



## Contribution to the study of therapeutic virtues of the local date extracts from the oasis of Ouargla (Northern East Algerian Sahara)

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### Abstract

The object of this study is the phytochemical characterization and evaluation of the antioxidant, anti-hemolytic and antibacterial activity of extracts from three cultivars of the most abundant dates in Ouargla oasis: Ghars, Deglet-Nour, and Degla-Beida harvesting at "Tmar" stage (The last stage of date ripening). The Antioxidant activity was determined by the phosphomolybdic acid method and the reducing power test. The antihemolytic activity was evaluated in vitro by red blood cells' capacity to resist the hydrogen peroxide attack. The antibacterial activity was tested in vitro by the diffusion disc method against six Gram-positive and Gram-negative pathogenic bacteria strains. The two realized antioxidant activity tests showed a strong antioxidant activity of the ethyl acetate extracts of the Ghars cultivar with  $38,75 \pm 18,03$  mg ascorbic acid equivalents/100 g of dates. The study of the anti-hemolytic activity of dates revealed that the three studied cultivars of dates have an anti-hemolytic effect. Besides, the sensitivity test of the bacterial strains showed the activity of some extracts of the three date cultivars vis-a-vis the four strains: *Enterococcus faecalis*, *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus*. The maximum inhibition was obtained with ethyl acetate extract from dates of the cultivar Deglet-Nour ( $11,5 \pm 0,50$  cm diameter of inhibition) against *Bacillus cereus*. The results of this study show that the local dates produced in Ouargla oasis constitute a natural source of substances with biological activities. It is, therefore, necessary to sustainably preserve the traditional phoenicultural heritage of this oasis, which is in deterioration these days.

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## Introduction

*Phoenix dactylifera* L., commonly named as the date palm tree. It is a species from the Saharan region. In addition to its ecological and social role, its fruits are considered a basic food for the people in these regions. In 2013, the global production of dates was estimated at 7,627,624,40 tonnes (FAOSTAT, 2015). The Algerian production in 2015 was estimated at 6,006,960 quintals, all combined varieties, including 1,296,344 quintals in the oasis of Ouargla (DSA, 2015) (Fig. 1).

The date, the fruit of the palm tree is constituted of pericarp, endocarp, and seed. During its development the date pass by five stages of ripening named: Hababouk, Kimri, Khalal, Routab and Tmar. Through this development, there is a Change in its physicochemical and biochemical characteristics (Munier, 1973). The physico-chemical and biochemical characteristics and the nutritional value of the dates were reported in several investigations by Sawaya *et al.* (1983), Sayah and Ould El Hadj (2010) and Ismail and Altuwairki (2016).

This fruit is rich in sugars, especially glucose and fructose. It can also be a good source of fibers, vitamins, and minerals. But, it contains low content of proteins and fats. Mansouri *et al.* (2005), Boudries *et al.* (2007) and Telli (2009) study the presence of polyphenols, flavonoids, and carotenoids in date which the content is modified during ripening stages of the date.

Recently, several studies have been carried out on the antioxidant activity of dates. The researchers showed the antioxidant activity of dates was attributed mainly to polyphenols. However, the researches on the anti-hemolytic and antimicrobial activity of dates remain modest and particularly made on Tunisian dates (Masmoudi-Allouche *et al.*, 2016), Moroccan dates (Bouhlali *et al.*, 2016), and Saudi Arabian dates (Ismail and Altuwairki, 2016).

The oasis of Ouargla offers an important varietal diversity of dates. There are three main date cultivars

in Ouargla: Deglet-Nour, Ghars, and Degla-Beida. The objective of this study is to search for secondary metabolites of dates of the oasis of Ouargla and to evaluate their antioxidant and anti-hemolytic activities. The objective is also consisting to test in vitro the antibacterial activity of some date extracts against bacterial strains with GRAM positive and GRAM negative.

## Material and methods

Three date cultivars, Degla-Beida (DB), Deglet-Nour (DN), and Ghars (G) were chosen in this study.

They were harvested from the farm of Ouargla University at Tmar stage. The dates were harvested in November 2018. The samples were placed in glass jars and stored at 4 °C until analysis.

### Bacterial strains

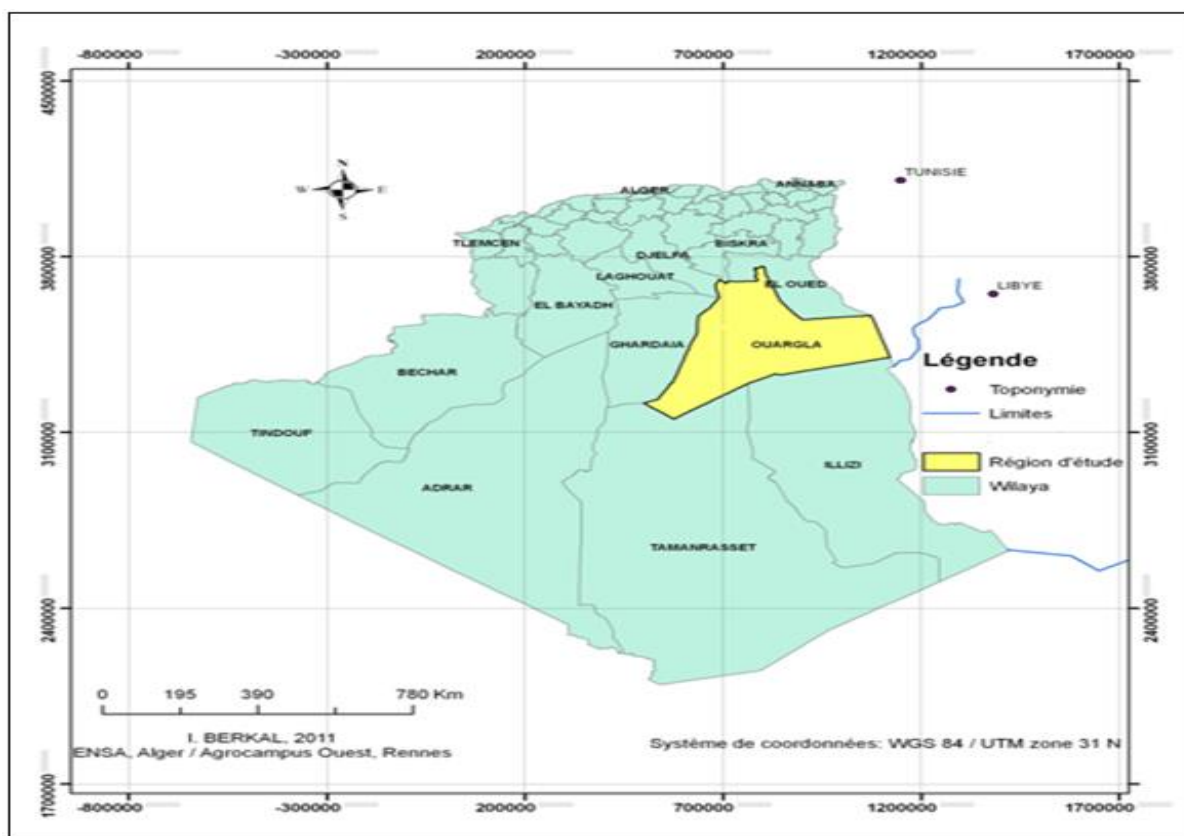
Six bacterial strains were tested, three are GRAM negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 et *Proteus mirabilis* ATCC 35659) and the three others are GRAM positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 et *Bacillus cereus* ATCC 10876). The strains were obtained from the Microbiology laboratory at Mohamed Boudiaf hospital in Ouargla.

### Preparation of date extracts

Date extracts are obtained by maceration of 100 g of the pulp of dates in 300 ml of a mixture of methanol/water (80/20) (V/V) for 24 hours. After filtration, the solvent was evaporated under reduced pressure at 40 °C using a rotary evaporator (Mansouri *et al.*, 2005). The crude extracts were dissolved in distilled water, then successively exhausted (V/V × 3) with three increasing polarity solvents, hexane (H), ethyl acetate (A) and n-butanol (n-b). After evaporation of the solvents, the dried extracts were stored at 4 °C until use.

### Phytochemical tests

Phytochemical tests were realized using classics characterization reagents (Table 1).



**Fig. 1.** The geographical location of Ouargla oasis (Idder *et al.*, 2014).

#### *Total antioxidant activity*

The total antioxidant activity of date extracts was evaluated by the phosphomolybdic acid method described by Prieto *et al.* (1999). Four (4) ml of phosphomolybdic reagent composed of a mixture of sulphuric acid (0,6 M), sodium phosphate (0,028 M) and ammonium molybdate (0,004 M) was added to 400 µl of the extract. After incubation in a water bath at 90 °C for 60 minutes, the absorbance was measured at 695 nm. Ascorbic acid was used as a standard and the results were expressed as milligrams of ascorbic acid equivalent per 100 grams of dates (mg AAE/100g).

#### *Reducing power test (FRAP: Ferric Reducing Antioxidant Power)*

The iron reduction of the date extracts was determined according to the method of Oyaizu (1986). One (1) ml of the extract at different concentrations was mixed with 2,5 ml of phosphate buffer (0,2 M, pH 6,6) and 2,5 ml of potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 minutes. Then, 2,5 ml of trichloroacetic acid

(10%) was added. The mixture was centrifuged at 650 rpm for 10 minutes. 2,5 ml of the supernatant was mixed with 2,5 ml of deionized water and 0,5 ml of iron chloride (0,1%). After 10 minutes of incubation, the absorbance was measured at 700 nm (Nabavi *et al.*, 2012). The results were expressed as milligrams of ascorbic acid equivalent per 100 grams of dates (mg AAE/100g)

#### *In-vitro antihemolytic activity*

The inhibition of erythrocytes hemolysis by the date extracts was evaluated by the method described by Ebrahimzadeh. To study the oxidative effect induced by H<sub>2</sub>O<sub>2</sub>, 2 ml of erythrocytes suspension (4%) was mixed with 0,5 ml of date extracts. The volume of the mixture was completed to 5 ml with phosphate-buffered saline. After incubation for 5 minutes, 0,50 ml of H<sub>2</sub>O<sub>2</sub> was added. The mixture was incubated at 25 °C for 240 minutes. The tubes were centrifuged at 2500 rpm for 10 minutes, and the absorbance was measured at 540 nm by spectrophotometer (Nabavi *et al.*, 2012). Ascorbic acid was used as a standard. The results were expressed as a percentage of hemolysis.

### Antibacterial activity

The antibacterial activity of date extracts was evaluated by the diffusion disc method. Wattman paper discs with 6 mm in diameter were impregnated with extracts and then deposited on the surface of inoculated plates using sterile forceps. DMSO impregnated discs (negative control) were also deposited on the surface of the plates. The plates were incubated at 37 °C for 24 h. The inhibition zones diameters were measured using a Vernier caliper.

### Statistical analysis

Analytical determinations of total antioxidant activity and antimicrobial activity were performed in triplicate and mean values are recorded. The values of

the different parameters were expressed as mean  $\pm$  standard deviation.

## Results and discussion

### Phytochemical tests

The results of the phytochemical tests carried out on the date extracts are reported in Table 2. The phytochemical tests showed the presence of flavonoids, tannins, terpenoids, cardiac glycosides, alkaloids, cardiac glycosides, coumarins and the absence of anthocyanins, saponins, Anthraquinones and volatile in the three cultivars of dates. Phytochemicals present in plants are generally responsible for their pharmacological and biological activities (Bruneton, 1999).

**Table 1.** Summary of phytochemicals and chemical identification reagents.

Phytochemicals	Chemical reagents	Reference
Flavonoids	Aluminium chloride (1%)	Khan <i>et al.</i> (2011)
Tannins	Iron chloride (1%)	Hussain <i>et al.</i> (2011)
Alkaloids	Mayer's reagent	Chitravadiya <i>et al.</i> (2009)
Anthocyanins	Sulphuric acid (10%)	Mangambu <i>et al.</i> (2014)
Coumarins	Ammonia (10%)	Bruneton (1999)
Terpenoids	Chloroform Concentrated sulphuric acid	Khan <i>et al.</i> (2011)
Saponins	Frothing test	Hussain <i>et al.</i> (2011)
Steroids	Acetic anhydride Chloroform	Khan <i>et al.</i> (2011)
Anthraquinones	Hydrochloric acid Benzene, Ammonia	Khan <i>et al.</i> (2011)
Cardiac glycosides	Chloroform Concentrated sulphuric acid	Harbone (1973)
Volatile oils	Sodium hydroxide Hydrochloric acid (10%)	Mojab <i>et al.</i> (2003)

The presence of phytochemicals in the studied dates shows that they may have a therapeutic interest.

### Total antioxidant activity

The highest total antioxidant activity is recorded by the ethyl acetate extract of Ghars cultivar with  $38,75 \pm 1,03$  mg ascorbic acid equivalent/100g of dates, then by the extract of ethyl acetate from Degla Beida cultivar with  $12,20 \pm 2,30$  mg ascorbic acid equivalent/100 g of dates (Fig. 2). The lowest antioxidant activity is presented by n-butanol extract

of Ghars cultivar with  $0,80 \pm 0,00$  mg ascorbic acid equivalent/100g of dates. In view of the results (88,59-180,08 mg gallic acid equivalent/100g dry matter) of Louaileche *et al.* (2015), the results of this study remain weak. According to Kechaou *et al.* (2013), the total antioxidant activity of six varieties of dates from Tunisia is between  $17,49 \pm 3,19$  and  $109,67 \pm 2,04$  mg ascorbic acid equivalent/g of fresh weight. Ali Haimoud *et al.* (2016) estimate values ranging from  $42,83 \pm 0,77$  to  $90,25 \pm 0,79$   $\mu\text{mol}$  of ascorbic acid/g of extract for Algerian dates. The antioxidant

activity of the studied dates may be provided by the phenolic compounds identified in the study of Sayah (2018). The obtained results of antioxidant activity

showed that the studied date cultivars constitute a source of molecules with antioxidant activity and can protect the body against oxidative stress.

**Table 2.** Phytochemical analysis of date fruit extracts.

Phytochemicals	Cultivars		
	Ghars	Deglet-Nour	Degla-Beida
Flavonoids	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Anthocyanins	-	-	-
Coumarins	+	+	+
Terpenoids	+	+	+
Saponins	-	-	-
Steroids	+	+	+
Anthraquinones	-	-	-
Cardiac glycosides	+	+	+
Volatile oils	-	-	-

+: presence; -: absence

#### Reducing power test (FRAP)

According to Fig. 3, the studied dates have a reducing power that varies from 0,17 to 11,25 mg ascorbic acid equivalent/100g of dates. Indeed, the highest antioxidant activity is that of the ethyl acetate extract of the cultivar Ghars. The reducing properties are associated with the presence of reductones (Sasikumar *et al.*, 2012; Kchaou *et al.*, 2013). Reducing power of the fruit extract seems to be due to the presence of polyphenols which may act in a

similar fashion as reductones by donating the electrons and reacting with free radicals to convert them into more stable products and terminal free radical chain reaction (Sasikumar *et al.*, 2012). Reducing power of the studied dates may be provided by the phenolic compounds. The antioxidant activity reported by Ali Haimoud *et al.* (2016) varies between 28,02 et 33,95  $\mu\text{mol Fe(II)}/100\text{g}$  of dry weight for the cultivars Ghars, Deglet-Nour and Degla-Beida from Algeria.

**Table 3.** Antibacterial activity (zone of inhibition in mm) of date extracts.

Cultivars	Extracts	Strains					
		<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Proteus mirabilis</i>	<i>Enterococcus faecalis</i>
Ghars	Hexane	7,50±0,50	8,50± 0,50	-	-	-	7,00±0,0
	Ethyl acetate	-	-	-	-	-	-
	n-butanol	-	-	-	-	-	7,50±0,50
Deglet-Nour	Hexane	7,00±0,00	9,75± 0,20	-	-	-	10,25±0,70
	Ethyl acetate	7,50±0,50	11,50± 0,50	6,75±0,20	-	-	7,50±0,50
	n-butanol	-	9,50± 0,50	7,50±0,50	-	-	9,50±0,50
Degla-Beida	Hexane	-	7,50± 0,50	-	-	-	7,50±0,50
	Ethyl acetate	6,75±0,20	9,00± 1,00	-	-	-	8,50±0,50
	n-butanol	-	8,00± 0,00	-	-	-	8,50±0,50

- : means resistant strains.

*In-vitro* Antihemolytic activity of date extracts Fig. 4 shows the protective effect of the extracts from the three studied dates on erythrocytes treated with H<sub>2</sub>O<sub>2</sub>. The studied dates stabilize differently the erythrocytes membrane. Indeed, the highest antihemolytic activity is recorded by the cultivar

Ghars with an inhibition percentage of 96,18%. Inhibition percentages of 65,64 and 67,93% are observed for the ethyl acetate extracts of the cultivars Deglet-Nour and Degla-Beida, also indicating the inhibitory effect on the hemolysis of these tow date cultivars.

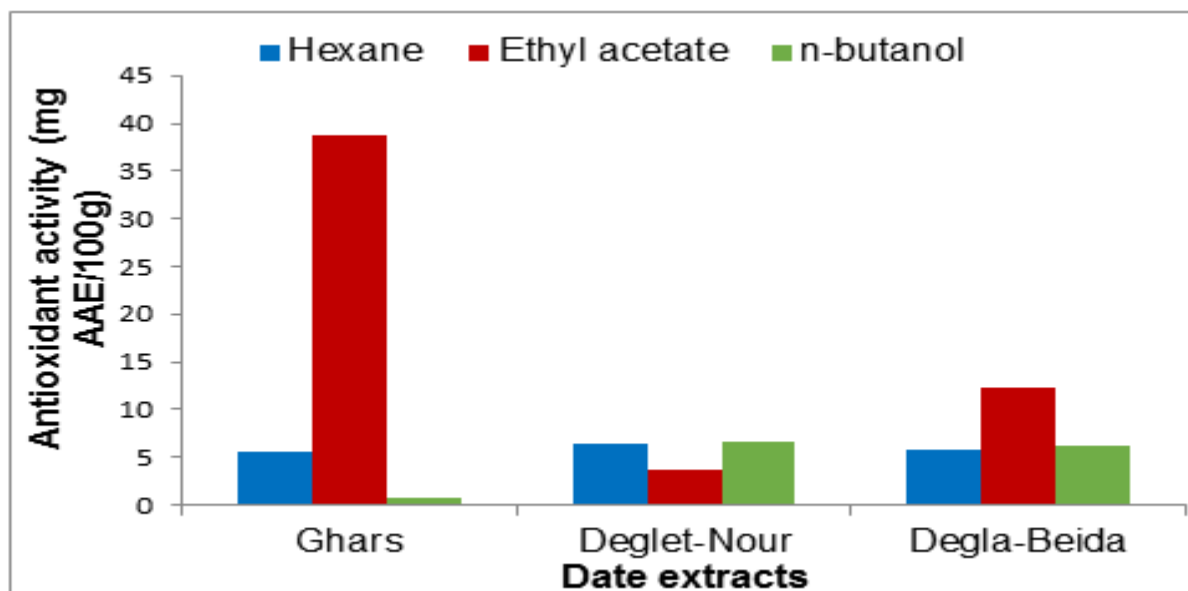


Fig. 2. Total antioxidant activity of date extracts.

The antihemolytic activity of the studied dates may be due to the presence of polyphenols, particularly the flavonoids and the tannins. The involvement of flavonoids in the stabilization of red blood cell membranes is confirmed in the study of Chaudhuri *et al.* (2007). Bouhlali *et al.* (2016) reported that the Moroccan dates presented a considerable antihemolytic activity (33,84%). They found a strong correlation between the antihemolytic activity of dates and their content of polyphenols ( $r = 0,69$ ) and flavonoids ( $r = 0,88$ ).

#### *Antibacterial activity of date extracts*

The antibacterial activity of the extracts from the three cultivars of dates Ghars, Deglet-Nour and Degla-Beida are represented in Table 3. The obtained results demonstrated that hexane, ethyl acetate and n-butanol extracts of the three cultivars of dates showed different inhibitory activities against bacterial growth depending on the bacterial strains. The tested date extracts exhibited antibacterial activity against Gram-positive bacteria more than Gram-negative

bacteria. However, *B. cereus* appears to be the most sensitive strain to date extracts compared to the other Gram-positive bacteria (Table 3). In addition, the ethyl acetate extract of the cultivar Deglet-Nour exhibited the highest antibacterial activity with the diameter of inhibition zone of  $11,50 \pm 0,50$  mm against *B. cereus*. These results can be explained by the difference in the chemical composition of the date extracts. The two pathogenic strains *Pseudomonas aeruginosa* and *Proteus mirabilis* are the most resistant germs to all date extracts. The results of the antibacterial activity of the ethyl acetate extracts of the present study are different from those obtained by Masmoudi-Allouche *et al.* (2016). Their results revealed that ethyl acetate extracts from Ruchdi, Deglet-Nour and Ftimi from Tunisia didn't show any effect on inhibiting the growth of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Enterococcus faecalis*. The antibacterial activity of the date extracts may be due to terpenoids and polyphenols (tannins, flavonoids) which play an important role in the protein

precipitation and the inhibition of microorganism enzymes (Naz *et al.*, 2007). The involvement of phenolic compounds from Algerian date extracts in their antibacterial activity was confirmed by Daas

Amiour *et al.* (2014). Therefore, the date extracts from the oasis of Ouargla, can be used as a source of antibacterial compounds and contribute in the future to the preparation of therapeutic interest products.

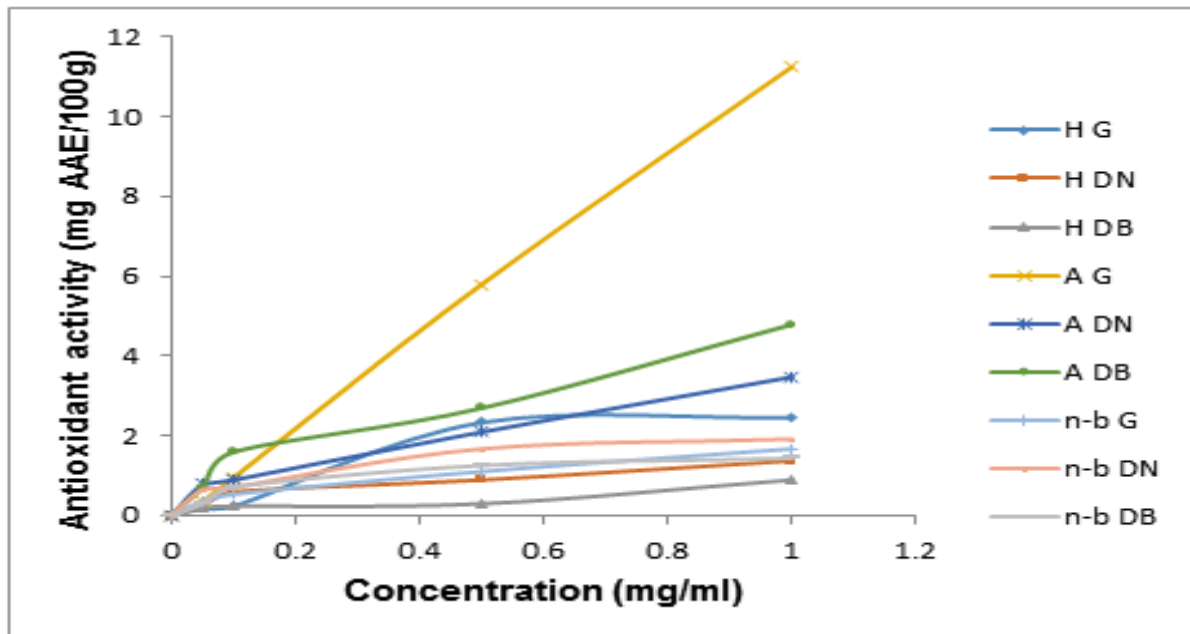


Fig. 3. Antioxidant activity evaluated by FRAP of date extracts.

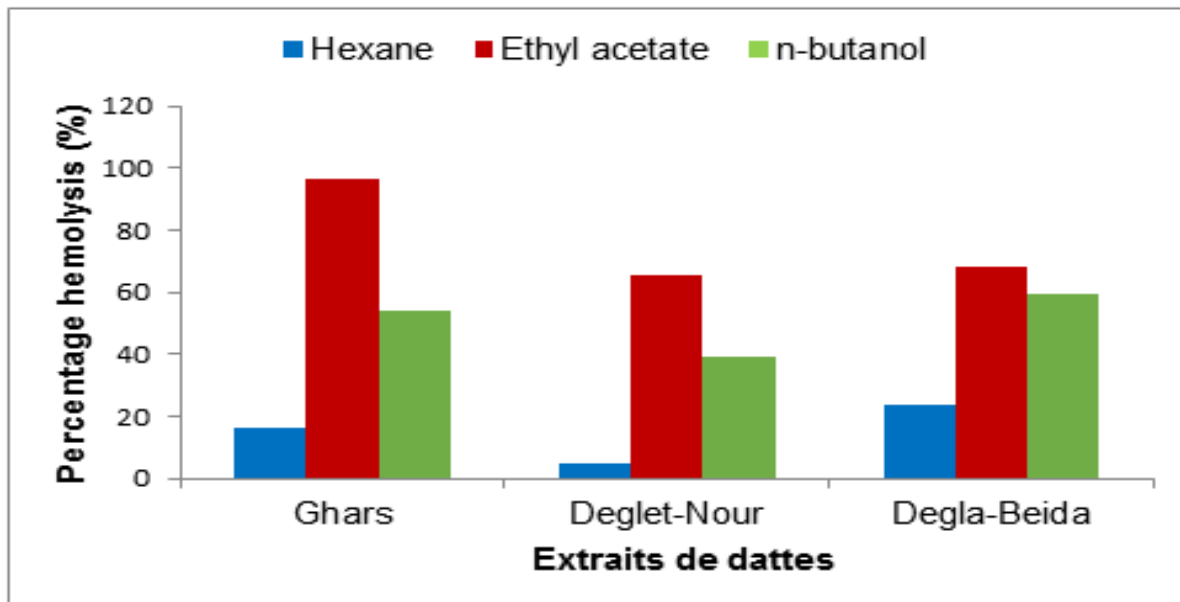


Fig. 4. Antihemolytic activity of date extracts.

### Conclusion

This study showed that extracts from the three cultivars of date Ghars, Deglet-Nour and Degla-Beida contain antioxidant phytochemicals. These extracts were effective in stabilizing the red blood cell membrane and can be used as a source of

antibacterial compounds. The compounds present in dates have antioxidant, anti-hemolytic and antibacterial activity. Therefore, the introduction of dates into the human diet can prevent the body from oxidative stress and against the development of certain diseases.

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