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ARTICLE

Antioxidant and antimicrobial activities of fruit juices and pomace extracts against acne-inducing bacteria

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ABSTRACT Acne vulgaris is the most common skin disease in the world, and the number of antibiotic resistant acne-inducing bacterial strains has been increasing in the past years. Natural substances from plants are promising candidates to treat this disease. In the present study, *in vitro* biological activity of the juice, as well as water and methanol extracts of the pomace, of 20 cultivated and wild fruits was investigated on 4 acne-inducing bacteria (*Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*). The MIC values of juices and pomace extracts (water and methanol) were determined by broth microdilution assays at pH 7 and at skin neutral pH 5.5. Total phenol content and radical scavenging capacity of the active juices and extracts was also determined. Red and purple berries revealed a substantial antibacterial and antioxidant effect but there was no strong correlation between the antioxidant and antimicrobial properties. *Staphylococcus* strains were the most sensitive to the juices, and *S. pyogenes*, to the methanol extracts. Among the bacteria tested, *P. acnes* proved to be the most insensitive species in this study. The growth inhibition effect of *Ribes uva-crispa* (gooseberry) juice was stronger at acidic pH (MIC 0.40 mg/ml) than at neutral pH (MIC 5.30 mg/ml). The antibacterial effect of the other fruits and berries showed no significant difference at the different pH values.

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KEY WORDS

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Propionibacterium
Staphylococcus
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pomace extract

Acne vulgaris is a common skin disease of humans caused by bacteria inducing non-inflammatory and inflammatory skin lesions (Leyden 1997; 2003). It is not a serious disease but often involves both physical scarring and social embarrassment (Lehmann et al. 2002; Harper 2004). *Propionibacterium acnes*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* have been recognized as acne-inducing bacteria (Harper 2004) with staphylococci and *P. acnes* being the most prevalent causes of the disease (Hassanzadeh et al. 2008). Numerous topical and oral drugs are available to treat acne vulgaris, but the number of strains resistant to common antibiotics such as erythromycin, kanamycin, neomycin or tetracycline has been increasing in the past years (Hassanzadeh et al. 2008; Swanson 2003). There is therefore a substantial demand for new compounds with antibacterial activity against acne-inducing bacteria. Natural substances promising such biological activity have been extensively studied, especially during the last decade (Gnan and Demello 1999; Rabanal et al. 2002; Chomnawang et al. 2005, 2007; Kumar et al. 2007).

In this study, *in vitro* biological activity of the juice, as well as water and methanol extract of the pomace (peels, seeds, and flesh remaining after juice pressing), of 20 cultivated and wild fruits was investigated on acne-inducing bacteria by broth dilution method.

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Materials and Methods

Bacterial isolates and their maintenance

Clinical isolates of *Staphylococcus aureus* (Szeged Microbial Collection, University of Szeged, Szeged, Hungary; SZMC 900), *Staphylococcus epidermidis* (SZMC 901) and *Streptococcus pyogenes* (SZMC 902) was maintained on T1 medium (4% beef extract, 4% peptone, 1% glucose, 1% yeast extract). *Propionibacterium acnes* (American Type Culture Collection, USA; ATCC 11827) was maintained on PA medium (1.5 % tryptone, 0.5% yeast extract, 0.5% NaCl, 0.5% beef extract, 0.3% glucose, 0.2% KH_2PO_4).

Plant material

The fruits and berries used were as follows. *Crataegus monogyna* Jacq., *Fragaria ananassa* Duch., *Prunus armeniaca* L., *P. avium* L., *P. avium* Gold, *P. cerasus* L., *P. persica* L., *Rubus fruticosus* L., *R. idaeus* L. from the family Rosaceae. *Ribes x nidigrolaria* Bauer, *R. nigrum* L., *R. rubrum* L., *R. uva-crispa* L. from the family Grossulariaceae. *Morus alba* L., *M. nigra* L. (Moraceae), *Berberis thunbergii* DC. cv. *atropurpurea* , *Mahonia aquifolium (*Pursh.) Nutt. (Berberi-

Table 1. Minimal Inhibitory Concentration (MIC, mg dry matter content / ml) of juices, water and methanol extracts at pH 7 and pH 5.5 after 48h incubation (J, juice; W, water extract; M, methanol extract).

		S. aureus		*S. epidermidis*		*S. pyogenes*		*P. acnes*	
		pH 7	pH 5.5	pH 7	pH 5.5	pH 7	pH 5.5	pH 7	pH 5.5
Berberidaceae									
Berberis thunbergii DC.	J	-[a]	-	-	-	-	-	-	-
	W	-	-	-	-	-	-	-	-
	M	-	-	14.73	14.73	0.83	0.83	15.73	15.73
Caprifoliaceae									
	J	-	-	-	-	5.45	5.45	-	-
Sambucus nigra L.	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-
Cornaceae									
	J	-	-	-	-	-	-	-	-
Cornus mas L.	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	1.9	1.9	-	-
Grossulariaceae									
	J	-	-	13.49	11.09	-	-	-	-
Ribes rubrum L.	W	-	-	5.13	5.13	-	-	-	-
	M	-	-	-	-	-	-	-	-
	J	-	-	14.29	14.29	-	-	-	-
Ribes x nidigrolaria Bauer	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-
	J	5.43	5.43	5.03	0.40	-	-	-	-
Ribes uva-crispa L.	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-
Rosaceae									
	J	-	-	-	-	-	-	-	-
Fragaria ananassa Duch.	W	-	-	-	-	-	-	-	
	M	-	-	-	-	6.06	6.06	-	-
	J	4.83	7.42	-	-	-	-	-	-
Prunus armeniaca L.	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-
	J	-	-	-	-	15.6	15.6	13.87	14.65
Prunus avium L.	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	6.13	6.13	-	-
	J	3.32	3.32	3.52	3.52	-	-	-	-
Rubus fruticosus L	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-
	J	-	-	12.35	9.18	-	-	-	-
Rubus idaeus L.	W	-	-	-	-	-	-	-	-
	M	-	-	-	-	-	-	-	-
Gentamycin		16[b]		8		2.0		1.0	
Ampicillin		> 64		> 64		0.5		1.0	

[a]No inhibition effect at the highest concentration. [b] MIC for control antibiotics (µg /ml).

daceae), *Sambucus nigra* L., *S. alba* Raf. (Caprifoliaceae), and *Cornus mas* L. (Cornaceae).

Commercial fresh fruits were purchased on a local market (Szeged, Hungary), *M. alba, M. nigra, B. thunbergii, M. aquifolium, S. nigra, S. alba* were harvested in the neighbourhood of Szeged, and *C. mas* in the Mátra mountains. Harvested plants were identified using the Plant Identification Manual of Hungarian Vascular Plants (Simon 2004) or by the kind help of Dr. E. Mihalik (University Botanical Garden, Szeged).

Extraction methods

Fruit juices were freshly pressed and stored at -20°C. The remaining pomace was dried overnight at 60°C in an oven and then ground to powder. One gram of each powdered pomace was extracted 3 times with 10 ml of distilled water or methanol per cycle. The extracts were combined and evaporated to dryness at 100°C in an oven (water extracts) or at 35-40°C in a water bath (methanol extracts). The dry material was re-dissolved in 4 ml of distilled water (water extracts) or of 10% methanol-water solution (methanol extracts), and frozen in 1 ml aliquots. One aliquot from each extract was dried again and weighed for dry matter content calculation. The juices and extracts were diluted in the appropriate media for the tests.

Table 2. Total phenol content, radical scavenging capacity (of the tenfold dilution) and RC_{50} for DPPH of the juices and extracts with antibacterial activity. (J, juice; W, water extract; M, methanol extract.)

Plant species		Total phenol[a] (mg g^{-1})	Radical scavenging capacity (%)	RC_{50} (mg ml^{-1})[b]
B. thunbergii	M	171.29 ± 1.05	81.5 ± 8.4	0.17 ± 0.02
S. nigra	J	70.52 ± 4.24	79.9 ± 3.4	0.35 ± 0.01
C. mas	M	28.07 ± 0.04	80.4 ± 0.50	1.11 ± 0.08
R. rubrum	J	14.81 ± 0.47	82.3 ± 9.8	1.51 ± 0.22
	W	14.20 ± 0.04	59.2 ± 6.7	1.11 ± 0.09
R. x nidigrolaria	J	18.87 ± 0.08	84.3 ± 3.0	3.5 ± 0.87
R. uva-crispa	J	28.91 ± 0.78	91.0 ± 5.9	1.02 ± 0.01
F. x ananassa	M	51.03 ± 0.98	89.7 ± 8.9	1.01 ± 0.45
P. armeniaca	J	9.43 ± 0.22	37.8 ± 3.1	4.52 ± 0.35
P. avium	J	6.95 ± 0.06	17.8 ± 3.1	2.04 ± 0.69
	M	6.29 ± 0.12	28.7 ± 4.4	3.83 ± 0.24
R. fruticosus	J	61.24 ± 0.27	83.8 ± 8.7	0.85 ± 0.17
R. idaeus	J	21.52 ± 0.21	87.5 ± 6.6	0.88 ± 0.21

[a] Total phenol content is expressed as mg gallic acid equivalent per g dry mater content of juices or pomace extracts.
[b] RC_{50} is given as mg dry matter content per ml of juices or pomace extracts.

Determination of antibacterial effect by broth microdilution method

The *in vitro* antibacterial activities were determined by microdilution plate assay. Juice or extract dilutions were applied in a range of ten- to eighty-fold dilution, buffered to pH 7 or 5.5 with 0.165 M morpholino-propane sulfonic acid. In each well, 100 µl of diluted and sterile-filtered (0.45 µm, Millipore) juice or extract dilution was mixed with 100 µl bacterium cell suspension (10^5 cells/ml in the appropriate medium). The absorbance of the bacterial cultures was measured at 620 nm. Each test plate contained an uninoculated control (100 µl juice or extracts + 100 µl medium) the absorbance of which was subtracted from the absorbance of bacterial cultures containing the same juice or extract; a positive growth control, a medium-free sterility control for the juices or extracts, and a drug-free sterility control for the medium. The samples were tested in triplicate and the results were recorded after 48 h.

Determination of total phenolics

Total soluble phenolics were determined by the Folin-Ciocalteu method (Singleton and Rossi 1965). Before adding to the reagent solution, juices and extracts were diluted 1:10 or 1:100 with the appropriate medium (DW or 10% (v/v) methanol). Absorption was measured at 725 nm. Total phenolics were expressed as mg gallic acid equivalent per g dry matter content of juices or extracts.

Determination of radical scavenging capacity with DPPH (1,1-diphenyl-2-picrylhydrazil)

Radical scavenging capacity was measured spectrophotometrically at 515 nm. Tenfold dilutions of the juices and water and methanol extracts of pomace (0.2 ml) were added to a 1.2 ml solution of DPPH (100 µM in methanol). Control was prepared by adding 0.2 ml methanol instead of sample. After 30 min, the absorbance was measured at 515 nm. Radical scavenging capacity was determined by the following equation:

$$(A_{control} - A_{sample})/A_{control} \times 100.$$

Determination of RC_{50} values

RC_{50} was defined as the dry matter content of the sample, in milligrams per ml, required for decreasing the initial DPPH concentration by 50%, and was extrapolated from a dose-response curve (Valavanidis et al. 2004). Different dilutions (10, 50, 100, 150, 200x) of the juices and water and methanol extracts of pomace were added to a DPPH solution, and absorbance was measured as described above. Experiments were carried out in triplicate, and results were expressed as mean values ± standard deviation (SD).

Results

Approximately half of the tested juices or extracts had inhibitory potential against the investigated species (Table 1). The following plants had no effect on any of the bacteria: *C. monogyna, P. avium* Gold, *P. cerasus, P. persica, S. nigra, R. nigrum, M. aquifolium, M. alba* and *M. nigra*. Generally, the juices showed the broadest antimicrobial activity.

Staphylococci were inhibited by the juices of *Ribes* and *Rubus* species and *P. armeniaca*. *S. epidermidis* was sensitive to the methanol extract of *B. thunbergii* and to the water extract of *R. rubrum* pomace. It is worth to mention that this was the only case when an aqueous extract of pomace had any effect on the bacterial growth. *S. pyogenes* was more sensitive to methanol extracts than to juices; and especially to *B. thunbergii* and *C. mas* pomace with MIC values of 0.83 and 1.9 mg/ml, respectively. *P. acnes* was the most insensitive bacterium, its growth was inhibited only by the methanol extract of *B. thunbergii* and the juice of *P. avium.* Changes in pH had no or slight effect on the MIC values of the tested fruits; except for *R. uva-crispa* where acidic pH decreased the MIC value to less than one tenth of the value measured at neutral pH, suggesting the presence of undissociated antimicrobial phenolic and organic acids at pH 5.5.

Radical scavenging capacity of the juices and pomace extracts showing antibacterial activity had a broad range from 15.6% (*P. avium* juice) to 91% (*R. uva-crispa* juice) (Table 2.). There was no real correlation between antioxidant capacity and MIC values, suggesting that not only phenolic antioxidants are responsible for the antimicrobial effect.

Discussion

Antibacterial activity of berry juices has been demonstrated

against both Gram-positive and Gram-negative bacteria (Puupponen-Pimiä et al. 2001; Ryan et al. 2001; Cavanagh et al. 2003; Lee et al. 2003). Both cultivated and wild berries are rich in bioactive substances such as phenolic compounds including flavonoids, tannins, and phenolic acids, and have an important role in the maintenance of the human health. Berries of the *Ribes* genus contain benzoic acid, the flavonoid quercetin, and anthocyanins, all of which can have antibacterial action. The fruits in the genus *Rubus* are rich in ellagitannins which can permeabilize the outer cell membrane of Gram-negative bacteria (Puupponen-Pimiä et al. 2004). In the literature, no data were found on the antibacterial effect of the investigated *Ribes* and *Rubus* species on acne-inducing bacteria. In our experiments, however, berry juice and extracts had good antibacterial effect against the two staphylococci tested. On *S. epidermidis* (and on *Klebsiella pneumoniae*), *P. avium* fruit extracts exerted growth inhibition (Lee et al. 2003). In our study, juice and/or methanol extract of *P. avium* inhibited the growth of *S. pyogenes* and *P. acnes* but not *S. epidermidis*. For butanolic extract of *P. armeniaca*, inhibitory effect on the growth of Gram-negative and -positive bacteria was reported (Rashid et al. 2007). In our experiments, apricot juice had good antibacterial activity against *S. aureus.*

The difference in the antibacterial activity of juices and extracts may be due to their different components; soluble in aqueous and alcoholic media. Water extract contains the majority of anthocyanins, tannins, starches, saponins, polypeptides and lectins, while methanol extracts also contain polyphenols, lactones, flavones, and phenons (Cowan 1999). For example, the major alkaloid of *B. thunbergii*, berberine, has a proven antibacterial effect (Amign et al. 1969) and is rather non-polar. Accordingly, only the methanol extract of *Berberis* pomace showed antibacterial effect. In case of the other efficient methanol extracts (*P. avium, C. mas, F. ananassa*) the non-polar active compound is, as yet, unknown.

The effect of pH is very important in case of microbicidal acids of the fruits. These are membrane-active substances which damage the inner cell membrane in their undissociated form. They alter the membrane permeability of the microbial cell and acidify the cytoplasm (Puupponen-Pimiä et al. 2004). In our experiments, the antibacterial effect of juices and extracts was more or less independent from pH changes, suggesting that other, non-dissociable compounds were responsible for the growth inhibition.

The majority of reports on natural components against acne-inducing bacteria refer to medicinal plants. The effect of Thai (Chomnawang et al. 2005, 2007) and Indian (Kumar et al. 2007) herbal extracts against *P. acne* and *S. aureus* was investigated. Seven Indian and 13 Thai medical plants inhibited the growth of the bacteria, achieving best MIC values with *Garcinia mangostana* (0.039 mg/ml) and *Coscinium fenestratum* (0.049 mg/ml). There is also a study about the antimicrobial effect of *Hypericum* spp. on *S. aureus*, and *S. epidermidis* (Rabanal et al. 2002), where, similar to our results, only methanol and chloroform extracts but not aqueous extracts had antibacterial activity. Guava (*Psidium sp.*) aqueous leaf extract with MIC of 5.6 mg/ml inhibited the growth of *S. aureus* (Gnan and Demello 1999). In our study, the antimicrobial effect of commercial and wild fruits was investigated against these bacteria, and MIC values ranging from 0.40 to 15.73 mg/ml were found. Medicinal plants have lower values but by extraction of the active compounds from fruit juices or from the by-products of juice making the minimal inhibitory concentration could be decreased. Active ingredients from foodstuffs have the advantage being non-toxic and familiar to the human body. Their inhibitory potential on bacterial growth may be utilized in the development of natural drugs or cosmetics to treat acne vulgaris.

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