Industrial Crops & Products 111 (2018) 345–352

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Research Paper

Antimicrobial and antioxidant features of 'Gabsi' pomegranate peel extracts

Samira Kharchoufi[a], Fabio Licciardello[b], Laura Siracusa[c], Giuseppe Muratore[b], Mokthar Hamdi[a], Cristina Restuccia[b,*]

[a] Laboratory of Microbial Ecology and Technology, National Institute of Applied Sciences and Technology (INSAT), University of Carthage, Centre Urbain Nord, 2 Boulevard de la Terre, B.P. 676, 1080 Tunis, Tunisia
[b] Department of Agriculture, Food and Environment (Di3A), University of Catania, Via S. Sofia 100, 95123 Catania, Italy
[c] CNR-ICB, Consiglio Nazionale delle Ricerche-Istituto di Chimica Biomolecolare, Via Paolo Gaifami 18, 95126 Catania, Italy

ARTICLE INFO

Keywords:
Antioxidant activity
Bacteria
Fungi
Hydrolysable tannins
Polyphenols
Pomegranate peel

ABSTRACT

The work aimed at optimizing the extraction protocol of 'Gabsi' pomegranate peel (PP) and evaluating the total phenol content and profile, antioxidant activity and antimicrobial effect of the extracts obtained. Water and methanol as solvents were compared at different peel/solvent ratios; three different temperatures were also compared for the water extraction. Methanol pomegranate peel extract (MPPE) and water extracts (WPPE) were analysed for their DPPH antioxidant activity and polyphenol profile and content by HPLC/DAD and HPLC/MS. Different concentrations of MPPE and WPPE (ranging from 0.061 to 0.304 and from 0.072 to 0.361 g dry extract/mL, respectively) were also tested *in vitro* against *Penicillium digitatum*, *Pseudomonas putida* and *Saccharomyces cerevisiae* to determine the minimum inhibitory concentration (MIC). The MPPE and WPPE had comparable antioxidant activity (4081.43 and 3497.02 mmol Trolox/g, respectively). Hydrolysable tannins represented the main polyphenols in pomegranate peel, with punicalagin as the major compound. In general, MPPE dilutions tested in well-diffusion assays were more effective against targeted microorganisms compared with WPPE; indeed, only the highest WPPE concentration, was able to inhibit the growth of targeted microorganisms. Viability tests in liquid medium confirmed the highest antimicrobial capacity of MPPE, although even the WPPE was able to reduce *P. putida* and *S. cerevisiae* populations by up to 3.15 and 2.52 log CFU/mL, respectively.

1. Introduction

Pomegranate (*Punica granatum*) is a small tree originating from the Middle East and now extending throughout the Mediterranean, China, India, South Africa and America (Endo et al., 2010). 'Gabsi' is a Tunisian pomegranate cultivar well-known for its appreciable sensory quality and high value, representing approximately 35% of the total production (Emna, 2010). It has medium weight and size (330 g, 70–80 mm), red-colored juice and average juice yield exceeding 70 mL/100 g of arils (Zaouay et al., 2012). Popularity of pomegranate has increased tremendously especially in the last decade because of the proved antimicrobial, anti-viral, anti-cancer, potent anti-oxidant and anti-mutagenic effects of the fruit (Çam and Hısıl, 2010). Pomegranate fruit is composed of three parts: seeds, juice, and peel (Lansky and Newman, 2007); in particular, peels have been traditionally used as natural remedies against infectious diseases (Howell and D'Souza, 2013). In the traditional Chinese medicine, peels are considered as a powerful astringent and anti-inflammatory agent and are applied in the treatment of traumatic haemorrhage, ulcers and infections, and disorders of the digestive tract such as diarrhea and dysentery (Mo et al., 2013). In India, Tunisia, and Guatemala, water decoction of dried pomegranate peels is employed as a cure for aphthae and ulcers (Lansky and Newman, 2007).

Pomegranate peel represents about 40–50% of the total fruit weight (Çam and Hısıl, 2010; Gullon et al., 2016); it is produced as byproduct in huge amounts by the food industry and it is an important source of bioactive compounds (Li et al., 2006; Hasnaoui et al., 2014), including hydrolysable tannins, consisting of gallic acid and ellagic acid esters of core polyol molecules (Fischer et al., 2011; for an exhaustive review on tannins and their structures, please see Khanbabaee and Van Tee (2001). Examples of hydrolysable tannins present in pomegranate peel include hexahydroxydiphenic acid (HHDP) and its derivatives, ellagic acid and its derivatives, punicalin, and punicalagin, which is unique to this matrix (Çam and Hısıl, 2010; Romeo et al., 2015; Gullon et al., 2016). Mansour et al. (2013) investigated phenolic compounds present in 21 'Gabsi' pomegranate accessions by methanol and water

* Corresponding author.
E-mail address: crestu@unict.it (C. Restuccia).

http://dx.doi.org/10.1016/j.indcrop.2017.10.037
Received 24 October 2016; Received in revised form 21 September 2017; Accepted 22 October 2017

extraction, identifying gallic acid, ellagic acid, caffeic acid, *p*-coumaric acid, quercetin, and vanillic acid as the predominant compounds; they also proved the antibacterial activity of the extracts by the disc diffusion method against *Staphylococcus aureus*, *S. epidermidis*, *Salmonella typhimurium*, *Escherichia coli* and *Enterococcus faecalis,* with different efficacy depending on the 'Gabsi' accession.

Management of microbial spoilage throughout the food chain has a tremendous contribution to curtail pre- and post-production losses. In emerging trends of organic food production, natural antimicrobials have proved to be reliable alternatives to chemical fungicides, bactericides and pesticides (Aloui et al., 2014; Akhtar et al., 2015; Hintz et al., 2015). Therefore, the interest towards plant extracts, especially from byproducts such as fruit peels, with potential use in food preservation and pharmaceutical purposes, has greatly increased in recent years (Singh et al., 2002; Al-Zoreky, 2009).

This study, carried out on 'Gabsi', one of the most well-known and valuable pomegranate cultivars in Tunisia, aimed at comparing the total phenol content and profile, antioxidant activity and *in vitro* antimicrobial effectiveness against bacteria, yeasts and molds of methanol pomegranate peel extract (MPPE) and water pomegranate peel extract (WPPE).

2. Materials and methods

2.1. Chemicals

All solvents and reagents used in this study were high purity laboratory solvents by Carlo Erba (Milano, Italy); HPLC grade water and acetonitrile were obtained from VWR (Milano, Italy). Punicalagin (as mixture of isomers), gallic acid, ellagic acid, DPPH$^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl) and Trolox were purchased from Sigma-Aldrich s.r.l. (Milano, Italy).

2.2. Preparation of pomegranate peel extracts

'Gabsi' pomegranate fruits were rinsed with water and manually peeled. The collected peels were then dried at 35 °C for 2 days and maintained at −20 °C in vacuum-sealed packages until use. Five-gram portions of finely-powdered peels were mixed with 150, 75 and 50 mL of methanol or water, to obtain, respectively, methanol pomegranate peel extracts (MPPE) and water pomegranate peel extracts (WPPE). The mixtures were then stirred for 4 h at different temperatures (Table 1) prior to centrifugation at 7000*g* for 20 min at 4 °C. The clear extracts were membrane filtered (pore size 0.45 μm): then, MPPE were concentrated under reduced pressure at 40 °C and further dried in oven at 40 °C overnight, while WPPE were freeze-dried. Dried extracts were finally re-dissolved in 1.5 mL of distilled water.

2.3. Determination of total polyphenol content (TPC) and antioxidant activity by the DPPH$^{\cdot}$ radical scavenging method

TPC determination was carried out by the Folin–Ciocalteu method adapted to a microscale (Waterman and Mole, 1994; Arnous et al., 2002). Results were expressed as mg gallic acid equivalents (GAE)/g dry matter.

Antioxidant activity of pomegranate peel extracts was evaluated through the measurement of the free-radical scavenging capacity by the DPPH$^{\cdot}$ (2,2-diphenyl-1-picrylhydrazyl) assay (Hegedüs et al., 2011; Licciardello et al., 2015). An aliquot (900 μL) of methanolic DPPH$^{\cdot}$ solution (100 μM) was mixed with 100 μL of each sample. The mixture was shaken vigorously and left to react for 30 min in the dark at room temperature. The absorbance at 517 nm was recorded to determine the concentration of the remaining DPPH$^{\cdot}$. All measurements were performed in triplicate and results were expressed as mmol Trolox/g.

Table 1
Extraction conditions and relative codes for pomegranate peel extracts (PPE).

Sample code	Extraction solvent	Solvent/pomegranate peel powder (v/w)	Extraction temperature (°C)
MPPE1	methanol	30	25
MPPE2	methanol	20	25
MPPE3	methanol	15	25
WPPE1	water	30	40
WPPE2	water	30	55
WPPE3	water	30	75
WPPE4	water	20	75
WPPE5	water	15	75

2.4. HPLC/DAD and HPLC/ESI/MS analyses

Chromatographic analyses were carried out on an Ultimate 3000 UHPLC focussed instrument equipped with a binary high pressure pump, a photodiode array detector, a thermostated column compartment and an automated sample injector (Thermo Fisher Scientific, Inc., Italy). Collected data were processed through a Chromeleon Chromatography Information Management System v. 6.80. Chromatographic runs were all performed using a reverse-phase column (Gemini C_{18}, 250 × 4.6 mm, 5 μm particle size, Phenomenex, Italy) equipped with a guard column (Gemini C_{18} 4 × 3.0 mm, 5 μm particle size, Phenomenex, Italy). Pomegranate peel polyphenols were eluted with the following gradient of B (1% formic acid in acetonitrile) in A (1% formic acid in water): 0 min: 10% B; 20 min: 35% B; 25 min: 10% B. The solvent flow rate was 1 mL/min, the temperature was kept at 25 °C, and the injector volume selected was 10 μL. DAD analyses were carried out in the range between 700 and 190 nm. Quantification was carried out at 280 nm for HHDP and pedunculagin II using gallic acid (R_2 = 09999) as external standard; the same wavelength was used for the quantification of punicalagins using the corresponding standard (punicalagin, R_2 = 09997) to build the calibration curve. Ellagic acid and its derivatives were quantified at 360 nm using ellagic acid (R_2 = 09997) as reference.

ESI mass spectra were acquired by a Thermo Scientific Exactive Plu Orbitra MS (Thermo Fisher Scientific, Inc., Italy), using a heated electrospray ionization (HESI II) interface. Mass spectra were recorded operating in negative ion mode in the *m/z* range 120–1500 at a resolving power of 25000 (full-width-at-half-maximum, at *m/z* 200, RFWHM), resulting in a scan rate of > 1.5 scans/sec when using automatic gain control target of 1.0×10^6 and a C-trap inject time of 250 ms under the following conditions: capillary temperature 300 °C, nebulizer gas (nitrogen) with a flow rate of 60 arbitrary units; auxiliary gas flow rate of 10 arbitrary units; source voltage 3 kV; capillary voltage 82.5 V; tube lens voltage 85 V. The Orbitrap MS system was tuned and calibrated in positive modes, by infusion of solutions of a standard mixture of sodium dodecyl sulfate (Mr 265.17 Da), sodium taurocholate (Mr 514.42 Da) and Ultramark (Mr 1621 Da). Data acquisition and analyses were performed using the Excalibur software. Analyses were carried out in triplicate; results are reported in milligram (mg) of compound per gram (g) of vegetable material.

2.5. Targeted microorganisms

To test the antimicrobial efficacy of the extracts, *Pseudomonas putida*, *Saccharomyces cerevisiae* and *Penicillium digitatum*, belonging to Di3A (Department of Agriculture, Food and Environment, University of Catania) microbial collection, were chosen as representative of different microbial groups responsible for food spoilage. The microbial cultures were maintained on Potato Dextrose Agar (PDA; Oxoid, Basingstoke, UK) for *P. digitatum*, Nutrient Agar (NA; Oxoid) for *P. putida* and Sabouraud Dextrose Agar (SDA; Oxoid) for *S. cerevisiae* before antimicrobial tests.