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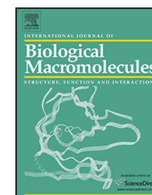

Phytochemical content, Fatty acids composition and antioxidant potential of different pomegranate parts: Comparison between edible and non edible varieties grown in Tunisia

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ABSTRACT

Few comparative studies were interested between edible and non-edible pomegranate varieties. In the present study, flowers, leaves, juice and peel of two pomegranate varieties, ornamental variety “Nana” (NV) and sweet variety “Tounsi” (TV) were compared for their phenolic contents and antioxidant activity. Seeds oils were compared also for their fatty acids (FA) composition.Results showed that peel and juice of NV contained higher content in tannins, Flavonoids and anthocyanins. In contrast, peel and juice of TV were more concentrated in carotenoids and polyphenols. Flowers extracts of TV contained more polyphenols and flavonoids. However leaves from NV provided more polyphenols, flavonoids and anthocyanins. Flowers and leaves proved to be the strongest antioxidants for both varieties. For FA composition, NV was more concentrated in Mono-unsaturated FA (MUFA) however TV contained more Di-unsaturated FA (DiUFA). *cis*-γ-Linolenic acid was the major FA in NV. Contrariwise punicic acid was the predominant in TV.

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1. Introduction

The pomegranate (*Punica granatum* L.), from Punicaceae family is one of the oldest cultivated fruit trees. It originated from the Middle East and was later dispersed to Mediterranean islands and countries, North Asia and Europe [1,2]. Recently, there has been an impressive increase of interest in pomegranate fruits considered as functional food which provided numerous medicinal and nutritional properties [3–6]. In fact, several studies revealed that pomegranate parts including arils, seeds, rind, flowers, bark and roots contain a wide range of bioactive constituents, including polyphenols (as tannins, ellagic acid, flavonoids, anthocyanins...), fatty acids, sterols, terpenoids and alkaloids [7–13]. Nutritionists, food scientists and consumers have payed tremendous attention to polyphenols as strong antioxidants due to their roles in the prevention of degenerative diseases, particularly cancers, cardiovascular and neurodegenerative diseases [14].

Pomegranate was characterized by high genetic diversity of morphological and biochemical quality traits. In fact, more than 500 pomegranate cultivars were identified worldwide [2] and usually, there are two pomegranate types: edible and ornamental types. Edible group is divided into sour, sour-sweet and sweet fruits according to juice taste or organoleptic proprieties [15]. Sourness was correlated with richness in organic acids such as malic and citric acids [16]. Sugars mainly fructose and glucose are known as the main sources of sweetness in pomegranate juice [17]. Ornamental types are often found in India, Russia, China and Turkmenistan. They produces large attractive flowers which has an ornamental significance [18]. Among them, dwarf pomegranate (*Punica granatum* L. var. *nana*) popularly planted in gardens as an ornamental plant. It differs from edible pomegranate by shorter height, orange and red color of flowers and smaller non-edible fruits [19]. Ornamental types possess significant therapeutic use since antiquity. In Unani system of medicine, flowers were used as medicines in various forms (decoction, powder, syrup, nasal drop...) in treat-

Abbreviations: TPC, total polyphenols concentration; TOPC, total O-diphenols concentration; TFC, total flavonoids concentration; TTC, total tannins concentration; TAC, total anthocyanins concentration; TCC, total carotenoids; NSO, Nana seeds oil; TSO, tounsi seeds oil; GAE, gallic acid equivalent; CEQ, catechin equivalent; TAE, tanic acid equivalent; DW, dry weight; FAMES, fatty acids methyl esters; SPSS, statistical package for the social sciences.

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ing wide variety of diseases. Hence, they are used as astringent, haemostatic, antihelminthic, stomachic, desiccant, cicatrizing etc. [19].

In Tunisia, pomegranate is one of the ancient fruit trees. Tunisia is considered as microgenecenter with numerous cultivars. Several studies were interested in pomegranate genetic diversity [20,21], fruit phytochemical composition [22] and phytochemicals from other tree parts [23]. Some studies were conducted to compare between sour and sweet pomegranate for their fruit quality [15]. But, no research was carried out on ornamental types. This prompted us to establish this study to quantify phenolic content (such as total phenols, orhtodiphenols, flavonoids, condensed tannins and anthocyanins) and non-phenolic compounds (carotenoids) and to determine antioxidant activity against DPPH radical and their reducing power of two pomegranate varieties: sweet pomegranate variety named "Tounsi" and non edible pomegranate variety "Nana".

2. Materials and methods

2.1. Plant sample preparation

Flowers, leaves and fruits were harvested from Tounsi and Nana pomegranate trees from two regions in Tunisia (Mahdia and Moknine). Fruits were washed and hand-peeled. Arils were squeezed using a commercial blender (Moulinex, France) to obtain juice. The extracted juice was centrifuged at 15000 rpm for 15 min. Then, the supernatant was recuperated and lyophilized. Seeds were manually washed, dried in the sun until constant weight and crushed to obtain fine powders. Flowers, leaves and fruit peel were sundried and powdered.

Extraction: 0.5 g of each sample (juice, flowers and leaves) was extracted with 10 ml of methanol in the dark for 24 h in a shaker at room temperature. The extracts were centrifuged at 15000 rpm for 15 min and the supernatants were filtered using 0.45 μm filter and stored at − 20 °C until further analysis.

Oil was extracted from pomegranate seeds of two studied varieties by the methods of soxhlet. About 30 g seeds were extracted with 200 ml of hexane at room temperature for 6 h. The solvent was removed by evaporation at 40 °C and the oil was flushed with nitrogen stream and stored at −20 °C in sealed tubes. Lipids contents were expressed as the percentage of lipids in the dry matter of seeds powder.

All samples were stored at + 4 °C until preparing methanolic extracts. For each variety, three trees were chosen as three replications (n = 3).

2.2. Total phenolic concentration (TPC)

TPC was quantified according to the method of Montedoro et al. [24] with slight modifications. Four hundred Microliter of each extract was combined with 1.6 ml water and 10 ml of diluted Folin-Ciocalteu reagent. After 1 min of incubation, 8 ml of sodium carbonate solution (7.5%) were added. The mixture was well shaked and incubated for 6 h. The absorbance was measured at 765 nm using a spectrophotometer (Lambda 25, UV/vis spectrometer). Calibration curve equation was determined using known gallic acid standards solutions. TPC was expressed as mg of gallic acid equivalent per g of dried weight (mg GAE/g DW).

2.3. Total ortho-diphenol concentration (TOPC)

TOPC was determined using the method described by Montedoro et al. [24]. In brief, 100 μl of sample were mixed with 1 ml of HCL (0.5N). Then, 1 ml of solution (a mixture of 10 g $NaNO_2$ + 10 g $NaMoO_4 \cdot 2H_2O$ in 100 ml water) and 1 ml of NaOH solution (1 M) were added. The mixture was incubated in the dark for 30 min and the absorbance was measured at 500 nm. TOPC was expressed as mg of tyrosol/g DW.

2.4. Total flavonoid concentration (TFC)

TFC was determined following the method of Zhishen et al. [25]. One ml of sample was mixed with 4 ml of H_2O and 300 μl of sodium nitrate solution (5%). The mixture was incubated for 5 min. After that, 0.3 ml of aluminum chloride solution (10% w/v) was added. After 6 min of incubation, 2 ml of sodium hydroxide 1 M was added. Then, 2.4 ml of distilled water was added to make volume up to 10 ml. Finally the mixture was shacked vigorously and the absorbance was read at 510 nm. The content of flavonoids was expressed as mg catechin equivalents (CEQ)/g DW.

2.5. Total tannin concentration (TTC)

TTC was determined according to Broadhurst and Jones [26] with minor modifications: 50 μl of sample were mixed with 1.5 ml of methanolic solution of vanillin (4%) and 750 μl of sulfuric acid H_2SO_4. The mixture was incubated in the dark for 15 min and the absorbance was measured at 500 nm. TTC was expressed as mg of tannic acid per g of dried weight (mg TAE/g DW).

2.6. Total anthocyanin concentration (TAC)

The quantification of TA was achieved according to Chung et al. [27]. In brief, 0.5 g of dried sample was extracted with 20 ml of acidified methanol (1% HCL) for 24 h at room temperature in the dark. The absorbance was measured spectrophotometrically at 530 nm and at 657 nm. Then, the absorbance was calculated as A530-(0.24 × A657), where A530 and A657 were, respectively, the absorbance in 530 nm and 657 nm. TTC was expressed as mg of cyaniding-3-glucoside equivalents per 100 g of dried weight according (Giusti and Wrolstad, 2001) using an extinction coefficient ϵ of 26.900 l/mol/cm at 530 nm and a molar mass (MW) of 449.2 g/ mol.

$TAC = (A \times MW \times DF \times V \times 100)/\epsilon \times 100$, where A = absorbance and DF = dilution factor.

2.7. Total carotenoid concentration (TCC)

Total carotenoids were quantified according to the method established by Talcott and Howard [28], with slight modifications. In fact, 2 g of powdered sample were extracted using 25 ml of acetone/ethanol (1:1, v/v) with 200 mg/L butylated hydroxytoluene (BHT) added. After extraction, sample was centrifuged at 1500*g* for 15 min at 4–5 °C. The supernatant was collected, and the remaining residue was re-extracted using the same method until the residue was colorless. Finally, the supernatant was brought to 100 ml with the extraction solvent, and the absorbance was measured at 470 nm. Total carotenoids were calculated following the equation (3) and expressed as milligrams per g of dry weight (DW):

$$\text{Totalcarotenoids} = Ab \times V \times 106/A1\% \times 100G \quad (3)$$

where Ab is the absorbance at 470 nm, V is the total volume of extract, A1% is the extinction coefficient for a 1% mixture of carotenoids at 2500, and G is sample weight (g).

2.8. DPPH free radical scavenging activity

The radical scavenging activity of different parts was determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical as a reagent according to Braca et al. [29] with minor modification and

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