Environmental and Experimental Biology (2013) 11: 195–199 *Original Paper*

Antimicrobial activity of extracts from fruits of *Aronia melanocarpa* and *Sorbus aucuparia*

Inga Liepiņa[1], Vizma Nikolajeva[1]*, Ida Jākobsone[2]

[1]Faculty of Biology, University of Latvia, Kronvalda Bulv. 4, Riga LV-1586, Latvia
[2]Faculty of Chemistry, University of Latvia, Kr. Valdemara 48, Riga LV-1013, Latvia

*Corresponding author, E-mail: vizma.nikolajeva@lu.lv

Abstract

Antimicrobial activity of extracts from fruits of wild rowan (*Sorbus aucuparia* L.) and cultivated black chokeberries [*Aronia melanocarpa* (Michx.) Elliott] harvested in Latvia was studied. Aqueous and ethanolic extracts were obtained from fresh, dried and frozen fruits. Extracts were also subjected to thermal processing (autoclaving or freezing). Results showed that both fruit extracts exhibited antibacterial activity against Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*, but they did not have antifungal influence. The extracts inhibited the growth of Gram-negative bacterium *Pseudomonas aeruginosa* but did not have influence on *Escherichia coli*. Processing changed the antibacterial activity, i.e., freezing reduced it. An increase of pH after drying or freezing of fruits was noted. This study indicated that black chokeberry and rowanberry grown in Latvia might be applicable in natural medicine and food as a source of antibacterial products.

Key words: antimicrobial activity; black chokeberry; fruit extracts; rowanberry; thermal processing.

Introduction

With increasing microbial resistance to antibiotics as well as with growing interest in eco-living lifestyles, increased attention is being paid to the natural antimicrobial compounds. The first scientific experiments on plant antimicrobial activity and chemical composition were documented by the second half of the 19th century (Zaika 1975). Inhibition of microbial growth has been discovered in a wide range of food plants, including various berries and fruits (Puupponen-Pimiä et al. 2005b; Nohynek et al. 2006).

Antimicrobial activity of plant extracts is based on phenolics (simple phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, coumarins), terpenoids and essential oils, alkaloids, lectins and polypeptides etc. The most abundant plant antimicrobial compounds are a group of phenolic compounds (Cowan 1999; Savoia 2012). Each plant species has its own complex of phenolics (Häkkinen 2000). Antimicrobial activity of phenolic compounds is dependent on pH (Friedmann, Jürgens 2000). The low pH of juices is caused by weak organic acids. However, only a weak correlation has been found between acidity of the samples and their antibacterial effect (Krisch et al. 2008).

Different plants belonging to various families possess strong antimicrobial properties (Puupponen-Pimiä et al. 2001; Chattopadhyay, Bhattacharyya 2007; Irkin, Korukluoglu 2007; Krisch et al. 2008). The Rosaceae family has several species with well-known antibacterial activity, such as cloudberries (*Rubus chamaemorus*), raspberries (*Rubus idaeus*), strawberries (*Fragaria ananassa*) (Puupponen-Pimiä et al. 2001; Puupponen-Pimiä et al 2005a), blackberries (*Rubus fructicosus*) and European rowan (*Sorbus aucuparia*) (Krisch et al. 2008).

In this study, the antibacterial and antifungal activity of culinary fruits of two Rosaceae species (cultivated black chokeberry *Aronia melanocarpa* and wild rowanberry *Sorbus aucuparia*) harvested in Latvia was investigated and influence of various methods of treatment of fruits and extracts was evaluated.

Materials and methods

Plant material

Two fruit-bearing plant species, black chokeberry *Aronia melanocarpa* (Michx.) Elliott and rowanberry *Sorbus aucuparia* L. were used. Fruits were harvested in September in Latvia. European rowan fruits were collected in a natural forest habitat from wild plants. Black chokeberry fruit was collected in a home garden. The fruits were washed with tap water, rinsed with distilled water, and then left to air-dry.

Preparation of extracts

Crude extracts were obtained from fresh, dried and frozen fruits. The extracts were obtained using distilled water or 50% solution of ethyl alcohol in water.

Extracts from fresh material were obtained using weighed black chokeberry and rowanberry, in amounts of 227 g and 200 g, respectively. Fruit material was finely ground in a mortar with a pestle. These samples were mixed with 100 mL of sterile distilled water or 50% ethanol, mixed and extracted at room temperature for 2 h. The slurry obtained was filtered through sterile muslin. As the loss on drying was 75% for rowanberry and 78% for black chokeberry, the concentration of obtained extracts was 0.5 g mL^{-1} of dry plant material.

Extracts from dried material were made from fresh fruit dried in an automatic dryer DR 435 CB (Bomann, Germany) at a temperature of 50 °C for five days. Loss on drying was 75% for rowanberry and 78% for black chokeberry. Dried materials were stored at 4 °C for 20 days and then the extraction was carried out. Ten grams of the dried material were mixed with 40 mL of water or 50% ethanol and left at room temperature for 2 h. Then, the macerated material was ground in a mortar, mixed with additional 40 mL of water or 50% ethanol and once again extracted at room temperature for 10 h. After that, the slurry was filtered through sterile muslin. The concentration of obtained extract was 0.2 g mL^{-1} of dry material.

Extracts from frozen material were prepared from fresh fruits frozen intact at a temperature of –20 °C. The samples were left frozen in closed tubes for 5.5 months and then defrosted before extraction. The extraction was carried out as described for dried material, in total for about 12 h. The obtained concentration was 0.2 g mL^{-1} of dry material.

Treatment of extracts

The obtained extracts were centrifuged (5804 R, Eppendorf) at 5000 rpm for 15 min. Then each of the supernatants was divided in three parts. One part was sterilized by filtration through microporous membrane (0.2 µm; Millipore), the second part was sterilized by autoclaving at 121 °C for 15 min, and the third part was frozen at –20 °C for 24 h. The autoclaved and membrane sterilized extracts were stored in the dark in the refrigerator at 4 °C until use.

Estimation of antimicrobial activity

Antimicrobial activity was determined by the agar well diffusion method (Perez et al. 1990). Microorganisms were obtained from the Microbial Strain Collection of Latvia (MSCL). Bacteria *Bacillus cereus* MSCL 330, *Escherichia coli* MSCL 332, *Pseudomonas aeruginosa* MSCL 331 and *Staphylococcus aureus* MSCL 334 as well as yeast *Candida albicans* MSCL 378 were used.

The test was performed on Muller-Hinton agar (Oxoid, UK) for bacteria and on Malt Extract agar (Difco, USA) for yeasts. Fresh inoculum of approximately 10^6 CFU (colony-forming units) mL^{-1} of tested microorganisms was used. Aliquots of 70 µL of each sample and control (distilled water and 50% ethanol) were applied into 6.0 mm diameter wells. After incubation of bacteria at 37 °C for 24 h and yeasts for 72 h, the inhibition zone diameter around the well was measured in millimeters and used to express the antimicrobial activity. Gentamicin (KRKA, Slovenia) 10 mg mL^{-1} was used as a positive control for bacteria and fluconazole (Diflucan, Pfizer Ltd., UK) 2 mg mL^{-1} was used for yeasts. Each sample was tested in triplicate using three individual Petri dishes and the final results were presented as the arithmetic average ± SD (standard deviation). Statistical analysis was done by analysis of variance. $P < 0.05$ was considered statistically significant.

Measurement of pH

The pH values of extracts were measured using a pH meter AD-1405 (Adrona, Latvia).

Results

All investigated extracts were acidic (Table 1). The pH values ranged from 3.0 (aqueous extract of rowanberry) to 4.4 (ethanolic extract of frozen black chokeberry). Dried and frozen plant extracts were on average 0.3 to 1.0 pH units less acidic than the respective extracts from fresh plants, while the dried and frozen plant extracts differed from each other by no more than 0.2 units. Values of pH in ethanolic extracts also were 0.2 to 0.8 units higher than in respective aqueous extracts.

Antimicrobial activity was tested against two Gram-positive and two Gram-negative bacteria and yeast *Candida albicans*. Results showed that the investigated fruits had antibacterial activity (Table 2), but they did not have an antifungal effect. The positive control (antifungal antibiotic fluconazole) had a 31-mm inhibition zone diameter. The extracts showed activity against Gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, and against Gram-negative bacterium *Pseudomonas aeruginosa*. The ethanolic extracts had equal or higher antibacterial activity

Table 1. Value of pH of aqueous and ethanolic extracts of fresh and treated fruits. Results are presented as mean value of triplicates. Standard deviation of all measurements was < 0.1. ^{a,b} means within a row followed by different superscripts differ, $P < 0.05$. ^{c,d} means within a column of aqueous and ethanolic extracts of black chokeberry or rowanberry followed by different superscripts differ, $P < 0.05$

Extract type	Fresh	Dried	Frozen
Aqueous extract of black chokeberry	3.2^{a}	$3.8^{b,c}$	$3.8^{b,c}$
Ethanolic extract of black chokeberry	3.4^{a}	$4.3^{b,d}$	$4.4^{b,d}$
Aqueous extract of rowanberry	$3.0^{a,c}$	$3.3^{b,c}$	$3.4^{b,c}$
Ethanolic extract of rowanberry	$3.3^{a,d}$	$4.1^{b,d}$	$3.9^{b,d}$

Table 2. Antibacterial activity of aqueous and ethanolic extracts of fresh and treated fruits, and fresh and treated extracts, expressed as inhibition zone diameter in millimeters. No activity was found against *Candida albicans*. 1, fresh extract; 2, autoclaved extract; 3, frozen extract. An amount of 70 μL of each extract were applied into 6.0 mm diameter agar wells. 0, no inhibition zone. *, the activity was higher in the ethanol than in the water ($P < 0.05$); n.d. not determined; ^{a,b} means within a column followed by different superscripts differ, $P < 0.05$

Fruit, treatment	Extract treatment	*Bacillus cereus*		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*	
		Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
Black chokeberry									
Fresh	1	0	0	0	0	0	0	9.0 ± 1.5^{a}	8.0 ± 2.0^{a}
	2	8.5 ± 0.5^{a}	10.0 ± 1.0^{b}	7.5 ± 0.5^{a}	$9.0 \pm 1.0^{a,b}$	0	0	9.0 ± 1.5^{a}	9.0 ± 1.5^{a}
	3	7.0 ± 0.5^{a}	7.5 ± 0.5^{a}	0	8.0 ± 1.0a*	0	0	12.0 ± 1.0^{a}	14.5 ± 1.5^{b}
Dried	1	0	$19.5 \pm 0.5^{c*}$	7.0 ± 0.5^{a}	$9.0 \pm 2.0^{a,b}$	0	0	0	0
	2	0	$10.5 \pm 0.5^{b*}$	0	$9.5 \pm 0.5^{a,b*}$	0	0	0	0
	3	0	$7.5 \pm 0.5^{a*}$	7.5 ± 0.5^{a}	8.0 ± 1.0^{a}	0	0	0	0
Frozen	1	0	$8.0 \pm 1.0^{a*}$	0	$7.0 \pm 1.0^{a*}$	0	0	0	0
	2	0	$10.0 \pm 0.0^{b*}$	0	0	0	0	0	0
	3	0	$7.0 \pm 1.0^{a*}$	0	$7.0 \pm 1.0^{a*}$	0	0	0	0
Rowanberry									
Fresh	1	10.0 ± 1.0^{b}	$8.5 \pm 1.5^{a,b}$	7.5 ± 0.5^{a}	7.0 ± 1.0^{a}	0	0	0	$9.0 \pm 1.5^{a*}$
	2	10.5 ± 1.5^{b}	11.5 ± 1.5^{b}	10.5 ± 1.5^{b}	11.5 ± 1.5^{b}	0	0	10.0 ± 1.5^{a}	10.0 ± 2.0^{a}
	3	$8.5 \pm 1.5^{a,b}$	10.0 ± 1.0^{b}	9.5 ± 0.5^{b}	$10.0 \pm 0.0^{a,b}$	0	0	$10.5 \pm 0.5^{a*}$	$14.0 \pm 1.0^{b*}$
Dried	1	0	$9.0 \pm 0.0^{b*}$	$8.0 \pm 2.0^{a,b}$	$9.0 \pm 1.0^{a,b}$	0	0	0	0
	2	7.0 ± 1.0^{a}	$9.0 \pm 1.0^{a,b}$	$8.0 \pm 2.0^{a,b}$	$8.0 \pm 2.0^{a,b}$	0	0	0	0
	3	0	$7.0 \pm 1.0^{a*}$	0	0	0	0	0	0
Frozen	1	7.0 ± 1.0^{a}	7.5 ± 1.5^{a}	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0
Gentamicin		32.0 ± 0.5^{c}	n.d.	32.0 ± 0.5^{c}	n.d.	31.0 ± 0.5^{c}	n.d.	32.0 ± 0.5^{c}	n.d.
Ethanol (50%)		n.d.	0	n.d.	0	n.d.	0	n.d.	0

than aqueous extracts.

Drying of fruits for use in extracts as well as thermal treatment (autoclaving, freezing) of extracts changed the antimicrobial properties, increasing or decreasing inhibition activity. Extracts of fresh fruits inhibited all of the tested bacteria with the exception of *E. coli* but extracts of dried or frozen fruits inhibited only Gram-positive bacteria. The freezing of fruits led to reduction of antibacterial activity. It was found that autoclaved or frozen extracts obtained from fresh fruits possessed equal or greater antibacterial activity than that of fresh extracts (Table 2). The freezing of extracts obtained from dried fruits reduced their antibacterial activity. Extracts of frozen fruits showed little antibacterial activity, and autoclaving or freezing reduced it even more, with the exception of autoclaved ethanolic extract of black chokeberry.

An association between pH value and level of antibacterial activity was not observed. Thus, the pH was not the determining factor for antimicrobial activity of extracts.

Discussion

The present study evaluated the antimicrobial activity of extracts obtained from fruits of two Rosaceae species: black chokeberry and rowanberry. Antimicrobial activity of plant extracts was detected against selected non-pathogenic or facultative pathogenic species of widely distributed bacteria and yeast *Candida albicans*. The extracts showed activity against *Bacillus cereus, Staphylococcus aureus* and *Pseudomonas aeruginosa* but no activity against *Escherichia coli* was observed. There are reports in the literature that black chokeberries inhibit *E. coli* (Valcheva-Kuzmanova, Belchev 2006), but this was not confirmed by our experiments. Ethanolic extracts demonstrated equal or larger antibacterial effects than aqueous extracts (Table 2). Also, according to the literature, better results usually have been obtained with alcoholic than with aqueous extracts (Krisch et al. 2008; Chitra et al. 2012). Ethanol is the most commonly used organic solvent, as the finished products can be relatively safely used (Low Dog 2009).

Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. The exceptional water-soluble compounds, such as polysaccharides and polypeptides, are commonly more effective as inhibitors of pathogen adsorption and would not be identified in the screening techniques commonly used (Cowan 1999).

Some experiments demonstrate a direct relationship between the phenolic content of plant extracts and the antimicrobial effect (Alberto et al. 2006). *Aronia melanocarpa* fruits are one of the richest plant sources of phenolic substances, mainly anthocyanins – glycosides of cyanin. Anthocyanins are water-soluble pigments accounting for the dark blue color of the fruits (Valcheva-Kuzmanova, Belcheva 2006; Kokotkiewicz et al. 2010). Anthocyanin content of berry fruits varies from 7.5 mg per 100 g fresh fruit in red currant (*Ribes rubrum*) to 460 mg per 100 g fresh fruit in *Aronia melanocarpa*. It is known that anthocyanins are active against various microorganisms, especially Gram-positive bacteria (Cisowska et al. 2011). Our results showed that Gram-positive bacteria are more susceptible to black chokeberry and rowanberry extracts than Gram-negative bacteria. It is possible that such an effect could be due to anthocyanins.

According to previous investigations, content of phenolics in rowanberries varies from 550 to 1014 mg per 100 g of fresh fruit (Hukkanen et al. 2006). However, the antimicrobial activity of rowanberries has previously been attributed to sorbic acid, with inhibitory effect especially against yeasts and molds (Brunnen 1985). It is known that chlorogenic acids (3- and 5-caffeoylquinic acid) contribute approximately 80% of the total phenolics in wild rowanberries. The content of chlorogenic acids reaches 340 mg per 100 g of fresh fruit. In comparison, the cultivated rowanberries contain less caffeoylquinic acids and more anthocyanins (Kylli et al. 2010).

Since fruits and their extracts are often treated before use, it was important to examine the effects of processing on the biological properties of the products. Aqueous and ethanolic extracts obtained from fresh, dried or frozen fruits demonstrated different pH and antibacterial activity. The same also applied to the treatment of extracts. The freezing of fruits led to reduction of antibacterial activity. Perhaps this was because of the higher concentration of fresh extracts in comparison with dried or frozen extracts. It is known that vacuum freeze-drying is the best industrial food preparation method of dehydrating berries to retain the color and flavour as well as most types of compounds (Reyes et al. 2011), but it is not available for household use.

The obtained results showed that pH was not the determining factor for antimicrobial activity of investigated extracts. At the same time, it is true that organic acids, for example ascorbic acid, possess a strong antimicrobial effect (Tajkarimi, Ibrahim 2011). It is known that an important role in ensuring plant antimicrobial properties is not only played by individual substances, but also by the whole complex of biologically active compounds. For example, ascorbic acid has a synergistic antimicrobial effect in combination with low pH (Giannuzzi, Zaritzky 1996), as does quince juice in combination with bilberry, black chokeberry or red currant juice (Babarikina et al. 2011).

Previous studies (Rakić et al. 2007; Ranilla et al. 2010) have shown that thermal treatment of fruits can increase the concentration of phenolic compounds. Their increase in frozen berries and fruits could be due to the fact that freezing breaks cell walls and therefore the cells can release more compounds after thawing. Phenolics can undergo also structural changes that alter antibacterial properties of juices and extracts (Puupponen-Pimiä et al. 2005b; Howard et al. 2012). Also, frozen berries stored long-term undergo structural and functional changes (Nohynek et al. 2006). Drying or freezing of cells also could mobilize compounds with buffering properties.

This study indicated that black chokeberry and rowanberry grown in Latvia might be applicable in natural medicine and food as a source of antibacterial products. Further studies are necessary to determine the individual biologically active constituents of these fruits.

Acknowledgements

This study was supported by the European Regional Development Fund (ERDF) (2010/0295/2DP/2.1.1.1.0/10/APIA/VIAA/134).

References

Alberto M.R., Canavosio M.A.R., Nadra M.C.M. 2006. Antimicrobial effect of polyphenols from apple skins on human bacterial pathogens. *Electron. J. Biotechnol.* 9: 205–209.

Babarikina A., Nikolajeva V., Babarykin D. 2011. Anti-*Helicobacter* activity of certain food plant extracts and juices and their composition *in vitro*. *Food Nutr. Sci.* 2: 868–877.

Brunnen U. 1985. Some antifungal properties of sorbic acid extracted from berries of rowan (*Sorbus aucuparia*). *J. Biol. Educ.* 19: 41–47.

Chattopadhyay R. R., Bhattacharyya S. K. 2007. Herbal spices as alternative antimicrobial food preservatives: An update. *Pharmacog. Rev.* 1: 239–247.

Chitra W., Calderon P., Gagnon D. 2012. Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. *J. Medic. Active Plants* 1: 60–68.

Cisowska A., Wojnicz D., Hendrich A. B. 2011. Anthocyanins as antimicrobial agents of natural plant origin. *Nat. Prod. Commun.* 6: 149–156.

Cowan M. M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12: 564–582.

Friedman M., Jürgens H. S. 2000. Effect of pH on the stability of plant phenolic compounds. *J. Agricult. Food Chem.* 48: 2101–2110.

Giannuzzi L., Zaritzky N. E. 1996. Effect of ascorbic acid in comparison to citric and lactic acid on *Listeria monocytogenes* inhibition at refrigeration temperatures. *Lebensm. Wiss.*

Technol. 29: 278–285.
Häkkinen S. 2000. *Flavonols and Phenolic Acids in Berries and Berry Products*. Dissertation. Kuopio University, Kuopio. 92 p.
Hukkanen A. T., Polonen S. S., Karenlampi S. O., Kokko H. I. 2006. Antioxidant capacity and phenolic content of sweet rowanberries. *J. Agric. Food Chem*. 54: 112–119.
Irkin R., Korukluoglu M. 2009. Growth inhibition of pathogenic bacteria and some yeasts by selected essential oils and survival of *L. monocytogenes* and *C. albicans* in apple-carrot juice. *Foodborne Pathog. Dis.* 6: 387–394.
Kokotkiewicz A., Jaremicz Z., Luczkiewicz M. 2010. *Aronia* plants: a review of traditional use, biological activities, and perspectives for modern medicine. *J. Med. Food* 13: 255–269.
Krisch J., Galgóczy L., Tölgyesi M., Papp T., Vágvölgyi C. 2008. Effect of fruit juices and pomace extracts on the growth of Gram-positive and Gram-negative bacteria. *Acta Biol. Szeged.* 52: 267–270.
Kylli P., Nohynek L., Puopponen-Pimiä R., Westerlund-Wikstrom B., McDougall G., Stewart D., Heinonen M. 2010. Rowanberry phenolics: compositional analysis and bioactivities. *J. Agric. Food Chem.* 58: 11985–11992.
Low Dog T. 2009. Smart talk on supplements and botanicals: Herbal teas versus tinctures; standardized extracts; green tea. *Alternat. Complement. Ther.* 15: 101–102.
Nohynek L.J., Alakomi H.L., Kähkönen M.P., Heinonen M., Helander I.M., Oksman-Caldentey K.M., Puupponen-Pimiä R.H. 2006. Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens. *Nutr. Cancer* 54: 18–32.
Perez C., Pauli M., Bazevque P. 1990. An antibiotic assay by the agar well diffusion method. *Acta Biol. Medic. Exp.* 15: 113–115.
Puupponen-Pimiä R., Nohynek L., Alakomi H.L., Oksman-Caldentey K.M. 2005a. The action of berry phenolics against human intestinal pathogens. *Biofactors* 23: 243–251.
Puupponen-Pimiä R., Nohynek L., Hartmann-Schmidlin S., Kähkönen M., Heinonen M., Määttä-Riihinen K., Oksman-Caldentey K.M. 2005b. Berry phenolics selectively inhibit the growth of intestinal pathogens. *J. Appl. Microbiol.* 98: 991–1000.
Puupponen-Pimiä R., Nohynek L., Meier C., Kähkönen M., Heinonen M., Hopia A., Oksman-Caldentey K.M. 2001. Antimicrobial properties of phenolic compounds from berries. *J. Appl. Microbiol.* 90: 494–507.
Reyes A., Evseev A., Mahn A., Bubnovich V., Bustos R., Scheuermann E. 2011. Effect of operating conditions in freeze-drying on the nutritional properties of blueberries. *Int. J. Food Sci. Nutr.* 62: 303–306.
Savoia D. 2012. Plant-derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol.* 7: 979–990.
Tajkarimi M., Ibrahim S. A. 2011. Antimicrobial activity of ascorbic acid alone or in combination with lactic acid on *Escherichia coli* O157:H7 in laboratory medium and carrot juice. *Food Control* 22: 801–804.
Valcheva-Kuzmanova S. V., Belcheva A. 2006. Current knowledge of *Aronia melanocarpa* as a medicinal plant. *Folia Med.* 48: 11–17.
Zaika L. L. 1975. Spices and herbs: their antimicrobial activity and its determination. *J. Food Safety* 9: 97–118.

Received 17 October 2013; received in revised form 19 November 2013; accepted 13 December 2013