

**PHYTOCHEMICAL ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL
ACTIVITIES OF LEAVES EXTRACT OF DATE PALM GROWN IN ALGERIA**

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ABSTRACT

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 -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: *Phoenix dactylifera*; leaves extract; polyphenol; antioxidant; reducing power.

1. INTRODUCTION

Phoenix dactylifera is a tree of the family *Areaceae* (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.

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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 *Phoenix dactylifera* is an important tree (1,2,3,4) for people and plays principal roles in social, environmental and economic sectors (5). As production, Algeria is one of the first producers of fruits of the date in the world; 500,000 t per year (6). In Africa, medicinal plants are traditionally 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 (7) in order to avoid the secondary effects undesirable (unwanted side-effects) of some synthetic chemical drugs. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease (8). 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 (9) or toxic (10).

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 (11). Many new studies confirmed the antimicrobial activity of polyphenols occurring in vegetable foods and medicinal plants and that they act as anti-allergic, antimicrobial, anti-inflammatory, vasoprotector and anti-tumour agents (12,13). The date is rich with phytochemicals like phenolic acids, sterols, procyanidins, flavanoids, carotenoids and anthocyanidin (14). The biological and pharmacological activities of date are very important; the dates have activities antimutagenic, antiviral, antifungal, antiherlipidemic and hepatoprotective (15) and they have high antioxidant activity (16). In all the studies carried out on the dates, in our knowledge, there is no scientific study and information of phytochemical, antioxidant capacity and antimicrobial activity concerning leaves extract of *Phoenix dactylifera*.

The present work is undertaken to estimate the chemical composition, antimicrobial and antioxidant effect of leaves extract of three varieties of *Phoenix dactylifera* growing in southeast of Algeria, and to evaluate any relationship between composition phytochemical and previous activities. As a result, new sources of antioxidant and antimicrobial agents can be

obtained from leaves extracts hoping that we open more research horizons.

2. EQUIPMENTS AND METHODS

Plant material

The aerial parts of *Phoenix 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 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)

The concentration of total polyphenols compounds in the extracts was estimated by the folin-ciocalteu method according to protocol reported by Moreira et al. (17) and Carlos Silva et al. (18) 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: $Y = 0.00778x + 0.26193$, $R^2 = 0.991$, x was the absorbance and Y was the gallic acid equivalent. All results presented are means (\pm SEM) and were analyzed in three replications.

Estimation of total flavanoid content (TFC)

Several authors describe the method for determination of total flavanoid concentration by aluminum colorimetric assay (19). Briefly, a 500 μ L 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: $Y=0.00035 x + 0.00188$, $R^2=0.988$. Total flavanoid was expressed in milligram catechin equivalent CE/ g per dry plant powder.

Estimation of total flavonol content

The quantification of the total flavonol content performed by the method of Daniels et al. (20) and Mazza et al. (21) with small modification, 25 μ L of the crude extracts was added to 25 μ L HCl (0.1 %) in 95 % ethanol, all were mixed with 500 μ l 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: $Y=0.00321 x + 0.02013$, $R^2=0.997$.

DPPH radical scavenging activity

The radical scavenging activity using free-radical DPPH assay determinate with the method described by Hatano et al. (22) and Falleh et al. (23). 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 IC_{50} ($\mu\text{L}/\text{mL}$), the antiradical dose required to cause 50 % and calculated by the following equation:

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of control at 30 min, A_1 is the absorbance of the sample extract at 30 min. All results presented are means (\pm SEM) and were analyzed in three replications.

Reducing power assay

The reducing power of methanolic extract was determined according to the method described by Gulcin et al. (24). 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 trichloroacetic 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.

Estimation of total antioxidant activity

The total antioxidant capacity of the crude extract of leaves was evaluated by the method described by Prieto et al. (25), 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 methanol, 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.

3. ANTIMICROBIAL ACTIVITY ASSAYS

Microorganisms

Eleven bacteria and three fungi strains were used in this study; the bacterial cells assayed included two gram-positive, *Staphylococcus aureus* ATCC (26) and *Bacillus cereus* ATCC 14579. For the gram-negative, nine bacterial strains were used, *Escherichia coli* ATCC 35218, *Salmonella arizona* DM 35605, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* HM 627626, *Pseudomonas aeruginosa* HM 627575, *Pseudomonas aeruginosa* ATCC 15442, *Pseudomonas aeruginosa* HM 627579, *Pseudomonas putida* HM 6227611 (27), and *Agrobacterium tumefaciens* B6 C58 (28). For the fungal strains, *Phytophthora nicotina*,

Pyrenophora teres F, *Candida albicans* ATCC 90026. All strains were obtained from the Laboratory of Waste Water Treatment, Centre of Research and Technologies of Water (Tunisia).

Incubation conditions

Nutrient agar was used culture medium for bacteria, incubated for 24 h at 37 °C (29) and yeasts were cultured in sabouraud dextrose agar (SDA; 4 % dextrose, 2 % neopeptone and 1.7 % agar) for 24-48 h at 30 °C.

Disc diffusion assay

Methanol extracts of *Phoenix dactylifera* were dissolved in methanol-water 80 % for a final concentration 50 mg/mL and filter-sterilized through a 0.45 membrane filter. The antimicrobial activity was estimated by method of disc diffusion (29), 100 µL of suspension for each microorganism 10⁸ colony-forming units (CFU)/mL of bacteria and 10⁴ (CFU)/mL of fungi were put in the plastic petri plates containing 20 mL of nutrient agar for bacteria and sabouraud dextrose agar for fungi, after they were placed in the petri sterilized filter paper disc (6mm in diameter) and were soaked with 15 µL of the 50 mg/mL of each methanolic extracts (150 µg/disc). The methanol 80 % was used as a negative control and polymyxine B it was the positive control, prepared with the same procedure described above except that the methanol extract was substituted by 15 µL of positive control at 50 mg/mL, the diameter of the inhibition zone around each disc was measured for three replicates.

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.

4. RESULTS AND DISCUSSION

Extract yield

The methanol is a solvent extract which have significant amounts of polyphenols compounds and used in several recent studies, for Penna et al. (30) and Edziri et al. (31) it is the best

solvent of antimicrobial substances compared with the other solvents and given an elevated antioxidant activity (32).

The results of extract yield for each variety of *Phoenix 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. For Selma Dziri et al. (33), the mass yield obtained for methanolic extract of leaves *A. roseum var. odoratissimum* about 6.3 % and Qing-Feng et al. (34) found 16.1 % for methanolic extract of *Rhizoma Smilacis Chinae*.

Table 1. Mass yield of leaves obtained by methanol 80% of three varieties of *Phoenix 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 \pm standard deviation of three independent experiments. Values with different row are significantly ($P < 0.05$).

Total polyphenol, flavanoid and flavonol

The total polyphenol content of methanol extract of three varieties of *Phoenix 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 (35), 70.07 mg GAE/g DW for *M. edule* (23). Mansouri et al. (36) 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. (14) 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 flavanoid components have a remarkable activity against several Gram-positive bacteria, such as *Staphylococcus aureus* and Gram-negative, such as *Escherchia coli* (11).

The mean values of total flavonol 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 Deglet 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 *Phoenix 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, for these reasons antioxidant and antimicrobial activity were determined.

Table 2. Total polyphenol, flavanoid and flavonol of methanolic leaves extract of *Phoenix 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	93.42 ± 2.75	28.57 ± 0.73
Hamraya	156.46 ± 5.43	90.79 ± 4.02	24.58 ± 0.24

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

Antioxidant assay

The DPPH radical scavenging activity of methanolic extract leaves of the three varieties of *Phoenix dactylifera* is presented in Table 3. For crude extract of Ghars variety obtained the

higher value ($IC_{50}=2.98\pm 0.08 \mu\text{g/mL}$), the intermediate value found in Deglet Nour ($IC_{50}=3.74 \pm 0.07 \mu\text{g/mL}$) and the lowest amount obtained in Hamraya variety ($IC_{50}= 4.83 \pm 0.10 \mu\text{g/mL}$). If we compare these values with other methanolic extracts of leaves, Edziri et al. (44) we find ($IC_{50}= 230.5\pm 0.3 \mu\text{g/mL}$) for *Petroselinum sativum* and ($IC_{50}= 600.1\pm 0.1 \mu\text{g/mL}$) for *Beta vulgaris var cicla*. The antioxidant capacity of different varieties of *Phoenix dactylifera* is higher than the positive control BHT ($IC_{50}= 11.7\pm 0.3 \mu\text{g/mL}$), this antioxidant capacity free radical scavenger DPPH related with the quantity of total polyphenol composition (37,38). The relationship is related to their ability to antioxidant activity, free radical scavenger (38). Moreover, the antimicrobial activity of these compounds according in plants has been extensively investigated against a wide range of microorganisms (39) it is found responsible for the strong antioxidant, antibacterial and antifungal activity.

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 (40). 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 ($EC_{50} = 13.28 \pm 0.05 \mu\text{g/mL}$), the intermediate value obtained from Deglet Nour extract ($EC_{50} = 32.73 \pm 1.35 \mu\text{g/mL}$) and the lowest value founded in Hamraya extract ($EC_{50} = 42.26 \pm 2.04 \mu\text{g/mL}$). The reducing power of all extracts were significantly higher than those of standard antioxidant (BHA, $EC_{50} = 62.43 \pm 2.55 \mu\text{g/mL}$) and chlorogenic acid ($EC_{50} = 49.41 \pm 2.37 \mu\text{g/mL}$). These results reveal that there is a relationship between the concentration of polyphenol and values of reducing power for methanolic extracts, the results are shown in Table 3.

The total antioxidant activity of methanolic extracts of leaves range from 324.45 ± 11.43 to $206.21 \pm 9.14 \text{ mg GAE/g DW}$, the high values are $324.45 \pm 11.43 \text{ mg GAE/g DW}$ for Ghars leaves extract, then 218.15 ± 7.55 for Deglet Nour leaves extract and finally $206.21 \pm 9.14 \text{ mg GAE/g DW}$ for Hamraya leaves extract. These results exhibit strong values and confirmed the high antioxidant activity of leaves extract of *Phoenix dactylifera* founded in DPPH, - Carotene and reducing power. Chaira et al. (14) 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.

Table 3. DPPH radical scavenging activity (IC₅₀ in µg/ml), reducing power (EC₅₀ in µg/ml) and total antioxidant activity (mg GAE/g DW) power *Phoenix dactylifera* leaves extract and authentic standards (BHT in IC₅₀, BHA and chlorogenic acid in EC₅₀ 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	

Antimicrobial activity

The results of the antibacterial activity of methanol extracts of studies tree against a set of Gram-positive (*Staphylococcus aureus* ATCC and *Bacillus cereus* ATCC 14579), and Gram-negative (*Escherchia coli* ATCC 35218, *Salmonella arizona* DM 35605, *Pseudomonas aeriginosa* ATCC 27853, *Pseudomonas aeriginosa* HM 627626, *Pseudomonas aeriginosa* HM 627575, *Pseudomonas aeriginosa* ATCC 15442, *Pseudomonas aeriginosa* HM 627579 and *Pseudomonas putida* HM 6227611) are summarised in Table 5. All methanolic extracts of leaves were active on all bacteria tested except one (*Agrobacterium tumefaciens* B6 C58), the Gram-positive bacteria appeared more sensitive than the bacteria of gram-negative. The zone diameter of inhibition ranged from 19.8 ± 0.8 mm at 17.4 ± 0.8 mm, the higher antibacterial activity founded in *Staphylococcus aureus* ATCC (19.8 ± 0.5 mm) from leaves extract of Ghars and the lowest sensitivity obtained from *Pseudomonas aeriginosa* ATCC 27853 (10.5 ± 0.3) from leaves extract of Hamraya. This antibacterial activity is higher than the positive control polymyxine B, the zone diameter of inhibition ranged from 12.5 ± 0.5 mm at 8.5 ± 0.3 mm.

Table 4. Antibacterial activity of methanolic extracts leaves of different varieties of *Phoenix dactylifera*

Microorganisms	Diameter of zone inhibition			
	Ghars extract	Deglet Nour extract	Hamraya extract	polymyxine B
<i>Staphylococcus aureus</i> ATCC	19.8 ± 0.5	17.4 ± 0.8	14.4 ± 0.6	12.5 ± 0.5
<i>Bacillus cereus</i> ATCC 14579	19.3 ± 0.6	16.5 ± 0.5	14.5 ± 0.5	11.0 ± 0.4
<i>Escherchia coli</i> ATCC 35218	14.7 ± 0.5	12.8 ± 0.6	11.5 ± 0.6	08.5 ± 0.0
<i>Salmonella arizona</i> DM 35605	17.7 ± 0.7	15.2 ± 0.5	14.7 ± 0.5	10.5 ± 0.5
<i>Agrobacterium tumefaciens</i> C58B6	na	na	na	08.5 ± 0.3
<i>Pseudomonas aeruginosa</i> ATCC 27853	16.7 ± 0.6	15.6 ± 0.4	15.2 ± 0.6	11.5 ± 0.0
<i>Pseudomonas aeruginosa</i> HM 627626	14.6 ± 0.5	13.2 ± 0.5	13.5 ± 0.4	10.4 ± 0.5
<i>Pseudomonas aeruginosa</i> HM 627575	14.2 ± 0.5	13.5 ± 0.6	13.1 ± 0.4	11.5 ± 0.5
<i>Pseudomonas aeruginosa</i> ATCC 15442	16.8 ± 0.3	15.6 ± 0.6	13.5 ± 0.0	12.0 ± 0.4
<i>Pseudomonas aeruginosa</i> HM 627579	15.5 ± 0.2	15.5 ± 0.4	14.2 ± 0.0	12.5 ± 0.3
<i>Pseudomonas putida</i> HM 6227611	15.7 ± 0.5	14.2 ± 0.5	13.5 ± 0.5	11.8 ± 0.5

na: not active

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

5. 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 *Phoenix 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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