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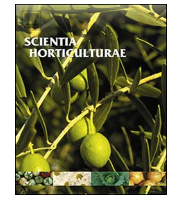

Volatiles from different plant parts of *Punica granatum* grown in Tuscany (Italy)

Rose Vanessa Bandeira Reidel*, Pier Luigi Cioni, Luisa Pistelli

Dipartimento di Farmacia, Università di Pisa, Via Bonanno 6, 56126 Pisa, Italy

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ABSTRACT

The volatiles of different pomegranate plant parts were extracted using headspace solid phase micro-extraction (HS-SPME) and analyzed for the first time by gas chromatography-mass spectrometry (GC–MS) and flame ionization detector (GC-FID). All the data were submitted to multivariate statistical analysis evidencing many differences amongst the selected plant parts. Sesquiterpene hydrocarbons were the most abundant class in the aroma of leaves and in all the flower organs where the main constituents were β-caryophyllene, (*E*, *E*)-α-farnesene and *trans*-α-bergamotene. Stem aroma showed similar content of sesquiterpene hydrocarbons and non-terpenes with α-humulene as main compound. The aroma of all aril parts showed high amount of non-terpenes with the prevalence of aldehydes and esters.

1. Introduction

Pomegranate (*Punica granatum* L.) is a tree belonging the *Punicaceae* family and has more than 500 varieties distributed originally from Central Asia, but extending in Southern Asia Mediterranean area and Europe, North and South America. This plant has deciduous, obovate and coriaceous leaves with bright red flowers. Fruits are berries delimited by a pericarp that contain numerous seeds named arils, surrounded by a translucent pulp and suspended by a white and astringent membrane (Pignatti, 1982; Kumari et al., 2012). This ancient tree was one of the first domesticated fruit being part of the many mythological beliefs (Still, 2006). In Italy, this species was cultivated since ancient times in wet areas especially in Apulia for both food and industrial uses (Russo et al., 2012).

The edible part of the fruits, known as aril, are rich in acids, sugars, vitamins, polysaccharides, polyphenols, minerals and many other bioactive phytochemicals which are responsible of its medicinal benefit, used in treatment and prevention of serious chronic diseases such as cardiovascular disease, diabetes and cancer (Haque et al., 2015; Miguel et al., 2010). Pomegranate fruits are consumed directly as freshy seeds or used to prepare juice, jams, wine or as flavouring and colouring agents. Juice can be used as a homemade remedy to treat anemia and dysentery (Rao et al., 2015). Likewise, this ancient plant has great ethnomedicine value with the utilization of all its parts: barks, bark stems and roots are used as anti-parasitic remedies, expectorant, astringent and mouthwash (Baumann, 2007; Rao et al., 2015; Amjad et al., 2017) while the pericarp is employed to treat gastrointestinal and renal problems (Merzouki et al., 2000; Jouad et al., 2001; Jamila and Mostafa, 2014). Leaves are used in diarrhea and externally for skin problems (Merzouki et al., 2000; Tangjitman et al., 2015; Amjad et al., 2017), while seeds and flowers have anti-inflammatory effect (Baumann, 2007; Khan et al., 2015).

Studies from the literature reported many biological activities of *P. granatum* such as antioxidant (Shubert et al., 1999; Graziano et al., 2016), anti-inflammatory (Colombo et al., 2013), anticancer (Lansky and Newman, 2007; Syed et al., 2007; Izquierdo-Vega et al., 2017; Sharma et al., 2017), anti-parasitic (Williams et al., 2016), analgesic (Nadia et al., 2017), spasmolytic and spasmogenic (Ali et al., 2017), antimicrobial (Wafa et al., 2017), antifungal (Rongai et al., 2017), neuroprotective (Morzelle et al., 2016), anti-cholinesterase and anti-hyperglycemic (Bekir et al., 2016).

The increased production and consumption of pomegranate related to its high nutritional value and considerable health benefits led to the publications of several scientific studies concerning its organoleptic characteristics. The aroma profile gives an important contribution in the consumer acceptance of the fruit and its derivatives contributing to the current success of pomegranates in the world industrial trade. The aroma profile of pomegranate juice from five commercial samples produced in the USA, analyzed by SPME, was a combination of various terpene derivatives mainly represented by β-caryophyllene, α-farnesene and limonene (Vázquez-Araújo et al., 2011). Mayuoni-Kirshinbaum et al. (2012) found similar volatile pattern in the juice made with the same pomegranate cultivar from Israel market. The same authors evidenced the increase of sesquiterpene volatiles during the

* Corresponding author.
E-mail address: rvanessa.reidel@gmail.com (R.V. Bandeira Reidel).

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prolonged storage of commercial pomegranates together with the reduction of their sensory quality (Mayuoni-Kirshinbaum et al., 2013). Moreover, the juice aroma of fruits from nine Spanish cultivars of pomegranate was rich in hexanal, limonene, *trans*-2-hexenal and *cis*-3-hexenol (Melgarejo et al., 2011). Another recent paper reported non-terpene derivatives as abundant volatiles, especially alcohols, in the juice and ground seeds without pulp obtained of five pomegranate cultivars from Turkey (Güler and Gül, 2016). Tripathi et al. (2014) isolated and identified 3-octen-1-yl acetate, α-terpineol, hexanol, ethyl acetate, *trans*-3-hexen-1-ol and acetoin as principal volatiles of pomegranate arils extracted by high vacuum distillation and solvent extraction with diethyl ether. Finally, a recent review evidenced that the pomegranate fruit flavor is commonly constituted by a mixture of many volatiles especially alcohols, aldehydes and terpenes (Mayuoni-Kirshinbaum and Porat, 2014).

The present study deals with the investigation of the aroma profile of different organs from a wild *P. granatum* plant grown in Italy collected during its ontogenetic development to contribute to its chemotaxonomic characterization and to improve its agricultural practices and food trade. This is the first report on the aroma profile of distinct parts of *P. granatum.*

2. Materials and methods

2.1. Plant material

Stems (*Ste*), flower buds (*Fb*), stamens (*Sta*), fruit exocarp (*Ex*), leaves (*Le*), dried seeds without pulp (*DS*), entire fresh seeds with pulp (*FS*) and squeezed fresh seeds (*SFS*) were obtained from a wild plant grown around Pisa (43°44′19.2″N 10°27′41.3″E). All the plant parts were analyzed separately. *Punica granatum*, investigated in the present study (Fig. 1), was an old wild shrub (about 50 years old) collected in the open countryside where no other plants of the same species were present. The plant was identified by one of the authors (Pier Luigi Cioni) and a voucher specimen (Erbario Generale – N.A. 5501 *Punica granatum*/3) deposited at the Herbarium of the Botanical Garden (University of Pisa, Italy).

2.2. Solid phase micro extraction analyses (SPME)

The analyses of the volatile organic compounds (VOCs) from different plant organs were performed using fresh plant material by Supelco SPME device coated with polydimethylsiloxane (PDMS, 100 μm). All samples were analyzed immediately after harvesting. The fresh plant material was introduced separately into a glass vial (5 ml, filled up with 1.5 ml of sample) covered with aluminum foil and left to equilibrate for 20 min at room temperature. Multiple experiments were conduct to find the best condition of SPME analysis and then we performed a single measurement. After the equilibration time, the fibre was inserted into the vial and exposed to the headspace for 20 min at room temperature. Then, the fibre was withdrawn into the needle and transferred to the injector of the GC and GC–MS system, where the fibre was desorbed for 30 min.

2.3. GC-FID analysis

The GC analyses were accomplished with an HP-5890 series II instrument equipped with dual FID and two silica capillary columns (30 m x 0.25 mm; film thickness: 0.25 μm), a HP-Wax and a DB-5. The oven temperature was programmed isothermal at 60 °C for 10 min and then rising from 60 °C to 220 °C at 5 °C/min; injector temp., 250 °C; detector temp., 250 °C; carrier gas, N_2 (2 ml/min).

2.4. GC/MS analysis

GC–MS analysis were carried out with a Varian CP-3800 gas chromatograph equipped with a DB 5 capillary column (30 m x 0.25 mm; coating thickness: 0.25 μm) and a Varian Saturn 2000 ion trap mass

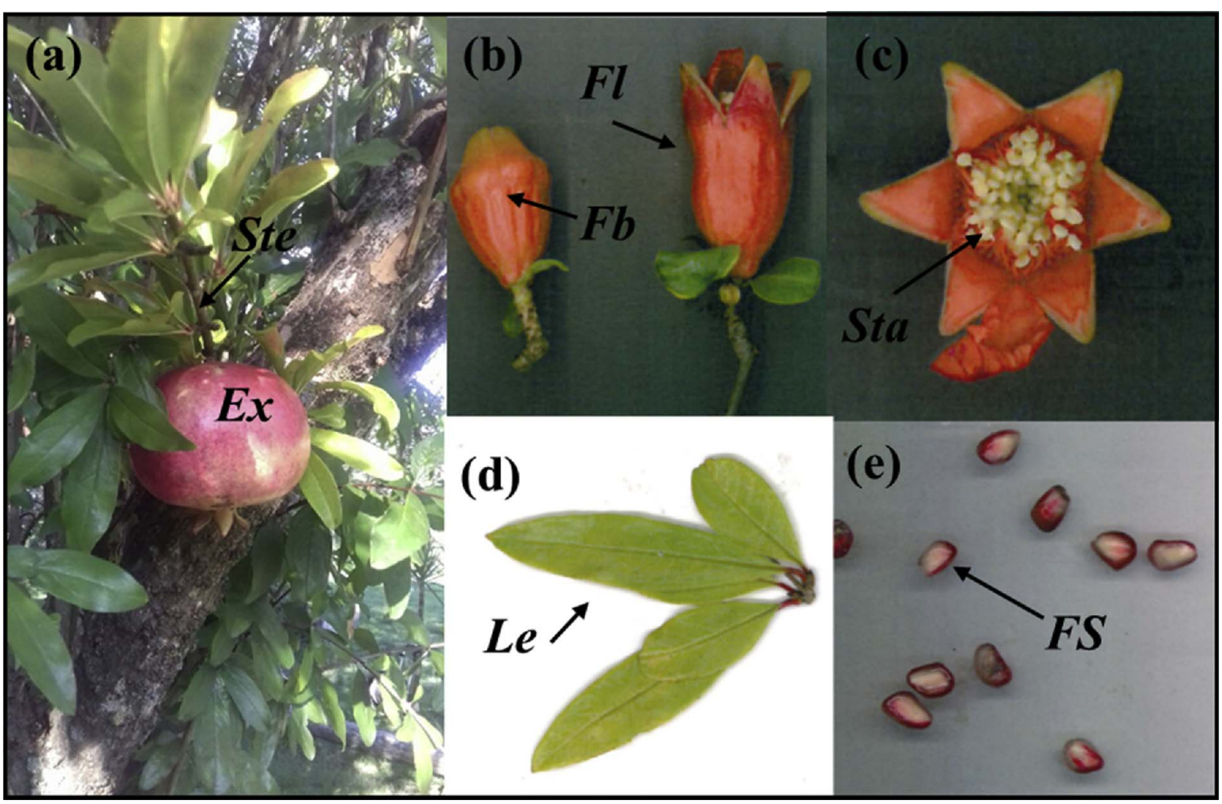

Fig. 1. Parts of *Punica granatum* analyzed: (a) Stems (*Ste*), exocarp (*Ex*); (b) both flower stages of development, flower bud (*Fb*) and blossoming flower (*Fl*); (c) Stamens (*Sta*); (d) leaves (*Le*); (e) fresh seeds (*FS*).