

Anticandidal effect of berry juices and extracts from *Ribes* species

Communication

Judit Krisch^{1*}, Lilla Ördögh², László Galgóczy², Tamás Papp², Csaba Vágvolgyi²

¹Institute of Food Engineering, Faculty of Engineering, University of Szeged,
H-6725 Szeged, Hungary

²Department of Microbiology, Faculty of Science and Informatics, University of Szeged,
H-6726 Szeged, Hungary

Received 25 September 2008; Accepted 3 November 2008

Abstract: The biological activities of fruit juices and pomace (skin, seeds) extracts from blackcurrant (*Ribes nigrum*), gooseberry (*Ribes uva-crispa*) and their hybrid plant (jostaberry, *Ribes x nidigrolaria*) were evaluated against the most frequently isolated twelve human pathogenic *Candida* species by broth dilution tests. The phenolic content of juice, water and methanol extracts were measured and the relationship with antifungal activity was assessed. Growth of the most *Candida* species was inhibited, with the exception of *C. albicans*, *C. krusei*, *C. lusitanae* and *C. pulcherrima*. *R. nigrum*, with the highest phenol content, was observed to have the highest anticandidal activity, indicating a positive correlation between phenol content and antifungal activity.

Keywords: *Candida* species • *Ribes* • Antifungal activity • Total phenolics

© Versita Warsaw and Springer-Verlag Berlin Heidelberg.

1. Introduction

Infections caused by *Candida* species are widespread throughout the world. Although *C. albicans* is the most common *Candida* species encountered as a cause of human infection, candidiasis due to non-albicans species is dramatically increasing. Antifungal therapy with synthetic chemical agents can lead to problems such as drug safety, resistance and various side effects. Thus there is a need to find naturally occurring substances with antifungal properties.

Polyphenols are secondary metabolites in plants that possess antioxidant activity, their role being protection against fungal and bacterial infections and environmental stress. Fruits, especially berries, are rich sources of polyphenols, substances with proven antimicrobial effects [1-3]. Phenolic compounds described from the members of the *Ribes* genus include phenolic acids (e.g. p-coumaric acid), flavonols

(quercetin, isoquercitrin, myricetin and kaempferol) [4,5], anthocyanins and proanthocyanidins [6-9]; anthocyanins being the dominant group in dark *Ribes* species. The use of *R. nigrum* and *R. uva-crispa* properties for medicinal purposes has been documented throughout history. *R. nigrum* fruits are often used to support the immune and digestive systems as the juice contains a polysaccharide-rich substance, designated cassis polysaccharide (CAPS), having macrophage-stimulating and antitumor activity [10]. A crude extract of *R. nigrum* fruit displayed antiviral activity against herpes [11] and influenza [12] viruses. *R. uva-crispa* is known to have laxative and astringent properties.

In our experiments freshly pressed berry juices, as well as water and methanol extracts of *R. nigrum*, *R. uva-crispa* and *Ribes x nidigrolaria* were used to evaluate antifungal activity against 12 human pathogenic *Candida* strains. Extracts were made from the skin and seed fraction (pomace) remaining after juice pressing. This fraction is dark coloured and has high content

* E-mail: krisch@mk.u-szeged.hu

Strain	<i>Ribes nigrum</i>			<i>Ribes uva-crispa</i>			<i>Ribes x nidigrolaria</i>			Flu ^b
	juice	water extract	MetOH extract	juice	water extract	MetOH extract	juice	water extract	MetOH extract	
<i>C. albicans</i> (ATCC 10231)	^a	-	-	-	-	-	-	-	-	> 128
<i>C. glabrata</i> (CBS 138)	10.38	3.85	-	5.63	-	4.60	-	-	2.91	64
<i>C. guilliermondii</i> (CBS 566)	-	-	6.13	-	-	-	-	-	-	8
<i>C. inconspicua</i> (CBS 180)	-	2.82	4.22	-	-	-	-	-	3.33	64
<i>C. krusei</i> Z76(CBS 573)	-	-	-	-	-	-	-	-	-	> 128
<i>C. lipolytica</i> (CBS 6124)	-	5.15	-	-	-	4.63	-	-	4.67	16
<i>C. lusitaniae</i> (CBS 6936)	-	-	-	-	-	-	-	-	-	> 128
<i>C. norvegica</i> (CBS 4239)	-	3.62	-	-	-	-	-	-	10.98	> 128
<i>C. parapsilosis</i> (CBS 604)	-	2.76	4.41	4.06	-	-	-	-	3.56	2
<i>C. pulcherrima</i> (CBS 5833)	-	-	-	-	-	-	-	-	-	128
<i>C. tropicalis</i> (CBS 94)	-	3.75	7.16	-	-	-	-	-	4.23	> 128
<i>C. zeylanoides</i> (CBS 619)	-	1.83	-	-	-	-	-	-	5.68	> 128

Table 1. Anticandidal activity (Minimal Inhibitory Concentration, MIC; mg dry matter content/ml) of berry juices, and of water and methanol extracts of the pomace.

^a no growth inhibition at the highest concentration

^b Fluconazole (MIC, µg/ml) for reference

of phenolic phytochemicals. We hypothesize that such waste products can provide compounds with antimicrobial activity.

2. Experimental Procedures

2.1 Microorganisms

Candida albicans was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA), and the other *Candida* strains (*C. glabrata*, *C. guilliermondii*, *C. inconspicua*, *C. crusei*, *C. lipolytica*, *C. lusitaniae*, *C. norvegica*, *C. parapsilosis*, *C. pulcherrima*, *C. tropicalis*, *C. zeylanoides*) from the Centraalbureau voor Schimmelcultures (CBS, Utrecht, The Netherlands). Inocula were prepared in RPMI1640 medium (Sigma-Aldrich, St. Louis, MO, USA) in concentration of 10⁵ cells/ml.

2.2 Fruits and extraction methods

The plants investigated included black currant (*Ribes nigrum* L.), gooseberry (*Ribes uva-crispa*; syn: *R. grossularia*), and jostaberry; the latter is generally described as a hybrid between *R. nigrum* and *R. uva-crispa* but in fact it is the result of a complex breeding (*Ribes x culverwellii*, *Ribes nidigrolaria*, *Ribes x nidigrolaria* or *R. nigrum x uva-crispa*). Fresh fruits were purchased at a local market (Szeged). Fruit juices were freshly pressed and stored at -20°C. The remaining pomace was dried overnight at 60°C in an oven and then grounded 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 redissolved in 4 ml distilled water (water extracts) or 1% methanol-water solution (methanol extracts), and frozen in 1 ml aliquots. One sample from each extracts was dried

	Juice	Water extract	MetOH extract
<i>Ribes nigrum</i>	68.03 ± 1.13	178.39 ± 1.80	145.60 ± 8.36
<i>Ribes uva-crispa</i>	40.42 ± 7.70	17.25 ± 1.01	24.54 ± 0.38
<i>Ribes x nidigrolaria</i>	21.50 ± 1.22	45.86 ± 1.25	50.51 ± 1.49

Table 2. Total phenol content of juices and extracts (μg gallic acid equivalent/mg dry weight).

Results are means of three independent measurements.

again and weighed for dry matter content calculation. Juices and extracts were diluted in the appropriate media for the tests.

2.3 Determination of anticandidal activity by broth microdilution assay

In vitro antifungal activities were evaluated by microdilution plate assay in RPMI1640 medium (Sigma-Aldrich, St. Louis, MO, USA), buffered with 0.165 M morpholino propanesulfonic acid (pH 7) according to National Committee for Clinical Laboratory Standards (CLSI) recommendations [13]. In each well, 100 μl of diluted and sterile-filtered (0.45 μm , Millipore, USA) juice or extract was mixed with 100 μl cell suspension (10^5 cells/ml). Each test plate contained an uninoculated control, a positive growth control, a medium-free control and a drug sterile control. The assays were performed in triplicates and were read after incubation for 24 hours at 37°C without shaking.

2.4 Determination of total phenolics

Total soluble phenolics were determined by the Folin-Ciocalteu method [14]. Juices and extracts were diluted 1:100 before incubation at room temperature. Absorption was measured at 725 nm in a Beckman DU-65 spectrophotometer (Beckman Instruments Inc., Fullerton, CA, USA). Total phenolics were expressed as μg gallic acid equivalent/mg dry weight of juices or extracts.

3. Results and Discussion

Berry juices and extracts exhibited anticandidal activity against 8 *Candida* species (*C. glabrata*, *C. guilliermondii*, *C. inconspicua*, *C. lipolytica*, *C. norvegica*, *C. parapsilosis*, *C. tropicalis* and *C. zeylanoides*). On the contrary, *C. albicans*, *C. krusei*, *C. lusitanae* and *C. pulcherrima* were insensitive to each of the investigated extracts and juices. The MIC values for juices and extracts varied from 2.82 mg/ml to 10.98 mg/ml of dry matter content (Table 1).

The phenol content was higher in the dark coloured pomace fraction of *R. nigrum* and *Ribes x nidigrolaria* than in the juices, and the water and methanol pomace extracts exhibited good anticandidal effect (Table 1). The juices showed no inhibitory effect on *Candida* growth, except for *C. glabrata* and *C. parapsilosis*, two increasingly emerging pathogens frequently associated with biofilm formation [15,16].

Unripe, green *R. uva-crispa* (which in Hungary is the preferred form for consumption) had the relatively lowest phenol content (Table 2) and also the weakest antifungal effect. Our results however, indicate no significant relationship between phenol content and anticandidal activity (the linear correlation of the two sets of data was very poor; $R^2=0.4468$).

Literature on the antifungal activity of fruits is limited and most published research deals with the action of etheric oils from herbs and medicinal plants. However, there has been some recent publications on antimicrobial, including anticandidal, activity of nordic berries and phenolic extracts [17,18]. Growth inhibition on *C. albicans* was observed with cloudberry (*Rubus chamaemorus*), raspberry (*Rubus idaeus*) and strawberry (*Fragaria x ananassa*), but not with *R. nigrum*, supporting the results presented here. Published data on the antifungal activity of berries against other *Candida* species appears to be absent in the current literature.

In our study we made an attempt to fill the gap in literature data about the anticandidal activity of fruits or pomace extracts on non-*albicans* *Candida* species. We found that polyphenol-rich pomace of *Ribes* species, the by-product of fruit juice manufacturing, may be a source of natural agents against some *Candida* species. In the future efforts will be made to identify the main components responsible for the anticandidal effect in pomace extracts.

Acknowledgements

This work was financially supported by the grants GVOP-3.1.1-2004-05-0471/3.0 and ETT 214/2006. T. P. holds a János Bolyai Research Scholarship from the Hungarian Academy of Sciences.

References

- [1] Cavanagh H.M., Hipwell M., Wilkinson J.M., Antibacterial activity of berry fruits used for culinary purposes, *J. Med. Food*, 2003, 6, 57-61
- [2] Maatta-Riihinen K., Oksman-Caldentey K.M., Berry phenolics selectively inhibit the growth of intestinal pathogens, *J. Appl. Microbiol.*, 2005, 98, 991-1000
- [3] Puupponen-Pimia R., Nohynek L., Meier C., Kahkonen M., Heinonen M., Hopia A., et al., Antimicrobial properties of phenolic compounds from berries, *J. Appl. Microbiol.*, 2001, 90, 494-507
- [4] Ehala S., Vaher M., Kaljurand M., Characterization of phenolic profiles of Northern European berries by capillary electrophoresis and determination of their antioxidant activity, *J. Agric Food Chem.*, 2005, 53, 6484-6490
- [5] Hakkinen S., Heinonen M., Karenlamp S., Mykkanen H., Ruuskanen J., Torronen R., Screening of selected flavonoids and phenolic acids in 19 berries, *Food Res. Internat.*, 1999, 32, 345-352
- [6] Jordheim M., Måge F., Andersen Ø.M., Anthocyanins in berries of *Ribes* including Gooseberry cultivars with high content of acylated pigments, *J. Agric. Food. Chem.*, 2007, 55, 5529-5535
- [7] Nakajima J-I., Tanaka I, Seo S., Yamazaki M., Saito K., LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries, *J. Biomed. Biotech.*, 2004, 5, 241-247
- [8] Slimestad R., Solheim H., Anthocyanins from black currants (*Ribes nigrum* L.), *J. Agric. Food Chem.*, 2002, 50, 3228-3231
- [9] Zadernowski R., Naczek M., Nesterowicz J., Phenolic acid profiles in some small berries, *J. Agric. Food. Chem.* 2005, 53, 2118-2131
- [10] Takata R., Yamamoto R., Yanai T., Konno T., Okubo T., Immunostimulatory effects of a polysaccharide-rich substance with antitumor activity isolated from black currant (*Ribes nigrum* L.), *Biosci. Biotech. Biochem.*, 2005, 69, 2042-2050
- [11] Suzutani T., Ogasawara M., Yoshida I., Azuma M., Knox Y.M., Anti-herpesvirus activity of an extract of *Ribes nigrum* L., *Phytother. Res.*, 2003, 17, 609-613
- [12] Knox Y.M., Suzutani T., Yoshida I., Azuma M., Anti-influenza virus activity of crude extract of *Ribes nigrum* L., *Phytother. Res.*, 2003, 17, 120-122
- [13] Clinical and Laboratory Standards Institute (CLSI), Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved Standard Second Edition (M27-A2), 2002
- [14] Singleton V.L., Rossi J.A. Jr., Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *Am. J. Enol. Viticult.*, 1965, 16, 144-158
- [15] Fidel Jr. P.L., Vazquez J.A., Sobel J.D., *Candida glabrata*: review of epidemiology, pathogenesis, and clinical disease with comparison to *C. albicans*, *Clin. Microbiol. Rev.*, 1999, 12, 80-96
- [16] Weems J.J. Jr., *Candida parapsilosis*: epidemiology, pathogenicity, clinical manifestations, and antimicrobial susceptibility, *Clin. Infect. Dis.*, 1992, 14, 756-766
- [17] Rauha J-P., Remes S., Heinonen M., Hopia A., Kahkonen M., Kujala T., et al., Antimicrobial effect of Finnish plant extracts containing flavonoids and other phenolic compounds, *Internat. J. Food Microbiol.*, 2000, 56, 3-12
- [18] Nohynek L.J., Alakomi H-L., Kahkonen M.P., Heinonen M., Helander I.M., Oksman-Caldentey K-M., et al., Berry phenolics: antimicrobial properties and mechanisms of action against severe human pathogens, *Nutrit. Cancer*, 2006, 54, 18-32