Bioactivity of Algerian palm dates *Phoenix dactylifera* L.

Fatma Mihoub, Freha Gourchala, Safia Lakhdar-Toumi

Ibn Khaldoun University, Tiaret, Algeria

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Corresponding author:

Fatma Mihoub
E-mail:
mihoub2@yahoo.fr

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Abstract

Introduction. The current study was conducted in order to evaluate *in vitro* the phytochemical profile and antibacterial activity of Algerian palm dates *Phoenix dactylifera L.*

Materials and methods. Qualitative and quantitative (total polyphenols, flavonoids and antioxidant activity)phytochemical analysis were performed on aqueous and methanolic extracts of seven varieties of dates as the evaluation of antibacterial activity against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 strains.

Results and discussion. Phytochemical screening of aqueous and methanolic extracts showed the presence of several families of chemical compounds such as catechin tannins, saponins and terpenoids in the seven varieties of dates. The screening of these extracts reveals slight qualitative differences with a greater presence of saponins in the aqueous extract while terpenoids are present in large quantity in the methanolic extract. A quantitative characterization of extracts showed significantly high levels (P<0.05) in the methanolic extract from: 85.8±0.8 to 275 ± 0.07 GAE/100g, 36.9 ± 0.3 to 70.1 ± 0.9 QE/100g and 18.5 ± 0.9 to 58.5 ± 0.5% vs. 66.1±0.2 to 189±0.09 GAE/100g, 29.1±0.5 to 50.8±0.6 QE/100g and 14.7±0.4 to 41±0.1% in aqueous extracts for total polyphenols, flavonoids and reducing power respectively. The susceptibility of bacterial species to various extracts of *Phoenix dactylifera* L. fruits by agar well diffusion assay showed a maximum inhibition zone diameter (IZD) of 43.0 ± 1.0 and 26.3 ± 1.5 mm for the methanolic extract of the variety *Tamesrit* against *S. aureus* and *E. coli* strains respectively. The minimum inhibitory concentrations (MIC) ranged from 0.08 g / mL for the methanolic extract of the variety *H'mira* against *E. coli* to less than 0.04 g/mL for the methanolic extracts of the varieties *Tamesrit, Akerbouch* and *Bent Kbala* against *S. aureus.*

Conclusion. Given the interesting contents of polyphenols, flavonoids and antioxidant activity in addition to the inhibitory power of date extracts, we can conclude that this product could be an excellent source of antioxidants and bioconservatives in food preparation.

Introduction

The use of antibiotics is currently contested due in part to their high costs and potential toxicological risks and on the other hand, their frequent consumption could lead to resistance of strains and be responsible for therapeutic failures [1, 2]. Secondary metabolites are natural plant compounds, available at low cost. Due to their natural antioxidant and antibacterial effects, they can constitute pharmacological alternatives for the prevention and the treatment of several pathologies [3, 4]. For thousands of years, dates, fruits of *Phoenix dactylifera* L., are known to have many medicinal properties and in countries ranging from the Near East to the Middle East are used in traditional medicine as protectors and curatives [5-7]. Moreover, this fruit having many favorable biological properties as antioxidant, antimicrobial…. [8, 9]. In Algeria, the date occupies a very important place with an average annual production estimated at 1029596 tons; this allowed him to occupy the world's third largest producer of date [10]. But its valuation and exploitation as sources of natural bioactive substances endowed with antibacterial activity which presents an interest in the field of biopharmaceuticals are very limited. The objective of this study is to evaluate *in vitro* bioactivity of seven varieties of Algerian dates on phytochemical profile and antibacterial activity against two bacterial strains (*Staphylococcus aureus, Escherichia coli*) frequent in human pathology and often responsible for food poisoning.

Materials and methods

Plant material

Seven (07) date palm cultivars at the "*Tmar*" stage (maturity stage) locally known as: *H'mira, Adhem Fgig,BouzrourAkerbouch, Bentkbala, Ghars* and *Tamesrit* from the region of Ghardaia (southern Algeria) were used for the experimentation. 10 kg of each variety harvested (season 2017) in the same locality were provided by farmers. After cleaning, samples were placed in hermetic bags and stored at 4 °C until analysis.

Extract preparation

The pulps of each variety previously cleaned and ground, constitute the raw material for extracts. For aqueous extract, 10g of each variety are mixed with 100 mL of distilled water and continuous stirred for 2 h at room temperature then filtered. The methanolic extract is prepared by mixing 10g of pulps to 50 mL of methanol/water (80:20, V/V), placed under slow stirring for 24 h at room temperature then reduced under pressure in a rota-vapor to remove methanol.

Phytochemical analysis

Phytochemical analysis on aqueous and methanol/water extracts of dates was determined. Some phytochemical groups have been characterized, according to the methods described by Ciulei [11], tannins by the $FeCl_3$ test; alkaloids per Dragendorff reagent, the saponins per froth test, terpenoïds by Salkowski reaction (to 0.5 g of the extract were added to 2 ml of chloroform and 3 ml of concentrated sulfuric acid H_2SO_4 where a reddish-brown color indicates the presence of terpenoids) [12] and anthocyanins using HCl and NH_4OH test

[13]. The total phenolics contents were determined using Folin-Ciocalteau reagent according to Singleton and Rossi method [14] using gallic acid as standard. The results are expressed in mg of Gallic Acid Equivalent (GAE)/100g of fresh weight. The flavonoids amount is determined according to Lamaison and Carnat [15] method using quercitin as standard; the results are displayed in mg of Quercitin Equivalent /100g of fresh weight. The antioxidant activity is performed according to the FRAP method [16], the results obtained are expressed in mg of vitamin C per 100 g of the fresh fruit.

Antibacterial susceptibility assay

Bacterial strains. Two ATCC reference bacterial strains were used for the antibacterial susceptibility assay of different date extracts: *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 stored at -20 °C in glycerol.

Agar well diffusion assay and determination of MIC. The potential antibacterial activity of the aqueous and methanolic extracts of the pulp of the different cultivars was evaluated *in vitro* by the standard agar well diffusion assay [17]. From a culture of the bacterial suspension of 18 hours at 37°C, an inoculum adjusted to 0.5 Mac Farland density (a dilution of 1/10th and 1/100th of the bacterial suspension respectively of *S. aureus* and *E. coli* was performed in sterile distilled water before inoculation [18]) was seeded uniformly by tight streaks with a sterile cotton swab on the surface of the Petri dish (90 mm) containing 20 mL of Muller Hinton agar. Four wells/dish of 6 mm diameter each were perforated in the agar with the tip of a sterile Pasteur pipette and filled with 100 µL of aqueous or methanolic palm dates extracts (0.20 g/mL). All plates were incubated for 24 h at 37°C. The antibacterial activity is evaluated by measuring the diameter of the inhibition zone (IZD) formed around the well. The percentage of growth inhibition is calculated by the formula: I% = IZD/PDD*100 (IZD: Inhibition Zone Diameter, DD: Petri Dish Diameter=90mm) [19]. For assessment of minimum inhibitory concentration (MIC), the incorporation technique in agar is used [20] with some modifications. A concentration range of the aqueous and methanolic crude extracts was prepared respectively in water and methanol/water to have final concentrations of 0.04, 0.08, 0.12, 0.16 and 0.20 g/mL. 2 mL of each extract were incorporated in 18 mL of Muller Hinton agar maintained liquid and all is well stirred. After the agar plates completely solidified, 100 µL of the bacterial suspension already adjusted under the same conditions described above are inoculated by swabbing at the surface of the dish and all is incubated for 24 h at 37°C. The CMI is defined as the lowest concentration at which there is no visible growth [21]. The solvents used for the preparation of extracts (distilled water and methanol/water) are used as negative controls. While some antibiotics are used as positive controls to check their effects on the growth of each germ and are compared to the critical values of the diameters of

the zones of inhibition data by CA-SFM/EUCAST [22].These are: Tetracycline (30µg/disc), Ceftazidime (30µg/disc), Chloramphenicol (30µg/disc), Ampicillin (10µg/disc), Fosfomycin (200µg/disc), Cefazolin (30µg/disc) and Colistin sulfate (10µg/disc).

Statistical analysis

Each assay was carried out in triplicate. All experiments are expressed as mean ± standard deviation (SD). The data were treated using the STATISTICA software (Version 8) by the ANOVA test followed by the Duncan test for multiple comparisons.

Results and discussion

Phytochemical characterization of date extracts

Table 1

Phytochemical screening of aqueous and methanolic date extracts

Varieties	Saponins	Catechic tannins	Terpenoids	Anthocyans	Alkaloids
	Aqueous extract				
Ghars	+++	++	+	-	-
Tamesrit	+++	++	++	-	-
AdemFgig	+ + +	+	++	-	-
Akerbouch	+ + +	+ + +	+ +	-	-
BentKbala	+ + +	+ + +	+ +	-	-
Bouzror	++	+ + +	+ +	-	-
H'mira	+ +	+ + +	+ +	-	-
Methanolic extract					
Ghars	++	++	++	-	-
Tamesrit	++	++	+++	-	-
AdemFgig	+ +	+	++ +	-	-
Akerbouch	+ +	+ + +	+ + +	-	-
BentKbala	+ +	+ + +	+ + +	-	-
Bouzror	+	+ + +	+ + +	-	-
H'mira	+	+ + +	+ + +	-	-

+++ Very intense, ++ intense, + weak, − absent

Qualitative phytochemical analysis (Table 1) detected the presence of saponins, tannins and terpenoids in (methanolic/aqueous) date extracts.

The results depended on the reactions, going from clearly to weakly positive. These observations are similar to those reported by others authors indicating that *Phoenix dactylifera* fruit is a potential source of secondary metabolites [23-24]. A comparison of date extracts composition found that methanolic extracts presented greats levels of terpenoids than aqueous ones witch possessed more saponins, while both extracts had the same tannins contents. These results indicated the influence of the extraction solvent on the total content of secondary metabolites. Similar findings were obtained by others studies [25-27]. In contrast, anthocyans and alkaloids were absents in all date extracts.

Quantitative analysis of the composition on secondary metabolites showed a significant difference between the seven date cultivars extracts contents (Table 2). *Tamesrit* variety gave the highest levels of flavonoids and total polyphenols, followed by *Akerbouch* and *BentKbala* varieties thus reflecting interesting antioxidants activities with 58.5, 50.2 and 40.8% respectively. The aqueous extract revealed a low content of antioxidant compounds compared to the metanolic extract. Moreover, the antioxidant activity is inversely proportional to the richness in antioxidant compounds on the aqueous extract however, it seems to be more proportional to the flavonoids levels on the methanolic extracts; theses phytochemicals are a group of polyphenolic compounds, which have multiples pharmacological properties, they

presents strong antioxidant power [7] and could participate also in antibacterial activity [28-31].

Table 2

Polyphenols, flavonoids contents and antioxidant activity of aqueous and methanolic date extracts

Compounds / Varieties	Polyphenols (mg GAE/100g)	Flavonoids (mg QE/100g)	Antioxidant activity (%)
Aqueous extract			
Ghars	76.5±0.7^{d}	30.9±0.9^{e}	36.9±0.1^{b}
Tamesrit	189±0.09^{a}	46.7±0.3^{b}	14.7±0.4^{e}
Adhem Fgig	66.1±0.2^{e}	29.1±0.5^{f}	41±0.1^{a}
Akerbouch	123±1.1^{b}	50.8±0.6^{a}	16.4±0.8^{d}
Bent Kbala	121±0.9^{b}	36±0.7^{d}	17.8±0.7^{d}
Bouzrour	88.5±0.8^{c}	41±0.2^{c}	28.9±0.9^{c}
H'mira	89.2±0.6^{c}	31.2±0.1^{e}	29.7±1.2^{c}
Methanolic extract			
Ghars	103±1.1^{e}	41±0.3^{e}	21.3±1.3^{e}
Tamesrit	275±0.07^{a}	67.2±1.2^{a}	58.5±0.5^{a}
Adhem Fgig	85.8±0.8^{f}	36.9± 0.3^{f}	18.5±0.9^{f}
Akerbouch	173±0.6^{ab}	70.1±0.9^{a}	50.2±0.4^{b}
Bent Kbala	172±0.2^{b}	50±0.9^{c}	40.8±2.5^{c}
Bouzrour	117.5±0.5^{d}	55±1.0^{b}	36.2±0.9^{d}
H'mira	123 ± 1.1^{c}	44.3±0.9^{d}	23.7±0.3^{e}

The means followed by the letters a, b, c, d, e and f in the same column are significantly different ($p<0.05$).

3.2. Antibacterial activity of date extracts

The antibacterial activity of the aqueous and methanolic extracts of the seven date varieties against two pathogenic bacterial (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) revealed that the aqueous extracts have not expressed inhibition zones around the wells, whereas methanolic extracts showed a strong antibacterial activity against the two bacterial strains. Our results are in a good concordance with some studies like [32-33]. The difference between the antibacterial activities could be explained by the nature and the concentration of the metabolites content in each type of extract. Indeed, the ability to extracting and solubilizing phytomolecules is very different and depends strongly on the solvent type [34-35]. According to Cowan [36], it could be deduced that the antibacterial substances contained in dates are more soluble in methanol.

Moreover, the active extracts were significantly more effective ($P<0.05$) on *S. aureus* (IZD: 15 to 43 mm) than on *E. coli* (IZD: 11 to 27 mm) (Table 3).The resistance of Gram-negative bacteria may be due to the presence of lipopolysaccharides in their cell wall, thus preventing the possible active components of date extracts to crossing and lysing their cytoplasmic membranes [25, 37].

Table 3

Antibacterial activity of methanolic dates extracts compared to antibiotics

Varieties	IZD* (mm)		Antibiotics	IZD* (mm)	
	E. coli	***S. aureus***		***E. coli***	***S. aureus***
Ghars	18.7 ± 0.6^{d}	26.3 ± 1.5^{cd}	Cefazolin	24.6 ± 1.1^{b}	32.6 ± 0.7^{a}
Tamesrit	26.3 ± 1.5^{ab}	43.0 ± 1.0^{a}	Colistin sulfate	13.0 ± 1.7^{e}	16.0 ± 2.1^{d}
AdemFgig	24.7 ± 1.2^{b}	24.3 ± 2.1^{d}	Tetracycline	10.0 ± 1.4^{f}	26.0 ± 1.2^{c}
Akerbouch	27.0 ± 1.0^{a}	35.3 ± 3.1^{b}	Ampicillin	21.0 ± 0.6^{c}	25.0 ± 1.0^{c}
BentKbala	25.3 ± 0.6^{ab}	29.3 ± 0.15^{c}	Fosfomycin	35.0 ± 1.5^{a}	29.0 ± 2.5^{b}
Bouzror	22.0 ± 1.0^{c}	27.0 ± 1.0^{cd}	Ceftazidime	6.0 ± 0.1^{g}	6.0 ± 0.0^{f}
H'mira	11.0 ± 1.0^{e}	15.0 ± 0.1^{e}	Chloramphenicol	20.3 ± 0.4^{d}	7.6 ± 0.0^{e}

* Mean values ± SD, n=3 ($p<0.05$), IZD: Inhibition Zone Diameter. The Means followed by the letters a, b, c, d, e, f and g in the same column are significantly different ($p<0.05$)

The methanolic extract of *Tamesrit* variety which was significantly ($p<0.05$) the most effective, showed the maximum IZD against *S. aureus* (43 mm: nearly 50% of the population were inhibited) and *E. coli* (26.3 mm: nearly 30% of the population were inhibited) (Table 4).

Table 4

Minimum inhibitory concentration (MIC) and percentages of growth inhibition of methanolic dates extracts

Varieties	***E. coli***						
	Concentration of extract (g/mL)					MIC (g/mL)	I%
	0.20	0.16	0.12	0.08	0.04		
Ghars	-	-	-	-	+	0.08	20.8
Tamesrit	-	-	-	-	+	0.08	29.2
AdemFgig	-	-	-	-	+	0.08	27.4
Akerbouch	-	-	-	-	+	0.08	30.0
BentKbala	-	-	-	-	+	0.08	28.1
Bouzror	-	-	-	-	+	0.08	24.4
H'mira	-	-	-	+	+	0.12	12.2
Varieties	***S. aureus***						
	Concentration of extract (g/mL)					MIC (g/mL)	I%
	0.20	0.16	0.12	0.08	0.04		
Ghars	-	-	-	-	+	0.08	29.2
Tamesrit	-	-	-	-	-	<0.04	47.8
AdemFgig	-	-	-	-	+	0.08	27.0
Akerbouch	-	-	-	-	-	<0.04	39.2
BentKbala	-	-	-	-	-	<0.04	32.6
Bouzror	-	-	-	-	+	0.08	30.0
H'mira	-	-	-	-	+	0.08	16.7

+: presence of growth, -: no growth, I%: The percentage of growth inhibition

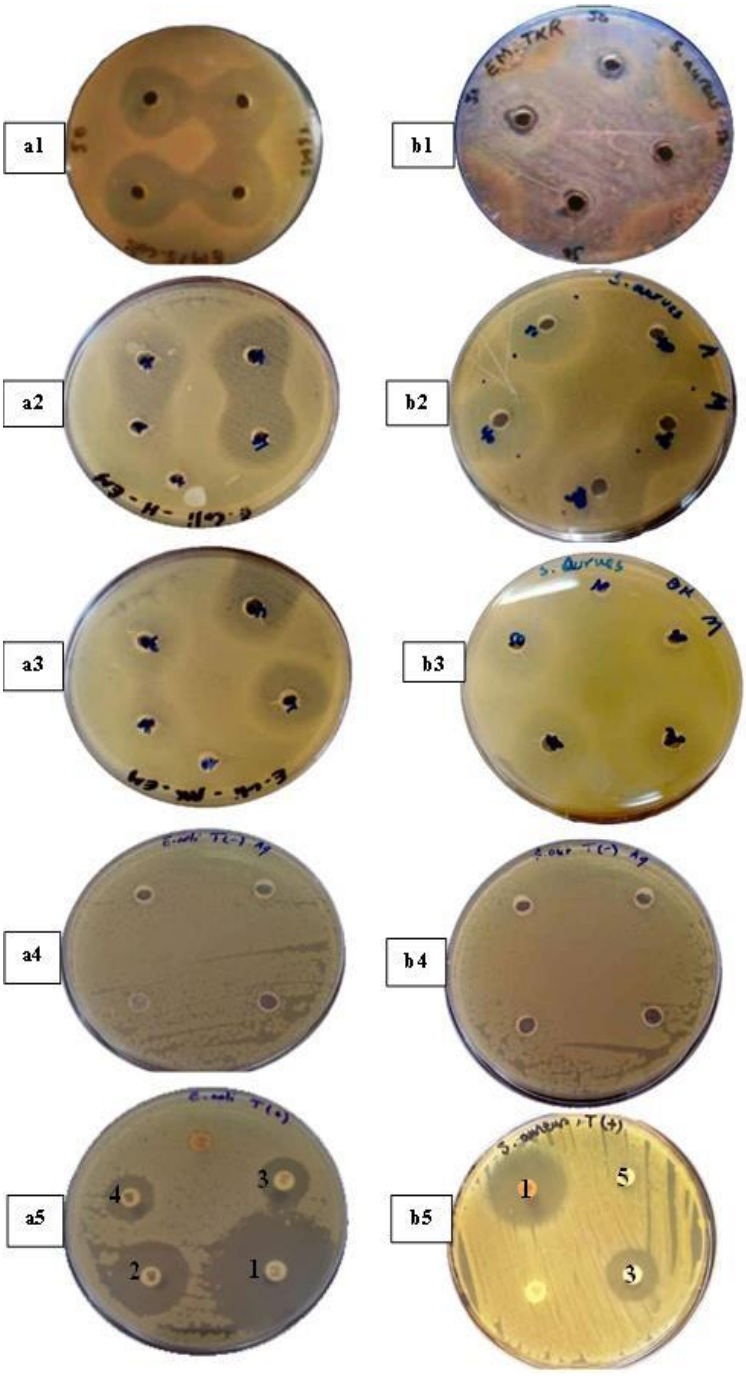

Figure 1. Antibacterial activities of methanolic extracts of some date's varieties compared with negative (a4, b4) and positive (a5, b5) controls against *E. coli* (a) and *S. aureus* (b) strains

Designation in Figure 1

Antibiotics for positive control: 1: Fosfomycin, **2:** Cefazolin, **3:** Colistin sulfate, **4:** Tetracyclin, **5:** Chloramphenicol
a1, a2 and a3: effect of *Tamesrit, Akerbouch* and *Bent Kbala* respectively against *E. coli*
b1, b2 and b3: effect of *Tamesrit, Akerbouch* and *Bent Kbala* respectively against *S. aureus*

Minimum inhibitory concentration (MIC) values below 0.04 g/mL are noted for *Tamesrit*, *Akerbouch* and *Bent Kbala* extracts against*S. aureus* (Table 4). Whereas for both strains, a MIC of 0.08g/mL is recorded for the methanolic extracts of majority of dates cultivars excepting *H'mira* extract which showed a MIC of 0.12g/mL (I% = 12.2%) to inhibit the growth of *E. coli*.

Extracts of *Akerbouch* and *Bent Kbala* varieties gave an interesting IZD for Gram positive bacteria of 35.3 to 29.3 respectively mm and for Gram negative bacteria of 27 to 25.3 mm respectively (Table 3, Figure 1). A study conducted on the bacterial activity of three Algerian varieties *Ghars*, *Deglet Nour* and *Mech Degla* on some bacterial species gave IZDs of 7.5 to 9.5 mm against *E. coli* which are clearly lowers to the diameters we obtained with all methanolic extracts on the same germ [38].However a study of the antibacterial activity of methanolic extracts of Saudi dates gave IZDs of 16 ± 0.20 mm on *S. aureus* and 11 ± 0.00 mm on *E. coli*[35];these values correspond to the minimum IZDs found for our varieties. In another study, the antibacterial activity of methanolic Nigerian dates extracts gave IZDs of 12 to 18 mm against *E. coli* [39]; values comparable to those obtained with *H'mira* and *Ghars* cultivars extracts in the present study which exhibit the lowest antibacterial activity.

On the other hand, for the control antibiotics used, Fosfomycin and Cefazolin gave a maximum inhibition diameter of 32.6 mm and 35 mm against *S. aureus* and *E. coli* respectively. The interest of the results we obtained is that all the antibiotics showed an antibacterial activity comparable to that of dates extracts or even less than some (Table 3). Effectively, the methanolic extract of *Tamesrit* variety (IZD=43 mm) was more active (P<0.05) than all antibiotics against *S. aureus* (IZD maximum of 32.6 mm for cefazolin). The results of this study showed that *S. aureus* were highly resistant to Chloramphenicol and ceftazidime while *E. coli* resisted to ceftazidime and tetracycline versus the active methanolic extracts of the different date cultivars. The presence of flavonoids, saponins, terpenoids and tannins, may be involved in the bioactivity of plant extracts, these chemical groups have been demonstrated in other studies conducted on the evaluation of antibacterial activity [29].The studied dates extracts (Table 1) contain heterogeneity of chemical compounds that could explain the antibacterial activity observed.

Conclusion

The sensitivity of the studied bacteria (*S. aureus* and *E. coli*) was more apparent for the extracts of *Tamesrit* variety followed by those of *Akerbouch* and *Bent Kbala*, this result is very important because it provides an answer element for the choice of some dates varieties for a possible therapeutic use. The date could be used as a bioconservative in the food industry which gives added value to this fruit playing both a role of preservative and stabilizer during food storage by their antibacterial effects but also able to improve their organoleptic and nutritional qualities by its contribution in interesting elements such as the polyphenolic compounds and the antioxidant power, so it would be a very interesting functional food ingredient. The difference between the antibacterial activity of the two date extracts (aqueous

and methanolic) is difficult to explain because both extracts contained all sought metabolites, but not in the same proportions. So, identification and quantification of active components is necessary and could shed more light on the difference in the biological activities of dates extracts.

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