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Algerian date palm (*Phoenix dactylifera* L.) fruit cultivars: HPLC fingerprinting and antibacterial activity

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Abstract:
The abusive use of antibiotics causes the destruction of intestinal flora and the proliferation of antibiotic-resistant pathogens. Date palm is used in traditional medicine in the Saharan regions due to its biological properties.
The study aimed to identify the phytochemical composition and assess the antibacterial activity of the methanolic extracts of three date cultivars from Algeria. Their total phenolic, flavonoid, and flavonol contents were measured spectrophotometrically. The phytochemical screening was conducted by HPLC fingerprinting using twenty-three pure phenolic compounds as standards. The antibacterial activity against pathogenic bacterial species was assessed using the disk diffusion method.
The colorimetric methods showed that the total phenolic, flavonoid, and flavonol contents ranged from 2.13 ± 0.09 to 2.67 ± 0.02 mg GAE/100 g DW, 1.33 ± 0.21 to 1.55 ± 0.13 mg CEQ/100 g DW, and 0.41 ± 0.23 to 0.47 ± 0.05 mg REQ/100 g DW, respectively. HPLC fingerprinting showed that the extracts of date cultivars served as an excellent source of bioactive compounds (gallic acid, tannic acid, ferulic acid, vanillin, caffeine, quercetin, luteolin, rutin, aspegenin, isorhamnetin, and hesperidin). They also exhibited an antibacterial potential with an inhibition zone diameter ranging from 8.40 to 12.50 mm.
The results clearly demonstrate the antibacterial potency of date palm fruits, which could be attributed to their considerable content of phenolic compounds such as gallic acid, rutin, quercetin, and luteolin.

Keywords: *Phoenix dactylifera* L., high-performance liquid chromatography fingerprinting, phenolic compounds, flavonoids, flavonol, secondary metabolites

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INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is an important fruit crop for the populations of the Middle East and North Africa. This fruit has great nutritional and economic importance [1, 2].

Since antiquity, the date and its pits have been used in traditional medicine in the Saharan regions, more precisely in the oases, where the date palm was cultivated [3]. Dates have high energy values and are rich in reductive and easily assimilated sugars, minerals (selenium, potassium, calcium, magnesium, manganese, and iron) and vitamins (A, B, and C). In addition, this fruit is endowed with numerous health benefits resulting from the mixture of secondary metabolites (polyphenols, anthocyanins, carotenoids, tannins, procyanidins, sterols, flavonols, flavones, anthocyanidins, isoflavones, phytoestrogens, phenolic acids, cinnamic acid derivatives, and volatile compounds) [4]. The date palm has been the subject of phytochemical, pharmacological, and nutritional research [5–8]. Various studies have determined the physicochemical composition of dates but scarce field research has only focused on phenolic components and involved only a few varieties of this fruit. These bioactive compounds are gaining increasing interest, given their important biological properties [9, 10].

This study was part of a program to valorize the Algerian flora through the search for new compounds or active ingredients. We aimed to determine phenolic compounds and to assess the antibacterial effect of methanolic extracts from three cultivars (Hamraya, Figheth, and Tamajort) of the date palm (*P. dactylifera*) fruits growing in the El-Oued region (Algeria).

STUDY OBJECTS AND METHODS

Plant material collection. Three date palm fruit cultivars (*Phoenix dactylifera* L.) were collected from the El-Oued region (South-East of Algeria) at the final stage of fruit ripeness, at the beginning of the 2012 harvest season. They are locally named Hamraya (33'34'30,09N, 6'49'43,11E), Figheth (33'35'15,56N, 6'49'36,83E), and Tamajort (33'33'39,99N, 6'47'27,73E).

Preparation of extracts. The preparation of methanolic extracts was performed according to the research by Biglari *et al.* [11]. In particular, 100 mg of the edible parts of each cultivar were macerated in 300 mL of methanol-water (4:1, v/v) at room temperature under continuous shaking for 5 h. The mixture was filtered and concentrated to obtain crude extracts, which were kept at 4°C until used.

Total phenolic content assay. The total phenolic content was determined by the spectrophotometric method described by Al-Farsi *et al.* [12]. For this, 200 µL of each extract was added to 1.5 mL of the Folin-Ciocalteu reagent. The solutions were mixed and incubated in the dark for 5 min. Then, 1.5 mL of sodium bicarbonate (60 g/L) was added to the reaction medium. After 90 min of incubation at room temperature, the absorbance of all extracts was measured with a UV-visible spectrophotometer at 725 nm against the blank without extract. The phenolic content was expressed as milligrams of gallic acid equivalent per 100 g dry weight (mg GAE/100 g DW) based on a calibration line constructed from the standard solution of gallic acid.

Total flavonoids assay. Total flavonoids were determined by the method described by Biglari *et al.* [11]. For this, 4 mL of distilled water and 1 mL of each extract were added to 0.3 mL of 5% sodium nitrite ($NaNO_2$) and 0.3 mL of 2% aluminum chloride ($AlCl_3$) in methanol. After incubation for 5 min at room temperature, 2 mL of 1% sodium hydroxide (NaOH) in methanol was added. The mixture was diluted to 10 mL with distilled water. The absorbance of the resulting mixture was measured directly with a UV-visible spectrophotometer at 510 nm against the blank. The flavonoid contents were expressed as milligrams of catechin equivalent per 100 g dry weight (mg CEQ/100 g DW) based on a calibration line constructed from the standard solution of catechin.

Total flavonols assay. To determine total flavonols, 500 µL of each methanolic extract was added to 500 µL of 2% aluminum chloride ($AlCl_3$) and 500 µL of 5% sodium acetate (CH_3COONa). The absorbance was determined at 440 nm after incubation for 2.5 h at room temperature. The flavonol contents were expressed as milligrams of rutin equivalent per 100 g dry weight (mg REQ/100 g DW) based on a calibration line constructed from the standard solution of rutin [13].

HPLC analysis of phenolic compounds. To determine phenolic compounds, 10 mg of powdered extracts of *P. dactylifera* were dissolved in 10 mL of methanol to a final concentration of 1 mg/mL. The solution was filtered through a 0.45-µm syringe filter for sterilization. Then, 1 mg of each standard was dissolved individually in 1 mL of methanol and sterile-filtered through a 0.45-µm syringe filter before subjecting to high-performance liquid chromatography (HPLC). The analysis of phenolic compounds in various extracts was carried out using HPLC coupled with a visible UV multi-wavelength detector under the following operating conditions:

– steel column: 25×0.46 cm;
– stationary phase: C18;
– elution solvent: methanol:acetonitrile (30:70 v/v);
– wavelengths: 220, 280, 300, and 365 nm; and
– injection loop capacity: 20 µL.

To identify the compounds, 23 pure phenolic compounds were used as standards, namely rutin, naringin, quercetin, luteolin, isorhamnetin, 2,5-dimethyl hydroxycinnamic acid, 3,4,5-trimethoxybenzoic acid, 3,4,5-trimethoxycinnamic acid, ferulic acid, gallic acid, m-anisic acid, o-anisic acid, syringic acid, transcinnamic acid, 3,4-dimethoxycinnamic acid, 2,5-dihydroxycinnamic acid, apigenin, caffeine, vanillin, tannic acid, naringenin-7-O-glucoside, hesperidine, and caffeic acid. The peaks were identified by comparing the retention time of the standard compounds with that of different peaks obtained in the HPLC analysis of the extracts [13].

Antibacterial activity. The bacterial strains used to evaluate the antibacterial potentials of the date extracts included three Gram-positive (*Staphylococcus aureus* ATCC25223, *Bacillus spizizenii* ATCC6633, and *Listeria monocytogenes* ATCC15313) and three Gram-negative (*Pseudomonas aeruginosa* ATCC27853, *Escherichia coli* ATCC8739, and *Salmonella typhimurium* ATCC14028) strains. The bacterial strains were obtained from the Pasteur Institute of Algiers (Algeria). All the bacteria were grown on nutrient agar at 4°C.

The antibacterial potentials of the methanolic date extracts were determined using the agar disc diffusion method [14]. The bacterial strains were cultured in a nutrient broth for 24 h and diluted with sterilized peptone water. Suspensions of the tested microorganisms (0.5 McFarland units) were spread onto the media plates. Sterile filter paper disks of 6 mm in diameter were impregnated with 20 µL of each extract. Methanol was used as a negative control, while pure standard antibiotics (streptomycin and cefazolin) at a concentration of 1 mg/mL were used as a positive control. The plates were incubated at 37°C for 24 h. The zones of inhibition appearing around the disks were measured and recorded in mm. All the tests were performed in triplicate.

Statistical analysis. The results were given as mean ± standard deviation (SD). The analysis of variance (ANOVA) was used to look at the differences in mean values. Turkey's test was used to determine statistically significant differences ($p < 0.05$).

Table 1 Yields and bioactive contents of methanolic extracts of date cultivars (*Phoenix dactylifera* L.)

Cultivars	Yield, % w/w	Total phenolic contents, mg GAE/100 g DW	Flavonoid contents, mg CEQ/100 g DW	Flavonol contents, mg REQ/100 g DW
Hamraya	27.28 ± 0.23[b]	2.13 ± 0.09[a]	1.55 ± 0.13[a]	0.47 ± 0.05[a]
Figheth	33.14 ± 0.04[a]	2.46 ± 0.17[a]	1.49 ± 0.17[b]	0.43 ± 0.32[a]
Tamajort	32.02 ± 1.29[a]	2.67 ± 0.02[a]	1.33 ± 0.21[b]	0.41 ± 0.23[a]

[a–b]: values (mean ± standard deviation, n = 3) in the same column sharing different letters are significantly different ($p < 0.05$)

Table 2 Phenolic compounds detected in methanolic extracts of *Phoenix dactylifera* L. by HPLC at 220 and 280 nm

	220 nm		280 nm	
	Phenolic compounds	Retention time, min	Phenolic compounds	Retention time, min
Hamraya	3,4,5-Trimethoxybenzoic acid	10.721	Gallic acid	3.260
	m-Anisic acid	12.437	Tannic acid	3.270
			Caffeine	6.405
			Naringenin-7-o-glucoside	10.387
			Trans-cinnamic acid	13.821
			2,5-Dimethyl hydroxycinnamic acid	14.823
			Hesperidin	15.070
Figheth	3,4,5-Trimethoxybenzoic acid	11.174	Gallic acid	3.193
	m-Anisic acid	11.936	Tannic acid	3.270
			Caffeine	6.354
			Naringenin-7-o-glucoside	10.338
			Trans-cinnamic acid	13.915
			2,5-Dihydroxycinnamic acid	14.823
			Hesperidin	15.070
Tamajort	not detected	–	Gallic acid	3.182
			Tannic acid	3.399
			Caffeine	6.358
			Naringenin-7-o-glucoside	10.345
			Trans-cinnamic acid	13.900
			2,5-Dimethyl hydroxycinnamic acid	14.834
			Hesperidin	15.158

RESULTS AND DISCUSSION

Bioactive content. The results shown in Table 1 correspond to the yield and bioactive contents (phenolic, flavonoid, and flavonol amounts) of the methanolic extracts of three date cultivars (*Phoenix dactylifera* L.) from Algeria. As we can see, the Figheth and Tamajort cultivars had the highest yields. There was no difference in the total phenolic content ($p > 0.05$) between the extracts of Tamajort, Figheth, and Hamraya. The Hamraya extract had the maximum flavonoid amount, compared to the Figheth and Tamajort cultivars (Table 1).

Finally, we found no significant differences ($p > 0.05$) between the flavonol contents of the Hamraya, Figheth, and Tamajort varieties.

The yield varies according to several parameters: the plant material studied (particle size), the physicochemical characteristics of the solvents used, and the solvents' polarity. It also depends on storage conditions, duration, harvest period, the method, and extraction conditions [13].

To the best of our knowledge, there are no publications on the phenolic and flavonoid compounds of the date cultivars under study analyzed by using HPLC coupled with a visible UV multi-wavelength detector (220, 280, 300, and 365 nm). However, our findings were in accordance with those of Biglari *et al.* who showed that the total polyphenol contents of dates from Iran ranged from 2.89 to 6.64 mg GAE/100 g DW [11]. In addition, the study by Alam *et al.* on the extracts of 26 date varieties from the United Arab Emirates and Pakistan found total phenols ranging from 46 to 397 mg GAE/100 g fresh weight (FW) [15].

Kadum *et al.* tested the ethanolic extracts of five date varieties (Ajwa, Anbara, Piyarom, Rabbi, and Deglet Nour) from Malaysia [16]. They found that the flavonoid amounts varied from 38.63 to 57.07 mg RE/100g DW.

The results in this study were consistent with our previous study, where flavonoids and flavonols of the dates from Algeria varied from 1.06 ± 0.12 to 4.23 ± 0.29 mg CEQ/100 g DW and from 0.44 ± 0.10 to 1.43 ± 0.15 mg REQ/100 g DW, respectively [17].

In the study by Benmeddour *et al.*, the flavonol amounts of date cultivars from Biskra region (Algeria) ranged between 6.73 and 36.64 mg REQ/100 g DW [13].

Several factors can affect the bioactive content of plants. Indeed, studies have shown that these are

Table 3 Phenolic compounds detected in methanolic extracts of *Phoenix dactylifera* L. by HPLC at 300 and 365 nm

	300 nm		365 nm	
	Phenolic compounds	Retention time, min	Phenolic compounds	Retention time, min
Hamraya	Caffeic acid	7.062	Rutin	8.664
	Vanillin	8.604	o-Anisic acid	9.668
	Ferulic acid	9.266	Luteolin	12.776
	3-Hydroxy-4-methoxycinnamic acid	9.677	Quercetin	12.810
	3,4,5-Trimethoxycinnamic acid	12.816	Aspegenin	14.497
	3,4-Dimethoxycinnamic acid	14.882	Isorhamnetin	15.059
Figheth	Caffeic acid	7.080	Rutin	8.779
	Vanillin	8.771	o-Anisic acid	9.740
	Ferulic acid	9.356	Luteolin	12.344
	3-hydroxy-4-methoxycinnamic acid	9.669	Quercetin	12.830
	3,4,5-Trimethoxycinnamic acid	12.902	Aspegenin	14.756
	3,4-Dimethoxycinnamic acid	14.950	Isorhamnetin	15.059
Tamajort	Caffeic acid	7.076	Rutin	8.651
	Vanillin	8.568	o-Anisic acid	9.732
	Ferulic acid	9.356	Luteolin	12.675
	3-Hydroxy-4-methoxycinnamic acid	9.816	Quercetin	12.814
	3,4,5-Trimethoxycinnamic acid	12.898	Aspegenin	14.403
	3,4-Dimethoxycinnamic acid	14.935	Isorhamnetin	14.745

extrinsic factors (such as geographical and climatic factors), genetic factors, and the degree of the plant's maturation [12, 18].

HPLC analysis of phenolic compounds. The phenolic compounds of the date methanolic extracts were detected by HPLC at 220, 280, 300, and 365 nm. The retention times of the standard phenolic compounds were compared with the peaks of the chromatograms of the extracts. At 220 nm, 3,4,5-trimethoxybenzoic acid and m-anisic acid were detected only in the methanolic extracts of Figheth and Hamraya. At 280 nm, gallic acid, tannic acid, caffeine, naringenin-7-o-glucoside, trans-cinnamic acid, 2,5-dimethyl hydroxycinnamic acid, and hesperidin were detected in the methanolic extracts of Hamraya, Figheth, and Tamajort (Table 2).

The chromatographic analyses at 300 nm identified caffeic acid, vanillin, ferulic acid, 3-hydroxy-4-methoxycinnamic acid, 3,4,5-trimethoxycinnamic acid, and 3,4-dimethoxycinnamic acid in all the date extracts. The qualitative analysis at 365 nm revealed the presence of rutin, o-anisic acid, luteolin, quercetin, aspegenin, and isorhamnetin in all the extracts studied (Table 3).

Numerous studies have identified polyphenolic compounds in *P. dactylifera* extracts by HPLC analysis. Indeed, the study by Souli *et al.* identified and quantified trans-ferulic and syringic acids as major phenolic compounds in most Tunisian date cultivars [19].

The chromatographic analyses of the extracts of Al-Qasim cultivated in Saudi Arabia revealed the presence of 3,30-di-O-methyl ellagic acid, 7-methoxy-quercetin-O-hexose isomers, caffeic acid, ferulic acid, isomers of quercentin-rutinoside, kaempferol methylether, p-hydroxybenzoic acid, phytol, punicalagin, and quercetin-3-O-glucoside (isoquercitrin) [20].

Dhaouadi *et al.* quantified phenolic compounds in the aqueous extract of the Deglet-Nour cultivar grown in Tunisia [21]. In their study, the amounts of coumaric, gallic, vanillic, cinnamic, 3,4-dicaffeoylquinic, 5-O-caffeoyl shikimic, caffeoyl-quinic, and caffeic acids were 23.03, 6.79, 2.55, 35.79, 13.86, 17.70, 285, and 9.81 mg/100 g, respectively. Similarly, Kchaou *et al.* established a phenolic profile of the hydroacetonic extracts of three Tunisian date cultivars, including gallic, vanillic, caffeic, syringic, coumaric, ferulic, and sinapic acids, as well as rutin [22]. El Sohaimy *et al.* tested four Omani date varieties by HPLC. The separation showed the presence of gallic, coumaric, caffeic, vanillic, and syringic acids with respective contents of 19.14, 1.67, 1.75, 0.27, and 0.37 mg/100 g [23].

In a study by Shahdadi *et al.*, aqueous and ethanolic extracts from Egyptian date cultivars contained 7.51 and 5.28 μg/g of gallic acid, 2.85 and 1.79 μg/g of tannic acid, and 0.15 and 0.22 μg/g of ferulic acid, respectively [24]. In contrast, the study reported the absence of cinnamic acid. The differences between our results and those reported in literature can be attributed to the geographic origin of the fruits. The phenolic compounds in *P. dactylifera* extracts may also differ because of the extraction solvent [22, 25, 26].

Antibacterial activity. The methanolic extracts of date fruits were screened for their antibacterial activities against six pathogenic bacterial species (Table 4). Theantibacterial activity was recorded when the inhibition zone was greater than 6 mm. The results of antibacterial screening revealed significant antibacterial activity. The inhibition zone diameters ranged from 08.40 ± 0.00 to 12.50 ± 1.00 mm. Streptomycin and cefazolin, which were used as positive experimental

Table 4 Inhibition zone diameters for the methanolic extracts of date cultivars against pathogenic bacterial species

Pathogenic bacterial species	Inhibition zone diameters, mm				
	Cultivars			Antibiotics	
	Hamraya	Figheth	Tamajort	Streptomycin	Cefazolin
Staphylococcus aureus ATCC638P	8.40 ± 0.00	9.30 ± 0.50	10.50 ± 0.50	19.16 ± 0.76	25.40 ± 0.00
Bacillus spizizenii ATCC6633	10.40 ± 0.60	12.50 ± 1.00	9.00 ± 0.50	22.80 ± 1.31	31.50 ± 0.00
Listeria monocytogenes ATCC15313	10.00 ± 1.00	10.50 ± 0.60	n.a.	29.50 ± 1.50	27.40 ± 0.00
Pseudomonas aeruginosa ATCC27853	n.a.	8.00 ± 0.50	n.a.	25.10 ± 2.59	34.20 ± 0.00
Escherichia coli ATCC8739	8.40 ± 0.70	10.30 ± 0.00	n.a.	28.83 ± 1.04	25.60 ± 0.00
Salmonella typhimurium ATCC14028	10.50 ± 0.60	8.500 ± 0.002	10.20 ± 0.35	23.53 ± 1.28	28.80 ± 0.00

* n.a. – no activity revealed; values are presented in mean ± SEM (n = 3)

controls against all bacterial strains assayed, produced an inhibition zone diameter ranging from 19.16 ± 0.76 to 34.20 ± 0.00 mm, while no inhibitory effect could be observed for methanol used as a negative control.

Among the three extracts, Figheth was the most effective against *Bacillus spizizenii* ATCC6633, with the largest zone of inhibition (12.50 ± 1.00 mm), while Hamraya showed the smallest inhibition zone diameter (08.40 ± 0.00 mm) against *Staphylococcus aureus* ATCC638P. Table 4 shows that the methanolic extract of Tamajort had no effect on *Listeria monocytogenes* ATCC15313, *Pseudomonas aeruginosa* ATCC27853, and *Escherichia coli* ATCC8739.

Among natural substances widespread in medicinal plants, flavonoids and organic acids belong to the promising groups of bioactive compounds with strong antibacterial potency [27].

Alshwyeh tested the methanolic extracts of Saudi Arabian native date palm (Ajwa, Khalas Alkharj, and Al-Qasim) cultivars against *Streptococcus pnuemoniae*, *Bacillus subtilis*, *Klebsiella pneumonia*, *S. aureus*, and *E. coli* [20]. They found that the extracts displayed a broad spectrum of inhibitory effects against these pathogenic bacteria.

The inhibitory activity of plant extracts against the growth of microorganisms was attributed to the presence of phenolic compounds [28]. These antibacterial compounds act essentially by enzyme inhibition of DNA gyrase and disturb the function of bacterial cell membranes, retarding the growth and multiplication of bacteria.

Different antibacterial mechanisms of plant flavonoids were reported by Faegheh *et al.* and Górniak *et al.* [29, 30]. In particular, flavonoids inhibit nucleic acid synthesis, cytoplasmic membrane function, energy metabolism, biofilm attachment, and porin on the cell membrane, as well as alter membrane permeability and attenuate pathogenicity.

Abdullah *et al.* tested the antibacterial potential of hot aqueous and methanolic extracts of Ajwa date fruit against Gram-negative bacteria (*Salmonella typhi*, *E. coli*, *Vibrio cholera*, and *Shigella flexneri*) [31]. They found that the methanolic extract showed a higher antibacterial activity than the aqueous extract, suggesting that different extraction methods yield different phytochemicals producing the bactericidal effect.

Flavonoids cause increased permeability of the internal bacterial membrane and disruption of membrane potential. According to Usman Amin *et al.*, the ring of flavonoids may play a role in intercalation or hydrogen binding with nucleic acid base stacks, which may explain their inhibitory action on the synthesis of DNA and mRNA [32].

Flavonoids are known for their powerful antioxidant power. Indeed, they could potentially have an effect on iron chelation, which prevents the intracellular penetration of the co-factor Ca^{2+} into the bacterial cell [33].

CONCLUSION

Our study was designed to determine the phytochemical composition and the antibacterial potential of the methanolic extracts of three cultivars of date palm fruits. HPLC revealed the presence of gallic acid, tannic acid, caffeine, naringenin-7-o-glucoside, trans-cinnamic acid, 2,5-dimethyl hydroxycinnamic acid, hesperidin, caffeic acid, vanillin, ferulic acid, 3-hydroxy-4-methoxycinnamic acid, 3,4,5-trimethoxycinnamic acid, 3,4-dimethoxycinnamic acid, rutin, o-anisic acid, luteolin, quercetin, aspegenin, and isorhamnetin in all the date cultivars. The results showed that the extracts serve as an excellent source of bioactive compounds (polyphenols, flavonoids, and flavonols) and exhibit antibacterial potency with an inhibition zone diameter ranging from 8.40 ± 0.00 to 12.50 ± 1.00 mm. The results clearly demonstrate the antibacterial activity of date palm fruits, which could be attributed to their considerable content of natural compounds. Thus, they can replace the antibiotics which are restricted because of several side effects.

CONTRIBUTION

S. Ali Haimoud and R. Allem conceived and designed the analysis; contributed data and analysis tools;

and performed the analysis. S. Ali Haimoud collected the data and wrote the paper.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests related to the publication of this article.

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