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ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF WASTE PRODUCTS REMAINING AFTER JUICE PRESSING

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ABSTRACT

Pomace (skin, seeds and flesh) remaining after juice pressing is rich in anthocyanins and other phenolic phytochemicals and could have potential antimicrobial and antioxidant effects. In our study, aqueous and methanol extracts made from the pomace of six fruits, used widely in juice making (*Fragaria x ananassa*, *Prunus cerasus, Ribes nigrum, R. rubrum, Rubus idaeus, R. fruticosus),* were tested for antimicrobial activity on Gram-positive and Gram-negative bacteria (*Bacillus cereus, B. subtilis, Campylobacter jejuni, E. coli, Salmonella* Typhimurium *and Serratia marcescens)* and on pathogenic *Candida* species (*C. albicans, C. krusei, C. glabrata, C. parapsilosis, C. pulcherrima,)* by broth dilution assay. Total soluble phenol content was measured by the Folin-Ciocalteu method and antioxidant capacity was determined using DPPH.

C. jejuni and E. coli were the most sensitive bacteria especially to the methanol extracts. The growth of *C. albicans, C. krusei* and *C. pulcherrima* was not inhibited by any of the extracts. In general, *Ribes* and *Rubus* species were efficient inhibitors. Phenol content and antioxidant capacity was high in the dark colored pomace (up to 50.4 µg gallic acid equivalent/mg dry matter content and 83%, respectively).

Keywords:
pomace, antimicrobial, antioxidant, phenol

1. INTRODUCTION

Phenolic compounds naturally present in fruits have antioxidant activity and can protect the plant from environmental stress and fungal or bacterial infections. The main classes of polyphenols are phenolic acids, flavonoids including anthocyanins, stilbenes, lignans and complex polymers. Waste products remaining after juice processing (peel, seeds, stems, flesh) are good sources of these ingredients. There is an increasing interest to use pomace for the extraction of natural antioxidants and other value added products [1, 2, 3, 4]. The extracted phenolic compounds of pomace could also have antibacterial and antifungal properties [5, 6]. There is a consumer demand for foodstuffs with minimal or no added synthetic preservatives. Natural antioxidants with antimicrobial activities can be used for enhancing food quality and shelf life. Beside food industry, agriculture and medicine can also have benefits from pomace-derived bioactive compounds as natural antifungal and antibacterial agents.

In the present study, the *in vitro* biological activities of water and methanol extracts of pomace (peels, seeds, flesh) remaining after pressing the juice of six fruits were investigated on food-spoiling and human pathogenic bacteria (*Bacillus cereus, B. subtilis, Campylobacter jejuni, E. coli, Salmonella* Typhimurium *and Serratia marcescens)* and on pathogenic Candida species (*C. albicans, C. krusei, C. glabrata, C. pulcherrima, C. parapsilosis)* by broth dilution method.

2. MATERIALS AND METHODS

2.1. Strains and culture conditions

Bacillus subtilis ssp. subtilis BD170, B. cereus var. mycoides ATCC 9634, Escherichia coli SZMC 0582, Serratia marcescens SZMC 0582, and a clinical isolate of *Salmonella* ser. Typhimurium were grown on T1 medium (10 g glucose, 4 g beef extract, 4 g peptone, 1 g yeast extract, 1 L water). *Campylobacter jejuni* were cultured on Campylobacter blood-free selective agar medium (CCDA; Charcoal cefoperazone deoxycholate agar, Merck). The yeasts *Candida albicans* ATCC10231, *C.*

glabrata CBS 138, *C. krusei* CBS573, , *C. parapsilosis* CBS604, and *C. pulcherrima* CBS 5833 were grown on RPMI1640 medium (Sigma).

2.2. Fruits and extraction methods

Fruits investigated in this study were *Fragaria ananassa* (strawberry), *Prunus cerasus* (sour cherry), *Ribes nigrum* (black currant), *Ribes rubrum* (red currant), *Rubus fruticosus* (blackberry) and *Rubus idaeus* (raspberry). Fresh fruits were purchased on a local market (Szeged). Fruit juices were freshly pressed, and the remaining pomace was dried overnight at 60°C and then grounded to powder. One gram pomace was extracted with 3 x 10 ml of distilled water or methanol. The extracts were evaporated to dryness at 100°C in an oven (water extracts) or at 40 °C in a water bath (methanol extracts). The dry material was dissolved in 4 ml distilled water (water extracts) or 10 % methanol-water solution (methanol extracts), and frozen in 1 ml aliquots at -20°C. One sample from each extract was dried again and weighed for dry matter content calculation. The extracts were diluted in the appropriate media for the tests.

2.3. Determination of antibacterial and antifungal effect by broth dilution method

Absorbance of the bacterial or yeast cultures was measured at 620 nm in the presence of the fivefold diluted extract. In each well, 100 µl of diluted and sterile-filtered (0.45 µm, Millipore) extract was mixed with 100 µl cell suspension (10^5 cells/ml) containing the appropriate medium (final dilution of the tested extract was tenfold). Each test plate contained a positive growth control. The samples were tested in triplicate and the results were recorded after 48 h.

2.4. Determination of total soluble phenol content

Phenol content was measured by the Folin-Ciocalteu method at 725 nm. Calibration was made with gallic acid and results are given as µg gallic acid equivalent/mg dry matter content of the extract.

2.5. Determination of antioxidant capacity

Antioxidant capacity was measured at 517 nm using DPPH (1,1 diphenyl-2 picrylhydrazyl) and determined using the formula: % capacity=$[(A_{control} - A_{extract})/A_{control}] \times 100$.

3. RESULTS AND DISCUSSION

3.1. Antibacterial and antifungal effect

Best results were obtained with the dark coloured pomace of *Ribes nigrum* and *Rubus fruticosus*. Both aqueous and methanol extracts of these fruits reduced the growth of almost all bacteria in this study, and *S.* Typhimurium growth was completely inhibited by *R. nigrum* pomace extracts. In general, methanol extracts had stronger inhibitory effect than water extracts. The most insensitive bacterium was the Gram-negative *S. marcescens*, and the most sensitive one, the also Gram-negative *C. jejuni* which was inhibited by all of the investigated extracts (Table 1).

The components present in aqueous and alcoholic extracts are partly dissimilar. Water extract contains the majority of anthocyanins, tannins, starches, saponins, polypeptides and lectins of the pomace, while methanol extracts, in addition, polyphenols, lactones, flavones, and phenons [7]. Phenolic and organic acids acidify the cytoplasm of microorganisms, while certain small hydrophobic molecules, called permeabilisers, can disintegrate the outer membrane of Gram-negative bacteria causing bactericidal effects. Puupponen-Pimia and co-workers [8] found that raspberry extracts caused permeabilisation of *Salmonella* membrane. We hypothesized that the presence of such permeabilisers found in methanol extracts can cause the better growth inhibition effect of these extracts.

Table 1. Growth inhibition effect of pomace extracts on bacteria

	Gram positive bacteria				Gram negative bacteria							
	B. subtilis		*B. cereus*		*E. coli*		*S. marcescens*		*S.* Typhi-murium		*C. jejuni*	
	W	M	W	M	W	M	W	M	W	M	W	M
Fragaria ananassa	4	1	4	1	4	3	4	4	n.d	n.d	n.d	n.d
Prunus cerasus	4	4	2	4	3	1	4	1	2	0	1	1
Ribes nigrum	1	1	4	0	1	1	1	4	0	0	0	2
Ribes rubrum	4	4	4	4	4	1	4	1	1	1	1	1
Rubus fruticosus	1	1	1	0	4	1	4	2	3	2	1	0
Rubus idaeus	4	4	3	4	4	2	4	1	4	2	1	0

0 - no growth; 1 - growth<25 %; 2 - growth<50 %; 3 - growth<75%; 4 - growth>75%. Control growth is taken as 100%. W: water extracts; M: methanol extracts. n.d. - no data.

Candida species showed lower sensitivity to the pomace extracts. The growth of *C. albicans, C. krusei* and *C. pulcherrima* was not inhibited by any of the extracts. *R. nigrum* and *R. fruticosus* water extracts reduced the growth of *C. glabrata*, and inhibited totally the growth of *C. parapsilosis*. *F.*

ananassa and *R. idaeus* had inhibitory effect on both sensitive Candida species while *P. cerasus* and *R. rubrum* had no effect on any of the yeasts (Table 2).

Similar data on the non-sensitivity of *C. albicans* to plant extracts can be found in the literature [9, 10]. Our results with *C. parapsilosis* were, however, in contrast to those where this and seven other yeast species showed resistance to berry water extracts and juices [11].

Table 2 Growth inhibition effect of pomace extracts on Candida species

	C. albicans		*C. krusei*		*C. glabrata*		*C. pulcherrima*		*C. parapsilosis*	
	W	M	W	M	W	M	W	M	W	M
Fragaria ananassa	4	4	4	4	2	4	4	4	1	4
Prunus cerasus	4	4	4	4	4	4	4	4	4	4
Ribes nigrum	4	4	4	4	1	4	4	4	0	1
Ribes rubrum	4	4	4	4	4	4	4	4	4	4
Rubus fruticosus	4	4	4	4	2	4	4	4	0	4
Rubus idaeus	4	4	4	4	1	4	4	4	2	4

0 - no growth; 1 growth<25 %; 2 - growth<50 %; 3 - growth<75%; 4 - growth>75%. Control growth is taken as 100%. W: water extracts; M: methanol extracts

3.2. Phenol content and antioxidant capacity

Phenol content of the water and methanol extracts can be seen in Table 3. *R. nigrum* had the highest and *F. ananassa* the lowest value. Antioxidant capacity ranged from 66 % (*R. rubrum*) to 83 % (*R. nigrum*) (Fig.1). It seems that there is no firm correlation between the soluble phenol content and antioxidant capacity. In spite of this, higher phenol content resulted in better inhibitory effect against sensitive bacteria and yeasts. Linear regression between phenol content and antibacterial effect showed R^2 value of 0.6197 for water extracts and 0.5438 for methanol extracts.

Table 3. Soluble phenol content of pomace given as µg gallic acid equivalent/mg dry matter content of extracts

	Water extract	Methanol extract
Fragaria ananassa	13.61 ± 0.17	17.0 ± 0.33
Prunus cerasus	31.43 ± 0.35	27.22 ± 1.16
Ribes nigrum	49.70 ± 0.10	50,44 ± 0.06
Ribes rubrum	28.16 ± 0.13	20.54 ± 0.66
Rubus fruticosus	23.88 ± 0.61	46.50 ± 2.50
Rubus idaeus	29.71 ± 0.71	36.72 ± 3.70

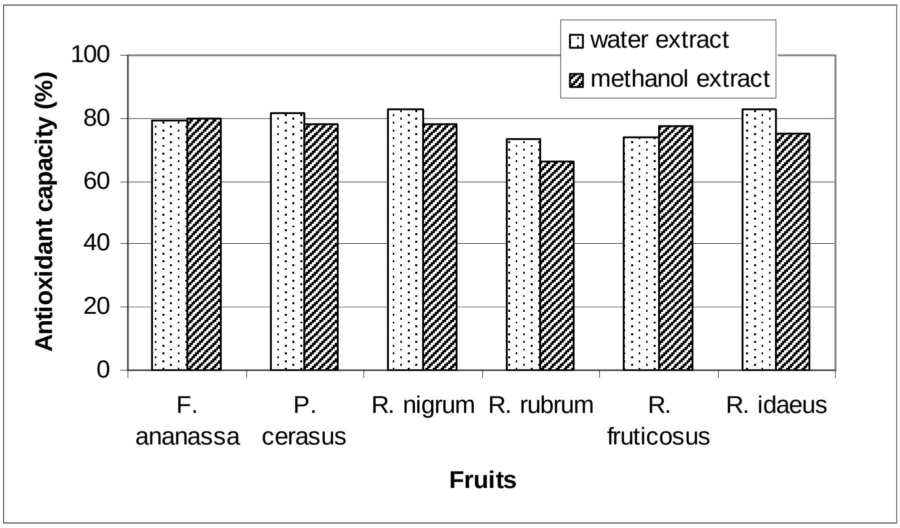

Fig. 1 Antioxidant capacity of fruits pomace extracts

Pomace remaining after juice processing is not a waste product. It is rich in extractable bioactive compounds, and can be used in various ways in the food industry as source of natural antioxidants and/or preservatives. There is also a perspective to use the antimicrobial capacity of pomace in agriculture and medicine. Our results suggest that especially dark coloured pomace, extracted with solvents for hydrophobic molecules, are good candidates as antimicrobial agents.

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