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In vitro assessment of singlet oxygen quenching capacity and phenolic contents of some extracts from Algerian date seeds “*Ali Ourached*”

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Abstract

Several natural compounds are known to exhibit direct significant antioxidant properties against two various responsible pathogenic related to oxidative stress (Free radicals and singlet oxygen). In this study, aqueous and hydromethanolic extracts of *Ali Ourached* date seeds were evaluated and compared the total polyphenols and flavonoids contents, as well as their ability to scavenge the free radicals and quench singlet oxygen. Among these two extracts of *Ali Ourached* date seeds, the highest level of total phenolic content was observed in the hydromethanolic extract (362.66 mg GAE/g extract), whereas the average flavonoid content was 488.26 mg EQ/g of extract as compared to aqueous extract 185.14 mg EQ/g of extract. The methanol extract exhibited higher DPPH and ABTS radical scavenging activity with the IC₅₀ values of 262.90 and 141.99 μ g/ml, respectively and correlated with polyphenols and flavonoids content. The highest total antioxidant capacity of hydromethanolic extract was 80.37 mg/g ascorbic acid at concentration 140 μ g/ml and the lowest 14.99 mg/g ascorbic acid at low concentration. Singlet oxygen quenching ability of aqueous and hydromethanolic extracts of *Ali Ourached* date seeds was 7.64 and 8.88 % at 120 mg/ml concentration.

Keywords: Date seeds, Polyphenols, Antioxidant, Free radicals, Singlet oxygen.

Introduction

The date palm (*Phoenix dactylifera* L.) is especially considered as the most ancient cultivated plant for humans in the Middle East and North Africa and is highly well known worldwide (El Hilaly et al., 2018). The fruit of these palms “dates” not only considered as the most traditional food, but they also have valuable nutritional and economic returns to the population of these regions (Ardekani et al., 2010), where is formed a fleshy pericarp and seed (pit) that constitute between 10% and 15% of the date fruit weight (Bijami et al., 2020). Date seeds are known as waste/ useless products, sometimes used for animal feed are also considered as a source of oil and a coffee substitute (Khan et al., 2016). Today, several studies including those examined on various parts of date palm have shown that they have a healthy potential (Al-Farsi et al., 2007). Considering that, date seeds contain many beneficial bioactive compounds (Hmidani et al., 2020). Thus, utilization of such compounds which are of natural origin is very important for the dietary management of certain diseases, which an increased possibility of eliminating special diseases caused by oxidative stress (Masmoudi-Allouche et al., 2016). Numerous studies have shown that the date seeds possess anticancer, anti-inflammatory, antimicrobial,

antimutagenic, antioxidant, gastroprotective, hepatoprotective, immune–stimulant and nephroprotective activities (Ahmed et al., 2016). However, in vitro and in vivo studies relating to the date seeds are somewhat limited. The aim of the present study is to investigate the phytochemical compositions and antioxidant property of the aqueous and hydromethanolic extracts of *Ali Ourached* date seeds, as a possible source of antioxidant and bioactive compounds

Preparation of extracts:

The fine powder date seeds of *Ali Ourached* variety was transferred to two Eppendorf tubes containing of 50 ml capacity. Eppendorf tube contained 5 g of powdered seed and 30 ml of water or 80% methanol was added to it. The mixtures were stirred using an orbital shaker-incubator for 12 h at room temperature. The aqueous and hydromethanolic extracts were then centrifuged at 13500 rpm for 5 min and filtrated using filter paper. The combined hydromethanolic filtrate was concentrated at 28°C under vacuum using a rotary evaporator. The Aqueous and hydromethanolic extracts subsequently to freeze were tempered at –80 °C for 48 h prior to freeze-drying using a freeze-dryer. The percent yield of aqueous extract of *Ali Ourached* date seeds is 7.736 % and the hydromethanolic extract yielded a mass weighing 0.4782 g and extractive value was found to be 9.564 % w/w.

Total Phenolic content:

The total phenolic content of *Ali Ourached* date seeds aqueous and hydromethanolic extracts was determined spectrophotometrically using Folin–Ciocalteu assay (Al-Farsi et al., 2005). Briefly, 0.75 mL of diluted Folin-Ciocalteu reagent (10%) was added to a stock solution containing 0.1 mL of each extract or standard. After 5 min, a volume of 0.75 mL of sodium carbonate solution (60 g/L) was added to each mixture and mixed thoroughly. The reaction mixture was allowed to react for 90 min at room temperature. After incubation, the absorbance was measured at 725 nm using UV–Vis spectrophotometer. Gallic acid was used as a standard and TPC of the two extracts were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract).

Total Phenolic content:

The total flavonoid content of each extract of the extracts (aqueous and hydromethanolic extracts) was quantified by the Trichloro-aluminum method (Kim et al., 2003). 0.5 ml of each extract was mixed with 0.15 ml of sodium nitrite (5%, w/v), followed by 0.3 ml of AlCl₃ (10%, w/v) ethanolic solution. The mixture was vigorously stirred and the absorption at 510 nm was

read after 5 min at room temperature. Quercetin was used as a standard and the results of TFC have been given in mg quercetin equivalent per gram of extract (mg EQ/g of extract).

In Vitro Antioxidant Studies:

DPPH radical scavenging activity:

Radical scavenging activity of aqueous and hydromethanolic extracts against stable DPPH[•] (2,2-diphenyl-1-picrylhydrazylhydrate) was determined spectrophotometrically and measured by slightly modified method of Blois (Blois, 1958).

To each 0.04 ml of each extract, 1 ml of methanol solution of DPPH ($6 \times 10^{-5} \text{M}$) was added. The obtained mixture was immediately shaken and incubated for 30 min in obscurity at room temperature. The absorbance was measured at 517 nm.

The same procedure was repeated for Ascorbic acid as a negative control at various concentrations (ranging from 20 to 140 $\mu\text{g/ml}$).

The radical scavenging activity was expressed as the radical scavenging percentage using the following formula where; A_{control} and A_{sample} are the absorbance of the control and the tested extract solution with DPPH solution, respectively.

$$I (\%) = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

ABTS radical cation scavenging activity:

The antioxidant potential was measured by ABTS assay that measures the relative ability of antioxidant substances of aqueous and hydromethanolic extracts to scavenge the blue-green ABTS^{•+} generated in the aqueous phase (Re et al., 1999). The ABTS^{•+} was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate for 16 h in the dark, at room temperature, before UV measurements. Before usage, the ABTS^{•+} stock solution was diluted to get an absorbance of 0.700 ± 0.005 at 734 nm with 80 % ethanol. Briefly, 0.975 ml diluted ABTS solution was added to 0.025 ml of diluted sample to react in the dark at room temperature for 6 min and then the decrease in absorption was measured at 734 nm. The same procedure was repeated for Ascorbic acid as a negative control at different concentrations (ranging from 15 to 105 $\mu\text{g/ml}$). The percentage inhibition by extract or control was calculated using equation:

$$I (\%) = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

Where A_{control} and A_{sample} are the absorbance of the control and the tested extract solution with ABTS solution, respectively.

Calculation of 50% inhibition concentration:

The optical density obtained with each concentration of the aqueous and hydromethanolic extracts / negative control solution (Ascorbic Acid) was plotted by taking concentration ($\mu\text{g/ml}$) on X-axis and percentage inhibition (%) on Y-axis. The graphs were extrapolated to find the concentration of sample required to scavenge 50% of ABTS and DPPH.

Singlet oxygen quenching activity

Oxidation of DPIBF by the singlet oxygen generated by a peroxidase, H_2O_2 and halide system and reading the absorbance at 420 nm (Sachindra et al., 2008). The reaction was carried out in acetate buffer solution (0.05 M, pH 4.5) containing 1 unit of peroxidase, 0.25 mM KBr, 0.25 mM H_2O_2 and 0.1 mM DPBIF solubilized in 0.05% Triton X-100. All the reagents except hydrogen peroxide were mixed in a quartz cuvette and the reaction was initiated by the addition of H_2O_2 into the reaction mixture. The change in absorbance was monitored at 420 nm at 30 s interval for 5 min. The quenching % was measured from the reduction in absorbance in presence and absence of the extract.

Total antioxidant capacity

The total antioxidant capacity of aqueous and hydromethanolic extracts was evaluated by the phosphomolybdenum method (Prieto et al., 1999). The assay was performed by mixing 0.1 ml from each sample extract or standard with 1 ml of reagent solution (0.6 M sulfuric acid, 0.028 M sodium phosphate and 0.004 M ammonium molybdate). Each mixture tube containing the reaction solution was incubated at 95°C for 90 min. Then, the absorbance value of the solution was measured at 695 nm. The total antioxidant activity of each extract expressed as the number of gram equivalent of ascorbic acid. The calibration curve was determined using various concentrations of 20, 40, 60, 80, 100, 120 and 140 $\mu\text{g/ml}$.

Results

The content of phenolic compounds in aqueous and hydromethanolic extracts, determined from regression equation of calibration curve ($y=6.9806x-0.086$, $R^2=0.9998$) and expressed in mg quercetin equivalent per gram of extract. It is found that hydromethanolic extract has the highest total phenolic content (362.66 mg GAE/g extract). The lowest amount of phenolic content was found in aqueous extract (118.63 mg GAE/g extract) (Table 1). The amount of polyphenols extracted using 80% methanol has much higher in comparison to that obtained on extraction with water. Many reports are available which demonstrate the usefulness of phenolic compounds as one of the main groups of compounds in various biological activities,

particularly in antioxidant activity. For this reason, it is logical to calculate their total amount in date seeds extracts

Total flavonoid content of the aqueous and hydromethanolic extracts was calculated from the regression equation of calibration curve ($y=1.9571x+0.025$, $R^2 = 0.999$) and mg quercetin equivalent per gram of extract. Results revealed that the flavonoid content was highest in 80% methanol extract (185.14 mg EQ/g of extract). Lowest value of flavonoids was observed in aqueous extract (488.26 Total flavonoid content).

Table 1: Total Phenolic and Flavonoid content of *Ali Ourached* date seeds

Variety	Extract	Total phenolic content (mg GAE/g of extract)	Total flavonoid content (mg EQ/g of extract)
<i>Ali Ourached</i>	Aqueous	118.63 $\pm$ 1.01	185.14 $\pm$ 2.17
	Hydromethanolic	362.66 $\pm$ 1.76	488.26 $\pm$ 9.07

The DPPH scavenging activity of the aqueous and hydromethanolic extracts tested is presented in Table 2. The DPPH radical scavenging activity of the two extracts was found to be linearly dose dependent with hydromethanolic extract showing higher scavenging activity (262.90 μ g/ml) compared to aqueous extract (559.53 μ g/ml). However, when compared with that of ascorbic acid, the scavenging activity of these two extracts was lower.

The hydromethanolic extract of *Ali Ourached* date seeds showed higher ABTS radical scavenging activity (141.99 μ g/ml) compared to aqueous extract (475.28 μ g/ml). These results suggest that the aqueous and hydromethanolic extracts are apparently good free radical scavengers and could thus be beneficial in the treatment of many diseases by probability to inhibit autoxidation of lipids (Aliyu et al., 2015).

Table 2: 50% Inhibition concentration (IC₅₀) of aqueous and hydromethanolic extracts of *Ali Ourached* date seeds against DPPH and ABTS.

Extracts/Compound		IC ₅₀ (μ g/ml)		% quenching
		DPPH	ABTS	Singlet oxygen
<i>Ali Ourached</i> date seeds	Aqueous extract	559.53 $\pm$ 7.49	475.28 $\pm$ 7.06	7.64 $\pm$ 0.07
	Hydromethanolic extract	262.90 $\pm$ 1.83	141.99 $\pm$ 0.81	8.88 $\pm$ 0.26

Ascorbic acid	73.80 ± 1.23	73.95 ± 1.03	-
Control	-	-	25.06 ± 0.64

In this study, in absence of the extract the absorbance of DPIBF was reduced by 25.06 % in 5 min, while the reduction was only 7.64 and 8.88 % in presence of 120 $\mu\text{g/ml}$ of aqueous and hydromethanolic extracts, respectively (Figure 2). As singlet oxygen is generated in variety of biological systems, and with absorption light by photosensitization (Kanofsky et al., 1988), the solutions to quench the singlet oxygen are natural antioxidants, this is due to the physical and chemical mechanisms (Mukai et al., 1993).

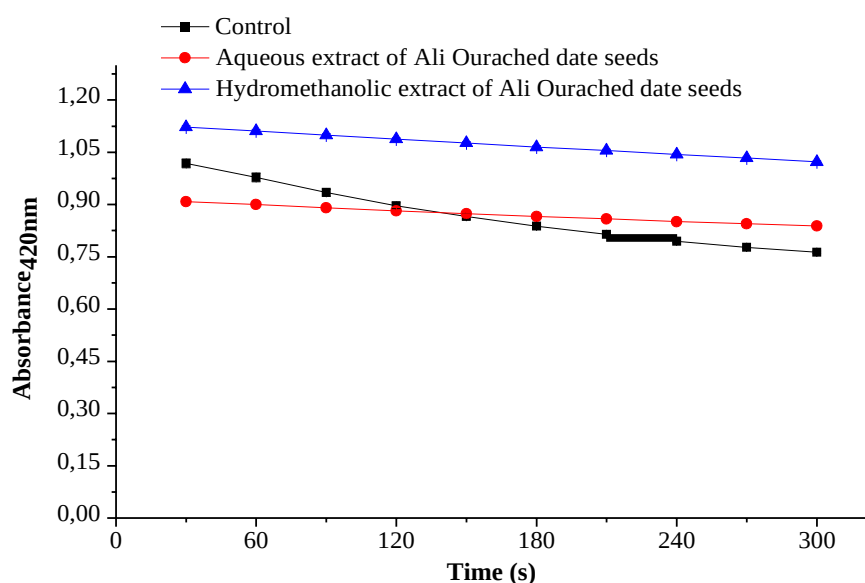


Figure 1: Reduction in absorbance at 420 nm of DPIBF as influenced by the concentration of aqueous and hydromethanolic extracts of *Ali Ourached* date seeds

The results indicate higher TAC of the hydromethanolic extract of *Ali Ourached* date seeds compared to aqueous extract. The hydromethanolic extract possesses significant total antioxidant capacity equivalent to 14.99 and 80.37 mg/g ascorbic acid at low and higher concentration (20 and 140 $\mu\text{g/ml}$, respectively). Among the tow extracts, the aqueous extract showed the lowest (51.32 mg/g ascorbic acid) activity at concentration 140 $\mu\text{g/ml}$ (Figure 1). This suggests the potential comparable antioxidant constituents of the aqueous and hydromethanolic extracts of *Ali Ourached* date seeds because antioxidant capacity of ascorbic acid has been used as a reference standard with which plant extracts with potential antioxidants are compared (Godavari et al., 2018).

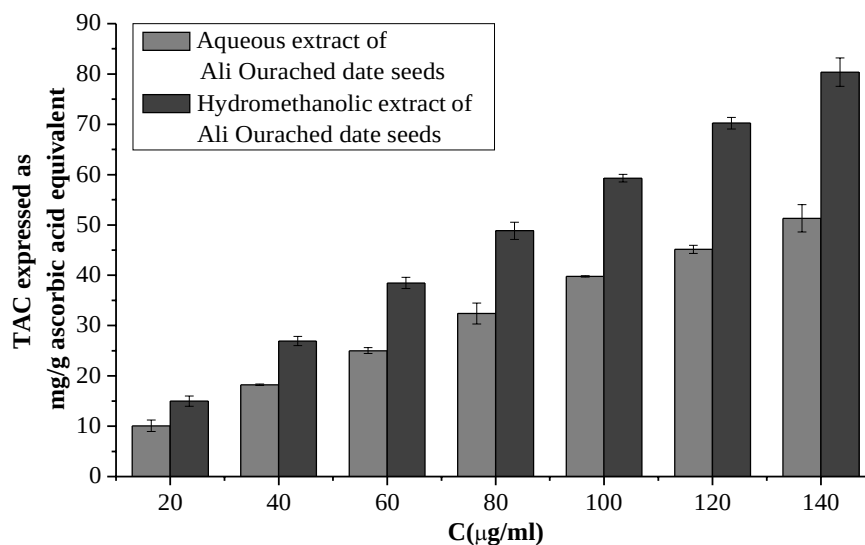


Figure 2: Total antioxidant capacity of aqueous and hydromethanolic extracts of *Ali Ourached* date seeds. Data are presented as mean value $\pm$ standard deviation SD (n = 3)

Conclusion

This study indicated that the date seeds (variety of *Ali Ourached*) examined possess radical scavenging and singlet oxygen quenching property. At present, in Algeria, the main use of date seeds is as animal feed and other secondary uses such as coffee and oil. As date seeds are known to contain antioxidative substances and variety of biomolecules of health benefits, increased attempts should be made towards their better utilization in medicinal preparations or food.

Références

- 1- El Hilaly, J., Ennassir, J., Benlyas, M., Alem, C., Amarouch, M. Y., & Filali-Zegzouti, Y. (2018). Anti-inflammatory properties and phenolic profile of six Moroccan date fruit (*Phoenix dactylifera* L.) varieties. *Journal of King Saud University-Science*, 30(4), 519-526.
- 2- Ardekani, M. R. S., Khanavi, M., Hajimahmoodi, M., Jahangiri, M., & Hadjiakhoondi, A. (2010). Comparison of antioxidant activity and total phenol contents of some date seed varieties from Iran. *Iranian journal of pharmaceutical research: IJPR*, 9(2), 141.
- 3- Bijami, A., Rezanejad, F., Oloumi, H., & Mozafari, H. (2020). Minerals, antioxidant compounds and phenolic profile regarding date palm (*Phoenix dactylifera* L.) seed development. *Scientia Horticulturae*, 262, 109017.

- 4- Khan, S. A., Al Kiyumi, A. R., Al Sheidi, M. S., Al Khusaibi, T. S., Al Shehhi, N. M., & Alam, T. (2016). In vitro inhibitory effects on α -glucosidase and α -amylase level and antioxidant potential of seeds of *Phoenix dactylifera* L. *Asian Pacific Journal of Tropical Biomedicine*, 6(4), 322-329.
- 5- Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., & Al-Rawahy, F. (2007). Compositional and functional characteristics of dates, syrups, and their by-products. *Food chemistry*, 104(3), 943-947.
- 6- Hmidani, A., Bourkhis, B., Khouya, T., Ramchoun, M., Filali-Zegzouti, Y., & Alem, C. (2020). Phenolic profile and anti-inflammatory activity of four Moroccan date (*Phoenix dactylifera* L.) seed varieties. *Heliyon*, 6(2), e03436.
- 7- Masmoudi-Allouche, F., Touati, S., Mnafigui, K., Gharsallah, N., El Feki, A., & Allouche, N. (2016). Phytochemical profile, antioxidant, antibacterial, antidiabetic and anti-obesity activities of fruits and pits from date palm (*Phoenix dactylifera* L.) grown in south of Tunisia. *Journal of Pharmacognosy and Phytochemistry*, 5(3), 15.
- 8- Ahmed, A., Bano, N., & Tayyab, M. (2016). Phytochemical and therapeutic evaluation of date (*Phoenix dactylifera*). A review. *J Pharm Altern Med*, 9, 11-17.
- 9- Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005). Compositional and sensory characteristics of three native sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of agricultural and food chemistry*, 53(19), 7586-7591.
- 10- Kim, D. O., Jeong, S. W., & Lee, C. Y. (2003). Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food chemistry*, 81(3), 321-326.
- 11- Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199-1200.
- 12- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, 26(9-10), 1231-1237.
- 13- Sachindra, N. M., & Bhaskar, N. (2008). In vitro antioxidant activity of liquor from fermented shrimp biowaste. *Bioresource technology*, 99(18), 9013-9016.
- 14- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry*, 269(2), 337-341.
- 15- Aliyu, A. B., Ibrahim, M. A., Musa, A. M., Musa, A. O., Kiplimo, J. J., & Oyewale, A. O. (2013). Free radical scavenging and total antioxidant capacity of root extracts of *Anchomanes difformis* Engl.(Araceae). *Acta Pol Pharm*, 70(1), 115-21.

- 16- Kanofsky, J. R., Hoogland, H., Wever, R., & Weiss, S. J. (1988). Singlet oxygen production by human eosinophils. *Journal of Biological Chemistry*, 263(20), 9692-9696.
- 17- Mukai, K., Itoh, S., Daifuku, K., Morimoto, H., & Inoue, K. (1993). Kinetic study of the quenching reaction of singlet oxygen by biological hydroquinones and related compounds. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 1183(2), 323-326.
- 18- Godavari, A., & Amutha, K. (2017). In vitro Antidiabetic Activity of *Garcinia mangostana* by Enzymatic Inhibition Assay. *Research Journal of Pharmacy and Technology*, 10(2), 508-512.