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BIOACTIVITY OF BLACKBERRY (*Rubus fruticosus* L.) POMACE: POLYPHENOL CONTENT, RADICAL SCAVENGING, ANTIMICROBIAL AND ANTITUMOR ACTIVITY

Dragana D. Četojević-Simin[1], Aleksandra S. Ranitović[2], Dragoljub D. Cvetković[2], Siniša L. Markov[2], Milica N. Vinčić[2], Sonja M. Djilas[2]*

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

A large volume of industrial waste of biological origin is produced annually worldwide, causing a serious disposal problem even though it is a huge source of beneficial compounds which may be used for their high nutritional and bioactive properties. The bio-potential of blackberry pomace (waste) obtained after juice separation from the Čačanska bestrna and Thornfree cultivars was evaluated. Higher amounts of total and monomeric anthocyanins, total phenolics, and total flavonoids were found in Thornfree pomace extract and demonstrated stronger 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (2.12 mmol TEAC g^{-1}) than Čačanska bestrna pomace extract (1.03 mmol TEAC g^{-1}). Both extracts highly increased apoptosis/necrosis ratios in all investigated cell lines. The highest cell growth inhibition effects (EC_{50}=52.5 - 64.7 µg mL^{-1}) and the highest increase of apoptosis (AI=12.2) were obtained in breast adenocarcinoma cell line. Both blackberry pomace extracts inhibited the growth of all tested microorganisms. In the reference and wild bacterial strains MIC and MBC/MFC were achieved in the 0.39-25 mg mL^{-1} and 0.78-25 mg mL^{-1} range, respectively. Blackberry cultivar pomaces are rich source of phytochemicals with significant health promoting properties that could be further utilized as beneficial food ingredients.

KEY WORDS: blackberry pomace; DPPH radical scavenging; cell growth; bactericidal activity; fungicidal activity

INTRODUCTION

During the production of juices, concentrates, jams, and jellies, the generated berry fruit pomace consists of the pulp, peel and the seeds (1). Huge volumes of waste are produced in the food industry after fruit processing worldwide, causing a serious disposal problem (2). Berry fruit pomace contains high levels of essential minerals, vitamins C

* Corresponding author: Dragana Četojević-Simin, University of Novi Sad, Faculty of Medicine, Oncology Intitute of Vojvodina, Dr Goldmana 4, 21204 Sremska Kamenica, Serbia, e-mail: cetojevic.dragana@onk.ns.ac.rs

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and E, fatty acids, fibers and polyphenols such as anthocyanins, phenolic acids, flavanols, and tannins (1, 3), which could be further utilized due to their proven health benefits. Blackberry pomace can even be used after extended frozen storage (1).

Rubus fruticosus (Linnaeus) is a perennial shrub famous for its fruit which is traded globally due to its delicious taste. The leaves are used in traditional phytotherapy in Mediterranean countries for the treatment of wounds, sores, scratches, gum inflammations, ulcers and sore throat (4-6). Berry fruits have a high content of bioactive compounds that possess antioxidant, anticarcinogenic, vasodilatory and antimicrobial activity (7, 8). Compared to other berries, blackberries contain high levels of ellagitannins and abundance of healthy antioxidants such as flavonoids, salicylic acid, ellagic acid, and fiber that have also been recognized for their antitumor effects (9-11).

Beside their nutritional value, fruits, vegetables, herbs, and spices have been traditionally used to cure various human ailments (6). Increased consumption of fruits and vegetables is recommended in dietary guidelines worldwide for their beneficial health effects (9, 12, 13). Further, broad use of antibiotics to cure microbial infections has made bacteria and fungi resistant to common commercial antibiotics (14). Natural products as antimicrobials are a promising alternative to control foodborne bacterial infections. Previous results concerning the antibacterial activity of berry fruits showed that raspberry fruit extract inhibited growth of *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus*, *Staphylococcus saprophyticus* and *Listeria monocytogenes* (15, 16). On the other hand, blackberry juice stimulated growth of beneficial *Lactobacillus* strains (10). Apart from the inhibition of bacteria growth, blackberry extract inhibited the early stages of herpes simplex virus type 1 (HSV-1 replication and had potent virucidal activity (17).

In this study, the juice processing by-product, the pomace extracts from two blackberry cultivars, Čačanska bestrna and Thornfree were used to determine the contents of total phenolics, total flavonoids, and total and monomeric anthocyanins. Radical scavenging activity was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Antitumor *in vitro* activity, as well as cell death, were also evaluated. Antimicrobial activity was evaluated in a panel of reference and wild strains.

MATERIAL AND METHODS

Pomace extraction and sample preparation

The blackberry cultivars Čačanska bestrna and Thornfree were obtained from ″Alfa RS″, Lipolist, Serbia and were stored at -20 °C prior to analysis. The blackberry pomace was prepared by pressing the unfrozen fruits through a cheesecloth. The yields of the Čačanska bestrna and Thornfree pomaces were 228 g kg^{-1} and 276.4 g kg^{-1}, respectively.

They pomaces were extracted with 80% methanol aqueous solution containing 0.05% acetic acid, and lyophilized according to a previously reported procedure (16). The yields of the lyophilized blackberry pomaces from Čačanska bestrna and Thornfree cultivars were 86.5 g kg^{-1} and 90.3 g kg^{-1}, respectively.

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The lyophilized extracts were re-dissolved in sterile distilled water to obtain 10 mg mL^{-1} stock solution for the evaluation of antioxidant and antimicrobial activity, or in DMSO (dimethyl sulphoxide) to obtain 500 mg mL^{-1} stock solution for the evaluation of cytotoxic activity and cell death. The extracts were investigated in the concentration ranges from 0.0017-0.0833, 0.005-2.5 and 0.195-25 mg mL^{-1} in the radical scavenging, cell growth and antimicrobial assay, respectively.

Polyphenol antioxidants content and DPPH radical scavenging activity

Total phenolics content (TPh) in blackberry pomace extracts was determined spectrophotometrically according to the Folin-Ciocalteu method (18), by calibrating against gallic acid and expressing the results as gallic acid equivalents (GAE) in g kg^{-1} of dried extracts and fresh pomace. Total flavonoids (TFd) content was measured by the aluminum chloride spectrophotometric assay (19), determined from the regression equation of the rutin calibration curve, and expressed as rutin equivalents (RE) in g kg^{-1} of dried extracts and fresh pomace. The total and monomeric anthocyanins content (TAc, MAc) was estimated spectrophotometrically using the pH single and differential method according to Lee et al. (20). Anthocyanins were quantified as cyanidin-3-glucoside equivalents (CyGE) using an extinction coefficient of 26,900 in L $mol^{-1}cm^{-1}$, and resulting values were expressed in terms of cyanidin-3-glucoside equvalents in g kg^{-1} of dried extracts and fresh pomace.

The DPPH radical scavenging activity (SA) of blackberry pomace extracts was determined according to a previously described procedure (16).

Cell lines, cell growth activity and cell death detection

Cell growth activity was evaluated *in vitro* in human cell lines: HeLa (cervix epitheloid carcinoma), MCF7 (breast adenocarcinoma), HT-29 (colon adenocarcinoma) and MRC-5 (fetal lung).

Cell growth activity was determined after 48 h exposition time according to the previously described procedure (16), using colorimetric sulforhodamine B (SRB) assay (21).

Apoptosis and necrosis were detected using the Cell Death Detection ELISA^{PLUS} kit, Roche (Version 11.0). The cell death detection experiments were performed in all cell lines and for the most active extracts obtained in the cell growth experiments (16). Depending on the extract and the cell line used, the concentrations were in the 420-700 μg mL^{-1} range (for the 2 h exposition time).

Test microorganisms and antimicrobial assays

The antimicrobial activity was evaluated using the reference and wild strains (^{w}) of Gram-positive and Gram-negative bacteria (16) and yeasts (*Saccharomyces cerevisiae* 112 Hefebank Weihenstephan and *Candida albicans* ATCC 10231). The bacterial and yeast strains were stored at -80 °C in Nutrient broth supplemented with 20% (v/v) glyce-

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rol or Sabouraud Maltose broth (both Himedia, Mumbai, India) with 10% (v/v) glycerol, respectively.

Screening of the antimicrobial activity was performed by disk diffusion method (22). The minimal inhibitory (MIC), minimal bactericidal (MBC) and Minimal fungicidal (MFC) concentrations were determined by microdilution method as previously described (16). The yeasts were grown at 25 °C on Sabouraud maltose agar (Himedia, Mumbai, India). After 48 h incubation, the colonies were enumerated by viable count.

The antibiotic (30 μg cefotaxime and 10 μg clavulanic acid per disc, Bioanalyse®, Ankara, Turkey) and antifungal agent (cycloheximide solution, 0.03 g mL^{-1}, Sigma-Aldrich, Co. St. Louis, USA) were used as controls.

Statistical analysis

The results of polyphenol antioxidants content and radical scavenging activity were expressed as mean ± SD of three experiments (n=3). The results of cell growth activity were obtained in two independent experiments, each performed in quadruplicate (n = 8). In the cell death experiments the results for treatments and negative control were obtained from the pooled quadruplicates (n=4) that were evaluated in duplicate (n=2) according to manufacturer's recommendation. The antimicrobial assays were performed in duplicate, i.e. with two tested bacterial or yeast suspensions prepared for each strain, in three repetitions (n=6).

A comparison of the group means and the significance between the groups were verified by one-way ANOVA using OriginPro 8 SRO (OriginLab Corporation, Northampton, USA). Statistical significance was set at $p < 0.05$, unless noted otherwise.

RESULTS AND DISCUSSION

Polyphenol antioxidants of blackberry pomace

As mentioned above, two blackberry cultivars (Čačanska bestrna and Thornfree), which are usually consumed as fresh and processed fruits in Serbian diet, were used to prepare pomace. As a by-product in juice processing, the blackberry pomace consists of the skin, seeds and part of pulp and contains significant amount of polyphenol antioxidants especially anthocyanins (23). The screening of the polyphenolic antioxidants of blackberry pomace extracts - the total phenolics (TPh), total flavonoids (TFd), total and monomeric anthocyanins (TAc and MAc) content was carried out using spectrophotometric assays (Table 1).

The obtained results show that the pomace of both blackberry cultivars are a rich source of antioxidant compounds. Significantly higher amounts of TAc and MAc ($p<0.001$), TPh ($p<0.01$) and TFd ($p<0.001$) were found in the Thornfree pomace extract. This is in accordance with the results of Da Fonseca Machado et al. (24), who reported that the content of total phenolics and monomeric anthocyanins were 7.36 g GAE kg^{-1} and 1.02 g CyGE kg^{-1} fresh residue of blackberries. Also, Laroze et al. (25) established that high amounts of total polyphenols were recovered from pressing pomace of black-

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berry (270 g GAE kg^{-1} extract), blueberry (172 g GAE kg^{-1} extract) and cranberry (138 g GAE kg^{-1} extract) (27). Compared to raspberry pomace extracts (16) the blackberry pomace extracts (Table 1) showed higher contents of total phenolics (26-44 vs. 75-88 g GAE kg^{-1}), total flavonoids (22-25 vs. 40-45 g RE kg^{-1}), and total anthocyanins (2-4 vs. 7-12 g CyGE kg^{-1}).

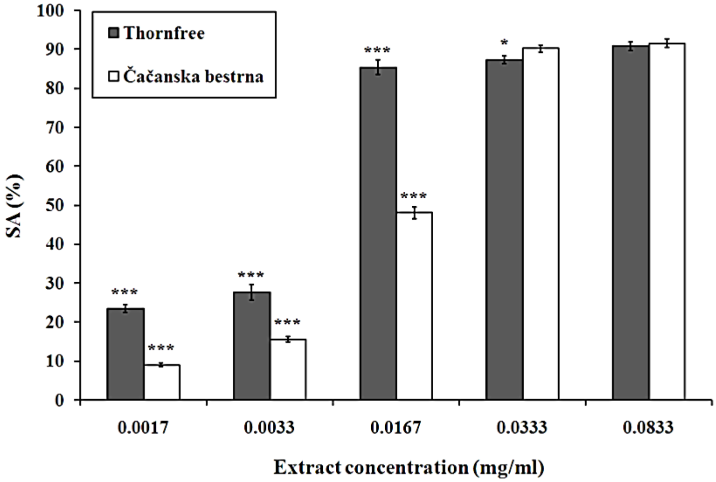
Table 1. Contents of total phenolics, total flavonoids, total and monomeric anthocyanins (TAc and MAc) in pomace extracts (PE) and fresh pomace (FP) of the blackberry cultivars

Blackberry cultivar	TPh (g GAE kg^{-1})		TFd (g RE kg^{-1})		TAc (g CyGE kg^{-1})		MAc (g CyGE kg^{-1})	
	PE	FP	PE	FP	PE	FP	PE	FP
Thornfree	88.28±3.48**	7.97±0.31**	45.51±2.16*	4.11±0.20*	12.61±0.48***	1.14±0.04***	11.49±0.48***	1.04±0.04***
Čačanska bestrna	75.50±3.25**	6.53±0.28**	39.89±1.73*	3.45±0.15*	6.81±0.32***	0.59±0.03***	5.90±0.27***	0.51±0.02***

Means in the same column differ significantly at level * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$

DPPH radical scavenging activity

In the present study, a method based on the DPPH radical scavenging was applied to assess the *in vitro* antioxidative activity of blackberry pomace extracts. Lower concentrations (0.0017-0.0167 mg mL^{-1}) of the Thornfree blackberry pomace extract exhibited significantly high scavenging activity (p<0.001) (Figure 1).



*SA values are means of three replicate analysis ± SD. Means for the same concentration of blackberry pomace extracts applied differ significantly at the level * $p < 0.05$ or *** $p < 0.001$

Figure 1. DPPH radical scavenging activity* of blackberry pomace extracts

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Based on DPPH radical scavenging activity, TEAC (trolox equivalent antioxidant capacity) value of the pomace extract from both blackberry cultivars was determined (Table 2). The Thornfree extract expressed significantly higher ($p<0.001$) TEAC value than that of the Čačanska bestrna.

Table 2. Antioxidant activity of pomace extracts and fresh pomace of the blackberry cultivars

Blackberry cultivar	DPPH	
	PE (mmol TEAC g^{-1})	FP (µmol TEAC g^{-1})
Thornfree	2.12 ± 0.07***	191.71 ± 6.57***
Čačanska bestrna	1.03 ± 0.03***	89.19 ± 3.01***

Means in the same column differ significantly at the level ***$p < 0.001$

It has been shown that berry fruits are rich in anthocyanins and other antioxidants, and have high antioxidant activity. Among berry fruits, blackberry stands out by exhibiting the highest antioxidant activity and the highest levels of phenols, flavonoids, anthocyanins and carotenoids (26, 27). Also, many studies reported the fruit pomace contained abundant phenolic compounds which indicated that this by-product might be useful as a raw material for creating new value-added food, pharmaceuticals, and cosmetic formulations. According to the DPPH assay results, the apple pomace presented good antioxidant activity (6.36 g TE kg^{-1} dry weight) (28). Zhou et al. (29) reported that the antioxidant activity of the pomaces from five cultivars of bayberries (*Myrica rubra*) contained high amounts of phenolic compounds, being in the range 65.7-91.0 g TEAC kg^{-1} dry weight. In the study of Galván D'Alessandro et al. (30) high amounts of total polyphenols (>70 g GAE kg^{-1} dry basis), and especially of anthocyanins (>13 g CyGE kg^{-1} dry basis) were recovered from *Aronia sp.* waste, and the obtained extracts exhibited very high antioxidant capacity (DPPH, >450 µmol TEAC g^{-1} dry basis). Da Fonseca Machado et al. (24) analyzed the blackberry juice industrial residues and found that the antioxidant activity, measured by DPPH method, was in the range 29.04-76.03 µmol TE g^{-1} fresh residue.

In vitro antitumor activity

The most pronounced cell growth effects, lowest EC_{50} values, and the most favorable non-tumor/tumor ratios were observed in the breast adenocarcinoma cell line (MCF7). In the breast cell line the EC_{50} values reached 52.5 and 64.7 µg mL^{-1} for Čačanska bestrna and Thornfree, respectively (Table 3). The EC_{50} values were slightly higher indicating lower activity in the cervix and colon tumor cells, as well as in the cells derived from the healthy tissue (in the range of 66-87.3 µg mL^{-1}). The Čačanska bestrna extract was slightly more active than Thornfree ($p>0.05$) in all cell lines (Table 3).

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Table 3. EC_{50} values* (μg mL^{-1}) of blackberry pomace extracts obtained after 48 h in human cell lines

Extract	HeLa	NT/T Hela	MCF7	NT/T MCF7	HT-29	NT/T HT-29	MRC-5
Thornfree	80.14±8.44 b,d	**1.09**	64.71±11.66 b,c	**1.35**	85.99±7.78 b,e	**1.02**	87.28±8.35 b,e
Čačanska bestrna	69.31±7.77 a,d	**1.03**	52.48±10.59 a,c	**1.37**	66.02±9.60 a,d	**1.09**	71.70±8.74 a,d

*Values represent mean ± SD of eight measurements (n=8). NT/T non-tumor/tumor EC50 ratio
Means with different letters within each column (a-b) and within each row (c-e) differ significantly ($p<0.05$)

Compared to raspberry pomace extracts investigated for the same activity (16), the blackberry pomace extracts from Čačanska bestrna and Thornfree cultivars showed higher activity ($p>0.01$) towards colon carcinoma, but also for the cell line derived from healthy tissue, while the activity towards breast and cervix carcinoma was comparable or slightly lower ($p>0.05$). The non-tumor/tumor EC_{50} ratios that were obtained for both blackberry pomace extracts in the breast (NT/T = 1.3) and colon (NT/T=1.1) adenocarcinoma cells (Table 3) were more favorable than for raspberry (NT/T=0.8) pomace extracts (16).

The blackberry press residues from the Čačanska bestrna and Thornfree cultivars are rich source of anthocyanins (especially cyanidin 3-glucoside), and possess good antioxidative activity, reducing power and α- glucosidase inhibition potential that correlated highly with the contents of anthocyanin compounds (23). Anthocyanins have been reported to induce phase II enzimes which may inactivate carcinogens activated by phase I enzymes, and inhibit possible DNA damage by the carcinogens (31, 32).

Blackberry anthocyanins are thought to suppress cancer cell growth by modifying cell signaling pathways and expression of activated protein 1 (AP-1) and nuclear factor κB (NFκB) that regulate cell proliferation and cell cycle control (32). The inhibition of the cancer cell proliferation by berry juices did not involve caspase-dependent apoptosis, but appeared to involve cell cycle arrest that was demonstrated by down-regulation of the expression of cdk4, cdk6, cyclin D1 and cyclin D3 (33).

Apoptosis and necrosis

In the MCF7 and HeLa cell lines both extracts increased apoptosis (EFA) and decreased necrosis (EFN), while in the HT-29 and MRC-5 cell lines they decreased both parameters (Table 4). The overall increase in the A/N ratio compared to the control was the most pronounced for the Thornfree extract in MCF7 (A/N > 31), and was favorable for both extracts in all other cell lines (A/N > 1) (Table 4). Compared to raspberry pomace extracts for the same activity (AI=1.0-3.1) (16), the blackberry pomace extracts Čačanska bestrna and Thornfree showed non-selective action towards all investigated cell lines and significantly higher ($p<0.01$) apoptotic increases (AI=1.4-12.2). The very high apoptotic increase was obtained by Thornfree pomace extract in the breast carcinoma cell line (A/N=32 and AI=12.2), and it was two-fold higher than in the cell line derived from healthy tissue (T/NT=1.82; Table 4).

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Table 4. Apoptosis and necrosis obtained after 2 h in the human cell lines

		Thornfree	Čačanska bestrna	DMSO
MCF7	EFA	4.56b	1.23a	-
	EFN	0.37a	0.89a	-
	A/N	31.82c	3.65b	2.61a
	AI	**12.19**b	**1.40**a	-
	NT/T	**1.82**b	**0.25**a	-
HT-29	EFA	0.75a	0.80a	-
	EFN	0.11a	0.12a	-
	A/N	3.91b	3.96b	0.58a
	AI	**6.74**a	**6.83**a	-
	NT/T	**1.01**a	**1.26**a	-
HeLa	EFA	1.90a	2.28a	-
	EFN	0.36a	0.34a	-
	A/N	1.53b	1.95b	0.29a
	AI	**5.28**a	**6.72**b	-
	NT/T	**0.79**a	**1.24**a	-
MRC-5	EFA	0.72a	0.65a	-
	EFN	0.11a	0.17a	-
	A/N	1.47b	1.19b	0.22a
	AI	**6.68**b	**5.41**a	-

Means with different letters (a-c) within each row differ significantly ($p<0.05$). EFA - enrichment factor for apoptosis, EFN - enrichment factor for necrosis, A/N - apoptosis/necrosis ratio, AI - apoptotic increase, NT/T - non-tumor / tumor apoptotic increase, DMSO - Dimethyl sulfoxide

The blackberry pomace extracts were significantly more active towards colon adenocarcinoma cell line (IC_{50}= 70-90 µg mL^{-1}; Table 3) and showed significantly higher apoptotic increases (up to AI=12; Table 4) compared to raspberry pomaces (IC_{50}=160-190 µg mL^{-1} and AI=3) (16) in all investigated cell lines.

Although the cell growth experiments showed that the Čačanska bestrna pomace extract exhibited a slightly higher activity towards all cells lines, it should be pointed out that higher non-tumor/tumor ratios (NT/T; Table 3) and more favorable proapoptotic properties in comparison with the activity towards cells derived from the healthy tissue (T/NT ratio; Table 4) were obtained for the Thornfree extract in breast adenocarcinoma cells.

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Antimicrobial activity

The results of antimicrobial activity obtained using disk diffusion method (Table 5) show that the blackberry pomace extracts from Čačanska bestrna and Thornfree cultivars inhibited the growth of all tested bacteria, but did not show activity against tested yeasts. The blackberry extracts displayed comparable antibacterial potential against majority of tested bacteria except against *E. coli* ATCC 10536 and *P. aeruginosa*^{w}, which were more susceptible toward Čačanska bestrna than Thornfree (inhibition zones were significantly different; $p<0.05$). Among bacterial strains the most resistant were wild strains of *Salmonella* sp., *S. saprophyticus* and *Bacillus* sp. The zones with reduced growth appeared around the disks for these strains, indicating bacteriostatic activity of the extracts. The inhibition zones of positive controls (antibiotics and antifungal agents) were two to three times higher than the zones of pomace extracts (Table 5).

The values for MIC, MBC and MFC ranged from 0.39 to >25 mg mL^{-1}, from 0.78 to >25 mg/mL, and from 9.38 to >25 mg mL^{-1}, respectively (Table 5). The strains of *Staphylococcus* and *P. aeruginosa*^{w} were the most susceptible, whereas *C. albicans* was the most resistant to the Čačanska bestrna extract. The MIC and MBC values for the Čačanska bestrna cultivar were lower than for Thornfree in all tested strains except for *E. coli*, referring to its higher antimicrobial potential. *E. coli* was the most susceptible, while yeasts and *Salmonella* strains were the most resistant to the Thornfree extract (MBC was ≥12.5 mg mL^{-1}). The most resistant strain to the activity of both extracts were *Bacillus sp*., for which either high growth inhibition concentrations were needed (25 mg mL^{-1}) or MBC values were not reached in the tested concentration range (Table 5). It is worth to emphasize that both blackberry pomace extracts inhibited growth of the strains of *Salmonella* and *L. monocytogenes*, which are major causative agents for over half of the foodborne illnesses. Control of these foodborne enteric pathogens is a challenge for public health agencies and food industry (10, 34).

Although there are several studies referring to antimicrobial potential of blackberry fruit, leaves and stem extracts (5, 10, 14), only a few studies of blackberry pomace extracts have been published to date. In those studies blackberry pomace extract showed moderate activity only against *Serratia marcenscens* and *Bacillus* strains (35) or did not show any antibacterial activity (36). The antibacterial potential of raspberry pomace extracts from two cultivars grown in Serbia (MBC were in the range 0.29-0.78 mg mL^{-1}) was demonstrated by Četojević-Simin et al. (16), and exhibited higher potential compared to blackberry pomaces against the same panel of bacterial strains.

Different mechanisms and synergistic or additive effects between organic acids, phenolic acids, tannins, and other components of the berry extracts are proposed to be responsible for their antibacterial activity (34, 37). The hydrophobicity of the components - polymeric phenolics or organic acids (citric, benzoic, malic, etc.) enables them to stack and accumulate in the microbial cell membrane. Organic acids, in their undissociate state can diffuse through the cell membrane, where they dissociate. That causes the increase of permeability and weakening of microbial membrane integrity, acidification of the cytoplasm, inhibition of the enzymes, damage of structural proteins and DNA, and leakage of the intracellular constituents, leading to cell death (34, 38, 39). Another potential mecha-

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nism is the dissociation of phenolics at the plasma membrane, which leads to hyper-acidification at the plasma membrane interface and inhibition of ATP syntesis (34).

Table 5. Antimicrobial activity of the blackberry pomace extracts

Group	Tested strain	Disk diffusion method[a]		MIC/MBC(MFC) (mg mL^{-1})[b]		Controls (antibiotic/ antifungal agent)[a]
		Čačanska bestrna	Thornfree	Čačanska bestrna	Thornfree	
G (–) bacteria	*Escherichia coli*	17* (0.82)	15* (1.41)	0.78/1.56	0.78/1.56	30 (1.0)
	Escherichia coli[w]	13.5 (3.11)	13.5 (1.29)	0.78/1.56	3.125/6.25	25.33 (0.58)
	Salmonella typhymurium	16.75 (3.2)	14.25 (1.71)	1.17/1.56	9.38/12.5	33.6 (0.85)
	Salmonella sp.[w]	19.25 (3.3)[c]	15 (2.45)[c]	1.17/1.56	6.25/12.5	27.83 (0.76)
	Pseudomonas aeruginosa	14.75 (1.26)	13 (0.82)	0.39/0.78	4.69/6.25	20.17 (0.76)
	Pseudomonas aeruginosa[w]	16* (0.82)	14* (0.82)	0.78/1.56	1.56/3.125	15.33 (0.58)
G (+) bacteria	*Staphylococcus aureus*	11 (3.56)	10.25 (3.3)	0.39/0.78	1.56/3.125	34.3 (0.75)
	Staphylococcus saprophyticus[w]	16.75 (0.5)[c]	12 (2.94)[c]	0.59/0.78	3.125/6.25	24.0 (0.5)
	Bacillus cereus	19.5 (3.7)	16.75 (0.96)	25/>25	18.75/25	34.5 (1.5)
	Bacillus sp.[w]	17.5 (2.89)[c]	14.25 (0.96)[c]	18.75/25	>25	39.33 (0.58)
	Listeria monocytogenes[w]	15.25 (1.71)	13.5 (1.29)	0.78/1.56	4.69/6.25	14.25 (0.55)
Yeast	*Saccharomyces cerevisiae*	nd	nd	4.69/6.25	9.38/12.5	>38
	Candida albicans	nd	nd	>25	>25	15.33 (0.58)[c]

* Means in the same row differ significantly at level $p<0.05$ (disk diffusion method); a Mean of diameter of the inhibition zone (mm; including disc, 6 mm) with standard deviation in parentheses; b Since all replicates for MIC /MBC (MFC) had equal values, standard deviations were not presented; c Zone of reduced growth; nd - inhibition zone was not detected; w - wild strain

CONCLUSION

The obtained results indicate that the blackberry pomace is a rich source of polyphenol antioxidants possessing high antioxidant activity. Higher amounts of total and monomeric anthocyanins, total phenolics and total flavonoids were found in the Thornfree pomace extract. The highest non-tumor/tumor ratios and more favorable proapoptotic properties were obtained for the Thornfree extract towards breast adenocarcinoma cells. Both blackberry pomace extracts inhibited the growth of all tested microorganisms. The blackberry processing by-products of the Čačanska bestrna and Thornfree cultivars are a rich source of phytochemicals with significant health promoting properties that could be used as beneficial food ingredients, and make impact on the consumer confidence on the safety of the products and profitability of the food processing industry.

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БИОАКТИВНОСТ ТРОПА КУПИНЕ (*Rubus fruticosus* L.): САДРЖАЈ ПОЛИФЕНОЛА, АНТИРАДИКАЛСКА, АНТИМИКРОБНА И АНТИТУМОРСКА АКТИВНОСТ

Драгана Д. Четојевић-Симин[1], Александра С. Ранитовић[2],*
Драгољуб Д. Цветковић[2], Синиша Л. Марков[2], Милица Н. Винчић[2], Соња М. Ђилас[2]

[1] Универзитет у Новом Саду, Медицински факултет, Институт за онкологију Војводине, Др Голдмана 4, 21204 Сремска Каменица, Србија
[2] Универзитет у Новом Саду, Технолошки факултет Нови Сад, Булевар Цара Лазара 1, 21000 Нови Сад, Србија

Огромне количине индустријског отпада се производе сваке године широм света, док његово одлагање представља значајно финансијско оптерећење. Индустријски отпад се може искористити као извор једињења која поседују изузетну хранљиву вредност и биоактивне особине. Одређен је биолошки потенцијал тропа купине, добијен након издвајања сока сорти Чачанска бестрна и Thornfree. Екстракт сорте Thornfree садржао је више концентрације укупних и мономерних антоцијана, укупних фенола и флавоноида и показао јачу DPPH активност хватања слободних радикала (2,12 mmol TEAC g^{-1}) у поређењу са сортом Чачанска бестрна. Оба екстракта су значајно повећала однос апоптоза/некроза на свим испитаним ћелијским линијама. Највећа инхибиција ћелијског раста (EC_{50}=52,5 - 64,7 μg mL^{-1}) и највећи пораст апоптозе (AI=12,2) добијен је на ћелијској линији аденокарцинома дојке. Оба екстракта су инхибирала раст свих испитаних микроорганизама. У референтним и „дивљим" бактеријским сојевима MIC су постигнуте у опсегу од 0,39-25 mg mL^{-1}, а MBC/MFC у опсегу од 0,78-25 mg mL^{-1}. Троп купине је богат извор фитохемикалија са особинама значајним у исхрани и за промоцију здравља, а које се могу искористити као додаци храни.

Кључне речи: троп купине; DPPH тест; ћелијски раст; бактерицидна активност; фунгицидна активност

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