Journal of King Saud University – Science (2016) 28, 136–142

King Saud University
Journal of King Saud University – Science
www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Evaluation of antioxidant, antihemolytic and antibacterial potential of six Moroccan date fruit (*Phoenix dactylifera* L.) varieties

Eimad dine Tariq Bouhlali [a,b,*], Mohamed Bammou [a], Khalid Sellam [a], Mohamed Benlyas [a], Chakib Alem [b], Younes Filali-Zegzouti [a]

[a] *Team of Biology, Environment and Health, Faculty of Science and Technology, Errachidia, Morocco*
[b] *Team of Biochemistry of Natural Substances, Faculty of Science and Technology, Errachidia, Morocco*

Received 29 August 2015; accepted 10 January 2016
Available online 16 January 2016

KEYWORDS

Date fruit;
Antioxidant;
Anti-hemolytic;
Antibacterial

Abstract The research aimed to examine the antioxidant, anti-hemolytic and antimicrobial activities of six Moroccan date fruit varieties. Estimation of total phenolic and flavonoid contents revealed that, *Bousrdoun* (537.07 mg GAE/100 g DW) and *Jihl* (208.53 mg RE/100 g) had the highest phenolic and flavonoid contents, respectively. Among the date fruit varieties tested for antioxidant activities, *Jihl* had the highest activity compared to other varieties. It had an inhibitory concentration (IC_{50}) value of 2.05 g/L for DPPH scavenging activity and a ferric reducing power of (860.89 µmol TE/100 g DW). As well as a high protective effect against AAPH-induced erythrocyte hemolysis with a hemolysis half-time of 210.99 min.

The antibacterial capacity of various extracts was investigated against Gram positive (*Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 29213, *Staphylococcus aureus* ATCC 25923) and Gram negative bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella abony* NCTC 6017). The *Bousrdon* and *Jihl* extracts were found to be more potent inhibitory activities with MIC values ranging between 2.5 mg mL^{-1} and 10 mg mL^{-1} for all bacterial strains tested.

These results suggested that date fruit extract, especially *Jihl* and *Bousrdon* extract, is not only an important source of antioxidants, which possess a high protective effect of membrane against free radical, but also a potential source of antibacterial components.

* Corresponding author at: Biochemical of Natural Resources Laboratory, Faculty of Science and Technology, Errachidia 52000, Morocco. Tel.: +212 698984535.
E-mail address: bouhlali.eimad@gmail.com (E.T. Bouhlali).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

Date fruit is one of the oldest known fruit crops in southeast Morocco, where it is considered as an ideal food because of its edible sweet fruit, energy boosters and hunger pacifiers also, due to its many medicinal properties. Date fruit contains a wide range of chemical compounds such as phenolic acid,

http://dx.doi.org/10.1016/j.jksus.2016.01.002
1018-3647

flavonoids, anthocyanins, procyanidins, carotenoids, and sterols, which is known to have too many health benefits (Baliga et al., 2011).

The pulp and the pit of date fruit have been used in traditional medicine for the prevention of a wide range of infectious diseases. The ethnomedicinal inspection showed that people in southeast Morocco used date fruit to treat hypertension and diabetes (Tahraoui et al., 2007). The date fruit is known in folk medicine for its laxative effect and it is also used to regulate the urine and in vaginal pessaries, to improve sexual potency and fertility, also to cure abdominal complaints, vomiting, asthma, bronchitis, fever, fatigue, tuberculosis, wandering of the mind and loss of consciousness (Aamir et al., 2013; Ammar et al., 2009).

Pre-clinical researches that were done using date fruit extract show a wide spectrum of health benefits such as antioxidant, antimutagenic, antimicrobial, anticancer, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, and immunostimulant activities (Baliga et al., 2011).

The target of this research is to investigate the antioxidant, anti-hemolytic and antibacterial potential of six Moroccan date varieties.

2. Materials and methods

Six Moroccan date varieties locally known as *Bouskri*, *Bousrdon*, *Bousthammi*, *Boufgous*, *Jihl* and *Majhoul* considered to be of premium quality in the south-east Morocco were obtained at *Tamr* stage (October 2014) from The Errachidia National Institute for Agricultural Research. The samples were rinsed, pitted and stored at −20 °C until extraction and analysis.

2.1. Preparation of rich polyphenol extracts

The rich Phenolic compound extract was prepared according to the method of Bouhlali et al. (2015c). Briefly, 30 g of pitted and crushed date fruit was extracted with 150 mL methanol–water (4:1, v/v), at 35 °C for 12 h using an orbital shaker-incubator. The mixture was then filtered and the filtrate was concentrated under reduced pressure at 40 °C until the total evaporation of solvent using a rotary evaporator. The results of methanolic crude extract were kept at −20 °C in dark glass bottles until use.

2.2. Measurement of total polyphenolic contents

The total phenolic contents of date fruit extract were measured according to the method of Bouhlali et al. (2015b). Briefly, 500 µL of Folin–Ciocalteu reagent (10-fold diluted with water) was mixed with 100 µL of date fruit solution, and then 400 µL of aqueous sodium carbonate solution (7.5% w/v) was added. The mixture was allowed to stand for 60 min at room temperature and the absorbance measured at 765 nm. The calibration curve was prepared using Gallic acid. The total phenolic compounds were expressed as Gallic acid equivalent in mg/100 g dry weight (DW) of date fruit.

2.3. Measurement of flavonoid content

The total flavonoid content of date fruit extract was determined by the method of Kim et al. (2003). One mL of date fruit solution was mixed with 4 mL of distilled water. Then 300 µL of aqueous sodium nitrite solution (5%) was added, followed by 300 µL of aqueous aluminum chloride solution (10%). Test tubes were incubated for 5 min at room temperature, and then 2 mL of sodium hydroxide (1 M) was added to the mixture and then the final volume was made upto 10 mL with distilled water. The mixture was vortexed and the absorbance was determined at 510 nm. The absorbance was calibrated to a standard curve of Rutin solution and the results were expressed as mg Rutin equivalents (RE)/100 of dry weight (DW) of date fruit.

2.4. Measurement of total condensed tannins

The total condensed tannins were determined using the method described by Heimler et al. (2006). Concisely 400 µL of the date fruit extract was mixed with 3 mL of methanolic solution of vanillin (4%) and 1.5 mL of concentrated hydrochloric acid. The absorbance was measured at 500 nm after incubation at room temperature for 15 min. Catechin was used to prepare a calibration curve, and the results were expressed as mg CE (catechin equivalents)/100 g of dry weight (DW) date fruit.

2.5. ABTS radical scavenging assay

The ABTS assay was done using the method of Re et al. (1999). The ABTS radical cations ($ABTS^{+}$) were produced by reacting aqueous solution of ABTS (7 mM) with an aqueous solution of potassium persulphate (2.45 mM). The mixture was left at an ambient temperature in the dark for 12–16 h before use, the resulting solution was diluted with distilled water to obtain 0.700 ± 0.005 at 734 nm in the absorbance. 30 µL of the sample added to 3 mL of the ABTS solution was allowed at an ambient temperature. After 6 min the absorbance at 734 nm was recorded immediately. Trolox solution was used to prepare the standard curve and the total antioxidants were expressed as mmol of Trolox equivalents per 100 g of dry weight (DW) of date fruit.

2.6. DPPH radical scavenging activity

Radical scavenging activity of the date fruit extract against stable DPPH was evaluated as described by Blois (1958) method with slight modifications. The reaction mixture contained 100 µL of date fruit extract at a different concentration and 1.9 mL of methanolic DPPH (0.3 mM). The resultant mixtures were left at room temperature for 20 min and the absorbance was measured at 517 nm. IC_{50} (concentration providing 50 % inhibition) values were calculated from the plotted graph of scavenging activity against the concentrations of the samples.

$$\%\ \text{inhibition} = \frac{(\text{Abs(control)} - \text{Abs(sample)})}{\text{Abs(control)}} \times 100$$

Abs control is the absorbance without extract; Abs sample is the absorbance of the extract or standard.

2.7. Ferric reducing antioxidant power assay

The ferric reducing activity of the date fruit extract was estimated based on the method of Benzie and Strain (1999). The FRAP reagent was prepared by mixing 50 mL of acetate buffer

(0.3 M) at (pH 3.6), 5 mL tripyridyltriazine (TPTZ) solution 10 mM prepared in HCl (40 mM) and 5 mL of ferric chloride solution ($FeCl_3$) (20 mM). 2 mL of the freshly prepared FRAP reagent was added to 10 µL of extract. Then the absorbance was measured at 593 nm against the blank after 10 min at ambient temperature. Trolox was used to prepare the standard curve. The result was expressed as Trolox equivalent in mmol/100 g of dry weight (DW) of date fruit.

2.8. Evaluation of protective effect date fruit extract against AAPH induced erythrocyte oxidative hemolysis

The anti-hemolytic activity induced by a peroxyl radical initiator, AAPH was measured according to the method established by Bouhlali et al. (2015a). Two hundred microliters of Rabbit blood collected in heparin bulbs were mixed with 10 µL of date fruit extract, and then 600 µL of AAPH (10%) was added. The mixture was incubated at 37 °C. The absorbance of the mixture was measured at 450 nm every 5 min. The date fruit extract was replaced by Trolox and saline (0.9% NaCl) in the positive and the negative control respectively. The Protective effects of date fruit extract on free radical induced hemolysis of erythrocytes were estimated from the time required for half-hemolysis.

2.9. Antibacterial activity

2.9.1. Bacterial strains

The antimicrobial activity was evaluated against six selected bacteria associated with various forms of diseases, three Gram-positive: *Bacillus cereus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and three *Gram negative*: *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Salmonella abony* NCTC 6017. Microorganisms were obtained from the culture collection of The National Institute of Hygiene (Rabat).

2.9.1. Disc-diffusion method

The disc-diffusion test of date fruits extracts was carried out using the method described by the National Committee for Clinical Laboratory Standards (NCCLS, 2002) with slight modification. Briefly 250 µL of bacterial cell suspension (concentration of 10^8 CFU/mL prepared using an overnight culture growth incubated at 37 °C) was spread over the plates containing Mueller Hinton agar media, then a paper disc impregnated with 15 µL of the different date fruit extracts was placed on the surface of the media. Then the diameter of an inhibition zone was measured around the disks after incubation of Petri dishes for 24 h in 37 °C. Gentamicin (10 µg/disc) a known antibiotic drug was used to control the sensitivity of the tested bacteria. One other Petri dish was used as a negative control and contained the bacterial cell suspension without the test extract. This assay was done in triplicate, and the inhibition zones of the extract were compared with those of the reference antibiotic.

2.9.2. Minimum inhibitory concentration (MIC)

The antibacterial activity of the various date fruit extract was tested using the micro-dilution method of Drummond and Waigh (2000) with a slight modification. First of all, the resazurin solution was prepared by dissolving one tablet in 40 mL of sterile water and the bacterial culture was prepared by diluting an overnight culture of a test bacterium in 0.1% physiologic water to obtain 10^6 colony forming units/mL. Afterward 100 µL of bacterial culture was added in each numbered well followed by 100 µL of resazurin solution and then 100 µL of each serially diluted date fruit extract was added in the well. After incubation in 37 °C for 6 h, the blue colored solution in microtiter plates indicates growth inhibition of bacteria. The negative control contained the sterile broth and resazurin solution, although the positive control is made up of broth culture and resazurin solution.

2.10. Statistical analysis

Statistical analysis was performed using StatView 5.0 software. The experimental results were reported as mean ± SE (standard error) ($n = 6$) on a dry weight for all experiments except antibacterial activity where the results were reported as mean ± SD (standard deviation) ($n = 3$). The analysis of variance (ANOVA) and the post hoc Bonferroni. ($p < 0.0018$) tests were used to compare the experimental groups. Pearson's correlation coefficient (r) was used to measure the association between two variables. Differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Phenolic content

The total phenolic contents of date fruit varieties were estimated using Folin–Ciocalteu's colorimetric method, which is a simple, rapid, reproducible and low-cost method. This method relies on the reduction of phosphomolybdic–phospho tungstic acid in an alkaline medium in the presence of phenolic compounds (Fu et al., 2011). Table 1 showed significant differences within the analyzed date fruit varieties ($p < 0.05$). The highest value of total polyphenols was determined in *Bousrdon* (537.07 mg GAE/100 g DW) and the lowest level was found in *Bouskri* (331.86 mg GAE/100 g DW). The polyphenolic content in this study was higher compared to the study of Hasnaoui et al. (2012) for the Moroccan varieties who found the total phenolic content ranged between 171.4 and 353.92 mg GAE/100 g DW. Our results confirm previous results established by Lemine et al. (2014) (405.5–661.1 mg GAE/100 g DW) for Mauritanian date cultivars and Benmeddour et al. (2013) (226–955 mg GAE/100 g DW) for Algerian date cultivars. These variations in the total phenolic contents may depend on varieties, maturity growing condition, storage conditions, fertilizer, soil type, season, geographic origin and amount of sunlight received may also be critical in this respect (Al-Farsi et al., 2007; Besbes et al., 2009).

3.2. Flavonoids content

Table 1 summarized that the flavonoid content in analyzed date fruits varied widely and ranged from 68.88 to 208.53 mg RE/100 g DW. *Jihl* cultivar showed the highest amount of flavonoid contents and *Bouskri* cultivar showed the lowest level. Our results are largely in good concordance

Table 1 Antioxidant activity, total phenolic content, total flavonoid and condensed tannin content of different date varieties from Morocco.

	Total phenolic content mg GAE/100 g DW	Total flavonoid content mg RE/100 g DW	Condensed tannins content mg CE/100 g DW	FRAP μmol TE/ 100 g DW	DPPH g of date/L
Boufgous	506.77 ± 23.95[a]	84.35 ± 2.95[a]	81.34 ± 2.64	627.99 ± 27.59	3.42 ± 0.08
Bouskri	331.86 ± 13.24	68.87 ± 2.14	57.56 ± 3.86	406.614 ± 14.31	6.25 ± 0.13
Bousrdon	537.074 ± 19.25	188.58 ± 4.45	92.14 ± 4.68	818.86 ± 21.91	3.11 ± 0.08
Bousthammi	441.851 ± 29.79	85.07 ± 100[a]	69.90 ± 3.97	530.23 ± 15.90	4.79 ± 0.12[a]
Jihl	495.269 ± 20.50[a]	208.53 ± 4.51	87.07 ± 5.29	860.89 ± 17.08	2.05 ± 0.03
Majhoul	398.228 ± 21.58	77.73 ± 3.65	64.29 ± 5.7	469.03 ± 17.99	5.25 ± 0.19[a]
Trolox	–	–	–	–	0.0101 ± 0.0019

Values in average ($n = 6$) ± SE. Averages, in the same column, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.001$).

with those previously announced by Benmeddour et al. (2013) and Lemine et al. (2014) who established that the total flavonoid content on dried matter of date fruit at the same stage of maturation "*Tamr*" ranged from 15.22 to 299.74 mg QE/100 g and 39.5 to 112.5 mg QE/100 g respectively. However, our observed results are very higher than those reported by Biglari et al. (2008) who found that the total flavonoid content in the Iranian date cultivars varied from 1.62 to 81.79 mg CE/100 g DW.

Flavonoids are known to be synthesized by plants in response to microbial infection; hence, it should not be astonishing that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms (Kumar and Pandey, 2013). Several scientific investigations showed that flavonoids possess a wide range of health benefits, which include oxidative stress such as anti-allergic, anti-inflammatory, analgesic, and cardio protective, hepatoprotective, anticancer, antiviral activities. This variability of therapeutic potentials depend on flavonoid structure (Agrawal, 2011; Kumar and Pandey, 2013).

3.3. Condensed tannin content

The determination of condensed tannin content was done using the vanillin–HCl assay, which is based on the measurement of red color that results when vanillin reacts with the meta-substituted ring of flavonols. However, the fact that any substituted monomeric flavanol can react with the vanillin makes this assay nonspecific for condensed tannin determination (Schofield et al., 2001). The results of condensed tannin content which showed significant differences among analyzed date fruit varieties is illustrated in Table1. *Bousrdon* cultivar showed the highest level of condensed tannins (92.141 mg CE/100 g DW) while the lowest level was observed in *Bouskri* cultivar (57.564 mg CE/100 g DW). Our results are lower than those reported by Benmeddour et al. (2013) who found that condensed tannin content varied between 82.81 and 525.06 mg CE/100 g DW.

3.4. Antioxidant activities

Date fruit contains a wide range of phenolic compounds, which have diverse antioxidant capacities. To better examine their antioxidant capacities, different assays are required. Hence, in this study the ferric reducing ability (FRAP) and free radical scavenging activity assay DPPH were used in this respect.

Simplicity, rapidity, reproducibility as well as the excellent relationship between the molar concentration of antioxidant and the ferric reducing ability, make FRAP the better assay to assess the antioxidant potential of food polyphenol compound (Müller et al., 2010). This method is based on the ability of electron-donating antioxidants to reduce at an acidic medium, a colorless ferric complex (Fe^{3+}–tripyridyltriazine) to blue-colored ferrous complex (Fe^{2+}–tripyridyltriazine) which had a maximum absorbance at 593 nm (Dudonne et al., 2009).

The result showed that all cultivars exhibited a good reducing power which is varied significantly ($p < 0.05$) from 406.61 to 860 μmol TE/100 g DW for *Jihl* and *Bouskri*, respectively. As shown in this study (Table 4), there is a strong positive relationship between the antioxidant activity measured by FRAP assay and the total polyphenol contents ($r = 0.871$), flavonoids content ($r = 0.948$), as well as condensed tannins ($r = 0.952$). Therefore, flavonoids are the principal provider to the antioxidant potential of date fruit extract.

DPPH (1,1-diphenyl-2-picrylhydrazyl) assay is one of the well-known and widely used methods for estimating antioxidant activity which is easy to perform and has an acceptable accuracy (Zhou and Yu, 2004). DPPH is a stable free radical, when it accepts a hydrogen radical or an electron from an antioxidant compound it becomes a stable diamagnetic molecule and loses its purple coloration which was measured at 517 nm.

The averages of free radical scavenging activity of date fruit cultivars using DPPH assay are displayed in Table 1, which showed a significant difference between examined date fruit varieties. Among these varieties *Jihl* cultivar (2.046 g of date/L) exhibited the highest level of scavenging activity based on DPPH assay with a lower IC_{50} value (IC_{50} value is the concentration of the sample required to inhibit 50% of radical) while *Bouskri* cultivar possessed the lowest level of antioxidant activity with a high IC_{50} (6.255 g of date/L). The scavenging activity of date fruit is still very low compared to the synthetic antioxidant (Trolox) which possessed an DPPH IC_{50} (0.0101 g of Trolox /L).

The analysis of correlation (Table 4) between phenolic, flavonoid and scavenging activity indicated a strong negative correlation. Phenolic content/DPPH ($r = -0.90$) and DPPH/ flavonoid content ($r = -0.842$) as well as DPPH/condensed tannins ($r = -0.942$). These strong negative correlations suggest that the phenolic compounds, especially flavonoid may be the main provider of the scavenging ability observed on date fruit.

3.5. Antihemolytic activity

The high membrane concentrations of polyunsaturated fatty acids (PUFA) and the hemoglobin redox reactions associated with O_2 transport, which are the potent promoters of reactive oxygen species make Erythrocytes the first targets for free radical attack (Nabavi et al., 2011), hence the need for their membrane protection. Table 2 illustrated the protective effect of date fruit extract on erythrocytes treated with a free radical generator (AAPH), which enhances membrane lipid peroxidation a key factor for cell lysis. Among analyzed date fruit varieties, *Jihl* exhibited considerable antihemolytic activity with a half time of hemolysis (210.99 min) more than the control (Trolox) with a half time of hemolysis (175.84 min), while Bouskri exhibited the lowest activity with a half time of hemolysis (158.70 min). The high positive correlation as shown in the Table 4 between flavonoid content and half time of hemolysis ($r = 0.887$) on the one hand, and phenolic content and the increase in half time of hemolysis ($r = 0.690$) on the other hand, justified the involvement of flavonoids and other polyphenols on the erythrocyte membrane stability as mentioned by various authors (De Freitas et al., 2008; Chaudhuri et al., 2007). Scavenging of lipid peroxyl radicals generated by AAPH seems to play a considerable part in antihemolytic activities as shown by a high correlation between antioxidant assays and antihemolytic activity (Table 4). The non-significant hemolysis observed when erythrocytes were treated only with date fruit extract as shown in the Table 3 can be justified as these extracts are nontoxic and harmless for the cells. However it possessed a membrane stabilizing effect that varied between 33.84% for *Jihl* and 0.44% for *Bouskri* compared to the control.

3.6. Antibacterial activity

The antibacterial screening of six Moroccan date fruit cultivars (*Bousrdon, Jihl*, *Boufgous*, *Bousthammi*, *Majhoul* and *Bouskri*) against *S. aureus*, *B. cereus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *S. abony* is shown in the Table 5. The antimicrobial activity of the samples was determined using the minimal inhibitory concentration (MIC) values for the tested bacteria.

All the tested date fruit extracts showed antibacterial activity except *Majhoul* and *Bouskri* extracts but this antibacterial effect is still lower compared to gentamicin (10 μg/disc) used as a reference antibiotic. This effect is varied between varieties and depends also on the tested bacterial strains.

Bousrdoun extract showed a higher antibacterial activity among analyzed varieties. The highest antibacterial activity of *Bousrdoun* was recorded against *S. abony* and against *B. cereus* with a MIC value of 2.5 mg mL^{-1}, the same antibacterial activity against *B. cereus* was shown using *Jihl* extract. The *Majhoul* and *Bouskri* varieties showed no activity against all bacterial strains tested (see Table 6).

The difference in the sensitivity of the studied bacterial strain against date fruit extract may be due to the change in their inherent structural or functional characteristics, which allows them to tolerate date fruit compounds. The nature and the combination of polyphenolic compounds present in the date fruit extract may also be critical in this respect. Several studies attributed the inhibitory effect of plant extracts against pathogenic bacteria to their polyphenolic compounds, precisely the ability of phenolic compounds to bind with the bacterial cell wall and then inhibit the bacterial growth (Sellam et al., 2013; Barbary et al., 2010; Baydar et al., 2004). These phenolics compounds may act through their ability to

Table 2 Antihemolytic activity of different date fruit extracts.

	Hemolysis half-time (min)	Protective effect (%)
Control	$175.84 \pm 4.04^{e,f}$	–
AAPH-blood	85.66 ± 3.46	–
AAPH-blood-*Boufgous* extract	$176.90 \pm 2.44^{a,b}$	206.51
AAPH-blood-*Bouskri* extract	158.70 ± 2.10	185.27
AAPH-blood-*Bousrdon* extract	$184.49 \pm 3.62^{a,c}$	215.37
AAPH-blood-*Bousthammi* extract	$177.44 \pm 2.59^{c,d,e}$	207.14
AAPH-blood-*Majhoul* extract	$170.37 \pm 4.67^{b,d,f}$	198.90
AAPH-blood-*Jihl* extract	210.99 ± 3.11	246.31
AAPH-Trolox 1%	182.17 ± 5.29	212.67

Values in average ($n = 6$) ± SE. Averages, in the same column, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.001$).

Table 3 Evaluation of hemolysis and membrane stabilizing effects induced by date fruit extract.

	Hemolysis half-time (min)	Membrane stabilizing effects (%)
Control	175.84 ± 4.04	
Blood-*Boufgous* extract	184.82 ± 1.47	5.11
Blood-*Bouskri* extract	176.62 ± 1.57	0.44
Blood-*Bousrdon* extract	195.03 ± 2.60	10.91
Blood-*Bousthammi* extract	186.34 ± 2.10	5.97
Blood-*Majhoul* extract	179.07 ± 3.37	1.84
Blood-*Jihl* extract	235.34 ± 3.17	33.84

Values in average ($n = 6$) ± SE. Averages, in the same column, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.001$).

Table 4 Correlation phenolic, flavonoid and condensed tannin content with antioxidant and antihemolytic activities.

	Polyphenols	Flavonoids	CT	FRAP	DPPH	AhE
Polyphenols	1					
Flavonoids	0.688	1				
CT	0.967	0.836	1			
FRAP	0.871	0.948	0.960	1		
DPPH	−0.900	−0.842	−0.942	−0.960	1	
AhE	0.690	0.867	0.770	0.890	−0.912	1

CT: Condensed Tannins; AhE: Antihemolytic effect.

Table 5 Antibacterial activity of six date fruit varieties against the bacterial strains based on the disc diffusion method.

	Bousrdoun	*Jihl*	*Boufgous*	*Bousthammi*	*Majhoul*	*Bouskri*	*Gentamicin*
Bacterial strains							
Gram-negative bacteria							
P.a ATCC 27853	12.33 ± 0.44	11.66 ± 0.44	10.66 ± 0.44	9.00 ± 0.66	Na	Na	17.66 ± 0.33
E.c ATCC 25922	12.33 ± 0.44	10.66 ± 0.44	10.66 ± 0.44	8.00 ± 0.66	Na	Na	19.00 ± 0.00
S.ab NCTC 6017	14.66 ± 0.44	11.66 ± 0.66	12.00 ± 0.00	10.00 ± 0.00	Na	Na	18.66 ± 0.22
Gram-positive bacteria							
B.c ATCC 29213	14.00 ± 0.00	12.00 ± 0.00	12.33 ± 0.44	10.00 ± 0.00	Na	Na	20.33 ± 0.22
B.s ATCC 6633	12.66 ± 0.44	11.66 ± 0.44	11.00 ± 0.00	9.33 ± 0.44	Na	Na	19.66 ± 0.33
S.a ATCC 25923	13.66 ± 0.44	11.66 ± 0.44	11.00 ± 0.66	7.66 ± 0.44	Na	Na	18.00 ± 0.00

P.a: *Pseudomonas aeruginosa*; *E.c*: *Escherichia coli*; *S.ab*: *Salmonella abony*; *S.a*: *Staphylococcus aureus*; *B.c*: *Bacillus cereus*; *B.s*: *Bacillus subtilis*. Na: Not active.

Table 6 MIC values of six date fruit varieties against the Bacterial strains tested.

Minimum inhibitory concentration (MIC) mg ml^{-1}						
	Bousrdoun	*Jihl*	*Boufgous*	*Bousthammi*	*Majhoul*	*Bouskri*
Bacterial strains						
Gram-negative bacteria						
P.a ATCC 27853	5.00	5.00	10.00	10.00	Nt	Nt
E.c ATCC 25922	5.00	10.00	10.00	> 10.00	Nt	Nt
S.ab NCTC 6017	2.50	5.00	5.00	10.00	Nt	Nt
Gram-positive bacteria						
S.a ATCC 25923	5.00	5.00	10.00	> 10.00	Nt	Nt
B.c ATCC 29213	2.50	2.50	5.00	10.00	Nt	Nt
B.s ATCC 6633	5.00	5.00	10.00	> 10.00	Nt	Nt

P.a: *Pseudomonas aeruginosa*; *E.c*: *Escherichia coli*; *S.ab*: *Salmonella abony*; *S.a*: *Staphylococcus aureus*; *B.s*: *Bacillus subtilis*; *B.c*: *Bacillus cereus*; Nt: Not tested.

precipitate protein and inhibit enzymes of microorganisms (Naz et al., 2007). Results showed a good association between the concentrations of polyphenols compounds and the antimicrobial activity.

4. Conclusion

The study revealed that compounds present in date fruit exhibit antioxidant, antihemolytic and antibacterial activities. The high correlation between these biological activities and antioxidant property has focused our attention on the role of phenol as the main responsible factor for these biological activities due to their antioxidant power.

References

Aamir, J., Kumari, A., Khan, M.N., Medam, S.K., 2013. Evaluation of the combinational antimicrobial effect of *Annona squamosa* and *Phoenix dactylifera* seeds methanolic extract on standard microbial strains. Int. J. Biol. Sci. 2 (5), 68–73.

Agrawal, A.D., 2011. Pharmacological activities of flavonoids: a review. Int. J. Pharm. Sci. Nanotechnol. 4 (2), 1394–1398.

Al-Farsi, M., Alasalvar, C., Al-Abid, M., Al-Shoaily, K., Al-Amry, M., Al-Rawahy, F., 2007. Compositional and functional characteristics of dates, syrups, and their by-products. Food Chem. 104 (3), 943–947.

Ammar, N.M., Lamia, T., Nabil, H., El-Sayed, M., Tom, J.M., 2009. Flavonoid constituents and antimicrobial activity of date (*Phoenix*

dactylifera L.) seeds growing in Egypt. In: Proceedings of 4th Conference on Research and Development of Pharmaceutical Industries (Current Challenges). Med. Aromat. Plant Sci. Biotechnol., 3, pp. 1–5.

Baliga, M.S., Baliga, B.R.V., Kandathil, S.M., Bhat, H.P., Vayalil, P. K., 2011. A review of the chemistry and pharmacology of the date fruits (*Phoenix dactylifera* L.). Food Res. Int. 44 (7), 1812–1822.

Barbary, O.M., El-Sohaimy, S.A., El-Saadani, M.A., Zeitoun, A.M. A., 2010. Antioxidant, antimicrobial and anti-HCV activities of lignan extracted from flaxseed. Res. J. Agric. Biol. Sci. 6, 247–256.

Baydar, N.G., Özkan, G., Sağdiç, O., 2004. Total phenolic contents and antibacterial activities of grape (*Vitis vinifera* L.) extracts. Food Control 15 (5), 335–339.

Benmeddour, Z., Mehinagic, E., Le Meurlay, D., Louaileche, H., 2013. Phenolic composition and antioxidant capacities of ten algerian date (*Phoenix dactylifera* L.) cultivars: a comparative study. J. Funct. Foods 5 (1), 346–354.

Benzie, I.F., Strain, J.J., 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. 299, 15–27.

Besbes, S., Drira, L., Blecker, C., Deroanne, C., Attia, H., 2009. Adding value to hard date (*Phoenix dactylifera* L.): compositional, functional and sensory characteristics of date jam. Food Chem. 112 (2), 406–411.

Biglari, F., AlKarkhi, A.F., Easa, A.M., 2008. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem. 107 (4), 1636–1641.

Blois, M.S., 1958. Antioxidant determinations by the use of a stable free radical. Nature 181, 1199–1200.

Bouhlali, E.D.T., Alem, C., Filali Zegzouti, Y., 2015a. Antioxidant and anti-hemolytic activities of phenolic constituents of six moroccan date fruit (*Phoenix dactylifera* L.) syrups. Biotechnol. Indian J. 12 (1), 45–52.

Bouhlali, E.D.T., Ramchoun, M., Alem, C., Ghafoor, K., Ennassir, J., Zegzouti, Y.F., 2015b. Functional composition and antioxidant activities of eight Moroccan date fruit varieties (*Phoenix dactylifera* L.). J. Saudi Soc. Agric. Sci. (in press)

Bouhlali, E.D.T., Alem, C., Ennassir, J., Benlyas, M., Nait-Mbark, A., Filali-Zegzouti, Y., 2015c. Phytochemical compositions and antioxidant capacity of three date (*Phoenix dactylifera* L.) seeds varieties grown in the South East Morocco. J. Saudi Soc. Agric. Sci. (in press)

Chaudhuri, S., Banerjee, A., Basu, K., Sengupta, B., Sengupta, P.K., 2007. Interaction of flavonoids with red blood cell membrane lipids and proteins: antioxidant and antihemolytic effects. Int. J. Biol. Macromol. 41 (1), 42–48.

de Freitas, M.V., Rita de Cássia, M.N., da Costa Huss, J.C., de Souza, T.M.T., Costa, J.O., Firmino, C.B., Penha-Silva, N., 2008. Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. Toxicol. In Vitro. 22 (1), 219–224.

Drummond, A.J., Waigh, R.D., 2000. The development of microbiological methods for phytochemical screening. Recent Res. Dev. Phytochem., 143–152

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M., Mérillon, J.M., 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J. Agric. Food Chem. 57 (5), 1768–1774.

Fu, L., Xu, B.T., Xu, X.R., Gan, R.Y., Zhang, Y., Xia, E.Q., Li, H.B., 2011. Antioxidant capacities and total phenolic contents of 62 fruits. Food Chem. 129 (2), 345–350.

Hasnaoui, A., Elhoumaizi, M.A., Borchani, C., Attia, H., Besbes, S., 2012. Physico-chemical characterization and associated antioxidant capacity of fiber concentrates from Moroccan date flesh. Indian J. Sci. Technol. 5 (7), 2954–2960.

Heimler, D., Vignolini, P., Dini, M.G., Vincieri, F.F., Romani, A., 2006. Antiradical activity and polyphenol composition of local Brassicaceae edible varieties. Food Chem. 99 (3), 464–469.

Kim, D.O., Jeong, S.W., Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chem. 81 (3), 321–326.

Kumar, S., Pandey, A.K., 2013. Chemistry and biological activities of flavonoids: an overview. Sci. World J. 2013, 1–16.

Lemine, M., Mint, F., Mohamed Ahmed, M.V.O., Ben Mohamed Maoulainine, L., Bouna, Z.E.A.O., Samb, A., Salem, A.O.M., 2014. Antioxidant activity of various Mauritanian date palm (*Phoenix dactylifera* L.) fruits at two edible ripening stages. Food Sci. Nutr. 2 (6), 700–705.

Müller, L., Gnoyke, S., Popken, A.M., Böhm, V., 2010. Antioxidant capacity and related parameters of different fruit formulations. LWT Food Sci. Technol. 43 (6), 992–999.

Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F., Eslami, B., Dehpour, A.A., 2011. Antioxidant and antihemolytic activities of *Ferula foetida* regel (Umbelliferae). Eur. Rev. Med. Pharmacol. Sci. 15 (2), 157.

Naz, S., Siddiqi, R., Ahmad, S., Rasool, S.A., Sayeed, S.A., 2007. Antibacterial activity directed isolation of compounds from *Punica granatum*. J. Food Sci. 72 (9), 341–345.

National Committee for Clinical Laboratory Standards, 2002. Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement, MI00-S12 (M2). NCCLS Wayne, PA.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 26 (9), 1231–1237.

Schofield, P., Mbugua, D.M., Pell, A.N., 2001. Analysis of condensed tannins: a review. Anim. Feed Sci. Technol. 91 (1), 21–40.

Sellam, K., Ramchoun, M., Alem, C., El-Rhaffari, L., 2013. Biological investigations of antioxidant-antimicrobial properties and chemical composition of essential oil from *Lavandula multifida*. Oxid. Antioxid. Med. Sci. 2 (3), 211–216.

Tahraoui, A., El-Hilaly, J., Israili, Z.H., Lyoussi, B., 2007. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia Province). J. Ethnopharmacol. 110 (1), 105–117.

Zhou, K., Yu, L., 2004. Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT Food Sci. Technol. 37 (7), 717–721.