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Research Article

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Date palm "deglet nour" (*Phoenix dactylifera*) fruit extracts: Functional components, antioxidant, anti-inflammatory activities, and gastroprotective effect

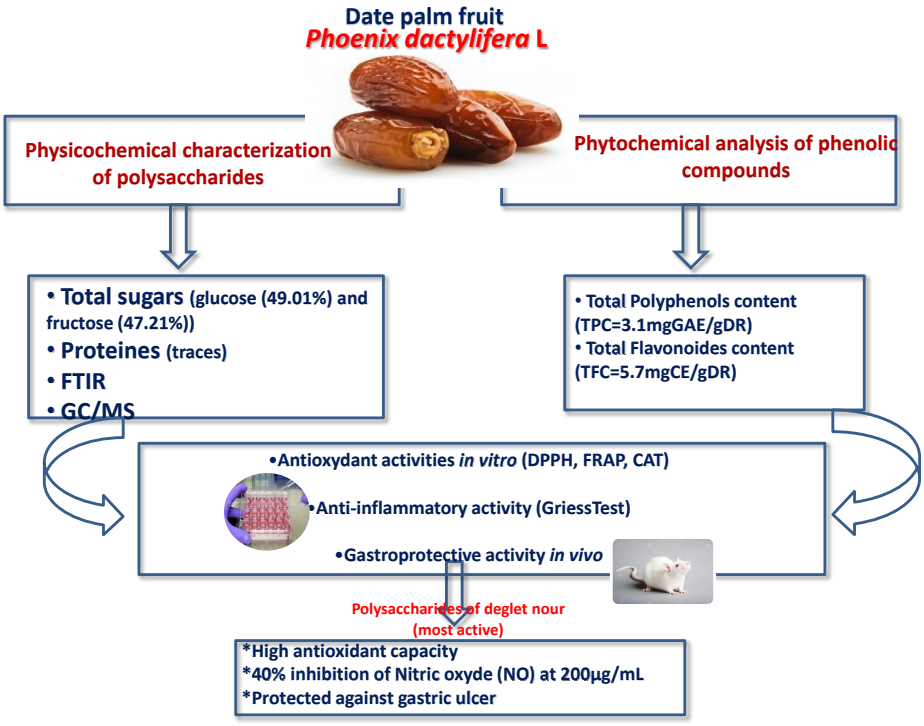
Rim BEN MANSOUR[1]*, Raja SERAIRI-BEJI[1,2], Riadh KSOURI[1] and Wided MEGDICHE-KSOURI[1].

[1]Laboratory of Aromatic and Medicinal Plants, Centre of Biotechnology of Borj Cedria, BP 901, Hammam-Lif 2050, Tunisia.
[2]High School of Health Sciences and Technics, BP 176, Bab Saadoun 1007, La Rabta, Tunisia.
Corresponding author*: rim_ben_mansour@hotmail.fr

Highlights

- Characterization of phenolic-rich extract (PRE) and Water-soluble crude polysaccharide extract (WCP) from *Phoenix dactylifera* fruits were investigated.
- Good antioxidant and anti-inflammatory properties were reported.
- Extracts showed a protective effect against an ethanol-induced gastric ulcers in rats
- Extracts counteracted EtOH-induced gastric lipoperoxidation and prevented the depletion of antioxidant enzyme activity.
- *Phoenix dactylifera* L. is an interesting food species with bioactive natural substances.

Graphical Abstract



Abstract
Deglet nour, is the most consumed variety of date palm fruit (*Phoenix dactylifera* L.) in the Middle East and North African countries and promotes the socio-economic development of growing areas. The fruit contains a high percentage of dietary fibers, salts, vitamins, and minerals, and the dates are widely used in traditional medicine to treat various ailments. In order to better discover this natural product, the purpose of this study was to evaluate the phytochemical properties of phenolic-rich extract (PRE) and Water-soluble crude polysaccharide extract (WCP) by determining the antioxidant and anti-inflammatory activities and the gastroprotective effect of these extracts against ethanol-induced oxidative stress in rats. The PRE mainly comprises of phenolic acids (gallic, ascorbic, ferulic, p-coumaric, caffeic, ellagic, chlorogenic, and syringic acids). Chemical analysis of WCP revealed that the extract was composed of 75% carbohydrates. The extracted polysaccharide comprises glucose (49.01 % mol) and fructose (47.21%). Both extracts exhibited high total antioxidant capacity (267 mg GAE/g DR). Polysaccharides are distinguished by a better anti-radical scavenging activity (IC50%=0.5mg/mL) and iron-reducing power (EC50%=0.98 mg/mL). The anti-inflammatory activity on macrophages RAW 267.4 showed that Deglet nour extracts decreased the nitric oxide (NO) production at an average of 40% at 200µg/mL. Concerning gastroprotective activity, we found that WCP and PRE, to a lesser degree, protected against alcohol-induced volume gastric juice and acidity decrease. More importantly, we showed that Deglet nour extracts and particularly WCP counteracted EtOH-induced gastric lipoperoxidation and prevented the depletion of antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). All these results can justify the utility of *Phoenix dactylifera* L. as interesting food species with bioactive natural substances that may be used in several fields, such as nutraceuticals and the agro-food industry.
Keywords: *Phoenix dactylifera*; functional compound; antioxidant activity; anti-inflammatory activity; gastroprotective effect.

Abbreviations
DPPH: 2,2-Diphenyl-1-picryl hydrazyl; **HPLC:** High Performance Liquid Chromatography**;** **NO:** Nitric Oxyde**;** **GC-MS:** Gas Chromatography-Mass Spectrometry**;** **FTIR:** Fourier Transform-Infrared Spectroscopy; **PRE**: phenolic rich extract; **WCP**: Water-soluble crude polysaccharide extract; **SOD**: superoxide dismutase; **CAT**: catalase; **GPx**: glutathione peroxidase.

1. Introduction
The date palm (*Phoenix dactylifera* L.) belonging to the Arecaceae (or Palmae) family has been an important cultivated crop for its edible fruit in the desert oasis of the Arab world for centuries. Various dates are found worldwide mainly, Alligh, Ajwa, Deglet nour, Hilali, Khalas, Khodry, Ruthana, Sukkary, Sefri, Segae, Munifi, Kenta, Eguwa, Garen Gaze, Limsi and Rochdi (Mrabet et al., 2012; Rahmeni et al., 2014). Tunisia is considered one of the date-producing countries and the first exporter (in value) of dates (Besbes et al., 2009). Tunisian dates capture 23% of the value of world trade and have more than 50% of the world's palm cultivars Deglet nour. According to the United Nations Food and Agriculture Organization (FAO), Tunisia is Europe's leading supplier of Deglet nour, providing around one-third of the total European market.
Date fruits are an ideal high-energy food (≈ 314 kcal/100 g of dates) rich in digestible sugars (45 to 80 %) mostly, glucose, sucrose and fructose sugar, dietary fiber (6.4 to 11.5 %), protein (2.3 to 5.6 %), vitamins (A, A1, B, B1, B2, B3, B5, B6, and C), essential amino acids, mineral

elements including potassium, boron, cobalt, copper, magnesium, manganese, selenium, and zinc and phenolic compounds (about 4 g /100 g) (Mrabet et al., 2012; Al-Shwyeh, 2019). Due to their high energy value and sugar content, dates offer a wide range of by-products. Today, date paste and flour are used in the food industry as a filling for pastries, biscuits and children's food, and yogurt (Benamara et al., 2004) and as a healthier substitute for sugar in cereals, puddings, baked goods, energy bars, ice cream and confections (Al-Farsi et al., 2005; Espirad, 2002).

Dates exhibit many beneficial health effects and are used to facilitate intestinal transit thanks to their high fiber content and play a preventive role against colorectal cancers, appendicitis, diverticulosis, varicose veins, and hemorrhoids. The date has also been shown to have a cholesterol-lowering effect (Al-Farsi et al., 2005). Energetic and rich in minerals, the fruit helps fight against anemia and demineralization. In decoction or infusion, dates treat colds. In gargle, they treat sore throats (Benchelah and Maka, 2006). Indeed, date concentrate could shield human bodies from oxidative injury and even weaken the impact of diarrheal movement and turned out to be compelling as neuroprotective against two-sided regular carotid artery impediment (Kumar and Yaashikaa, 2019). Among their various properties, dates are locally reputed to be useful against peptic ulcers and were widely consumed by Muslims during the fasting month of Ramadan, possibly to protect the gastric mucosa from the damaging effect of gastric acid (Al-Qarawi et al., 2005). Date pulp extract and palm sap stimulate gastrointestinal transit activity in rats and confirm their use in traditional Tunisian medicine for the treatment of constipation (Souli et al. 2018).

Several studies have revealed the effectiveness of polysaccharides and phenolics in crude extracts from plants as anti-inflammatory and gastro-protective agents (Wang et al., 2018). However, the date fruit is still poorly studied for these compounds and their antioxidant, anti-inflammatory, and anti-ulcer activities. For high quality and nutritional values and health-promoting potentialities, date fruit may be considered an emerging and potential candidate for developping health-promoting foods.

Considering these beneficial properties, we undertake this study to assess the *in vitro* antioxidant and anti-inflammatory activities of phenolic-rich extract and crude soluble polysaccharides from deglet nour variety and their influence on the incidence and severity of ethanol-induced gastric ulceration.

2. Materials and methods

2.1. Plant materials

The material used in this study is the fruit of *Phoenix dactylifera* L. of the variety Deglet nour, widely grown in southern Tunisia's palm groves. The choice of this variety was justified by its commercial value, taste quality, appearance, and creaminess. The fruits of the dates were pitted, and the fresh edible part was cut and dried at room temperature. The sample was milled using a grinder to obtain a paste and then stored at 4°C.

2.2. Extraction procedures

The date fruits were separated from the pits and dried at room temperature until mass stabilization and uniformly blended into a homogeneous paste using a stainless-steel blender.

For the crude soluble polysaccharides (WCP) preparation, dried fruit was defatted with petroleum ether for 48 h to remove lipids. The pretreated date was extracted three times with deionized hot water (m/10 v) under stirring for 5 hours at 80°C. The water extraction solution was separated from insoluble residue by filtration with Wattman paper and centrifugation (9000×g for 15 min) (Liu et al., 2014).

The supernatant was precipitated by adding of dehydrated alcohol to a final concentration of 80% (v/v) at 4°C for 24h. The resulting precipitate from the ethanol dispersion was collected and lyophilized to obtain the water crude soluble polysaccharides (WCP). The extraction yield (Y) percentage was calculated according to the following formula:

Y%= (Weight of lyophilized WCP (g) / Weight of deglet nour pulp powder (g)) *100.

The phenolic rich extract (PRE) was prepared by adding 30 mL of methanol (80%) to 3g of mashed date. After ultrasonic maceration for 30 min, the extract was filtered, evaporated, and finally lyophilized. The dry residue is weighed and stored at 4°C for subsequent analysis.

2.3. Assessment of the total phenolic and flavonoid contents

The total phenolic content (TPC) of PRE was assessed using a colorimetric assay based on the Folin-Ciocalteu reagent. A volume of 125 µL of PRE was added to 500 µL of H_2O and 125 µL of the Folin-Ciocalteu reagent (0.2 mol/L). After shaking, 1.250 µL of Na_2CO_3 (7%) was added. The final volume (3 mL) was then adjusted with H_2O. After incubation for 90 min at room temperature, the optical density (OD) at 760 nm was determined. Results were expressed as mg of gallic acid equivalent per g of dry extract (GAE/g DE) using a calibration curve (Ben Mansour et al., 2020). For total flavonoid content (TFC), 250 µL of PRE was mixed with 75 µL $NaNO_2$ (5%; w/v). Then, 150 µL of $AlCl_3/6H_2O$ (10%; w/v) and 500 µL of NaOH (1 M) were added after 6 min of incubation. After adjusting the volume to 2500 µL with H_2O, the absorbance was determined at 510 nm (Ben Mansour et al., 2020). TFC was expressed as mg (+)-catechin equivalent/g Dry Extract (mg CE g^{-1} DE).

2.4. Determination of total carbohydrate and protein contents

The total carbohydrate content of deglet nour fruit was determined according to the colorimetric assay adapted by Dubois et al. (1956) using the phenol-sulfuric acid method using galactose as standard. Briefly, 200 µL of the extract was homogenized with 200 µL of an aqueous phenol solution (5%). Then, 1 mL of concentrated sulfuric acid is immediately added into the reaction medium and heated in a water bath at 100°C for 5 min. The tubes are cooled in an ice bath and placed for 30 min in the dark. Absorbance was measured at 490 nm using a spectrophotometer. A standard range of glucose is achieved with concentrations of between 5 and 80 $mg.L^{-1}$.
Protein content was determined by the colorimetric method of Bradford (Bradford, 1976). Briefly, a volume of 2 mL of the extract is mixed with 988 µL of diluted Bradford's reagent (1/5 with sterile milli Q water) and incubated for 30 min in the dark at room temperature. The absorbance is read at 595 nm using a spectrophotometer (Perkin Elmer Lambda 40 UV/Vis Spectrophotometer).

2.5. Characterization of polysaccharides date deglet nour by Fourier Transform-Infrared Spectroscopy (FTIR) analysis

The infrared (IR) spectroscopy is a fast physical analysis method whose operating principle is based on the excitation of molecules by IR radiations of wave numbers between 4000 and 400 cm^{-1} using an FTIR spectrophotometer (PerkinElmer BX FTIR system spectrometer, PerkinElmer Company, Waltham, MA, USA). This technique makes it possible to reveal the presence of certain characteristic functional groups. The sample (2 mg) was incorporated into potassium bromide (KBr) powder and then pressed into a 1-mm pellet. The characteristic absorption bands of polysaccharides were recorded between 1200-950 cm^{-1} (Kacurakova et al., 2000).

2.6. Identification and quantification of monosaccharides by gas chromatography-mass spectrometry (GC/ MS)

GC-MS was used to identify and quantify the monosaccharides composition of the polysaccharides extracted from date fruits (*P. dactylifera* L.). The method of Guentas et al. (2001) was adopted. 1 mL of trifluoroacetic acid (2M) is added to 1 mg of the lyophilized polysaccharides. GC-MS (Agilent Technologies, 5975C inert MSD with its Triple-Axis Detector, Germany) was equipped with an electronic impact mass detector and an apolar capillary column (HP-5 ((5% Phenyl) -methylpolysiloxane)) (30m, 0.25mm, 0.25µm). The temperature rise program dedicated to the identification of the glycoside residues is as follows: 150°C for 1 min, followed by a gradient of 1°C/min up to 180°C and then a gradient of 2°C/min up to 250°C and finally a gradient of 15°C/min up to 300°C was maintained for 10 min. The extract is injected in the split mode under the following conditions (250°C., ratio: 20/1, flow rate: 18 mL/min). The temperature of the ionization source of the detector is 250°C and the quadrupole at 230°C. The ionization energy is set at 70eV. To quantify the constituent neutral oses of the polysaccharides, we have based on the total ion chromatography (TIC) profile to plot calibration curves for each trimethylsilyl monosaccharide standard.

2.7. Phenolic Compounds by HPLC

The phenolic compounds were identified using an HPLC system with a reversed-phase C18 analytical column of 4.6 x 100 mm and 3.5µm particle size (Zorbax Eclipse XDB C18). The DAD (Diode Array detector) was set to a scanning range of 200-400 nm. The temperature of the column was maintained at 25°C. The volume of injected extract was 2 µl and, 0.4 ml/min was the mobile phase flow rate. Mobile phase B was milli-Q water constituted of 0.1% formic acid, and mobile phase A was methanol. The optimized chromatographic condition was as follows: 0-5 min: 10% A- 90% B; 5-10 min: 20% A-80% B; 10-30 min: 30% A 70% B; 30-40 min: 50% A- 50% B; 40-45 min: 60% A- 40 % B; 45-50 min 70% A- 30% B; 50-55min: 90% A- 10% B; 55-60 min: 50% A- 50% B and at 60min 10% A- 90% B. Phenolic compounds identification were obtained by comparing their retention time and the UV spectra with those of pure standards**.**

2.8. Evaluation of antioxidant capacities of PRE and WCP *in vitro*

Total antioxidant capacity was assessed according to Koleva et al. (2002). Briefly, 100 µL of PRE or WCP was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixture was allowed to cool after incubation at 95°C for 90 min. The absorbance was measured at 695 nm, and TAC was expressed as mg gallic acid equivalent / g DE (mg GAE g^{-1} DE).

The antiradical capacity of PRE and WCP against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was assessed according to Boulaaba et al., (2019) method. Briefly, 250 µL of methanolic solution of stable radical DPPH (0.2 mM) was added to 1000 µL of increasing concentrations of PRE and WCP. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm. DPPH scavenging ability was expressed as IC_{50} (mg mL^{-1}), inhibiting the concentration of 50% of the synthetic radical. The inhibition percentage (IP %) of DPPH radical was calculated using the following formula:

$$IP\ (\%) = [(A_{control} - A_{sample})/A_{control}] \times 100$$

Ferric-reducing antioxidant power (FRAP) was focused on reducing the trivalent iron produced by the $FeCl_3$ (Ksouri et al., 2009). The intensity of the blue-green color was measured at 700 nm. Values were expressed as EC_{50} (mg/mL): the effective concentration of PRE and WCP corresponding to an OD = 0.5.

2.9. Murine macrophage cells culture and cytotoxicity test

The RPMI 1640 medium (10 % of fetal bovine serum (v/v), 100 U/mL of penicillin, and 100 µg/mL of streptomycin) was used to maintain RAW 264.7 cells (American Type Culture Collection) in the culture at 37°C in a humidified atmosphere of 5% carbon dioxide. The *in vitro* cytotoxicity of PRE and WCP was evaluated using the macrophage cells seeded in 24-well plates at 5×10^4 cells/well and allowed to attach. Then, cells were treated with increasing concentrations (50, 100, 150, and 200µg/mL) of PRE and WCP for 24h. Using crystal violet assay based on the proportionality of the absorbance at 540 nm of stained cells with viable cell numbers (Chiba et al., 1998), cell viability in each well was calculated as % of control.

2.10. NO production

RAW 264.7 cells were seeded in 24- well plates at 5×10^4 cells/well. After 24 h of incubation, cells were pretreated for one hour with 50, 100, 150, and 200µg/mL of PRE or WCP before 24h-stimulation with $1\mu g.mL^{-1}$ of lipopolysaccharide (LPS). Griess reagent (0.75%N (naphthyethylene) diamine, 0.8% sulfanilamide in 0.5N HCl) was used to determine the accumulation of nitrites in culture supernatant. The test was performed by mixing 100 µL of cells' supernatant with the same volume of Griess solution. The absorbance was then determined at 540 nm, and the nitrites content was calculated referring to the $NaNO_2$ standard curve (Aboura et al., 2017).

2.11. *In vivo* antiulcer and gastro-protective assessment

Healthy male Wistar rats weighing (180–220 g) were procured from Tunis Pasteur Institute and kept under standard conditions (20-22°C, 45-50% relative humidity; 12 h light: 12 h dark cycle). Animals were handled according to the guidelines of the Tunisian Society for the Care and Use of Laboratory Animals. The University of Tunisia Ethical Committee approved the study. Gastric ulceration was induced in rats orally with 0.2 mL of an ulcerogenic solution containing 0.3 M of HCl and 60% ethanol (HCl/EtOH). Groups (n=6) of rats were pre-treated 30 minutes before the ulcerogenic procedure in the following manner.

Group I: Control rat received vehicle solution; Group II: Rats received standard drug famotidine (20 mg/Kg); Group III: Rats treated with 50 mg/Kg of WCP; Group IV: Rats treated with 100 mg/Kg of PRE; Group V: Rats treated with 200 mg/Kg of PRE

After 21 days of treatment, animals are sacrificed and assessed for gastric mucosal damage. The stomach was opened along the greater curvature, and was washed under running water and the glandular portion of the stomach was examined. The length in mm of each lesion was measured under a dissecting microscope, and the ulcer index (UI) was measured as follows:

$$\text{Ulcer index (UI)}: \frac{\text{Average number of severity score x Percentage of animals with ulcers}}{\text{number of animals}}$$

The number of severity score for irritation and for ulcers were measured according to Lwoff (1971) following 5 points scale: 0 = neither ulcer nor irritation; 1 = irritation; 2 = 1 or 2 ulcers; 3 = 3 or 4 ulcers; 4 = >4 ulcers.

The other parameters were determined as follows:

The percentage of ulceration (%UP) = (UI *100) /3

The degree of protection (DP) = $PU_{(\text{control group})} - PU_{(\text{treated group with extract or famotidine})}$

The healing percentage (% HP) = $\frac{UI_{(\text{control group})} - UI_{(\text{treated group with extract or famotidine})}}{UI_{(\text{control group})}} \times 100$

Gastric secretions were assessed by pylorus ligation methods described by Shay et al. (1945). Extracts or famotidine were administered orally to 48 h fasted rats. One hour later, rats were lightly anesthetized by ether. The abdomen was opened, and the pylorus was ligated. The abdomen was closed by suturing. The animals were sacrificed 5 h later by an overdose of ether. The stomach was removed, and its content was subjected to measurement of gastric pH and volume (mL).

2.12. Assay of enzymatic antioxidants

The stomach tissues were washed with normal saline and homogenized in a Potter-Elvehjem glass homogenizer in ice-cold 0.15 M KCl to obtain a 20% homogenate. To the stomach homogenate, superoxide dismutase (SOD), Catalase (CAT), and glutathione peroxidase (GPx) activities were assessed respectively according to the method of Beyer and Fridovich (1987), Aebi (1984) and Flohe and Gunzler (1984).

2.13. Malondialdehyde (MDA) measurement

Malondialdehyde (MDA) measurement is consistent with the double heating method (Rtibi et al., 2015). Gastric mucosa was mixed with a solution containing (0.5 N HCl, 120 mM TBA buffered in 26 mM Tris), heated at 80°C for 10 min, and then quickly cooled in an ice bath. The absorbance of the upcoming chromophore was assessed at 532nm. The concentration of MDA levels was calculated using an extinction coefficient for the MDA-TBA complex of 1.56×105 $M^{-1}cm^{-1}$.

2.14. Statistical analysis

Data were subjected to a one-way analysis of variance for means of comparison, and significant differences were calculated according to Duncan's multiple range test. Data are reported as means ± standard error of the means. Differences at $p < 0.05$ were considered statistically significant. SPSS (version 11.0) was used to perform the statistical analysis.

3. Results and discussion

3.1. Evaluation of phenolic composition of deglet nour PRE

As shown in Table 1, total levels of phenolics (TPC) and flavonoids (TFC) in the PRE are respectively 3.1 and 5.7 mg GAE/gFW. The TFC amounts from Tunisian deglet nour corroborate with those found in seven different date varieties (Tazizaout, Akerbouche, Deglet-Nour, Ughterouss, Tantbouchte, Tafiziouine, and Tazerzait) harvested in Ghardaia-Algeria with values ranging from 2.49 to 8.36 mg EAG/100g FW (Mansouri et al., 2005) and with those found by Haimoud (2016) in different other varieties (3.50 and 6.53 mg EAG/100 g DW). On the other hand, as compared with other reports, the flavonoid contents are higher than those found by Haimoud et al. (2016) in the methanolic extracts of six varieties of *Phoenix dactylifera* (1.06 to 4.23 mg CE/100 g DW).

The HPLC chromatogram of PRE is shown in Figure 1. According to the phenolic profile, 15 phenolic compounds were identified by comparing their HPLC retention times and UV–Vis spectral data with those of authentic standards analyzed under identical conditions. We also identified 2 hydroxylated derivatives of benzoic acids (gallic, ellagic acids), 4 were cinnamic acid derivatives (caffeic, *p*-coumaric, ferulic, chlorogenic, protocatechuic acid ethyl ether acids), and 6 were flavonoids (catechin hydrate, epigallocatechin, catechol, epicatechin 3-*O*-gallate, isorhamnetin 3-*O*-Glucoside, chrysin).

The concentration of each compound was presented in Table 1. Epicatechin 3-*O*-gallate was the most prevalent detected compound (1.11µg/g of FW), representing 27% of the identified phenolic compounds (Table 1). The second prevalent compound in Deglet Nour PRE was the

gallic acid (0.74µg/gFW) and ascorbic acid (0.55µg/gFW). Other compounds are present in low amounts.

Table 1. Phenolic compounds identified and quantified by High Performance Liquid Chromatography (HPLC) from phenolic rich extract of deglet nour (PRE=Phenolic Rich Extract.

	TPC (mgGAE/gFW)		TFC (mgCE/gFW)	
PRE	3.1		5.7	
HPLC Identified compounds	**RT**	**CE**	**CC (R^2)**	**Quantification (µg/g FW)**
Ascorbic acid	5.2	y=1.56 x+2.4	1.00	0.55
Gallic acid	7.14	y=22. 28x+1.68	0.998	0.74
Catechol	12.84	y=3.632x+1.8	0.998	0.56
Catechin hydrate	14.3	y=3.632x+1.8	0.99	0.217
Chlorogenic Acid	15.75	y=9.02x-1.55	0.998	0.21
Epicatechin 3-*O*-gallate	16.77	y= 3.768x+1.52	0.998	1.11
Caffeic acid	17.42	y=23.49x+5.57	0.99	0.034
Syringic acid	18.28	y=22.34 x-1.56	0.99	0.03
P-coumaric acid	19.69	y=34.14 x+5.88	0.99	0.006
Ferulic acid	20.61	y=20.50 x-8.72	1.00	0.088
Luteolin 7-*O*-glucoside	20.93	y=7.43 x+13.16	1.00	0.25
Isorhamnetin 3-*O*-glucoside	21.1	y=38.958x-73.264	0.99	0.05
Protocatechuic acid ethyl ether	21.77	y=3.632x+1.8	0.99	0.081
Ellagic acid	22.27	y=8.819x+23.267	0.999	0.091
Chrysin	26.5	y=18.354x-189.52	0.99	0.096

TPC= Total Phenolic Content; TFC= Total Phenolic Content; CC= concentration; RT= Retention Time; CE=Concentration equation; FW= Fresh Weight).

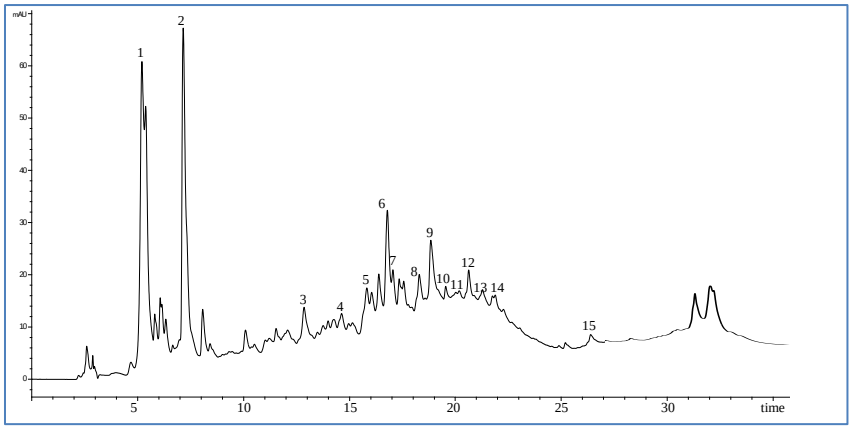


Figure 1. RP-HPLC chromatogram of phenolic-rich extract of deglet nour. Signal was collected at 280nm. Peak numbers corresponded to*:* 1: Ascorbic acid; 2: Gallic acid; 3: Catechol; 4: Catechin hydrate; 5: chlorogenic acid; 6: epicatechin-3-*O*-gallate; 7: Caffeic acid; 8, syringic acid; 9: *P*-coumaric acid; 10: ferulic acid; 11: Luteolin 7-*O*-glucoside; 12:Isorhamnetin 3-*O*-glucoside; 13: Protocatechuic acid ethyl ether; 14: Ellagic acid; 15: Chrysin.

3.1. Physical and chemical properties of water crude polysaccharides (WCP)

Elementary analyses were carried out to verify the suitable degree of purity of WCP. The yield of polysaccharides represents 4% of date's weight (Table 2). The phenol-sulfuric acid assay

showed that carbohydrate contents in WCP were 75 %. On the other hand, analysis of the percentage of protein in the polysaccharides extract shows that these compounds are traces.

Table 2. Yield, chemical and monosaccharide compositions of deglet nour polysaccharides (WCP)*.

Chemical composition	**WCP (%, W/W)**
Extraction yield	4± 0.21
Total sugar	75±1.10
Protein	traces
Neutral sugar	**Monosaccharide composition in WCP (%, mol)**
Arabinose	1.08± 0.09
Xylose	1.30± 0.10
Rhamnose	0.47± 0.00
Fructose	47.21±1.13
Galactose	0.93± 0.00
glucose	49.01± 1.42

*Values are expressed as means ± SD and three replicated independent determinations

In order to further characterize WCP extracted from deglet nour and to identify the fundamental groups present in its structure, Fourier transform-infrared (FTIR) spectroscopy was used as an important source of information for a quick evaluation of polysaccharides composition in vegetable samples (Kacurakova et al., 2000). The infrared spectrum of WCP is given in Figure 2.

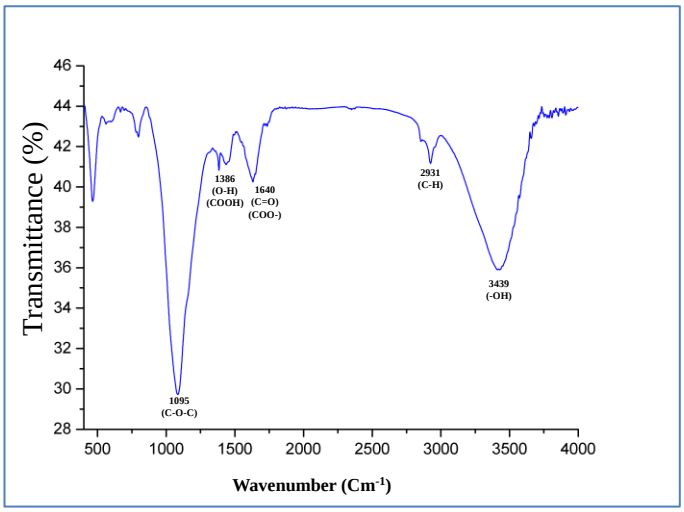


Figure 2. Ftir analysis of Deglet nour polysaccharids

A broad band at 3439 cm^{-1} was attributed to the stretching vibration of the hydroxyl group (-OH) following the polysaccharide' inter and intra-molecular interaction. A weak peak around 2931 cm^{-1} was assigned to the C-H symmetrical stretching vibrations attributed to the linkage of the methylene group (-CH 2^{-}) (Hammi et al., 2016). The detected band at 1640 cm^{-1} is typical of the carbonyl elongation (C=O) vibration of the carboxylate (COO^{-}) function, probably of uronic acids (Xu et al., 2016; Manrique and Lajolo, 2002), while the band between 1412 and 1430 cm^{-1} was associated with the stretching of the pectin methyl ester group (-OCH3). These probably suggested that some uronic acid carboxyls were esterified (Wang et al., 2016). The absorption band at 1386 cm^{-1} was assigned to the elongation vibration of the OH bond within (-COOH) groups (Xu et al., 2016). We found that an intense, centered band was detected at

1095 cm^{-1}, which is characteristic of the ether function (C-O-C) vibration of the glycosidic structure (Xu et al., 2016). The FTIR spectrum showed all typical absorption peaks associated with polysaccharides (Figure 2).

The neutral glycosyl residue composition of carbohydrates was detected by gas chromatography coupled to the mass spectrometer (GC-MS) after derivatization to their volatile forms by using the trimethylsilyl (TMS) method (Wang and Fang, 2004). As shown in Figure 3, multiple distinct peaks were obtained for the same monosaccharide (α and β anomeric forms of the pyranose and furanose conformations) due to the initial muta-rotational equilibrium of the sugars in the solution during the acid hydrolysis. For this reason, the injection of neutral sugar standards was essential for identifying monosaccharides based on their retention time and regarding the mass spectral database ((NIST MS) Spectral Library) to confirm the identification following their fragmentation of each sugar. The results showed that glucose (49.01% mol) and fructose (47.21% mol) were the most abundant neutral monosaccharides (Table 2). However, the lowest molar percentages were found in xylose (1.3 % mol), arabinose (1.08, mol), galactose (0.93%, mol), and rhamnose (0.47%, mol). There is no precise information on the exact composition of the polymers constituting the date fruits' soluble or insoluble fibers (Ghnimi et al., 2017). It is possible that the mainly polysaccharides are like pectin. Mustafa et al. (1986) found that pectin accumulates in the fruit until reaching a maximum in the Rutab stage, correlated with minimal activity of the enzyme pectin esterase.

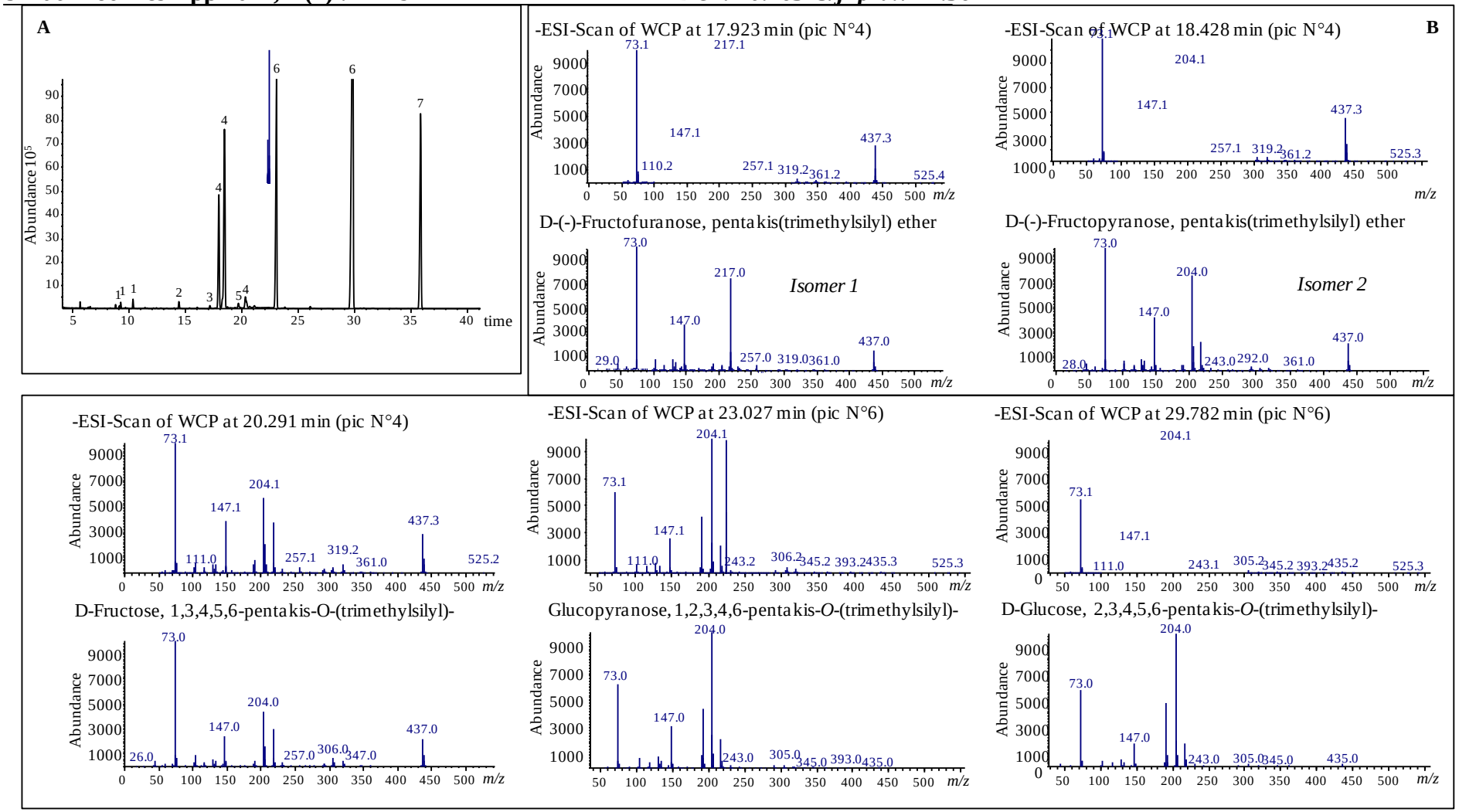


Figure 3. (A) Electron impact gas chromatographic profile (GC-EI-MS) of *O*-trimethylsilylated glycosides from Deglet nour: Identification of peaks: 1: arabinose; 2: xylose; 3: rhamnose; 4: fructose; 5: galactose; 6: glucose; 7:= myo-inositol (standard). (B) Extracted ion chromatogram (EIC) for each sugar with its related standard (time: minutes).

3.2. Evaluation of deglet nour PRE and WCP antioxidant and anti-inflammatory properties

Compounds like phenolics and polysaccharides are well known for their antioxidant activities due to their redox properties and radicals-reducing capacities (Ozgen et al., 2006). Total antioxidant capacity, DPPH, and FRAP assays are three tests with good repeatability frequently used for phyto extracts. As shown in the Table 3, PRE and WCP exhibited a wide range and a potential antioxidant capacity to quench radicals.

Table 3. Evaluation of *in vitro* antioxidant activity and effect of deglet nour phenolic rich extract (PRE) and water crude polysaccharides (WCP) on the LPS-induced Nitric Oxyde (NO) production in RAW 264.7 macrophages.

	PRE	WCP
Antioxidant activity		
TAA (mg GAE/g DR)	268a	267a
DPPH test($IC_{50\%}$ mg/mL)	0.7b	0.5a
Reducing power($EC_{50\%}$ mg/mL)	>2b	0.98a
NO inhibition (%)		
[50µg/ mL]	15.47	28.69
[100µg/ mL]	18.69	28.53
[150µg/ mL]	26.24	37.7

Both extracts showed a similar and high total antioxidant capacity at 267 mg GAE/g DR. Similar works demonstrated the same tendency. For example, the activity reported by Kchaou et al. (2013) on the aqueous, methanolic, acetonic, and ethanolic extracts of six date cultivars grown in Tunisia showed values in the order of 39.94, 44.50, 85.29, and 109.67 mg of ascorbic acid/g of extract. A significantly higher antiradical ($IC_{50\%}$=0.5 mg/mL) activity and a strong ferric reducing power ($EC_{50\%}$=0.98mg/mL) were observed in WCP as compared to the phenolic extract ($IC_{50\%}$=0.7mg/ml and $EC_{50\%}$=2mg/mL) (Table 3). Many studies reported that various plant polysaccharides exhibited notable antioxidant capacity and demonstrated a concentration-dependent scavenging activity by quenching DPPH radicals (Wang et al., 2012). The hydrogen-donating ability, measured using the DPPH test, appeared that several polysaccharides possessed hydrogen-donating capabilities and would act as antioxidants. The high ferric-reducing property of WCP was generally associated with electron-donating groups or hydrogen atoms (Wang et al., 2012). Accordingly, deglet nour polysaccharides might contain higher amounts of reductone, which could react with free radicals to stabilize and block radical chain reactions. Therefore, it was reported that other antioxidant substances were associated with polysaccharides, such as polyphenols (formation of a non-extractable complex between high molecular weight phenolics and polysaccharides), might contribute to the antioxidant activity (Nie and Xie 2011; Wang et al., 2016). Many other factors, such as molecular weight, galacturonic acid, and other chemical constituents contained in crude polysaccharides extracts (proteins, amino acids, phytosterol, ascorbic acid, thiamine, organic acids), were also supposed to play a role in their antioxidant activities (Cheung et al., 2012).

In the next part, we set out to investigate the ability of PRE and WCP to inhibit the production of nitric oxide (NO) produced by LPS-stimulated RAW264.7 cells. First, a cell viability assay was performed to ensure the non-cytotoxicity of the extracts. For the different used concentrations, PRE and WCP extracts do not affect macrophage cell viability (data not shown). LPS treatment drastically enhanced NO production by RAW264.7 cells. This high NO production can lead to oxidative damage, as NO acts with free radicals to produce the highly reactive peroxynitrite, leading to cell damage.

Interestingly, WCP and PRE at a lower extent significantly decreased LPS-induced NO production by RAW 264.7 in a dose-dependent manner. The anti-inflammatory property of deglet nour WCP is interesting, especially since the used concentrations are low compared to that used in literature (Kchaou et al., 2013). Various studies have demonstrated the anti-inflammatory properties of some polysaccharides. For example, in polysaccharides of *Sargassum hemiphyllum* at the concentration of 1, 2.5, or 5 mg/mL, the level of NO is respectively of the order of 25.70, 24.31 and 20.44 in LPS-induced RAW 264.7 cells (Hwang et al., 2011). On the other hand, the polysaccharides extract of *Chaenomeles speciosa* fruits can significantly inhibit the production of nitric oxide induced by LPS, particularly at a concentration of 4.5 mg/mL at levels close to those without induction of LPS (Zhang et al., 2014). In comparaison, Kang et al. (2011) demonstrated that the concentration of 100 µg/mL of *Ecklonia* polysaccharides was sufficient complete the NO inhibition.

3.3. Gastroprotective activity

Oral administration of the PRE at doses of 100 and 200 mg/kg, as well as WCP at a dose of 50 mg/kg and famotidine at a dose of 20 mg/kg, significantly inhibited gastric ulcer formation induced by ethanol/HCl (Table 4). Gastric lesions produced by the ulcerogenic mixture resulted in mucosa inflammation and several ulcers (UI=2.5), indicating the presence of several points and hemorrhagic furrows (% of ulceration = 89%). The positive control, famotidine (antihistamine H2), reduced the ulcer index from 2.5 to 1.5 and the ulcerative percentage from 89 to 50%, leading to the recovery of the gastric mucosa with a percentage of healing rating of around 51.2 %.

Table 4. Effect of administration of phenolic rich extract and soluble crude polysaccharides on gastric ulcer parameters in rats (n=6/group).

Parameters	Group 1 EtOH/HCl	Group 2 FAM	Group 3 PRE [100µg/mL]	Group 4 PRE [200µg/mL]	Group 5 WCP [50µg/mL]
UI	2.5c	1.5b	1.5a	1.2b	1.06ab
UP	89	50	50	43	40
PP	-	51.2	36.2	49.7	60
GP	-	40	40	52	57.6
GV	4.26d	2.66c	2.16b	2.06b	1.73a
GpH	2.06a	3.03b	2.76b	2.96b	3.4c

UI: Ulcer Index (mm); **GV**: Gastric Volume (ml); **GpH**: Gastric pH; **UP:** Ulceration Percentage, **PP**: Percentage of healing; **GP**: Guerison Percentage

The oral treatment with phenolic extract and polysaccharides reduced gastric lesions and promoted gastric protection but to different extent. WCP significantly reduced the index and the percentage of ulceration (UI=1.06, UP=40%) and showed a better gastric mucosal protective effect than the positive control group, with a healing percentage of up to 60%. Oral treatment with PRE at 100 and 200 mg/kg reduced these ulceration parameters to values similar to famotidine, with a better improvement at 200 mg/kg. The percentage of healing is also dose-dependent. Administration of 100 mg/kg protects mucosa by 36.2%, whereas the dose of 200 mg/kg was more effective and reached those of famotidine (49.7%).

In pylorus-ligated rats, the PRE, WCP and famotidine pretreatment reduced the gastric volume and acidity (pH) to different extent in HCl/EtOH-ulcerated rats (Table 4). Results indicated that ulceration caused by the ethanol/HCl mixture resulted in an increase of gastric juice volume to 4.25 mL, followed by an increase of acidity accompanied by a decrease of pH to 2.1. Famotidine and PRE-pretreated groups showed a similar response and decreased the gastric volume

significantly by around 40% compared to the ulcerated group. This decrease is also accompanied by a pH recovery of 3. WCP showed a more effective gastro-protective effect than famotidine and PRE, resulting in a 62% reduction in gastric volume compared to the ulcer group and a pH increase from 2.1 to 3.4.

The richness of methanolic extract by phenolic compounds recovered the volume and pH of gastric juice, and there is no significant difference between the two concentrations used. The beneficial effect of the extract is slightly better than the famotidine group but less effective than the polysaccharides extract. The results have shown that bioactive substances, namely the phenolic compounds and particularly the water-soluble polysaccharides of the date of deglet nour variety, possessed an antioxidant, anti-inflammatory, anti-ulcer and gastro-protective capacity. Considerable evidence shows that oxidative stress induces, with diverse degrees of importance, protein oxidation, lipids peroxidation, and nitrite release, causing the accumulation of reactive oxygen species, which are closely related to the gastric ulcer (Ben Mansour et al., 2022). As shown in Table 5, animals receiving PRE [100µg/mL] increased the gastric levels of MDA compared with the control group (receiving the standard diet). Notably, this increment was prevented in a dose-dependent way by the PRE pre-treatment (reduction of 65.56% and 57.05% for animals receiving PRE [200µg/mL] and animals receiving WCP [50µg/mL], respectively). Hence, the results suggested that PRE [100µg/mL] acted as a ROS scavenger. SOD, CAT, and GPx are part of the first line of defense against oxidative damage caused by ulcer injuries. Clearly, in ulcerated rats, a decrease in the activity of these enzymes was observed, compared with normal rats, while PRE (100 and 200µg/mL), WCP [50µg/mL], and famotidine significantly reversed the ethanol-induced changes in SOD, CAT and GPx levels (Table 5).

Table 5. Effect of extract and polysaccharides of deglet nour supplementation on SOD, CAT and GPx activities in plasma of ulcerated rats.

Groups	SOD (Umol)	CAT (Umol)	GPx (Umol)	MDA
Group 1	1.3	102.66	79.66	-
Group 2	1.92	156	102.16	-
Group 3	1.61	134.66	103.83	0.482
Group 4	1.98	157.5	129.83	0.316
Group 5	2.33	176	136.5	0.275

Group 1: control animals receiving the standard diet. **Group 2**: animals receiving famotidine; **Group 3**: Group 3: animals receiving PRE [100µg/mL] ; **Group 4**: animals receiving PRE [200µg/mL]; **Group 5**: animals receiving WCP [50µg/mL].

As far as we know, no previous studies evaluate the potential modulation of date polysaccharides on the activity of SOD, CAT, and GPx. This last result is original since no work has been done on this variety. The anti-ulcer capacity of polysaccharides and polyphenols has been demonstrated in several studies. Indeed, a diet enriched in fiber,particularly soluble fiber reduces the incidence of duodenal ulcers in humans. Since the impact of peptic ulcers is lower in populations with high fiber consumption, water-soluble polysaccharides offer duodenal and gastric protection through the reduction of gastric acid secretion, the inhibition of the volume of gastric juice and stimulation of mucosal cell proliferation (Magri et al., 2007). Many studies have shown that substances with antioxidant properties, such as polyphenolic compounds, especially flavonoids and phenolic acids, can protect against the adverse effects of ethanol/HCl on the stomach (Tadic et al., 2008). Since the methanolic extract has shown interesting antioxidant activity and an anti-inflammatory effect, it may havea gastro-protective effect (Tadic et al., 2008). The phenolic compounds in this extract could be labeled as responsible for producing such effects. Many studies have shown that certain flavonoids have

a gastroprotective effect through a decrease in the level of prostaglandins in the mucosa, an increase in the secretion of histamine by mast cells, and the inhibition of acid secretion and growth. In another study, Al Qarawi et al. (2005) showed that aqueous and ethanolic extracts of dates of the *Sukari* variety possessed the anti-ulcer activity and reduce the severity of ulceration by increasing the concentration of histamine and gastrin and decreased the rate of gastric mucin. Furthermore, Tadik et al. (2008) demonstrated that ethanolic extract of hawthorn berries (*Crataegus monogyna* Jacq. and *Crataegus oxyacantha*) provided significant protection against the ulcerogenic effect of absolute ethanol in the rat and that this effect was very close to that of Famotidine, a known anti-ulcer drug.

4. Conclusion

The results concerning natural substances from dates of the variety Deglet nour (polysaccharides and methanolic extract rich in phenolic compounds) were very active,particularly for their gastroprotective effect. These results may lead to new strategies using the fruit of *P. dactylifera* in treating gastric ulcers.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Author Contribution Statement

Rim BEN MANSOUR:. Experimentation, data curation, writing the original manuscript; **Raja SERAIRI-BEJI**: Contribution to investigation, data curation; **Riadh KSOURI**: Contribution to conceptualization and to resources; **Wided MEGDICHE-KSOURI**: contribution to data curation, supervision.

ORCID

Rim Ben Mansour: 0000-0002-1951-0938
Raja SERAIRI-BEJI : 0000-0003-2251-8444
Riadh KSOURI: 0000-0001-60042585
Wided MEGDICHE-KSOURI: 0000-0001-5497-0131.

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