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Research Article

Evaluation of Phenolic Content and Antioxidant Capacity of Ethanolic Extract for Selected Varieties of *Phoenyx dactylifera*

*[1,2] Laouini S. Eddine, [3]Segni Ladjel, [3]Ouahrani M.Ridha, [4]Gherraf N.Eddine, [5]Mokni Sonia

[1]*Department of Industrial Chemistry, Faculty of Science and Technology, Mohammed Kheider University, Algeria*
[2]*Vaolization and technology of resource Saharian laboratory, Faculty of Science and Technology, El Oued University, El Oued, Algeria*
[3]*Process engineering Laboratory, faculty of science and technology and material sciences, Kasdi Merbah University, Algeria*
[4]*Laboratory of Biomolecules and Plant, Breeding, Larbi Ben M'hidi University, Oum El Bouaghi 04000, Algeria*
[5]*Laboratory of Wastewater Treatment, Water Researches and Technologies Center, Technopark of Borj Cedria, BP 273.8050, Soliman, Tunisia*

ABSTRACT
The date palm (Phoenyx dactylifera) consisted, for the people of southern Algeria, as tree of providence. Dates and their extracts are also used for many centuries as a medicine against allergy, inflammation, constipation and gastroprotective; they also have a high antibacterial and antioxidant activity. However, no studies are conducted to evaluate the extract from the leaves of date palm (Phoenyx dactylifera) in point of view of phytochemical composition, antimicrobial and the antioxidant activity. In this study, we have determined the phenolic compounds, antioxidant and antimicrobial activity of methanolic extracts from three varieties of leaves tree. According to the results the leaves extracts have very important values for polyphenols (215.24 to 156.46 mg GAE / g DW) and high antioxidant activity (324. 45 to 206.21 mg GAE / g DW), DPPH (IC50 = 2.98 to 4.83 µg / ml) and β-bleaching test (IC50 = 133. 93 to 194. 12 µg / ml); also the three extracts reveal a considerable antimicrobial potency and antifungal considerable activity , the diameter of inhibition is 14.4 ± 0.6 mm for Hamraya, 19.8 ± 0.5 mm for Ghars and 17.4 ± 0.8 mm for Deglet Nour (concentration 50 mg / ml) from Staphylococcus aureus ATCC.

Key words: Phoenyx dactylifera, polyphenol, flavanoid, antioxidant, reducing power, DPPH

INTRODUCTION

Phoenyx dactylifera is a tree of the family Arecaceae (palms), subfamily Coryphoideae and order Arecales. It is widely found in Saharian oasis and considered as a dominant tree in this region. The fruit tree grows in its shade which provides us cover vegetables and foods. This tree has been known since antiquity; its origin is located in North Africa, the Sahara, west of India and the Persian Gulf region. Also it is widespread in all the hot spots from the Atlantic to the Red Sea. If we adapt the estimates based on the shape and organoleptic properties of fruits, there are more than 600 varieties of these fruit trees. For Muslims, all over the world, dates are of religious importance and are mentioned in several places in the Quran.

In Algeria the Phoenyx dactylifera is an important tree1, plays principal roles in social, environmental and economic sectors2. As production, Algeria is one of the first producers of fruits of the date in the world; 500,000 t per year 3. In Africa, medicinal plants are tradionally used; it was estimated over 80% of the population that they produce wide array of phytochemical; most of which are used, from the plant, as drugs source in order to avoid the secondary effects undesirable (unwanted side-effects) of some synthetic chemical drugs4. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease 5. Two synthetic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are more used in the food industry and may be responsible for liver damage and carcinogenesis or toxic 6,7.

For these reasons, it is necessary to focus on others natural antioxidants extract from plants. Several chemical compounds extracted from plant leaves, but the most important is the polyphenols, which are secondary metabolites ubiquitously distributed in all higher plants 8. The present work is undertaken to estimate the chemical composition and antioxidant effect of leaves extract of three varieties of Phoenyx dactylifera growing in southeast of Algeria, and to evaluate any relationship between composition phytochemical and previous activities. As a result, new sources of antioxidant agents can be obtained from leaves extracts hoping that we open more research horizons.

MATERIALS AND METHODS

Plant material: The aerial parts of Phoenyx dactylifera (leaves) of three trees were collected in March 2011 from Debila (Djedeida) located in Wilaya of El-Oued southeast Algeria (33° 07" 00" N 7° 11' 00" E) and were grown for

*Author for correspondence: Email: salah_laouini@yahoo.fr

Table 1: Mass yield of leaves obtained by methanol 80% of three varieties of *Phoenyx dactylifera*

Plant species	dry weight extract g/50 g of leaves powder	Yield (%) w/w
Gars	8.25±0.07	16.50±1.15
Deglet Nour	9.56±0.08	19.12±0.10
Hamraya	7.82±0.04	15.64±0.08

Results are expressed as the mean ±standard deviation of three independent experiments. Values with different row are significantly (P < 0.05).

six months before being used. This species was identified by Pr. Ouahrani M. Ridha Department of Chemistry, Kasdi Merbah University. The leaves were dried in well ventilated spaces at room temperature, powdered and sifted in a sieve (0.750 µm) before use.

Preparation of methanolic extracts: The powder of each plant material (50 g) was extract three times with 500 mL of 80 % v/v (methanol: water) during 48 h, stirred with condition 350 rpm and 35 °C using an orbital shaken. The extracts were filtered by Whatman N°.1. The filtrate was concentrated under reduced pressure at 40 °C by rotary evaporator (BUCHI R-210, Switzerland) to eliminate the methanol, and stored in -4 °C to give a crude extract yielding 8.25 g for Ghars, 9.56 g for Deglat Nour and 7.82 g for Hamraya, diluted in methanol and distilled water for next concentrations needed in this work.

Determination of total polyphenol content (TPC) 9,10: The concentration of total polyphenols compounds in the extracts was estimated by the folin-ciocalteu method with some modification. Briefly, a dilute solution of each extract in MeOH (1 mL) was mixed with 1 mL of folin-ciocalteu reagent, followed by 1 mL of a sodium carbonate (10 % w/v) after 4 min. The reaction mixture was incubated for 60 min at room temperature; the absorbance of reaction mixture at 700 nm was measured, the blank's prepared with the same procedure described above except that the samples solution was substituted by 1 mL of 80 % methanol. The concentration of total polyphenols in the extracts was expressed as mg gallic acid equivalent (GAE) per g of dry weight using UV-Visible (Shimadzu UV-1800, Japan) and the equation of calibration curve: Y= 0.00778x+0.26193, R2 =0.991, x was the absorbance and Y was the gallic acid equivalent. All results presented are means (±SEM) and were analyzed in three replications.

Estimation of total flavanoid content (TFC) 11: Several authors describe the method for determination of total flavanoid concentration by aluminum colorimetric assay. Briefly, a 500 µL aliquot of different extracts was mixed with 1.25 mL of distilled water and 0,075 ml $NaNO_2$ solution (5 %), after 5 min, the result mixture is added to 0.125 mL of a $AlCl_3$ solution (10 %), after 6 min, were mixed the precedent reaction with 0.5 mL of 1 M NaOH and 0.275 mL of distilled water H_2O. The absorbance was measured at 510 nm, using UV-Visible (Shimadzu UV-1800, Japan). Catechin was used to make the calibration curve and done in triplicate, the equation of calibration curve: Y=0.00035 x + 0.00188, R2 =0. 988. Total flavanoid was expressed in milligram catechin equivalent CE/ g per dry plant powder.

Estimation of total flavonol content 11,12: 25 µL of the crude extracts was added to 25 µL HCl (0.1 %) in 95 % ethanol, all were mixed with 500 µl HCl (2 %) and incubated for 30 min at room temperature and then the absorbance was measured at 360 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). The blank is prepared with the same procedure described above but we replace the simple extract by the quercetin. Total flavonol content was expressed as quercetin equivalent (QE)/ g of dry weight by using the equation of calibration curve: Y=0.00321 x + 0.02013, R2=0.997.

DPPH radical scavenging activity 13,14: The radical scavenging activity using free-radical DPPH assay. A 1 mL aliquot of each extract was added to 0.5 mL of a DPPH methanolic solution (7.8 mg DPPH in 100 mL methanol 100 %). The mixture was vigorously shaken and left to stand in the dark for 30 min at room temperature. The antioxidant activity was then measured by the decrease in absorption at 517 nm using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan)and corresponds to the extract ability to reduce the radical DPPH* to the yellow-coloured diphenilpicryldrazine. The antiradical activity was expressed as IC50 (µL/mL), the antiradical dose required to cause 50 % and calculated by the following equation:

DPPH scavenging activity (%) = (Ao− A1) / Ao x 100

Where Ao is the absorbance of control at 30 min, A1 is the absorbance of the sample extract at 30 min. All results presented are means (±SEM) and were analyzed in three replications.

Reducing power assay 15: 0.2 mL of sample extracts of different concentrations was added to 2.5 mL sodium phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferricyanide 1 %, the mixture incubated at 50 °C for 20 min. After this, 2.5 mL of tricloroacetic acid 10 % were added (10 %, w/v, in water) and centrifuged at 1000 rpm for 10 min at room temperature, the upper layer of solution 5 mL was mixed with 5 ml of distilled water and 1 mL ferric chloride 0.1 %, the absorbance measured at 700 nm again the blank using UV-Visible spectrophotometer (Shimadzu UV-1800, Japan), the extract concentration providing 0.5 of the absorbance (EC50) was calculated from the graph of measured absorbance. The values were expressed as mg per 1 L of leave extracts, all determinations were performed in triplicate.

Table 2: Total polyphenol, flavanoid and flavonol of methanolic leaves extract of *Phoenyx dactylifera*.

Plant species	Polyphenols (mg GAE/G DW)	Flavanoids (mg RE/g DW)	Flavonols (mg QE/g DW)
Ghars	215.24 ± 9.25	101.09 ± 4.35	39.21 ± 1.02
Deglet Nour	179.30 ± 4.21	093.42 ± 2.75	28.57 ± 0.73
Hamraya	156.46 ± 5.43	090.79 ± 4.02	24.58 ± 0.24

Data are expressed as means ± standard deviation of triplicate samples. Values with different row are significantly (P < 0.05).

Table 3: DPPH radical scavenging activity (IC_{50} in µg/ml), reducing power (EC_{50} in µg/ml) and total antioxidant activity (mg GAE/g DW) power *Phoenyx dactylifera* leaves extract and standards (BHT in IC_{50}, BHA and chlorogenic acid in EC_{50} µg/ml.

Plant species and standards	DPPH test	Reducing power	Total antioxidant activity
Ghars	2.98 ± 0.08	13.28 ± 0.05	324.45 ± 11.43
Deglet Nour	3.74 ± 0.07	32.73 ± 1.35	218.15 ± 07.55
Hamraya	4.83 ± 0.10	42.26 ± 2.04	206.21 ± 09.14
BHT	11.7 ± 0.30	−	−
BHA	−	62.43 ± 2.55	−
chlorogenic acid	−	49.41 ± 2.37	−

Data are expressed as means ± standard deviation of triplicate samples. Values with different row are significantly (P < 0.05).

Estimation of total antioxidant activity 16: Based on the reduction of Mo (VI) to Mo (V) by formation of the green phosphate/ M(V). In the appendorf tube, 0.3 mL of methanols extract 80 % known concentration was added to 2.7 mL mol of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath (Mammert D-91126 Schwabach FRG, Germany) at 95 °C for 90 min, the blank is prepared with the same procedure described above but we replace the volume of simple extract by 0.3 mL ethanol, the absorbance was calculated at 695 nm. The antioxidant capacity was expressed as mg gallic acid equivalent per gram of dry plant powder (me GAE/g DW). All determinations were performed in triplicate.

Statistical analysis: Data were expressed and were presented as mean values ± SD (standard deviations). All measurements were carried out in three experiments (all the analyses in the present study work which was done in duplicate determinations). Statistical calculations were carried out by OriginPro version 8 software (Prolab), Correlations were obtained by Pearson correlation coefficient in bivariate correlations. P values < 0.05 were regarded significant and P values < 0.01 were regarded very significant.

RESULTS AND DISCUSSION

Extract yield: The results of extract yield for each variety of Phoenyx dactylifera are mentioned in table 1, which shows the extraction yield (g/100 g dry weight), the Deglet Nour variety gives the highest yield (19.12±0,108 % w/ while the intermediate value (16.50±0.140 %) was obtained from the Ghars extract. the lowest value was found for Hamraya.

Total polyphenol, flavanoid and flavonol: The total polyphenol content of methanol extract of three varieties of Phoenyx dactylifera is shown in Table 2, the range was from 215.24 ± 9.25 to 156.46 ± 4.21 mg GAE/g DW. The higher amount of these compounds found in Ghars variety 215.24 ± 6.25 mg GAE/g DW, 179.30 ± 5.43 mg GAE/g DW in Deglet Nour and the lowest concentration obtained from Hamraya variety 156.46 ± 4.21 mg GAE/g DW, these concentrations significantly higher if are compared to other medicinal plants like G. multifolial 12.36 mg GAE/g DW and G. villosa 20.81 mg GAE/g DW 11, 70.07 mg GAE/g DW for M. edule 14. Mansouri et al., [17] estimated the polyphenol content of seven Algerian varieties of date and observed that they contain p-couramic, ferulic and sinapic acids, some cinnamic acid. Studies with three varieties of Omani dates have shown the presence of gallic acid, vannilic acid, syringic acid and ferulic acid. Recently works of Chaira et al., [8] have also observed that the total polyphenol in the Mermella variety date contain 0.54 mg/g of fresh weight.

The total flavanoid content in extracts presented in Table 2, the concentration of flavanoid significantly (P<0.05) is between 101.09 ± 4.35 to 90.79 ± 4.02 mg RE/g DW, the same for polyphenol. The higher concentration which as found in Ghars variety was 101.09 ± 4.35 mg RE/g DW, the second was Deglet Nour 93.42 ± 2.75 mg RE/g DW

and finally Hamraya at 90.79 ± 4.02 mg RE/g DW. The amount of flavanoid was highly considered if it was compared to those obtained in recent studies. For exemple, the total flavanoid content in Folium nelumbinis (Lotus leaf) 8.66 ± 0.36 mg RE/g DW and Folium mori (Mulberry leaf) 21.66 ± 6.89 mg RE/g DW (35).

The mean values of total flanvonol content varied from 39.21±1.02 to 24.58 ± 0.24 mg QE/g DW, the highest amounts were found in Ghars 39.21±1.02 mg QE/g DW, the moderate levels of flavonol content were also found in Dglet Nour variety 28.57 ± 0.73 mg QE/g DW and low value obtained from Hamraya 24.58 ± 0.24 mg QE/g DW. As it can be seen from Table 2, the flavonol contents varied among all selected varieties of Phoenyx dactylifera.

The quantity of phenolic compounds in leaves samples is greatly influenced by soil, water irrigation, environmental condition, genotype (cultivate/variety) agronomic practices (fertilization and pest management). The extracts of these trees showed high concentration of polyphenol, flavanoid and flavonol content.

Antioxidant assay: The DPPH radical scavenging activity of methanolic extract leaves of the three varieties of Phoenyx dactylifera is presented in Table 3. For crude extract of Ghars variety obtained the higher value (IC50=2.98±0.08 µg/mL), the intermediate value found in Deglet Nour (IC50=3.74 ± 0.07 µg/mL) and the lowest amount obtained in Hamraya variety (IC50= 4.83 ± 0.10 µg/mL). If we compare these values with other extracts of leaves, Edziri et al., [18] we find (IC50= 230.5±0.3 µg/mL) for Petroselinum sativum and (IC50= 600.1±0.1 µg/mL) for Beta vulgaris var cicla. The antioxidant capacity of different varieties of Phoenyx dactylifera is higher than the positive control BHT (IC50= 11.7±0.3 µg/mL), this antioxidant capacity free radical scavenger DPPH related with the quantity of total polyphenol composition 19,20. The relationship is related to their ability to antioxidant activity, free radical scavenger.

Fe+3 reductions are often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action, and can be strongly correlated with other antioxidant properties 21. The reducing power is confirmed by the change of yellow colour of the test solution to various shades of green and blue depending on the concentration of the plant extract, the high reducing power was obtained in methanolic extract of Ghars (EC50 = 13.28 ± 0.05 µg/mL), the intermediate value obtained from Deglet Nour extract (EC50 = 32.73 ± 1.35 µg/mL) and the lowest value founded in Hamraya extract (EC50 = 42.26 ± 2.04 µg/mL). The reducing power of all extracts were significantly higher than those of standard antioxidant (BHA, EC50 = 62.43 ± 2.55 µg/mL) and chlorogenic acid (EC50 = 49.41 ± 2.37 µg/mL). These results revel that there is a relationship between the concentration of polyphenol and values of reducing power for ethanolic extracts, the results are shown in Table 3.

The total antioxidant activity of ethanolic extracts of leaves range from 324.45 ± 11.43 to 206.21 ± 9.14 mg GAE/g DW, the high values are 324.45 ± 11.43 mg GAE/g DW for Ghars leaves extract, then 218.15 ± 7.55 for Deglet Nour leaves extract and finally 206.21 ± 9.14 mg GAE/g DW for Hamraya leaves extract. These results exhibit strong values and confirmed the high antioxidant activity of leaves extract of Phoenyx dactylifera founded in DPPH reducing power. Chaira et al., [8] estimated the total antioxidant capacity of Korkobb, Tunisian date fruit. The authors suppose that the highest level of flavanoid in this variety is responsible for the higher total antioxidant capacity, the results are presented in Table 3.

CONCLUSION

We think that the present study is the first to investigate the phytochemical composition, antioxidant and antimicrobial activity of methanolic extracts of three varieties of Phoenyx dactylifera grown in Southeast Algeria. This study shows that considerable variety exists between the three methanolic extracts of leaves of Ghars, Deglet Nour and Hamraya. We found high amount of total polyphenol, flavanoid and flavonol content, the Ghars variety exhibits the high amount of these compounds. On the other hand, the results of antioxidant activity tests present the strong capacity of three methanolic extracts, higher than the standard antioxidants (BHA, BHT and chlorogenic acid). Finally, all extracts show the high antimicrobial activity for the microorganisms tests (bacteria and fungi) exceeded most of the time the positive control. The good correlation found between activity and phytochemical contents indicates that effects observed could be attributed to phenolic compounds. This data suggest the strong potential of these extracts as a natural source of phenolic compounds, antioxidant and antimicrobial and may be considered in future to replace synthetic preservatives and drugs in pharmaceutical and food industry.

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