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RESEARCH ARTICLE

Phenolic profile, Antioxidant potential of date (*Phoenix dactylifera* Var. Degla Baidha and Deglet-Nour) seeds from Debila region (Oued Souf, Algeria)

Abdeldjabbar Messaoudi*, Messaouda Dekmouche, Zehour Rahmani, Cheyma Bensaci
Laboratory of Valorization and Promotion of Saharan Resources (VPRS). Univ. Kasdi Merbah Ouargla, BP 511 Route Ghardaia, Ouargla, Algeria -30000.
*Corresponding Author E-mail: **jabourimess2@gmail.com**

ABSTRACT:
The purpose of this study is to investigate the antioxidant property of aqueous and methanolic extracts of two date palm seeds (Degla Baidha and Deglet Nour) grown in Debila region (Oued Souf, Algeria). The date seed aqueous and methanolic extracts were studied for three in-vitro antioxidant activity (AA) by 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, ABTS radical scavenging assay, phosphomolybdenum method. The DSME of Degla Baidha showed the highest AA for the three methods cited previously (0.0824mg/mL, 0.1169mg/mL for IC50 value of DPPH and ABTS, respectively) and the total antioxidant capacity of the above-mentioned extract provided strong result at all concentrations. The phenolic and the flavonoid content of the two date seeds (Degla Baidha and Deglet-Nour) found changed between 1.5892–4.6591 mg GAE /100 g DW and 2.0642–6.0637 mg QE/100 g DW, respectively.

KEYWORDS: Antioxidant, total phenolic, date seed, DPPH, ABTS.

INTRODUCTION:
One of the human's most ancient cultivated fruit tree and the highly popular among worldwide for millennia, especially in the Middle East and North Africa (MENA), is the date palm (*Phoenix dactylifera* L., Arecaceae)[1]. The fruits of date palm (Dates) are a vital and staple element of the daily diet and the most traditional because they have valuable nutritional components and health benefits[2]. The valuable effects are not limited to the edible part of fruit (fleshy pericarp) only, but date seeds (pits), which are considered as disposed waste, are equi-important[3]. These seeds constitute approximately between 6.10% and 11.47% of date fruit weight and have been used previously as animal feed, making non-caffeinated coffee or a source of oil[4]. The date pits contain a wide range of nutritional functional compounds such as protein, fat, moisture, ash, dietary fiber and vitamins as well as important amounts of minerals and phenolic compounds[5,6,7,8].

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Several reports have indicated that date seeds as well as fleshy pericarp of fruit have been known to contain valuable bioactive compounds, mainly phenolic compounds[9]. Hence, the seeds of date might be considered a potential source of natural antioxidant[10,11,12], anti-inflammatory[13], antidiabetic[14], antimicrobial and anti-obesity[15].

Because there have been few studies assessing the in vitro activities effect of seeds dates grown in Oued Souf (Debila), this study was carried out to evaluate proximate, phenolic and flavonoid content, and evaluate the antioxidant activity.

MATERIAL AND METHODS:
Chemicals and reagents:
Folin-Ciocalteu reagent, Sodium carbonate, Gallic acid, sodium nitrite, Aluminum chloride, Quercetin, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Potassium persulfate, Sulfuric acid, Sodium phosphate, Ammonium molybdate, Methanol, Ethanol.

Collection of plant material:
The seeds of two date palm varieties locally grown in the south-east of Algeria (Debila- Oued Souf), namely Degla Baidha and Deglet-Nour which are listed among the top date palm cultivar produce of Algeria and are the most commonly consumed. The two date palm were collected in December 2019 at tamr stage.

Preparation of date seed extracts:
Two different cultivars of fresh date fruits (Degla Baidha and Deglet-Nour) of uniform size, free of physical damage and defects were chosen and then picked up by hand. The two date seeds were removed manually and air-dried in the laboratory at room temperature to prepare extracts.

Date seeds were coarsely powdered using HUKOER Portable Grain Grinder and finally passed through 1mm sieve to obtain a fine powder.

Approximately 5g of dry date seeds powder of each cultivar was extracted with 25mL of water and methanol–water (4:1, v/v) at room temperature for 12 h using an orbital shaker-incubator. After centrifugation at 13500rpm for 5 min and filtration using filter paper, then the methanol filtrates were evaporated at 40°C under vacuum using a rotary evaporator. Aqueous and methanolic extracts were further subjected to freeze-drying using a freeze-dryer and referred to as 'date seed aqueous extract, DSAE and date seed methanolic extract DSME'.

Phytochemical Composition:
Determination of total phenolic content:
The assay of total phenolic content in DSAE and DSME was carried out by the colorimetric method using Folin-Ciocalteu reagent and gallic acid as standard[16]. Briefly, 750µL of Folin-Ciocalteu reagent (diluted 10 times in water) was added to a solution containing 100µL of each extract or standard solution. The solution was mixed and after 5 min, 750µL ml of sodium carbonate (60g/L) solution was added to the mixture. The reaction mixture was left to incubate in the dark at room temperature for 90 min and the absorbance was measured at 725nm. The TPC was calculated by a standard gallic acid graph, and the results were expressed as milligram gallic acid equivalent per 100g dry weight (mg GAE/100g DW).

Determination of total flavonoid content:
The total flavonoid content in DSAE and DSME was determined by the colorimetric method using aluminum chloride reagent and Quercetin as standard[17]. About 500 µL of each extract or standard solution was mixed with 150µL of sodium nitrite (5%, w/v), followed by 300µL of aluminum chloride (10%, w/v) ethanolic solution. After incubation at room temperature for 5 min, the absorbance of the reaction mixture was measured at 510 nm. The TFC was calculated by a standard Quercetin graph, and the results were expressed as milligram quercetin equivalent per 100g dry weight (mg QE/100g DW).

In Vitro Antioxidant Studies:
DPPH Radical Scavenging Activity:
The free radical scavenging activity of DSAE and DSME was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH free radical[18]. Different dilutions of standard or the phenolic extract were prepared for each date seed variety. An aliquot of 40µL of diluted sample was added to 1mL of a methanol solution of DPPH (6×10^{-5}M). After mixing, the tubes were incubated for 30 min at room temperature in the dark; the absorbance of the samples was read at 517nm. The same procedure was repeated for Ascorbic acid as a positive control at various concentrations (ranging from 0.02 to 1.2mg/mL). The capability to scavenge the DPPH radical was evaluated using the following formula:

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

Where A_{blank} and A_{sample} are the absorbance of the blank and the tested sample with DPPH solution, respectively.

ABTS radical scavenging assay:
The relative ability of antioxidant substances of DSAE and DSME was based on the reduction of the blue-green ABTS•+ radical by electron- or hydrogen-donating[19]. The reagent ABTS radical cation (ABTS•+) was prepared by mixing a solution of 7mM ABTS and 140 mM potassium persulfate solution for 16 h in the dark. The stock solution of ABTS•+ was diluted just before assay to get an absorbance of 0.700 ± 0.005 at 734nm with 80% ethanol. Different dilutions of standard or the phenolic extract were prepared for each date seed variety. 25µL of diluted sample was added to 975µL diluted ABTS solution to react in the dark at room temperature for 6 min, and the decrease in the absorbance was measured at 734nm. The same procedure was repeated for Ascorbic acid as a positive control at different concentrations (ranging from 0.015 to 0.1025mg/mL). The percentage inhibition by date seed methanolic extract was calculated using equation:

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

Where A_{blank} and A_{sample} are the absorbance of the blank and the tested sample with ABTS•+ solution, respectively.

Total Antioxidant Using Phosphomolybdenum Assay:
The total antioxidant capacity of DSAE and DSME in the phosphomolybdenum method[20]. A 100µL from each

sample extract was combined with 1mL of reagent solution (600mM sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). A typical blank solution, containing all reagents except the test extract (Methanol in the place of extract), was prepared. After capping the tubes and incubation at 95°C for 90 min and then cooling at room temperature. Then the absorbance was monitored at 695nm. A calibration curve was prepared using a standard solution of ascorbic acid (0.02, 0.04, 0.06, 0.08, 0.10, 0.12 and 0.14mg/mL), and the results were expressed as the number of gram equivalent of ascorbic acid.

Statistical analysis:

Statistical analysis was performed using Origin 2019 from Origin Lab Corporation. Data are presented as mean ± standard deviation (SD).

RESULTS AND DISCUSSION:

Yield, total phenolic and flavonoid contents of date seed aqueous extract (DSAE) and date seed methanolic extract (DSME):

Yield, total phenolic and flavonoid contents of date seed extract using water or methanol (80%) as the extraction media (Table 1). It is evident that the DSME have a higher yield as compared to the DSAE. The highest yield was recorded at DSME of Deglet-Nour (10.734%) as compared to DSME of Degla Baidha (8.82%). While the lowest yield (7.128%) was obtained from DSAE of Degla Baidha. The extraction of phenolic compounds yield was significantly affected by variety, chemical nature of plant, extraction solvents and sample particle size[21]

The total phenol content (TPC) in DSAE and DSME of Degla Baidha and Deglet-Nour was determined using the Folin-Ciocalteu assay, expressed as miligram Gallic acid equivalent per 100gram dry weight (mg GAE/g 100 DW). The highest content on phenolic (4.6591mg GAE/100g DW) was observed in DSME of Deglet-Nour, whereas the lowest amount of phenolic (1.5892mg GAE/100g DW) was found in DSAE of Degla Baidha.

Total flavonoid content (TFC) of DSAE and DSME from Degla Baidha and Deglet-Nour was measured using aluminum chloride colorimetric methods expressed as miligram Quercetin equivalent per 100 gram dry weight (mg QE/g 100 DW). The highest amount of flavonoid (6.0637mg QE/100g DW) was present in DSME of Deglet-Nour, whereas the lowest content of flavonoid (2.0642mg QE/100g DW) was recorded in DSAE of Degla Baidha. This variation within may be owing to the extraction system as well as to the variety of date seed[22].

Table 1: Yield, total phenolic and total flavonoid contents in DSAE and DSME of Degla Baidha and Deglet-Nour

Varieties	Yield %		Total phenolic content (mg GAE/100 g DW) *		Total flavonoid content (mg QE/100 g DW) *	
	DSAE	DSME	DSAE	DSME	DSAE	DSME
Degla Baidha	7,128	8,82	1,5892±0,0171	3,7727±0,0485	2,0642±0,0381	4,9348±0,1607
Deglet-Nour	8,652	10,734	2,1477±0,0026	4,6591±0,0111	3,1248±0,1260	6,0637±0,0850

*Results are expressed as mean of 3 values ± standard deviation

Antioxidant activity:

Antioxidant capacity is a complex procedure usually happening through several mechanisms and is affected by numerous factors. Due to its complexity, it is necessary to perform various than one single method of antioxidant activity measurement to take into account the several mechanisms of antioxidant action.

Various methods are available for evaluation of antioxidant capacity, these assays were applied to scavenge and quench ROS which comprise free radicals such as ($(O_2^{\bullet-}$, $OH^{\bullet}$ and $RO^{\bullet}$) and non-free radicals like (H_2O_2 and $1O_2$)[23].

In this article, the DSAE and DSME of Degla Baidha and Deglet-Nour were subjected to a screening for the possible AA by three assays, namely DPPH and ABTS radicals scavenging activities and phosphomolybdenum method to their simplicity, stability and accuracy.

DPPH and ABTS radicals scavenging activities:

DPPH and ABTS assay based on the ability of antioxidant to scavenge 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$)[24]. A linear curve was obtained by plotting percentage (I%) of radical scavenging activity versus concentrations (mg/mL) to determine the IC50. The IC50 values of DSAE and DSME of Degla Baidha and Deglet-Nour or standards are shown in (Table 2).

The values of IC50 of DPPH radical scavenging activity ranged from 0.0824mg/mL to 0.3053mg/mL and the IC50 of ABTS radical scavenging activity varied from 0.1169 to 0.1488mg/mL.

In the presence of a potential antioxidant (an electron donor), the singlet electron of DPPH will be paired up, and the violet color of DPPH will turn into yellow while decreasing the intensity of the absorption at 517 nm[25].

The results showed that the antiradical activity was extraction solvents and cultivars dependent. DSME of Degla Baidha had the strongest DPPH scavenging capacity, whereas the lowest DPPH radical quenching was observed in the DSAE of Degla Baidha variety. The AA in the methanolic extracts of the two varieties decreases in the order: Degla Baidha> Deglet-Nour. The AA of DSAE and DSME was lower than those observed in Ascorbic acid (0.0738±0.0012mg/mL).

In the presence of a potential antioxidant, the reduction of the radical anion of ABTS by electron transfer or hydrogen atom transfer, and the blue-green of ABTS will turn into colourless form while decreasing the intensity of the absorption at 734nm.

The DSME of Degla Baidha showed the highest level of antioxidant activity (0.1169mg/mL), while DSAE of Deglet-Nour showed the lowest level (0.1488mg/mL). The DSAE and DSME decreases in the order: Degla Baidha> Deglet-Nour. The AA of DSAE and DSME was lower than those observed in Ascorbic acid (0.0739±0.0006mg/mL).

Phosphomolybdenum Method:

The total AA of DSAE and DSME of Degla Baidha and Deglet-Nour was measured using phosphomolybdenum method which was based on the formation of a green complex of phosphate/Mo(V) results from the reduction state of Mo from Mo (IV) to Mo (V) by the phenolic extract with a maximal absorption at 695nm.

The results showed higher TAC (expressed as ascorbic acid equivalent) of the DSME as compared to DSAE, except the low concentration 0.02mg/mL. The DSME of Deglet-Nour and Degla Baidha possesses significant total antioxidant capacity equivalent to 95,5605 and 88,4059mg/g ascorbic acid, respectively, at higher concentration. (Fig. 1)

Table 2: DPPH and ABTS scavenging activities

Varieties	DPPH (IC50mg/mL) *		ABTS (IC50mg/mL) *	
	DSAE	DSME	DSAE	DSME
Degla Baidha	0,3053±0,0078	0,0824±0,0012	0,1205±0,0042	0,1169±0,0038
Deglet-Nour	0,2276±0,0040	0,0972±0,0028	0,1488±0,0009	0,1178±0,0021

*Results are expressed as mean of 3 values ± standard deviation

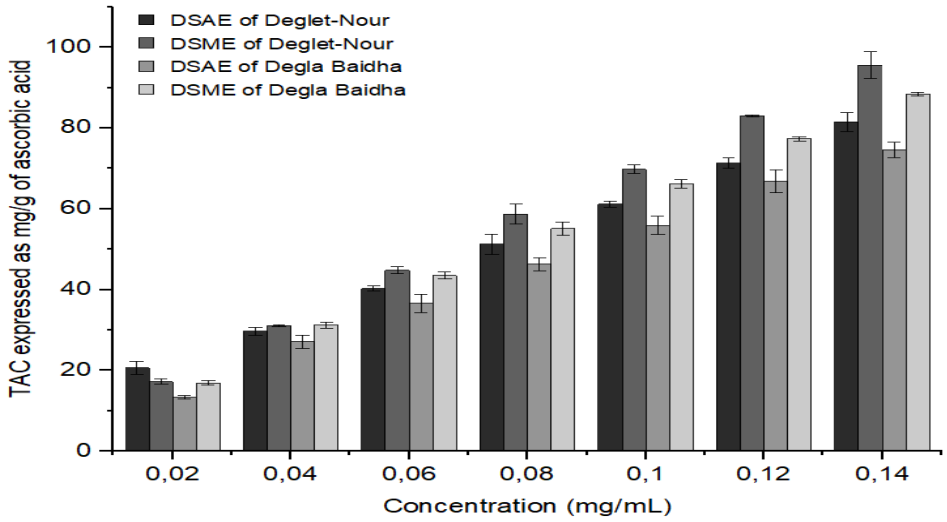

Fig. 1: Total antioxidant capacity of DSAE and DSME of Degla Baidha and Deglet-Nour

CONCLUSION:

This study revealed that date palm seeds contain various bioactive compounds (polyphenols, including flavonoids), which exhibited higher antioxidant activities. Therefore, date palm seeds can be considered a potent natural composition requiring a high valorization of this by-product using it as an ingredient to enhance some functional foods' nutritional value for human and animals' consumption, pharmaceutical industries, cosmetic and natural oils.

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