Food Chemistry 166 (2015) 407–413

Contents lists available at ScienceDirect

Food Chemistry

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

Bioactivity of Meeker and Willamette raspberry (*Rubus idaeus* L.) pomace extracts

Dragana D. Četojević-Simin [a,*], Aleksandra S. Velićanski [b], Dragoljub D. Cvetković [b], Siniša L. Markov [b], Gordana S. Ćetković [b], Vesna T. Tumbas Šaponjac [b], Jelena J. Vulić [b], Jasna M. Čanadanović-Brunet [b], Sonja M. Djilas [b]

[a] University of Novi Sad, Faculty of Medicine, Oncology Institute of Vojvodina, Dr Goldmana 4, 21204 Sremska Kamenica, Serbia
[b] University of Novi Sad, Faculty of Technology, Bul. Cara Lazara 1, 21000 Novi Sad, Serbia

ARTICLE INFO

Article history:
Received 20 January 2014
Received in revised form 8 May 2014
Accepted 11 June 2014
Available online 19 June 2014

Keywords:
Raspberry pomace
Free radical-scavenging activity
Antitumor activity
Apoptosis
Antibacterial activity

ABSTRACT

Taking into account the substantial potential of raspberry processing by-products, pomace extracts from two raspberry cultivars, Meeker and Willamette, were investigated. Total phenolic, flavonoid and anthocyanin contents were determined. Willamette pomace extract (EC_{50} = 0.042 mg/ml) demonstrated stronger 2,2-diphenyl-1-picrylhydrazyl DPPH radical-scavenging activity than did Meeker pomace extract (EC_{50} = 0.072 mg/ml). The most pronounced cell growth inhibition effect was obtained in the breast adenocarcinoma cell line, reaching EC_{50} values of 34.8 and 60.3 μg/ml for Willamette and Meeker extracts, respectively. Both extracts demonstrated favourable non-tumor/tumor cell growth ratios and potently increased the apoptosis/necrosis ratio in breast adenocarcinoma and cervix carcinoma cells. In reference and wild bacterial strains, minimal inhibitory concentrations (MIC) were achieved in a concentration range from 0.29 to 0.59 mg/ml, and minimal bactericidal concentrations (MBC) in a range from 0.39 to 0.78 mg/ml. The results indicate significant antioxidant, antiproliferative, proapoptotic and antibacterial activities of raspberry pomace and favour its use as a functional food ingredient.

1. Introduction

Berry fruits are renowned for their high concentrations of bioactive compounds such as anthocyanins, flavonols, catechins and hydroxybenzoic acids with demonstrated antioxidant, antimicrobial, anti-inflammatory, vasodilatory, antiproliferative and anticancer activities (Bobinaite, Viškelis, & Rimantas Venskutonis, 2012) and are thus considered as a top class of healthy food (Jimenez-Garcia et al., 2013; Mikulic-Petkovsek, Slatnar, Stampar, & Veberic, 2012; Paredes-Lopez, Cervantes-Ceja, Vigna-Perez, & Hernandez-Perez, 2010).

Raspberry (*Rubus idaeus* L.) is known as a rich source of dietary antioxidants, such as phenolic acids (ellagic acid and its conjugates, ellagitannins – lambertianin C and sanguiin H-6), flavonoids (flavan-3-ols and their oligomers, quercetin) and anthocyanins (cyanidin-3-sophoroside, cyanidin-3-(2-glucosylrutinoside), cyanidin-3-glucoside, pelargonidin-3-sophoroside, cyanidin-3-rutinoside, pelargonidin-3-(2-glucosylrutinoside), pelargonidin-3-glucoside, pelargonidin-3-rutinoside) (Bobinaite et al., 2012; Chen, Wang, Rosen, & Ho, 1999). Besides phenolic compounds, raspberries contain vitamin C, dietary fibres, α-tocopherol, tocotrienol, calcium, potassium, magnesium, carotenoids and linoleic acid (Lee, Dossett, & Finn, 2012; Zhang et al., 2010). Among antioxidant properties, raspberries have also shown other beneficial bioactivities, including anti-inflammatory, anti-proliferative (in human liver, breast, colon, and prostate cancer cells), antineurodegenerative, antiviral and antibacterial activities (Bobinaite et al., 2012; Nohynek et al., 2006; Pantelidis, Vasilakakis, Manganaris, & Diamantidis, 2007; Zhang et al., 2010). Not only the fruit, but also the raspberry leaves and roots have long traditions as medicinal agents. Infusions from the leaves are traditionally used for easing childbirth-related muscle spasms, morning sickness, colds, sour throats, diarrhoea, threat wounds, colic pain and as a uterin

Abbreviations: CyGE, cyanidin-3-glucoside equivalents; DMEM, Dulbecco's modified eagles medium; DMSO, dimethyl sulphoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; EC_{50}, half maximal effective concentration; EDTA, ethylenediamine tetraacetic acid; FCS, fetal calf serum; GAE, gallic acid equivalents; HeLa, cervix epithelioid carcinoma; HT-29, colon adenocarcinoma; MBC, minimal bactericidal concentration; MCF7, breast adenocarcinoma; MIC, minimal inhibitory concentration; MRC-5, human fetal lung; RE, rutin equivalents; SA, scavenging activity; SRB, sulphorhodamine B; TAc, total anthocyanin content; TFd, total flavonoid content; TPh, total phenolic content.

* Corresponding author. Tel.: +381 21 480 5588; fax: +381 21 661 3741.
E-mail address: ddaaggeerr@gmail.com (D.D. Četojević-Simin).

http://dx.doi.org/10.1016/j.foodchem.2014.06.063
0308-8146/

relaxant. The root of the plant is traditionally used for wound cleaning and relief of sore throats (Ryan, Wilkinson, & Cavanagh, 2001; Venskutonis, Dvaranauskaite, & Labokas, 2007).

Antimicrobial activity of plant phenolics has been intensively studied, and, in addition to controlling invasion and growth of plant pathogens, their activity against human pathogens has been investigated to characterise and develop new healthy food ingredients, medical compounds, and pharmaceuticals (Nohynek et al., 2006). So, raspberry juice and cordial have displayed growth inhibition of *Staphylococcus aureus*, *Escherichia coli*, *Mycobacterium phlei*, *Clostridium perfringens*, *Alcaligenes faecalis*, *Enterococcus faecalis*, *Shigella soneri* and three *Salmonella* serovars (Ryan et al., 2001). Raspberry fruit extracts cause death of the *Helicobacter pylori*, very strong inhibition of *Bacillus cereus*, *S. aureus* and *Staphylococcus epidermidis*, and strong inhibition of *Campylobacter jejuni* and *C. perfringens*. Strong antibacterial activity was attributed to phenolic compounds, especially the ellagitannin fraction (Nohynek et al., 2006). The first results of antibacterial activity of raspberry cultivars grown in Serbia, tested by the agar diffusion method, showed that raspberry fruit extracts inhibited growth of all tested Gram-positive and Gram-negative bacteria (Velićanski, Cvetković, & Markov, 2012).

The fragility and limited shelf-life contribute significantly to the nutritional and microbiological deterioration of raspberry, leading to diminished quality and health benefits (Bower, 2007). Consequently, small amounts of raspberries are consumed fresh or frozen. Greater amounts are processed to jam, juice, jelly, syrup and ingredients of various foods (Byamukama, Kiremire, Andersen, & Steigen, 2005). Juice pressing is a common way of industrial raspberry processing. During this processing, a considerable amount of raspberry pomace, which consists of the pulp/peel and the seeds, is generated. By-products of plant food processing, including raspberry pomace, represent a major disposal problem for the industry concerned, even though they are promising sources of compounds which may be used for their high nutritional and excellent technological properties (Schieber, Stintzing, & Carle, 2001).

Due to the favourable climate, Serbia is one of the largest manufacturers and exporters of raspberries (*R. idaeus* L.) in the world. Between 90% and 95% of cultivated raspberries in Serbia is the North American Willamette cultivar, characterised by a dark red colour, large, firm, nearly round berry, with excellent taste. The Meeker cultivar has a large, thimble-shaped, dark red berry with high sugar content and excellent flavour but is cultivated less favourably (Djurkovic, 2012).

Taking into account the renowned biological activity of raspberry and substantial potential of the plant processing by-products, in this study pomace extracts from two raspberry cultivars, Meeker and Willamette, were used to determine: (1) the total phenolic, flavonoid and anthocyanin contents (2) 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity (3) cell growth activity in a panel of human tumor and non-tumor cell lines (4) the mechanism of induced cell death i.e. apoptosis and necrosis and (5) minimal inhibitory and minimal bactericidal concentrations against reference and wild strains.

2. Material and methods

2.1. Pomace extraction

Two raspberry (*R. idaeus* L.) cultivars (Meeker and Willamette) were obtained from "Alfa RS", Lipolist, Serbia. Samples of the raspberry were stored at −20 °C prior to analysis. Raspberry pomace from both cultivars was obtained after juice separation. The yields of the Meeker and Willamette pomaces were 32.71% and 34.02%, respectively.

Samples of the raspberry pomaces (70 g) were extracted two times, for 60 min (560 ml) and 30 min (280 ml), at room temperature, using a homogenizer (Ultraturax, DIAX 900, Heidolph Instruments GmbH, Kelheim, Germany). The extraction was performed with 80% methanol aqueous solution containing 0.05% acetic acid. The obtained extracts were combined and evaporated to dryness under reduced pressure and lyophilised (Alpha 2-4 LSC Martin Christ, Osterode, Germany). The yields of the lyophilised raspberry pomace extracts from Meeker and Willamette cultivars were 9.98% and 9.43%, respectively.

2.2. Samples and standards

Lyophilised extracts were re-dissolved in sterile distilled water to obtain 25 mg/ml of stock solution for the evaluation of antioxidant and antibacterial activities, or in DMSO (dimethyl sulphoxide) to obtain a 500 mg/ml stock solution for the evaluation of cytotoxic activity and cell death. Extracts were investigated in the concentration ranges 0.005–0.2, 0.005–2.5 and 0.195–25 mg/ml in the radical-scavenging, cell growth and antimicrobial assays, respectively. For the evaluation of the cell growth activity of berry extracts, standard solutions and drugs were also tested. Ellagic and gallic acids and quercetin were diluted in DMSO. Vitamin C, Aspirin®, Doxorubicin® and Gemzar® were diluted in 9 mg/ml NaCl and sterilised using 0.22 μm syringe filters (Sartorius, Germany). Standard solutions were investigated in the concentration range 0.002–0.5 mg/ml and Gemzar®, Doxorubicin® and Aspirin® in the ranges 0.003–31.3 μg/ml, 0.006–58.1 μg/ml and 0.06–1 mg/ml, respectively.

2.3. Total phenolic, flavonoid and anthocyanin contents

Total phenolic content (TPh) in raspberry pomace extracts was determined spectrophotometrically according to the Folin–Ciocalteu method (Kalt, McDonald, Ricker, & Lu, 1999), calibrating against gallic acid and expressing the results as gallic acid equivalents (GAE) in mg per gramme of dried extracts. Total flavonoid content (TFd) was measured by the aluminium chloride spectrophotometric assay (Zhishen, Mengcheng, & Jianming, 1999), determined from the regression equation of the rutin calibration curve, and expressed as rutin equivalents (RE) in mg per gramme of dried extracts. The total anthocyanin content (TAc) was estimated spectrophotometrically, using the pH single method according to Lee, Durst, and Wrolstad (2006). Anthocyanins were quantified as cyanidin-3-glucoside equivalents (CyGE), using an extinction coefficient of 26,900, in l/mol × cm, and resulting values were expressed in terms of mg cyanidin-3-glucoside equivalents per gramme of dried extracts.

2.4. DPPH radical-scavenging activity

The DPPH radical-scavenging activity (SA) of raspberry pomace extracts was determined spectrophotometrically, using the modified DPPH method of Chen et al. (1999). Briefly, 1 ml of extract solution or 1 ml of distilled water (blank) was mixed with 2 ml of DPPH solution (2 mg of DPPH• was dissolved in 50 ml of methanol). The mixture was shaken vigorously and left at room temperature for 30 min, and then the absorbance was read at 517 nm, using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

The ability to scavenge the DPPH radicals (scavenging activity, SA) was calculated as $100 \times (ABL - AT)/ABL$ (%), where *ABL* is the absorbance of the blank, and *AT* is the absorbance of the treatment. The EC50 (half maximal effective concentration), defined as the concentration of extract required for 50% scavenging of DPPH radicals under experimental conditions employed, was used to