistical Analysis

The results were analyzed using statistical software (statistica version 5.5.fr). All values were expressed as mean ± standard deviation of three independent determinations. One-way analysis of variance (ANOVA) with LSD (Least Significant Difference) test was used to determine the significant differences ($p < 0.05$).

3. Result and Discussion

3.1. Total Phenolic Contents (TP)

Total phenolic contents of date fruits using water as extracting solvent are presented in figure 1. The TP contents of the investigated varieties ranged from 169.18 to 381.76 mg GAE/100g DM (127.97 to 334.58 mg GAE/100 g FW). Among the date varieties studied, significant differences ($P < 0.05$) were observed. The highest TP content was recorded in *Ourrous* variety while the lowest TP content was found in *Beid Lahmam* variety. The order of TP contents was as follows: *Ourrous*> *Deglet Nour*> *Tazerzeite*> *Tamjouherte*> *Takermoust*> *Tanslit*> *OutKbala*> *Beid Lahmam*. The results obtained in this study were higher than those of the Algerian date varieties analyzed by Mansouri *et al.* (2005): 2.49 to 8.36 mg GAE/100 FW. The results obtained by Biglari *et al.* (2008) for Iranian date varieties varied between 2.89 and 141.35 mg GAE/100 DM. The study conducted by Kchaou *et al.* (2013) using water as extracting solvent showed average levels close to our results for the Tunisian date varieties (160.98 to 222.23 mg GAE/100g FW). The differences observed could be related to many parameters such as the geographical origin, varieties, postharvest storage conditions and extraction duration and solvent.

3.2. Total Flavonoids (TF)

The flavonoid contents of the eight date varieties analyzed showed significant differences ($p< 0.05$). The TF contents ranged from 28.68 to 95.22 mg QE/100g DM (25.5 to 83.45 mg QE/100 g FW). The highest level of TF was detected in *Ourrous*, followed by *Deglet Nour*, while the lowest concentration was found in *Beid Lahmam* and *Outkbala* (figure 2). The results of this study were similar to those reported by Chibane *et al.* (2007) for three varieties of Algerian dates : *Frezza* (22.61 mg/100g FW), *Degla Beida* (27.43 mg/100g FW) and *Mesh Degla* (69.28 mg/100g FW). The results obtained by Biglari *et al.* (2008) for Iranian dates varied from 1.62 (*Jiroft*) to 81.79 mg/100g DM (*Kharak*). Benmeddour *et al.* (2013) reported that the flavonoid content of ten Algerian date samples ranged from 15.22 (*Deglet Nour*) to 299.74 mg/100 g DM (*Ghazi*).

3.3. Antioxidant Capacities

3.3.1. Total Antioxidant Capacity

As date fruit was an efficient antioxidant *in vitro* and in animal studies, it may be expected that its consumption would have a potent antioxidant activity in humans as well (Rock *et al.,* 2009). The antioxidant activity of the eight date varieties analyzed present significant differences ($p<0.05$). As showed in the figure 3, antioxidant activity recorded varied between 95.41 and 205.47 mg GAE/100g DM (88.59 and 180.08 mg GAE/100g FW). The results reported by Kchaou *et al.* (2013) for Tunisian date varieties, using water as the extracting solvent, ranged from 17.49 (*Bejo*) to 39.94 mg EAA/100g FW (*Deglet Nour*). According to Abbes *et al.* (2013), the antioxidant activity of three Tunisian date varieties *Allig*, *Kentichi* and *Deglet Nour* was of 118.75, 131.77, and 135.97 mg EAA/100g FW, respectively.

3.3.2. DPPH Radical Scavenging Capacity

The DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule. This radical is usually used as a substrate to investigate antioxidative potential of fruit extracts (Benmeddour *et al*., 2013; Kchaou *et al.*, 2013). Reduction capacity of DPPH radical is induced by antioxidants which yield an electron or a hydrogen atom (Fernandez-Agullo *et al.,* 2013). Data presented in figure 4 showed that the scavenging capacity against the DPPH radical by the eight studied date samples varied significantly ($p<0.05$). This antioxidant capacity ranged from 51.26% (*Outkbala*) to 89.08% (*Ourrous*). The current investigation revealed clearly that the varieties of date fruits have a strong capacity to scavenge the free radicals. Our results were in agreement with those obtained by Kchaou *et al.* (2013) for Tunisian date varieties (57.54% to 90.12%). However, according to Abbes *et al.* (2013), the antiradical activity recorded for Tunisian date fruits showed a wide variation ranging from 27.97 (*Allig*) to 76.40% (*Deglet Nour*).

3.4. Correlations

The scavenging capacity against DPPH radical used for determining antioxidant potential has been proven to exhibit a positive linear correlation (Table 1) with phenolic compound content, stating that these compounds contribute to the antioxidant capacity of the different date varieties. The statistical analysis revealed the presence of a highly significant correlation ($p<0.001$) between antioxidant activity and total phenolic ($r = 0.77$) and flavonoid contents ($r = 0.88$). These results are in agreement with those of Mansouri *et al.* (2005) and Biglari *et al.* (2008) who reported that phenolic compounds contribute significantly to the antioxidant capacities of date fruit.

4. Conclusion

The current investigation confirmed that date fruits can be considered as a potential natural source of bioactive phytochemicals such as phenolic compounds and flavonoids that play a major role in human health as free radical scavenger. Our findings showed statistical significant differences for both phenolic content and antioxidant potential of date fruit varieties, *Ourrous* being the best fruit. The date varieties which exhibited the strongest antioxidant

activity contained the highest levels of phenolic substances. These natural bioactive compounds can replace synthetic antioxidant in the food, pharmaceutical and cosmetic products.

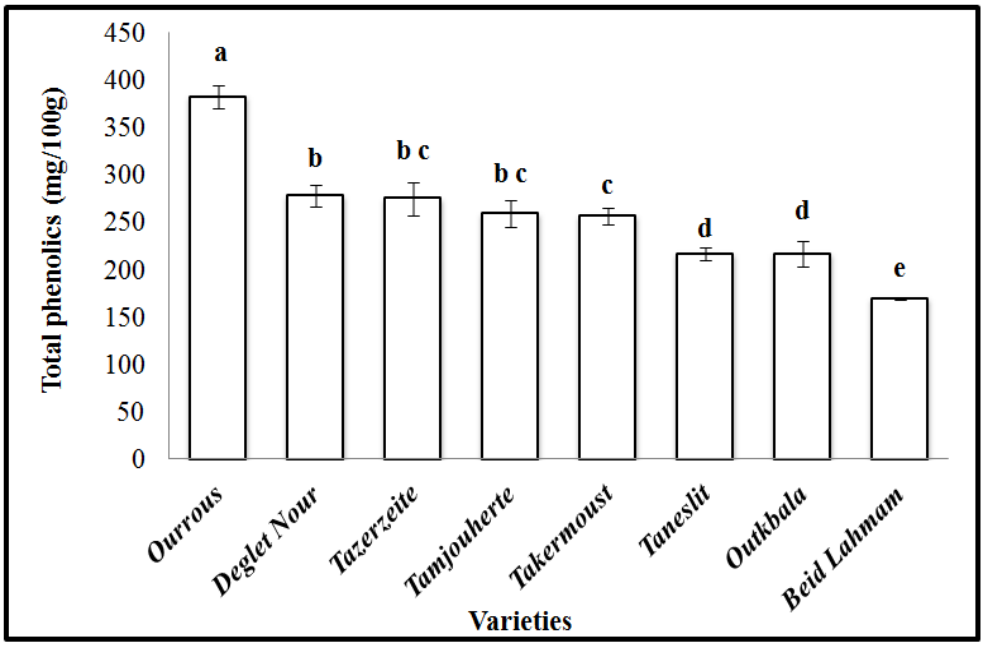

Figure 1. Total phenolic contents of the date varieties.

Different letters indicate statistically significant differences ($p < 0.05$) between varieties

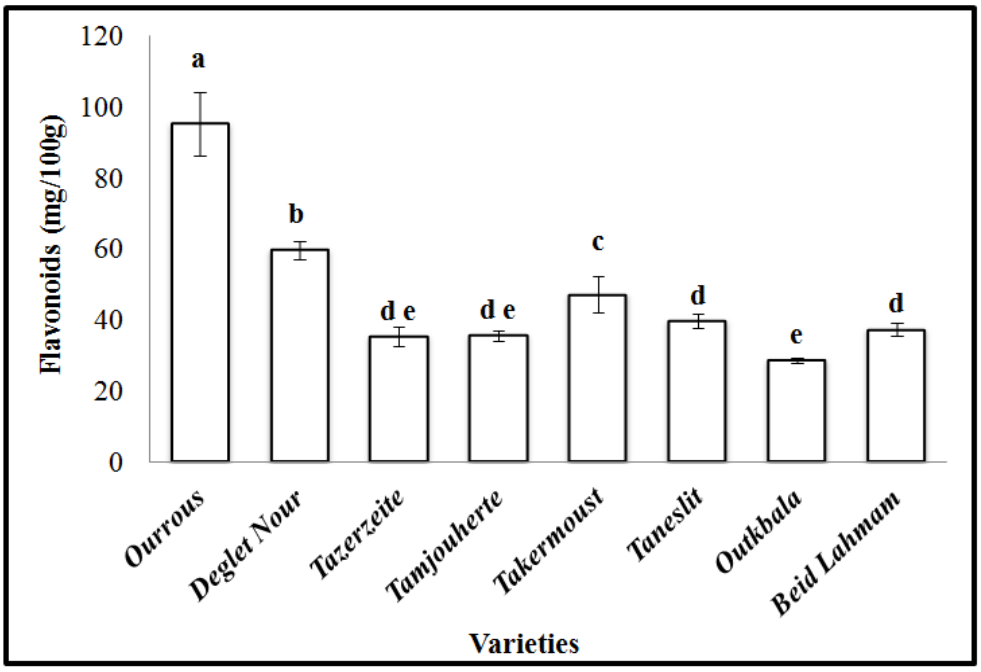

Figure 2. Flavonoid contents of the date varieties.

Different letters indicate statistically significant differences ($p < 0.05$) between varieties

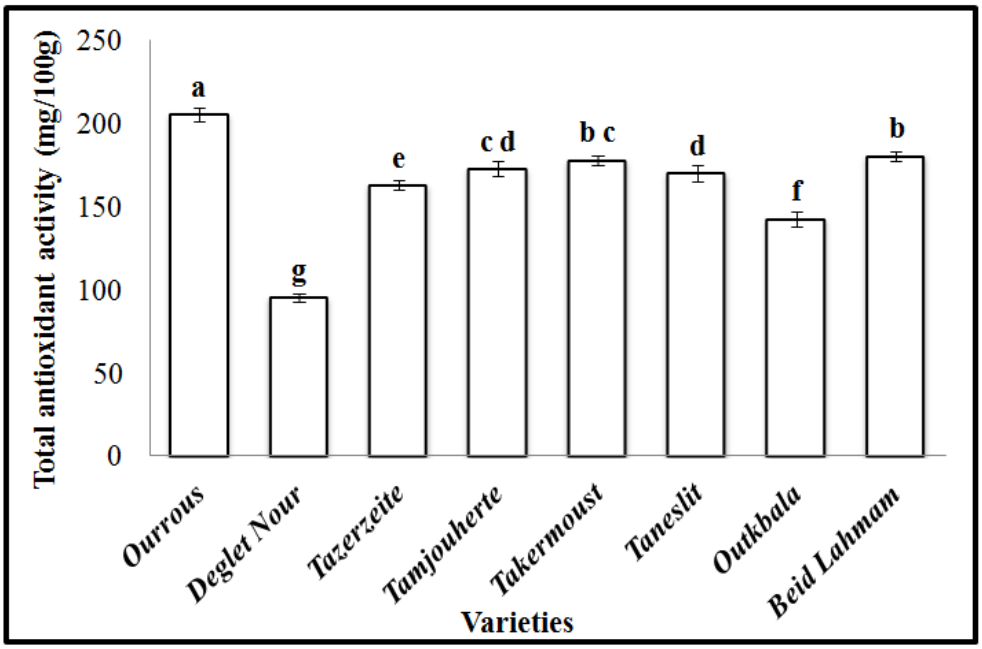

Figure 3. Total antioxidant activity of the date varieties.

Different letters indicate statistically significant differences ($p < 0.05$) between varieties

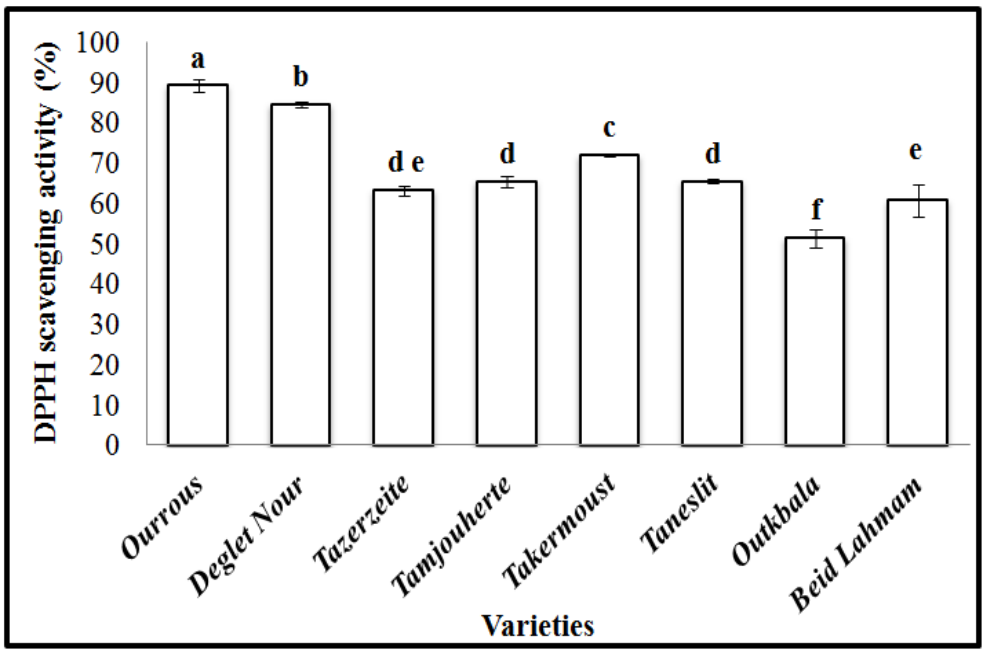

Figure 4. DPPH radical scavenging activity of the date varieties.

Different letters indicate statistically significant differences ($p < 0.05$) between varieties

Table 1. Matrix of correlation between the parameters analyzed.

Parameters	Correlation coefficient (r)	Equation
Phenolics - Flavonoids	0.83***	y = 0.291 x – 27.256
Phenolics - TAA	0.21	y = 0.108 x + 135.74
Phenolics - DPPH	0.77***	y = 0.158 x + 28.344
Flavonoids -TAA	0.25	y = 0.384 x + 145.21
Flavonoids-DPPH	0.88***	y = 0.528 x + 43.87

TAA: Total antioxidant activity;
*** Highly significant correlation ($p < 0.001$).

Acknowledgments

The authors are grateful to the Algerian Ministry of Higher Education and Scientific Research, and «Laboratoires Hors Murs», Montpellier, France, for financial support of this work. The authors are also thankful to Mr Boullahouat Nourredine and all persons who have provided the date fruit samples used in this study.

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