

## RESEARCH ARTICLE

# An investigation of algerian dates (*Phoenix dactylifera* L.); antioxidant, anti-inflammatory properties and phenolic compositions

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## ABSTRACT

Date fruit (*Phoenix dactylifera* L.) is not only known for its nutritional properties but also its therapeutic virtues. The objective was to investigate the phenolic compounds and evaluate the antioxidant and anti-inflammatory activities of pulp, seed, and whole fruit of Algerian dates. The total phenolic and flavonoid contents of ethanolic pulp (EP), seed (ES), and whole fruit (EF) extracts were measured using colorimetric methods. Phenolic acids and flavonoid profiles were determined by RP-HPLC-UV. The examination of the antioxidant potential was established by Free radical scavenging DPPH and FRAP assays. Anti-inflammatory activity of aqueous pulp (AP), seed (AS), and whole fruit (AF) of dates at 100, 200, and 300 mg/kg BW of each extract were carried out according to the experimental model of carrageenan-induced acute paw edema in mice, using diclofenac (50 mg/kg) as a reference product. Measurement of the percentage of inhibition of paw edema in mice as well as the histological study of the paw tissue, allowed us to assess the anti-inflammatory effect of the extracts investigated. Ethanolic pulp, seed, and whole fruit of date extracts contained total phenolic and flavonoids compounds at different concentrations as well as a diverse phenolic profile. The strongest antioxidant capacity was determined in ethanolic date seed extract. Concerning anti-inflammatory activity, the administration of aqueous pulp (100 and 200 mg/kg), seed (100mg/kg), and fruit (100, 200, and 300 mg/kg) of date extract induced a higher anti-inflammatory response from the fourth hour after carrageenan injection compared to the standard group treated with diclofenac. It is concluded that pulp, seed, and whole fruit of Algerian date possess antioxidant activity with phenolic and flavonoid contents and anti-inflammatory activity and dates with pulp and seed parts could be used as a natural bioactive substance for supporting anti-inflammatory therapies.

**Keywords:** Anti-inflammatory activity, antioxidant activity, *Phoenix dactylifera* L.

## INTRODUCTION

Inflammation is a normal process in our body; acute inflammation works to clear infections and promote the healing of wounds (Liu et al., 2019). It is a complex mechanism, made up of a cascade of sequential events in the tissue to eliminate the initial cause of cellular damage. Symptoms of inflammation are local redness, swelling, pain, heat, and loss of function (Virshette et al., 2019). Several chemical mediators such as bradykinins, prostaglandins, thromboxanes, complement proteins, histamine, and monokines activate and regulate inflammatory events that underline these manifestations (Bauri et al., 2015). The persistence of the inflammatory process increases the excessive production of reactive oxygen species (ROS)

(Hydroxyl radicals, peroxy radicals, and superoxide radicals), thus resulting in a state of oxidative stress (Tsai et al., 2015).

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most common synthetic drugs used in the world, for the treatment of inflammation, by inhibiting the cyclooxygenase (COX) arachidonic acid metabolism pathway that produces prostaglandins (Blackler et al., 2014). However, the use of NSAIDs is associated with many side effects, such as their unwanted effects on the gastrointestinal tract, kidneys, and cardiovascular system (Virshette et al., 2019). For this reason, a new therapeutic approach has been investigated for the development of herbal medicine for use in the treatment of inflammation without causing side effects.

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Received: 08 July 2021; Accepted: 23 August 2021

Indeed, plants are an important source of new bioactive substances, which lead to promising therapies.

Polyphenols are known for their anti-inflammatory effect by acting on the inflammatory process by modulating the activities of enzymes involved in the metabolism of arachidonic acid (phospholipase A2, COX, lipoxygenase (LOX)), nitric oxide synthases (NOS), modulating the secretion of other pro-inflammatory molecules (Hussain et al., 2016).

Date palm (*Phoenix dactylifera* L.) is one of the most important agricultural products in the Algerian Sahara. Date fruits are considered a high-energy food source (Hamad et al., 2015). They are composed of pulp and seed which constitutes 5,6% to 15% of date fruit weight (Abiola et al., 2018). In literature, parts of different date varieties are rich in flavonoids, carotenoids, phenolic acids, anthocyanins, tannins, and alkaloids but the phenolic composition of dates vary with variety, growth stage, and environmental conditions (El-Far et al., 2019; Qadir et al., 2019, Hussain et al., 2020). Several studies showed that different date palm varieties comprise gallic acid, catechin, p-coumaric acid, caffeic acid, ferulic acid, syringic acid, protocatechuic acid, vanillic acid, p-OH benzoic acid, cinnamic acid, quercetin, luteolin, rutin, apigenin, isoquercitrin as phenolic compounds and flavonoids (Hussain et al. 2020). The therapeutic properties of dates are attributed to their various phytochemical compounds. In many studies, it was shown that anti-inflammatory properties of dates related to its flavonoid and phenolic compounds such as ferulic acid, syringic acid, caffeic acid, and antioxidant behaviors (Al-Alawi et al., 2017; Qadir et al., 2019, Hussain et al., 2020). Several previous researches have reported that dates possess hepatoprotective, antidiabetic, neuroprotective, anticancer, and antimicrobial activities (Abiola et al., 2018; Pujari et al., 2014; Qadir et al., 2019). This study aimed to determine the total phenolic and flavonoid content, and phenolic profile as well as evaluate the antioxidant (DPPH, FRAP) and anti-inflammatory activities of pulp, seed, and whole fruit Algerian date extracts. Further, this could allow the use of Algerian dates as an eventual source of bioactive substances, which would be employed in therapy.

## MATERIALS AND METHODS

### Chemicals

Chemicals used in this study were of analytical grade. 2,2-diphenyl-1-picryl-hydrazyl (DPPH), Folin–Ciocalteu reagent, sodium carbonate, ethanol, methanol, aluminium nitrate, ammonium acetate, quercetin, diethyl ether, ethyl acetate, acetic acid, trichloromethane, acetonitrile, 2,4,6-Tripyridyl-s-triazine, hydrochloric acid, trolox, ferrous

sulphate heptahydrate, ferric chloride, formaldehyde, hematoxylin Harris, eosin, carrageenan and authentic phenolic standards used for RP-HPLC-UV (Gallic acid, protocatechuic acid, p-OH benzoic acid, catechin, caffeic acid, syringic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, luteolin, myricetin, resveratrol, daidzein, t-cinnamic acid, hesperetin, chrysin, pinocembrin, and caffeic acid phenyl ester (CAPE)) were provided from Sigma-Aldrich (St. Louis, MO, USA).

### Experimental design

In this study, the profile of phenolic compounds of ethanolic pulp, seed and whole fruit of dates extracts was determined by RP-HPLC-UV. The assays of potential antioxidant allowed us to attribute a strong antioxidant power of the ethanolic extract of date seeds. The results of the evaluation of the anti-inflammatory activity were revealed a higher effect of attenuation of the inflammatory response induced by injection of carrageenan of aqueous pulp, seed and whole fruit dates extracts. These findings could represent a potential support for pharmaceutical uses. Experimental design were shown in Fig. 1.

### Plant material and extract preparation

Date palm fruit (*Phoenix dactylifera* L.) of Deglet Noor variety was collected from Biskra, Algeria in October 2018. Three samples were used pulps (P), seeds (S), and whole fruits (F). Seeds were oven-dried for seven days at 50°C and then ground to obtain a fine powder. Pulps (EP), seeds (ES), and whole fruit (EF) were subjected to the maceration process with ethanol (1:4, w/v) at room temperature for 24h and were filtered.

The samples were mixed with distilled water (1:2 w/v) for aqueous extraction. Pulp aqueous extract (AP) was incubated at 5°C for 48h according to the method of Khalifa et al., (2018). Seed aqueous extract (AS) was obtained after 48h of incubation at 5°C (Al-Quarawi et al., 2008), solids of AS were centrifuged at 4000rpm/20mn (Sheikh et al. 2014). Whole Fruit aqueous extract (AF) was maintained for 1h30mn at 85°C with continuous stirring (Benyagoub et al., 2011),

### Phytochemical analysis

#### Total phenolic content (TPC)

The total phenolic contents of ethanolic extracts (EP, ES, EF) were determined by the colorimetric method using the Folin–Ciocalteu reagent and gallic acid as standard (Slinkard and Singleton, 1977). All tests were realized in triplicate. The total phenolic compounds were expressed as mg gallic acid equivalents (GAE) per 100g of sample (mg GAE/100g sample).

#### Total flavonoid content (TFC)

The total flavonoid contents of ethanolic extracts (EP, ES, EF) were carried out by the method of Fukumoto and

Mazza, (2000). This analysis was based on the colorimetric method and quercetin as standard. All tests were realized in triplicate. Results were expressed as mg of quercetin equivalents (QUE) per 100 g of sample (mg QUE/100g sample).

#### Identification of phenolic compounds by RP-HPLC-UV

All ethanolic crude extracts (EP, ES, and EF) were evaporated to dryness at 40°C under vacuum using a rotary evaporator (IKA RV 05 Basic, Germany). 10 ml of acidified water (pH=2) was added to the dry extracts. 15 ml of diethyl ether then 15 ml of ethyl acetate were used to purify phenolics from the extract by two solvent fractionation processes using an orbital shaker (HeidolphPromax 2020, Germany) for 15 minutes between stages. These phenolic extracts were concentrated under reduced pressure at 40°C using a rotary evaporator. The resultant extracts were diluted in 2 ml of pure methanol and then filtered through 0.45 µm PTFE syringe filters.

Phenolic acids and flavonoid profile of date fruit, pulp, and seed were determined using Reversed-Phase High-Performance Liquid Chromatography-Ultraviolet Spectrometry (RP-HPLC-UV) (Elite LaChrom Hitachi, Japan). Analyzes were performed using a reversed-phase C18 column (150 mm x 4.6 mm, 5µm; Fortis) and applying for a gradient program with acetonitrile, water, and acetic acid. A gradient program with 2% acetic acid in reservoir A (pure water) and 70-30% acetonitrile-pure water in reservoir B was used. In addition, the operation volume was optimized by adjusting the injection volume of the samples and standards to 20 µL, the mobile phase flow rate to 1.00 mL/min, and the column temperature to 30 °C in the column furnace (Can et al., 2015). The calibration values for all phenolic compounds were between 0.998 and 0.999. The results were expressed as mg per g of sample for date pulp, seed, and whole fruit. Measurements were done one time.

#### Evaluation of antioxidant activity

##### Ferric reducing antioxidant power (FRAP)

The reducing ability of ferric tripyridyltriazine (FE-III-TPTZ) complex was used for total antioxidant capacity assay of ethanolic extracts (EP, ES, EF) (Benzie and Strain, 1996). All tests were realized in triplicate. The results were expressed as mmol FeSO<sub>4</sub>·7H<sub>2</sub>O equivalent per 100 g of sample.

##### DPPH radical scavenging activity

Free radical scavenging capacity using 1,1-diphenylhydrazyl (DPPH) was carried out as described by Molyneux, (2004). All tests were realized in triplicate. The DPPH radical scavenging activity was determined by using Trolox as an antioxidant standard, and the values are expressed as

SC<sub>50</sub> (mg/mL), the concentration of the extracts resulting in 50% scavenging of DPPH radicals.

#### Evaluation of anti-inflammatory activity animals

One hundred and five (105) female NMRI mice weighing 28 ± 2g were obtained from the Pasteur Institute of Algeria. Animals were maintained under standard conditions (Temperature 25 ± 2°C, light-dark cycle 12:12 hr/day), and were given *ad libitum* access to water and standard food. *In vivo* experimental protocol was approved according to the guidelines established by the laboratory of pharmacognosy and Api-phytotherapy (Mostaganem University), following the instruction described by the Guide for the Care and Use of Laboratory Animals, and were approved by the Institutional Animal Ethics Committee (1205/c/08/CPCSEA, 21.04.08).

#### Acute toxicity test

An acute toxicity test was carried out according to the Economic Co-operation and Development (OECD, Essai No: 425 guidelines, 2008). Mice received intragastrically aqueous extracts (AP, AS, and AF) at 300, 1000, and 2000 mg/kg body weight for each sample, behavioural changes were observed over a period of 24h to 14 days for signs of acute or chronic toxicity.

#### Experimental groups

The anti-inflammatory activity of aqueous extracts was determined using the experimental model of carrageenan-induced acute paw edema in mice according to the method of Winter et al., (1962). Animals were divided into 6 groups and were given solution intragastrically as following:

Control group (C): 5 mice were given saline solution (0.9%)

Inflammatory group (IC): 5 mice were given saline solution (0.9%)

Date pulp group (AP): 15 mice were given aqueous extract of date pulps at 100 mg/kg (APD1), 200 mg/kg (APD2) and 300mg/kg (APD3). Each dose for 5 mice

Date seed group (AS): 15 mice were given aqueous extract of date seeds at 100 mg/kg (ASD1), 200 mg/kg (ASD2) and 300mg/kg (ASD3). Each dose for 5 mice

Date fruit group (AF): 15 mice were given aqueous extract of date fruit at 100 mg/kg (AFD1), 200 mg/kg (AFD2) and 300mg/kg (AFD3). Each dose for 5 mice

Standard group (STD): 5 mice were given diclofenac at 50 mg/kg.

One hour after treatment, 0.1ml of carrageenan solution (1%) was intraarticular injected in the sub-planter region of the right hind paw of all mice except the control group (C). The volume of edema was measured at the 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, and 6<sup>th</sup> hour after carrageenan administration. The percentage of edema inhibition was calculated using the following formula:

$$\text{Percentage inhibition (\%)} = (V_{IC} - V_t / V_{IC}) \times 100.$$

$V_{IC}$ : Increase in paw volume of the inflammatory control group

$V_t$ : Increase in paw volume of mice treated with different extracts.

### Histological study

Animals were anesthetized with trichloromethane by inhalation. Their paws were removed and fixed in 10% buffered formaldehyde solution. The paw tissues were dehydrated, cleared, and impregnated with paraffin. The solidified blocks were sectioned using microtome at 4  $\mu$ m thicknesses. The sections were deparaffinized and

stained with Harris's hematoxylin and eosin as nuclear and cytoplasmic stains (Marck, 2010). Morphological and anatomical changes in paw fragments were observed using a microscope and photographed at a magnification of 400X.

### Statistical analysis

Data of phytochemical analysis and antioxidant activity of date pulp, date seed, and whole date fruit expressed as SD but data of anti-inflammatory activity of date pulp, date seed, and whole date fruit were expressed as mean  $\pm$  SEM. Anti-inflammatory activity results were analyzed using a one-way ANOVA test followed by Turkey's multiple comparison tests.  $P \leq 0.05$  were considered statistically significant (\*\* $P \leq 0.001$ , \*\* $P \leq 0.01$  and \* $P \leq 0.05$ ).

## RESULTS

### Phytochemical analysis

The total phenolic contents in EP, ES, and EF were  $4.30 \pm 0.004$  mg GAE/100g FW,  $6967.30 \pm 2.100$  mg GAE/100gDW, and  $664.20 \pm 0.350$  mg GAE/100g FW respectively. In addition, the values of total flavonoid contents in EP and EF were not identified, for the ES it was  $1.35 \pm 0.006$  mg QE/100g DW as shown in Table 1.

The composition and concentration of phenolic contents were determined by HPLC-UV analysis. Eight phenolic acids (Gallic acid, protocatechuic acid, p-OH benzoic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, and t-cinnamic acid), ten flavonoids (Catechin, epicatechin, rutin, luteolin, myricetin, resveratrol, daidzein, hesperetin,

**Table 1: Total phenolic and flavonoid contents of date pulp, date seed, and whole date fruit. Values are mean $\pm$ SD, n=3**

Extracts	Phytochemical analysis	
	Total phenolics (mg GAE/100g )	Total flavonoids (mg QE/100g)
EP	$4.30 \pm 0.004$	ND
ES	$6967.30 \pm 2.100$	$1.35 \pm 0.006$
EF	$664.20 \pm 0.350$	ND

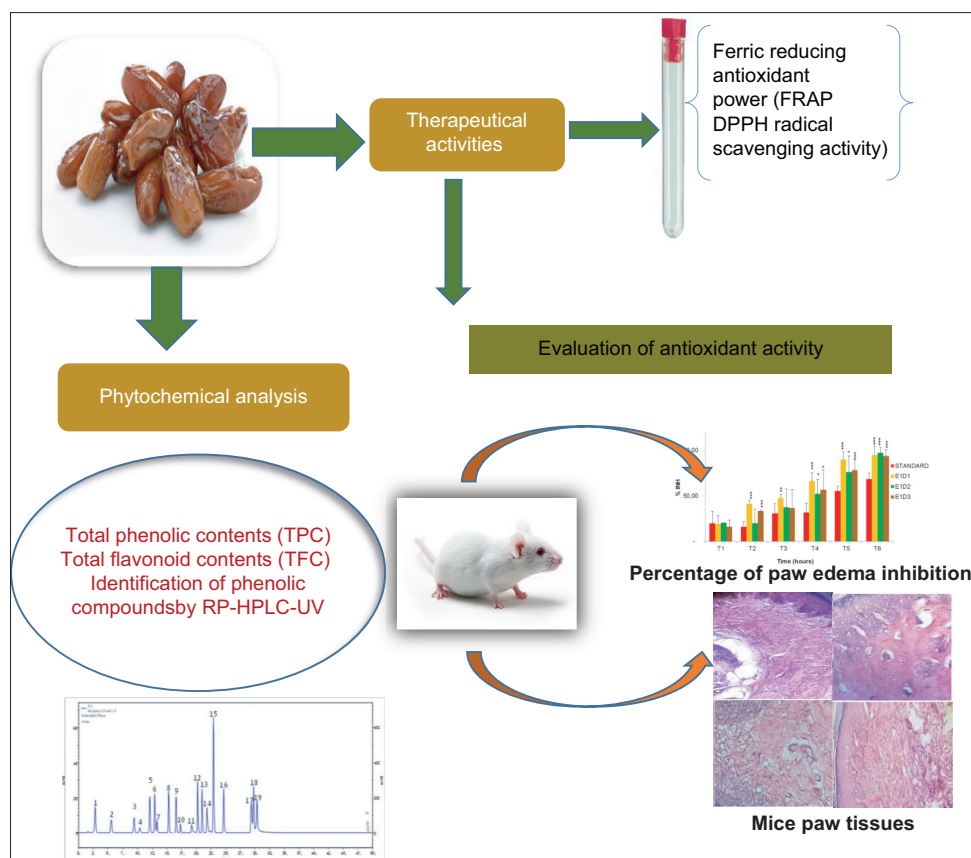
EP: Ethanolic extract of date pulp. ES: Ethanolic extract of date seed. EF: Ethanolic extract of whole date fruit. ND: Non detected.

**Table 2: Total phenolic compounds of date pulp, date seed, and whole date fruit by RP-HPLC-UV (mg/g of sample). Measurements were done one time, n=1**

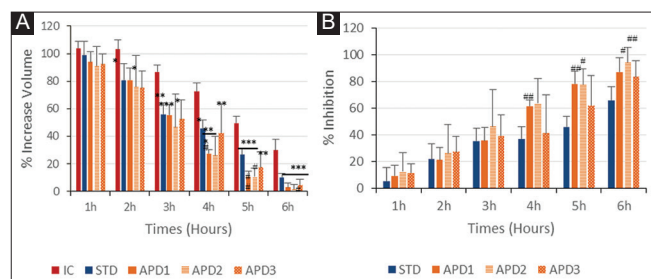
Standards	Date Pulp (mg/g)	Date Seed (mg/g)	Whole Date Fruit (mg/g)
Gallic Acid	11.013	11.154	12.144
Protocatechuic acid	3.213	80.148	27.905
p-OH Benzoic acid	2.928	3.863	3.138
Catechin	4.739	595.114	25.350
Caffeic acid	ND	29.223	7.315
Syringic acid	1.378	ND	1.769
Epicatechin	8.054	245.922	19.592
p-Coumaric acid	6.282	ND	6.925
Ferulic acid	9.375	ND	12.974
Rutin	ND	14.427	ND
Myricetin	ND	ND	ND
Resveratrol	ND	ND	ND
Daidzein	3.008	40.183	13.632
Luteolin	ND	ND	ND
t-Cinnamic acid	0.559	ND	0.661
Hesperetin	ND	ND	ND
Chrysin	29.748	35.753	28.894
Pinocembrin	3.655	6.132	3.154
CAPE	5.862	8.589	2.838

ND: Non detected





**Fig 1.** General diagram of the experiment.



**Fig 2.** Percentage of increase paw volume (A). Percentage of paw edema inhibition (B). Date pulp group (AP): APD1 (100 mg/kg), APD2 (200 mg/kg), APD3 (300 mg/kg). Standard group treated with Diclofenac (STD). Values represent means  $\pm$  SEM in each group. \* $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$  compared to inflammatory control (IC). # $P < 0.05$ ; ## $P < 0.01$  compared to STD.

chrysin, and pinocembrin) and one phenyl ester CAPE were identified and quantified in the investigated ethanolic extracts. All phenolic acids were identified in date fruit, seven of them were identified in date pulp except caffeic acid, just four phenolic acids (Gallic Acid, protocatechuic acid, p-OH benzoic acid, caffeic acid) were found in date seed. Concerning flavonoid contents, four were not found in the three samples (Myricetin, resveratrol, luteolin, hesperetin) in addition the Rutin was also absent in date pulp and whole date fruit. The highest amount of protocatechuic acid, p-OH benzoic acid, caffeic acid,

CAPE, catechin, epicatechin, rutin, daidzein, chrysin, and pinocembrin was detected in date seed and the highest amount of gallic acid, syringic acid, p-coumaric acid, ferulic acid, and t-cinnamic acid was detected in whole date fruit. Date seed had the strongest total phenolic composition as shown in Table 2.

### Antioxidant activity

Antioxidant activity was measured in the forms of FRAP, and DPPH radical scavenging activity. The determined values of DPPH and FRAP were different between samples; EF ( $0.219 \pm 0.010$  mg/ml;  $2.908 \pm 0.140$  mmol  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}/100\text{g FW}$ ), EP ( $35.235 \pm 2.920$  mg/ml,  $0.162 \pm 0.030$  mmol  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}/100\text{g FW}$ ) and ES ( $0.057 \pm 0.003$  mg/ml,  $70.301 \pm 15.000$  mmol  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}/100\text{g DW}$ ) as shown in Table 3.

### Acute toxicity

No behavioral disorders or mortality were observed in the mice after 14 days of administration of aqueous extracts (AP, AS, AF) at 300, 1000, and the highest dose of 2000 mg/kg.

### Anti-inflammatory activity

The percentage of increased paw volume in APD1 and APD3 groups indicated a significant decrease ( $p \leq 0.05$ ) at the second hour after the injection of the carrageenan

compared to the inflammatory control group (IC). From the fourth hour of the experiment, all groups (STD and AP) showed a significant decrease of paw volume until the sixth hour ( $p \leq 0.01$ ) of the experiment compared to (IC) (Fig. 2A). Mice treated with APD1 showed a significant increase in the inhibition of paw edema from the fourth hour ( $p \leq 0.01$ ) until the end of the experiment ( $p \leq 0.05$ ) compared to mice treated with diclofenac (STD). As for the APD2 group, it recorded a significant increase in the percentage of inhibition at the fifth ( $p \leq 0.05$ ) and sixth hour ( $p \leq 0.01$ ) after the induction of inflammation (Fig. 2B).

A significant decrease in paw volume in the ASD1 ( $p \leq 0.01$ ) and ASD3 ( $p \leq 0.05$ ) groups was observed at the first hour after induction of inflammation compared to the IC. At the second hour, this decrease had become significant for all groups (STD and AS) compared to the IC group and reached high significance at the 5<sup>th</sup> and 6<sup>th</sup> hour of the experiment (Fig. 3A).

The ASD1 group showed a significant increase ( $p \leq 0.05$ ) in the inhibition of edema after the first hour from the injection of carrageenan in comparison with the group

treated with diclofenac (STD). This difference in the inhibition of paw edema between ASD1 and STD groups showed a significant increase during the following five hours. At the sixth hour of the experiment, all groups (ASD1, ASD2, ASD3) showed a highly significant increase ( $p \leq 0.001$ ) in inhibition of paw edema compared to the STD group (Fig. 3B).

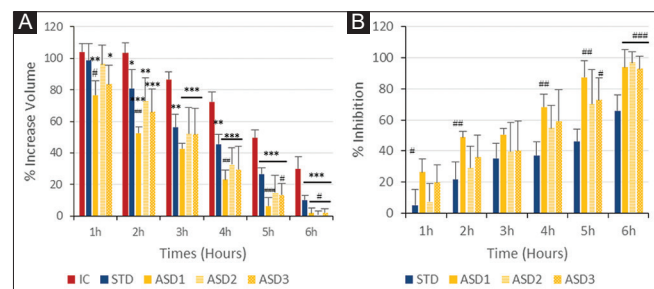
The AFD1 group presented a significant decrease in paw volume at the first hour compared to the IC group. A highly significant decrease was observed for all groups (STD and AF) from the third to sixth hour of the experiment (Fig. 4A). The percentage of paw edema inhibition in AF groups showed a significant increase starting from the fourth hour (AFD1:  $p \leq 0.05$ ; AFD2 and AFD3:  $p \leq 0.01$ ) compared to the group treated with diclofenac (STD). This increase in the inhibition of edema reached a highly significant difference ( $p \leq 0.001$ ) in the three doses studied at the fifth and sixth hours of the experiment (Fig. 4B).

### Histological study of paw tissue

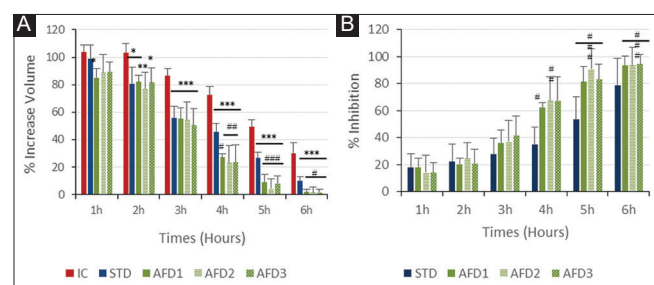
The histological examination revealed a different morphological appearance between the experimental groups.

The microscopic observation of the paw tissue in the control group (C) represented healthy tissue without inflammation. A microscopic analysis of the inflammatory group (IC) revealed a loose and clear connective tissue corresponding to the exudate, with an infiltrate of inflammatory cells (polynuclear) at the level of the epidermis and the interstitial dermis, associated with dilation of the capillaries and congestion engorged with red blood cells.

The standard group (STD) treated with diclofenac at 50 mg/kg presented attenuation of the edema with persistence at the level of some lesional areas accompanied by a slight leukocyte infiltrate. The paw fragments of mice treated with AF showed a significant regression of the inflammation and reduction of leukocyte infiltration as compared to the inflammatory control (IC). These observations were also found in the paw tissues of mice treated with AS. However, the APD3 dose didn't



**Fig 3.** Percentage of increase paw volume (A). Percentage of paw edema inhibition (B). Date seed group (AS): ASD1 (100 mg/kg), ASD2 (200 mg/kg), ASD3 (300 mg/kg). Standard group treated with Diclofenac (STD). Values represent means  $\pm$  SEM in each group. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  compared to inflammatory control (IC). # $P < 0.05$ ; ## $P < 0.01$ ; ### $P < 0.001$  compared to STD.



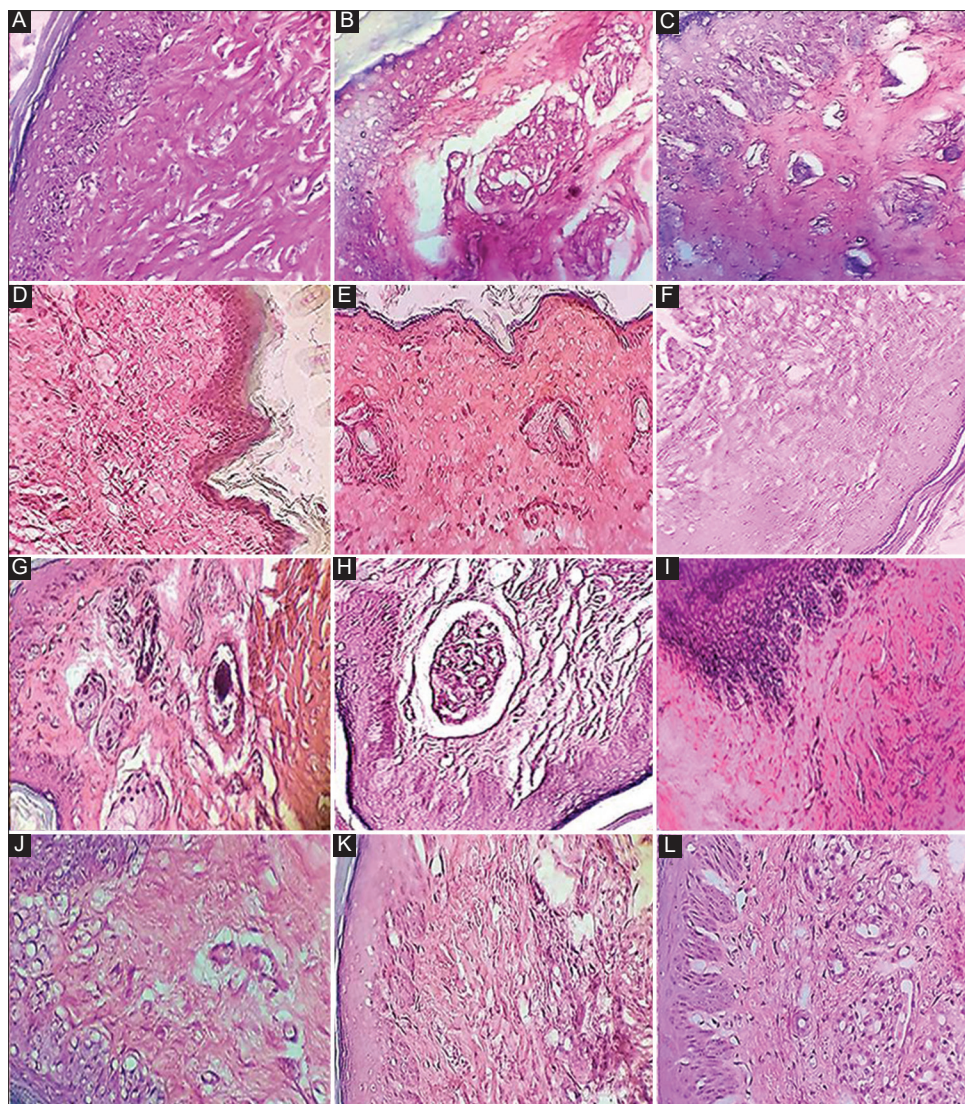
**Fig 4.** Percentage of increase paw volume (A). Percentage of paw edema inhibition (B). Date fruit group (AF): AFD1 (100 mg/kg), AFD2 (200 mg/kg), AFD3 (300 mg/kg). Standard group treated with Diclofenac (STD). Values represent means  $\pm$  SEM in each group. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$  compared to inflammatory control (IC). # $P < 0.05$ ; ## $P < 0.01$ ; ### $P < 0.001$  compared to STD.

**Table 3: Antioxidant activity of date pulp, date seed, and whole date fruit. Values are mean  $\pm$  SD, n=3**

	Antioxidant activity	
	DPPH SC <sub>50</sub> mg/ml	FRAP (mmol FeSO <sub>4</sub> .7H <sub>2</sub> O/100 g)
EP	35.235 $\pm$ 2.920	0.162 $\pm$ 0.030
ES	0.057 $\pm$ 0.003	70.301 $\pm$ 15.000
EF	0.219 $\pm$ 0.010	2.908 $\pm$ 0.140

EP: Ethanolic extract of date pulp. ES: Ethanolic extract of date seed. EF: Ethanolic extract of whole date fruit.





**Fig 5.** A representative photomicrograph of mice paw tissues stained with Haematoxylin and Eosin (H&E). At mag X40. A: control group (C); B: inflammatory group (IC); C: standard group (STD) treated with Diclofenac at 50mg/kg; D, E, F: Date fruit group (D: 100 mg/kg (AFD1), E: 200 mg/kg (AFD2), F: 300mg/kg (AFD3)). G, H, I: Date pulp group (G: 100 mg/kg (APD1), H: 200 mg/kg (APD2), I: 300 mg/kg (APD3)). J, K, L: Date seed group (J: 100 mg/kg (ASD1), K: 200 mg/kg (ASD2), L: 300mg/kg (ASD3)).

significantly reduce the inflammatory response, while the epidermal edema was partially suppressed and inflammatory cell infiltration persisted slightly at the inflamed sites in the paw tissues of treated groups APD1, APD2 compared to IC (Fig. 5).

## DISCUSSION

Several bioactive polyphenolic compounds are found in all parts of date (Pulp and seed) and these substances can give them potential therapeutic properties (Qadir, 2019). This study explored three distinct components; the identification and quantification of total phenolic and flavonoid compounds in pulp, seed, and whole fruit of date, determination of their antioxidant

capacity (DPPH, FRAP), and evaluation of their anti-inflammatory activity.

The total phenolic contents of EP obtained in our study agreed with previous results reported by Haimoud et al., (2016) in Algerian date (DegletNour), who found that TPC value was  $4.72 \pm 0.41$  mg GAE/100g DW for the methanolic extract of date fruit. Nevertheless, our data were higher than those reported by Al Harthi et al., (2015) who noted the TPC of ethanolic extract from Oman date ranged between 32.24 to 34.9 mg GAE/100 mg FW. Concerning the results of the total phenolics contained in the extract of the date fruit, they represented intermediate TPC values between the extract of date pulp and date seed. This result corresponds to the composition of 10% seed and 90% pulp, which form the date fruit.

Total flavonoid contents in EP were not identified in our study. Unlike Haimoud *et al.*, (2016) who reported that TFC of methanolic date extract was 3.48 mg CEQ/100g DW. Our data of the total flavonoid contents of ES were lowest than those reported by Metoui *et al.*, (2019) and Bouhlali *et al.*, (2017) who recorded  $2.31 \pm 0.01$  g CAE/100g DW,  $1.224 \pm 0.03$  to  $1.844 \pm 0.018$  g RE/100g DW respectively. Variations in the results of TPC and TFC amounts of date's pulp and seed may be probably owing to the variety, growing condition, maturity, geographic origin, storage conditions, and extraction methods (Ahmed *et al.*, 1995).

In our study, HPLC analysis of total phenolic compounds showed considerable variations among the samples. The phenolic acid profile detected in date pulp included four hydroxylated benzoic acid derivatives (Gallic Acid, protocatechuic acid, p-OH benzoic acid, syringic acid) and three cinnamic acid derivatives (p-Coumaric acid, ferulic acid, t-cinnamic acid). These results were more or less close to those recorded by Bouhlali *et al.* (2018) who detected seven phenolic acids (Caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, gallic acid, syringic acid, vanillic acid) in Moroccan date varieties. Hamad *et al.*, (2015) identified the major phenolic acids in Saudi Arabia date varieties, which were gallic acid, p-coumaric acid, and ferulic. Among the flavonoid compounds found in date pulp, chrysin was predominant followed by catechin and epicatechin. While rutin and luteolin were not present in the studied extract. On the other hand, the phenolic acids revealed in date seed represented three hydroxylated benzoic acid derivatives (Protocatechuic acid, gallic Acid, p-OH benzoic acid) and one cinnamic acid derivative (Caffeic acid). Among the analyzed flavonoid compounds in date seed, catechin was the predominant compound, followed by epicatechin, daidzein, and chrysin. About the phenolic profile determined in whole date fruit, it represented the total of phenolic acid and flavonoid compounds found in each part of date with intermediate amounts. This finding confirmed the mixture of phenolic compounds detected in the pulp and seed of date. Moreover, the difference in the presence and content of phenolic acids and flavonoids between our data (date pulp, date seed) and other studies may be explained by several parameters, including the variability of the studied cultivars and growing/extraction processing conditions (Al-Alawi *et al.*, 2017).

DPPH radical scavenging ability test is widely used to assess the free radical scavenging capacity of antioxidants. Our results revealed clearly that the ES had a strong free radical scavenging ability. Another study showed that the percentage of inhibition of Deglet Nour date seeds was  $41.32 \pm 0.01$  % (Metoui *et al.*, 2019). The strong antioxidant capacity measured in date seed extract (ES)

could be explained by its content of phenolic acids and flavonoids compounds. However, the lowest DPPH radical scavenging ability was measured in EP. Amira *et al.*, (2013) also noted this observation, who indicated  $EC_{50}$  DPPH values ranged from 0.93 to 1.91  $\mu$ g sample of Tunisian date seeds. Salomón-Torres *et al.*, (2019) reported that  $IC_{50}$  DPPH was 0.079 g/l in Mexican date pulp. The Amount of  $SC_{50}$  DPPH recorded in EF represented an intermediate level between EP and ES.

Other techniques are also used to assess antioxidant activity such as FRAP. Our FRAP results were in agreement with those obtained by  $SC_{50}$  DPPH for investigated samples. Therefore, a high level of correlation between  $SC_{50}$  DPPH and FRAP assays was observed. The data FRAP results in EP were much higher than those reported by Haimoud *et al.*, (2016) who studied DegletNour date fruit from Algeria ( $33.95 \pm 1.2$   $\mu$ mol Fe(II)/100g DW). In other parts, Bouhlali *et al.*, (2017) found values of FRAP assay varied between  $10.966 \pm 0.339$  to  $22.863 \pm 0.358$  mmol TE/100g DW in Moroccan date seeds. These results were lowest than those obtained by studied extract (ES). As for the EF extract, it regularly represents intermediate values between those provided by EP and ES extracts. Based on the data obtained in our research, the results showed that the pulp, seed, and fruit of dates have antioxidant activity at different levels and seeds have the highest antioxidant capacity.

The evaluation of the anti-inflammatory activity in our study was based on a very common test to elucidate the capacity of a compound to reduce induced local edema by injection of Carrageenan as an irritant into the paw of mice (Winter *et al.*, 1962). This method is supposed to be a biphasic event (Initial phase for 0-1h and Second phase for 1-6h) (Tsai *et al.*, 2015).

In the experiment, a high increase in the volume of mice paw was observed in the inflammatory control group (IC) at the first phase after the injection of carrageenan. This edema remained relatively unchanged from 1-3h and persisted during the experiment compared to the treated groups. This means that this phlogistic agent produced an acute inflammation that results from the sequential action of several mediators (El Abed *et al.*, 2018).

Nevertheless, a significant decrease of edema in mice paw was observed in the group treated with diclofenac at 50 mg/kg body weight at the second phase. This finding was similar to those recorded by El Abed *et al.*, (2018) using indomethacin as a reference product. Moreover, Bouhlali *et al.*, (2018) have noted a significant inhibition of paw edema by indomethacin from the fourth hour of the experiment. These results could be explained by the fact that the reference product (Diclofenac) belongs



to NSAIDs. These molecules are used to treat acute and chronic pain resulting from an inflammatory process.

In the AP group, the three doses (APD1, APD2, and APD3) led to significant attenuation in paw swelling precociously at the second phase of inflammation. The administration of aqueous extract of date pulp at 100mg/kg (APD1) and 200mg/kg (APD2) induced a higher anti-inflammatory response compared to the mice treated with the reference product (STD). Haimoud et al., (2016) and El Abed et al., (2018) reported that methanolic extract of date edible portion and aqueous ethanolic extract of date parthenocarpic respectively suppressed the increases of paw edema at the late phase of inflammation. Further, our finding allows us to confirm the contribution of the aqueous extract of date pulp at 100 and 200mg/kg to considerably reduce paw edema. The bioactive compounds contained in pulp date, such as phenol acids and flavonoids could be responsible for this effect, by interfering with the formation of inflammation mediators like prostaglandins and thromboxane (Zhang et al., 2013), thus inhibiting the expression of inflammatory cytokines such as IL-6, IL-8, IL-10, TNF- $\alpha$ , IGF-1 and the activities of superoxide dismutase (SOD), myeloperoxidase (MPO), NO and prostaglandin E2 (Kim et al., 2005; Al-Yahya et al., 2016, Vezza et al., 2016)

About AS group, administration of all studied doses induced a significant suppression of the inflammatory response at the first stage and during all phases of inflammation. Whereas, mice treated with aqueous date seed extract at 100 mg/kg (ASD1) revealed a strong anti-edema effect compared to mice treated with the reference product (STD). Bouhlali et al., (2018) reported that date seed extract induced a significant diminution in paw swelling at the second phase of inflammation. Therefore, our results lead us to conclude the anti-phlogistic effect of date seeds. This activity could be associated with phenolic acid contents in seeds of date, which can inhibit the production of NO, TNF- $\alpha$ , and IL-6 (Choi et al., 2017). Also, it has been shown that caffeic acid, catechin, epicatechin, rutin have anti-inflammatory effects in previous studies (Liu et al., 2014; Vezza et al., 2016; Abd Nikfarjam et al., 2017). On the other hand, the strong antioxidant potential revealed in date seeds could contribute to the reduction of the inflammation induced by the phlogistic agent (Saryono et al., 2018; El-Far et al., 2019).

Analysis of the results in the AF group demonstrated an enhanced potential for anti-inflammatory activity compared to the AP and AS groups. This was observed in the first phase of inflammation, paw edema was significantly suppressed by date fruit at all investigated doses. Moreover, mice treated with the extract of date fruit at 100, 200, and

300mg/kg showed a highly significant attenuation of the inflammatory response from the second stage than the one observed in the mice treated with the reference product (STD).

Histological examination agrees perfectly with the results previously shown. The microscopic observations of paw tissues corresponded to the macroscopic data of the paw swelling in all groups. The accentuated aspect of the inflammation in the inflammatory control group (IC) confirms the persistence of the edema. However, the histology of all investigated groups treated with the studied extracts (AP, AS, and AF) as well as with the reference product (STD) demonstrated minimal inflammation, which reflects the attenuation of paw edema in mice.

## CONCLUSION

The finding obtained from this study reveals the presence of several phenolic compounds at different concentrations in the ethanolic extracts of pulp, seed, and fruit of dates. These substances are considered bioactive molecules, could explain the potential antioxidant effect of the three studied extracts. Particularly, the ethanolic date seed extract (ES) has shown the strongest anti-free radical ability. On the other hand, all aqueous extracts of *Phoenix dactylifera* L. had induced a better anti-inflammatory response than the synthetic reference product (STD), especially aqueous date fruit extract (AF). This suggests that their mechanism of this activity could contribute to the inhibition of inflammatory mediators and pro-inflammatory cytokines. This study suggests that dates with pulp and seed parts could be used as a source of natural antioxidant and anti-inflammatory compounds. Further studies will be advisable to target with certainty the bioactive compounds of *Phoenix dactylifera* L. in the hope of understanding their exact role in process of the anti-inflammatory activity.

## ACKNOWLEDGEMENTS

The authors are grateful to Dr. Takerli (Anatomopathologist) for his collaboration to complete the histological interpretation. We also appreciate the help of Mr. Maazia (Department of Medicine, University of Mostaganem, Algeria) for the statistical study.

## Conflicts of interest statement

All authors declare no conflicts of interest.

## Author contributions

Mrs. Hadjer Chenini-Bendiab carried out the in vivo study. She performed statistical analyzes and histological studies. She also interpreted the results and wrote the manuscript

with Mrs. Meltem Uçar. Prof. Nouredine Djebli initiated this research and directed the entire experimental study. He also supervised the interpretation of the results and edited the manuscript with the first author. Mr. Yakup Kara took charge of the phytochemical analyzes as well as the evaluation of the anti-oxidant activity with the first author. Prof. Sevgi Kolayli contributed to the in vitro part of this study at the level of her laboratory.

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