*Agata Stobnicka[1], Elvira Jungfer[2], Małgorzata Gniewosz[1]

Composition and antibacterial properties of fresh cranberry (*Vaccinium macrocarpon* L.) juice**

[1]Department of Biotechnology, Microbiology and Food Evaluation, Warsaw University of Life Sciences, Poland
Head of Department: prof. Małgorzata Gniewosz, PhD
[2]Department of Nutrition and Food Sciences, University of Bonn, Germany
Head of Department: prof. Rudolf Galens

SKŁAD I WŁAŚCIWOŚCI ANTYBAKTERYJNE ŚWIEŻEGO SOKU Z ŻURAWINY WIELKOOWOCOWEJ (VACCINIUM MACROCARPON L.)

STRESZCZENIE

Celem niniejszych badań było określenie składu i właściwości antybakteryjnych surowego soku żurawinowego (Vaccinium macrocarpon L.). Działanie soku zostało sprawdzone w stosunku do bakterii typowych dla żywności (Staphylococcus aureus ATCC 25923, Bacillus subtilis ATCC 6633, Escherichia coli ATCC 25922 i Salmonella enteritidis ATCC 13076). Badany sok wykazał aktywność przeciwbakteryjną wobec wszystkich szczepów testowych. Najbardziej wrażliwy na jego działanie okazał się szczep Staphylococcus aureus ATCC 25923 (MIC = 2,3 mg/ml), a najbardziej oporny szczep Salmonella enteritidis ATCC 13076 (MIC = 19,2 mg/ml). Analiza składu soku metodą HPLC-UV-DAD-MS^n wykazała obecność m.in. procyjanidyn (dimery, trimery, tetramery), epikatechiny, kwasu chlorogenowego, glikozydów myrycetyny i kwercetyny. Wyniki te są obiecujące i dają nadzieję na potencjalne wykorzystanie soku żurawinowego w leczeniu zatruć pokarmowych i biegunki.

SŁOWA KLUCZOWE: SOK ŻURAWINOWY, VACCINIUM MACROCARPON, AKTYWNOŚĆ PRZECIWBAKTERYJNA

Introduction

Berry fruits are rich sources of bioactive compounds such as phenolic and organic acids which may hold antimicrobial activities (1). Cranberry (*Vaccinium macrocarpon* L.) is a polyphenolic-rich berry fruit native to North America, but also grown and popularly used in traditional folk medicine in Europe as a treatment of microbial infections. Cranberry fruits are important and willingly eaten ingredient of the diet as well, which offers important health benefits (2-4).

Staphylococcus aureus, *Bacillus subtilis*, *Escherichia coli* and *Salmonella enteritidis* are typical foodborne bacteria, which can cause food spoilage, food poisoning, stomach dysfunction and diarrhea. According to Tayel et al. (5), gastrointestinal disorders can be successfully treated with as conventional and as natural methods. However, consumers today are increasingly concerned not only about chemical residues in food, but also about side effects of chemical drugs and tend to choose natural food and natural methods of treatment, as well (6). Consequently development of natural antimicrobial compounds is on the rise. The objectives of the present research were to study (1) the antibacterial properties, including the bacteriostatic and bactericidal activity, of cranberry juice (*Vaccinium macrocarpon* L.) and HPLC–UVDAD-MS^n analysis (2) of the juice sample.

Materials and methods

Plant material

Fruits of *Vaccinium macrocarpon* L. („Pilgrim") (*Ericaceae*) were collected in Poland, (52.002°N, 20.937°E).

Preparing of the juice

Cranberry fruits were pressed in Omega 8006 juicer (Omega, USA). The juice was filtrated through Whatman paper No.1. The filtrate was stored in refrigerator at 4°C till further use.

Microorganisms

Reference bacterial strains were the Gram-positive (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633) and the Gram-negative (*Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076). All tested strains were obtained from American Type Culture Collection. All tested microorganisms were

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subcultured into Mueller-Hinton Agar (Btl, Poland) slants and were maintained at 4°C.

Antibacterial assay

The antibacterial assay was performed using the broth macrodilution method (7). A twofold serial dilution of the juice was prepared in Mueller-Hinton Broth (MHB) (Merck, Germany). For every experiment a negative control (distilled water, medium, inoculums) was included. Each test-tube received 2 ml of the medium, 10 μl of the inoculum (10^6 CFU/ml) and in sequence a proper amount of the juice (2000 μl; 1000 μl; 500 μl; 250 μl; 125 μl; 60 μl; 30 μl; 15 μl). Mentioned volumes of the juice correspond with following dry mass content: 38 mg; 19 mg; 9,6 mg; 4,8 mg; 2,4 mg; 1,2 mg; 0,6 mg; 0,3 mg. The content of each tube was mixed thoroughly with tube shaker and incubated at 37°C for 24 h. Microbial growth in each test-tube was determined by observing and comparing the test-tube with the negative control. The absence of microbial growth was interpreted as an antibacterial activity. The minimum inhibitory concentration (MIC) was the lowest concentration of the juice that prevented visible growth of bacteria. Minimum bactericidal concentration (MBC) was determined by plating 100 μl from each negative and the first positive growth test-tube on Mueller-Hinton Agar (Btl, Poland). Plates were incubated at 37°C for 24 h. MBC was defined as the lowest concentration yielding 0-50 colonies on the plate.

To avoid any interference into cranberry juice, the influence of pH on bacteria's growth was checked by adjusting pH of Mueller-Hinton Broth (MHB) (Merck, Germany), by the addition 0,1 M HCl, to the value detected by pH meter (Emerson, USA) in MIC tube. Then test-tube received 2 ml of the medium and 10 μl of the inoculum (10^6 CFU/ml). The content of each tube was mixed thoroughly with tube shaker and incubated at 37°C for 24 h. Microbial growth in test-tube was determined by observing and comparing the test-tube with the negative control.

Juice sample preparation with solid-phase extraction

Juice sample for analysis was prepared using the following solid-phase extraction (SPE) performed by using a Gilson ASPEC XL system (Automated Sample Preparation with Extraction Cartridges, Abimed, Langenfeld, Germany). Firstly, HR-XC SPE cartridge (500 mg, 3 ml cartridge, Macherey-Nagel, Düren, Germany) was conditioned with methanol and washed with UHQ water. The cartridge was loaded with 10 ml juice and washed with 0.1 n HCl. The polyphenols were eluted with 3 ml methanol.

HPLC-UVDAD-MS^n analysis

The analysis of the phenolic compounds by HPLC-UVDAD-MS^n was performed according to a method of Papagiannopolus et al. (8). The liquid chromatograph was a Summit system (Dionex, Idstein, Germany) consisting of a Degasys DG-1310 degasser (Uniflows, Tokyo, Japan), a P-580 A HPG pump, an ASI-100 T automated sample injector, an STH-585 column oven, and a UltiMate 3000 Diode Array Detector. Chromeleon software package Version 6.20 Build 531 was used for system control and data evaluation. Separation was carried out with an analytical column Nucleodur C18 Isis, 150 x 2 mm, 3 μm (Macherey-Nagel, Düren, Germany) temperated at 35°C. Solvents were UHQ water with 1 % acetic acid (v/v) (mobile phase A) and acetonitrile with 1% acetic acid (v/v) (mobile phase B). The injection volume was 10 μl and the chromatogram was monitored at 200-600 nm.

An LCQ Classic ion trap mass spectrometer equipped with an electrospray interface was coupled with the HPLC and controlled with Xcalibur software. All polyphenols were analysed in the negativ ion mode with following settings: source voltage,- 4.0 kV; sheath gas flow, 90; auxiliary gas flow, 60; capillary voltage, -10 V; capillary temperature, 300°C; first octapole offset, +4.0 V; interoctapole lens voltage, +30.0 V; second octapole offset, +10.0 V; ion trap DC offset, +10.0 V.

Polyphenols were identified using the UV spectral data and the molecular weight and fragmentation pattern from the mass spectrometer. Identification of individual compounds was conducted by comparison of characteristic mass fragmentation patterns with literature.

Results and discussion

Antibacterial activity of cranberry juice

In the present study, the cranberry juice (*V. macrocarpon*) showed the antibacterial activity against both Gram-positive (*Staphylococcus aureus* ATCC 25923 and *Bacillus subtilis* ATCC 6633) and Gram-negative (*Escherichia coli* ATCC 25922 and *Salmonella enteritidis* ATCC 13076) strains. Gram-negative bacteria were less sensitive than Gram-positive bacteria. The results summarized in table 1 demonstrate that the cranberry juice had the strongest antibacterial activity against *Staphylococcus aureus* ATCC 25923 (2.3 mg dry mass/ml) and the weakest against *Salmonella enteritidis* ATCC 13076 (19.0 mg dry mass/ml). MIC value for *Bacillus subtilis* ATCC 6633 was 4.8 mg dry

Table 1. Inhibition parameters (MIC,MBC) of the cranberry (*Vaccinium macrocarpon* L.) juice.

Microorganisms	Parameters	
	MIC (mg d.m./ml) (medium pH)	MBC (mg d.m./ml)
Staphylococcus aureus ATCC 25923	2.3 (6.96)	4.8
Bacillus subtilis ATCC 6633	4.8 (6.20)	4.8
Escherichia coli ATCC 25922	9.6 (4.98)	19.0
Salmonella enteritidis ATCC 13076	19.0 (4.26)	19.0
d.m. – dry mass		

mass/ml and for *Escherichia coli* – 9.6 mg dry mass/ml. Minimal bactericidal concentration had equal values for both Gram-positive strains (4.8 mg dry mass/ml) and for both Gram-negative strains (19.0 mg dry mass/ml).

The influence of pH on strains growth were summarized in table 2. Cranberry juice inhibited growth of tested strains (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633 and *Escherichia coli* ATCC 25922) at pH = 6.96, pH = 6.20 and pH = 4.98. As the results of checking influence of pH medium on bacterial growth showed, strain's growth was observed at mentioned pH. Strong antibacterial properties on these strains are probably not result of low pH medium, but come out from influence of substances other than acids, like quercetin, myricetin and procyanidins (9). However, inhibition of *Salmonella enteritidis* ATCC 13076 growth at pH = 4.26, was especially result from low pH medium.

The results are in accordance to Côte et al. (10), who finds cranberries may offer a line of defense against food poisoning with their ability to reduce the growth of *Salmonella* sp., *Escherichia coli, Staphylococcus aureus* and other types of bacteria found in food. Cranberry juice has also been reported to inactivate other foodborne pathogens such as *Listeria monocytogenes, Escherichia coli* O157:H7 and *Vibrio parahaemolyticus* (11-14). As a result it is anticipated that phytochemicals with adequate antibacterial efficacy can be used for the treatment of the bacterial infections (15). Cranberry juice is known to exhibit a range of antibacterial, anti-viral and pharmacological activities (16-18). According to Magariños et al. (19) *Staphylococcus aureus* ATCC 25923 was the most susceptible to cranberry juice inhibition among all tested strains.

Table 2. Influence of pH medium on bacteria's growth.

Microorganisms	Medium pH	Growth
Staphylococcus aureus ATCC 25923	6.96	+
Bacillus subtilis ATCC 6633	6.20	+
Escherichia coli ATCC 25922	4.98	+
Salmonella enteritidis ATCC 13076	4.26	–
+ normal growth, – no growth		

Composition of cranberry juice

The tentatively identified compounds are shown in table 3 and figure 1 shows the corresponding chromatogram monitored at 280 nm. As already described by Määttä-Riihinen et al. (20) and Gu et al. (21) epicatechin, procyanidin dimers (A- and B-type), trimers and tetramers could be shown in the cranberry juice. Different flavonols are detectable in the juice. According to Vvedenskaya et al. (22) the major flavonol glycoside in cranberries is quercetin as also show in this study and as well myricetin glycosides could be detected. The presence of the coumaroyl iridoid shown in peak 13 was already reported by Jensen et al. (23). In the group of phenolic acids caffeoyl hexoside (peak 2), chlorogenic acid (peak 3), and feruloyl quinic acid (peak 9) could be tentatively assigned. This is in accordance to Häkkinen et al. (24).

Conclusions

In conclusion, the present study demonstrates that cranberry juice (*V. macrocarpon*) has antibacterial effects against *S.aureus, B. subtilis, E.coli* and *S. enteritidis* and contributes to the knowledge of the natural antibacterial substances. The results provide promising baseline information for the potential use of cranberry juice for food poisoning and diarrhea treatment and may be further investigated as a natural solution for gastrointestinal disorders.

Table 3. Composition of the cranberry (*Vaccinium macrocarpon* L.) juice.

Peak No.	Tentatively identified compound	RT (min)	[M-H]-
1	Procyanidin tetramer	13.92	1152
2	Caffeoyl hexoside	17.5	341
3	Chlorogenic acid	18.53	353
4	Procyanidin dimer	19.19	575
5	Epicatechin	25.18	289
6	Unknown compound	26.16	–
7	Procyanidin trimer	32.42	863
8	Myricetin hexoside	38.18	479
9	Feruloyl quinic acid	40.15	367
10	Procyanidin trimer	41.08	863
11	Quercetin hexoside	48.51	463
12	Procyanidin trimer	50.35	863
13	Coumaroyl iridoid	51.57	537
14	Quercetin pentoside	53.5	433
15	Procyanidin trimer	54.73	863
16	Quercetin pentoside	55.39	433
17	Quercetin pentoside	57.45	433
18	Myricetin	59	317
19	Quercetin deoxyhexoside	60.37	447
20	Dimethoxymyricetin hexoside	63.25	507

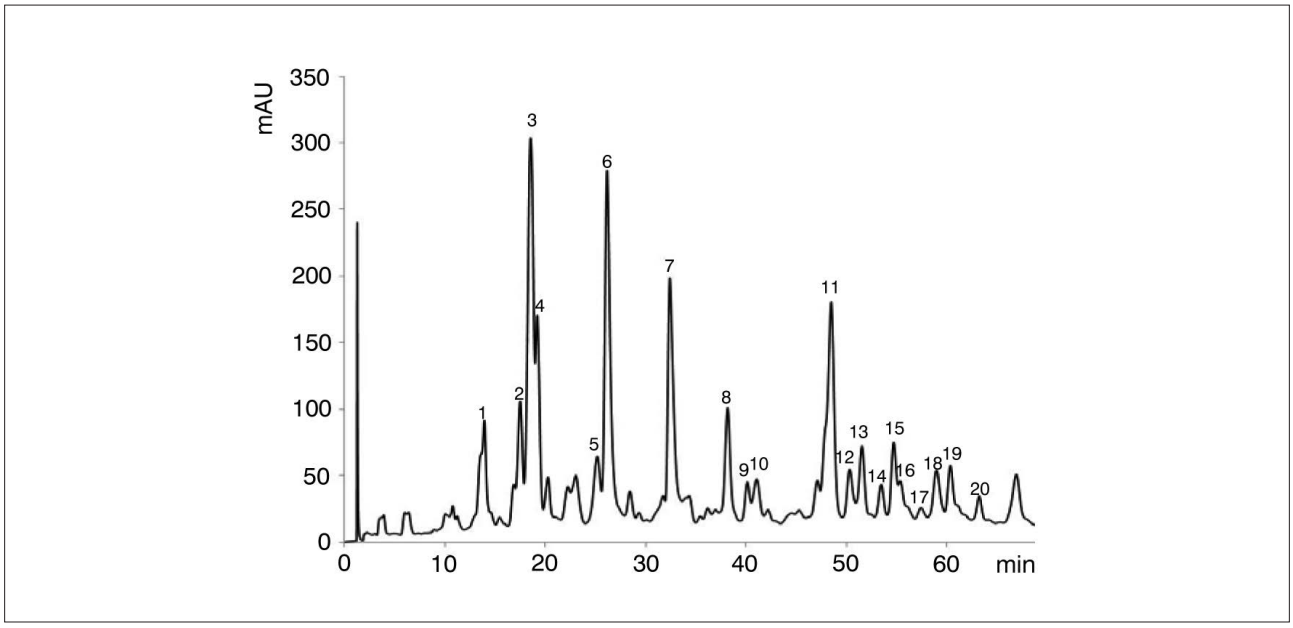

Fig. 1. HPLC Chromatogram of the SPE extract at 280 nm.

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Adres/address:
*Agata Stobnicka
Department of Biotechnology,
Microbiology and Food Evaluation
Warsaw University of Life Sciences
ul. Nowoursynowska 159, 02-776 Warszawa
tel.: +48 (22) 593-76-58, fax: + 48 (22) 593-76-81
e-mail: agatastobnicka@gmail.com