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Original article

Anti-inflammatory properties and phenolic profile of six Moroccan date fruit (*Phoenix dactylifera L.*) varieties

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ABSTRACT

Date fruit (*Phoenix dactylifera*) is widely used in Moroccan pharmacopoeia to treat inflammation and other diseases. For this purpose, six date fruit varieties (Boufgous, Bouskri, Bousrdon, Bousthammi, Jihl, and Majhoul) were assessed for their phenolics profile and anti-inflammatory activity. Total phenolic and flavonoid contents of these extracts were measured by the high-performance liquid chromatography (HPLC). Protein denaturation was assessed employing bovine serum albumin. Edema formation was induced in rat's right hind paw and mice's right ear using carrageenan and croton oil, respectively. Membrane stabilizing activity was estimated by the prevention of hypotonicity induced-erythrocyte membrane damage. The results showed that Gallic, Ferulic and Caffeic acids and Rutin were the most dominant among the analyzed polyphenolic compounds. With regard to anti-inflammatory activity, Bousrdoun showed highest NO scavenging ability (IC_{50} = 531.34 μg/mL), highest protein denaturation inhibition (IC_{50} = 408.64 μg/mL) as well as highest membrane stabilizing effect (IC_{50} = 483.61 μg/mL). However, Jihl exhibited highest reduction of ear edema (74%), while both Jihl and Bousrdoun exhibited similar and highest paw oedema (40.35%). Our findings indicate that among the investigated date varieties, Jihl and Bousrdoun are found to be the most active anti-inflammatory compounds. The difference in activities seems to be related to the variations in the phenolic and flavonoids content between date varieties.

1. Introduction

Inflammation is the ordinary response of body's immune system to harmful stimuli involving several mediators and immune cells (Larsen and Henson, 1983). The classical signs of inflammation include swelling, redness, pain, and fever (Williams and Maier, 1992; Nathan, 2002). The inflammation process can be classified as either acute or chronic. Chronic inflammation induces the over-production of several factors such as reactive oxygen (ROS), nitrogen species (RNS), cyclooxygenase (COX), and cytokines (Balkwill, 2009). These factors are associated with the incidence of several disorders such as atherosclerosis, diabetes, and sclerosis (Aggarwal, 2004).

Accordingly, medicinal plants have been widely investigated as alternative medicine to treat a variety of human disorders. In this context, *Phoenix Dactylifera* extracts have been largely studied for their therapeutic potential in animals (Middleton et al., 2000).

Date palm, a monocotyledon belonging to the *Palmaceae* family that includes more than 2000 species (Jain and Priyadarshan, 2009), has been one of the most important fruit crops in the arid districts of the Middle East and North Africa (Chao and Krueger, 2007). On the other hand, *Phoenix Dactylifera,* known as "Tmar or Tyni" in Moroccan pharmacopeia, is used to treat different diseases, mainly fever, inflammation, and hypertension (Tahraoui et al., 2007; Abdelrahman et al., 2012). Moreover, date fruit is

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characterized by a rich nutritional value because of its high energy content and carbohydrates (Myhara et al., 2000). Besides, date fruit contains high quantity of polyphenols; the most-studied natural compounds in a wide range of pharmacological activities (Eid et al., 2013; El Arem et al., 2014; Bouhlali et al. 2016b).

Therefore, this study aimed to investigate the anti-inflammatory activity of six varieties of Moroccan date fruit.

2. Materials and methods

2.1. Animals

Male Wistar rats (220–240 g) and male Swiss albino mice (25–30 g) were used in these experiments. The animals were obtained from the animal care facility at the Faculty of Sciences and Techniques, Errachidia, Morocco. They were divided into groups and housed in plastic cages under a 12-h light/dark cycle. This was done in a controlled temperature room (22 ± 2 °C), with free access to water and food. All studies were carried out according to ethical rules of animals' care as described by Zimmermann (1983).

2.2. Plant materials

The current study was performed using six Moroccan date varieties locally known as *Boufgous*, *Bouskri*, *Bousrdon*, *Bousthammi*, *Jihl* and *Majhoul*. The samples were given by Errachidia National Institute for Agricultural Research. They were rinsed, pitted, and stored at – 20°C until analysis.

2.3. Preparation of rich polyphenol extracts

The rich phenolic extract was prepared according to the method of Bouhlali et al. (2016a). Briefly, 30 g of pitted and crushed date fruit were extracted with 150 ml methanol–water (4:1, v/v), at 35 °C for 12 h using an orbital shaker-incubator. The mixture was then filtered and concentrated under reduced pressure at 40 °C until the total evaporation of solvent, using a rotary evaporator. The resulting extract were kept at −20 °C in dark glass bottles until use.

2.4. Identification and quantification of phenolic and flavonoid compounds

Phenolic acids and flavonoidic profiles of date fruit varieties were determined using the high-performance liquid chromatography (HPLC) analysis according to the method used by Zhang et al. (2013a). One g of date fruit extract, dissolved in 25 mL of acidified methanol solution (1 N HCl/methanol/water, 1/80/19, v/v/v), was ultrasonicated for 30 min. Then, the mixture was centrifuged at 1000 g for 15 min. Finally, 2 mL of the supernatant was filtered via a 0.45 μm filter prior to injection. Standards of seven phenolic acids (Caffeic, p-Coumaric, Chlorogenic, Ferulic, Gallic, Vanillic and Syringic acids) and three flavonoids (Luteolin, Quercetin, Rutin) were prepared at a concentration of 100 μg/ml.

Analytical separation was conducted on a Shimadzu liquid chromatograph system (Kyoto, Japan) equipped with an autosampler, dual pump (LC-20AB), a vacuum degasser, diode array detector (SPD-M10A), system controller (SCL-10A). The Restek C18 column (150 × 4.6 mm, 5μm particle size) (Bellefonte, USA) was used for polyphenolic separation at 40 °C. The binary mobile phase consisted of water-acetic acid (97:3, v/v) (eluent A) and acetonitrile (eluent B). Elution was performed at a flow rate of 1 mL.min^{-1} with the following gradient outline: 0–5 min, 0–8% solution B (linear gradient); 5–25 min, 8–25% solution B (linear gradient); 25–30 min, 25% solution B (isocratic elution); 30–50 min, 25–90% solution B (linear gradient). The injection volume was 20 μL, and the wavelengths of detection were set at 280, 320, and 350 nm. The components were identified based on the retention time and UV spectra of phenolic standards, and their quantities were determined using a calibration curve. The results were expressed as milligrams per 100 g of dried weight of date fruit.

2.5. Nitric oxide radical scavenging activity

Nitric oxide scavenging ability was assessed using the Griess reaction according to the method described by Balakrishnan et al. (2009). Five hundred microliters of sodium nitroprusside (10 mM) prepared in phosphate-buffered saline (pH 7.4) was mixed with 1mL of different date fruit extracts at various concentration (100–1500 μg/mL), and incubated at 25 °C for 150 min. After incubation, 1.5 mL of Griess reagent (1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 2.5% phosphoric acid) was added. The same method was repeated with equal volume of buffer and Trolox (20–120 μg/mL) as control and standard, respectively. The absorbance was determined at 546 nm after incubation for 30 min at room temperature. The scavenging ability of nitric oxide by date fruit extract and standards was enumerated using the following formula:

$$\text{NO scavenging activity}(\%) = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100$$

where Abs control is the absorbance without sample, and Abs sample is the absorbance of sample (extract or standard).

IC_{50} is the inhibitory concentration of date fruit extract that reduces 50% of the nitric oxide formation.

2.6. Inhibition of albumin denaturation

The inhibition of albumin denaturation was assessed using the method of Chandra et al. (2012). Briefly, 1 mL of 1% bovine serum albumin (prepared in phosphate-buffered saline, pH 6.4) was added to 1 mL of different date fruit extract at varying concentrations (200–1500 μg/ml). This mixture was left at room temperature for 20 min and then heated at 70 °C for 5 min. The resulting solution was cooled at room temperature and their turbidity was read at 660 nm. The same procedure was repeated with double-distilled water and indomethacin as control and standard, respectively. The inhibition percentage (IP%) of protein denaturation was calculated as follows:

$$\text{Percentage inhibition}(\text{IP}\%) = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs control}} \times 100$$

where Abs control is the absorbance without sample, and Abs sample is the absorbance of sample (extract or standard).

2.7. The membrane stabilization potential

The membrane stabilization potential was assessed using the method established by Murugan and Parimelazhagan (2014). The blood collected from healthy rats was mixed with an equal volume of sterilized Alsever's solution prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% of sodium chloride in distilled water. The resulting blood solution was centrifuged at 3000 rpm; packed cells were washed with an isotonic saline solution (9 g/L). The reaction mixture contained 1 mL of phosphate buffer, 2 ml of hypotonic saline solution (3.6 g/L), 0.5 mL date fruit extract of various concentrations (200–1500 μg/mL), and 0.5 mL of rat red blood cells at 10%. The mixtures were incubated at 37 °C for 30 min, and centrifuged at 3000 rpm. The same procedure was repeated using distilled water and indomethacin as control