Comparison of the contents of selected phenolic compounds in the fruit of *Vaccinium macrocarpon* Ait. and *Vaccinium oxycoccos* L.

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Summary

The contents of flavonoids and proanthocyanidins in the fruit of *Vaccinium macrocarpon* and *V. oxycoccos* have been determined by the methods recommended by Ph. Eur. V and the total content of phenolic compounds - by the Folin-Ciocalteu method. The content of flavonoids has been were 0.27% and 0. 09% calculated as hyperoside; the content of proanthocyanidins has been 2.05% and 0.92% as cyanidin chloride and the total phenolic compounds – 1.88% and 0.98% expressed as gallic acid of the fruit of *V. macrocarpon* and *V. oxycoccos*, respectively.

Key words: Vaccinium macrocarpon, Vaccinium oxycoccos, content of flavonoids, proanthocyanidins, total phenolic compounds

INTRODUCTION

The genus *Vaccinium* L. (Ericaceae), comprises about 450 species including *V. macrocarpon* Ait. (Cranberry, American Cranberry), *Vaccinium oxycoccos* L. (Small cranberry, marsh whortleberry), *V. microcarpos* (Turcz. Ex Rupr.) Schmalh., syn. V. *oxycoccos*. In natural conditions they grow in acidic and wet peatbog type soils in the cooler areas of the Northern Hemisphere.

V. macrocarpon is native to the northern and eastern parts of North America (Canada, United States). *V. oxycoccos and V. microcarpos* are grown in northern Europe and northern Asia, whereas *V. oxycoccos* also grows in the northern part of North America.

Cranberry is a small wintergreen dwarf shrub. In *V. oxycoccos* the egg-shaped leaves are small (5–10 mm long), the flowers are dark pink with a purple central spike, produced on finely hairy stems. The fruit is a small (about 1 cm in diameter), pale pink berry with a refreshing acrid acidic flavour. In *V. microcarpos* the leaves are more triangular and the flower stems are hairless. Some botanists include *V. microcarpos* within *V. oxycoccos*. The leaves of *V. macrocarpon* are larger than those of *V. oxycoccos* (10–20 mm long) and the red ball-shaped berries (2 cm in diameter) have a slightly apple-like taste. They ripen in June, July and August.

The ripe fresh, dried or frozen fruit is used for medicinal treatment and as a food products.

The phenolic classes identified in the fruit of *V. macrocarpon* include anthocyanins (glycosides of cyanidin and peonidin), flavonoids (mainly myricetin and quercetin glycosides) and their acylated derivatives, e. g. quercetin-3-(6"-benzoyl)-β-galactoside, quercetin-3-(6"-*p*-coumaroyl)-β-galactoside as well as methoxylated derivatives of glycosides of myricetin and quercetin [1-3] as well as proanthocyanidins. The cranberry proanthocyanidins are oligomers composed predominantly of epicatechin units which have unusual A-type linkages (minimum one A-type interflavan bound, but often multiple A-type interflavan linkages at each stage of polymerization) [4, 5].

The derivatives of benzoic and cinnamic acids (benzoic, *o*-hydroxybenzoic, cinnamic, *m*-hydroxybenzoic, *p*-hydroxybenzoic, *p*-hydroxyphenylacetic, phtalic, 2,3-dihydroxybenzoic, vanillic, *o*-hydroxycinnamic, 2,4-dihydroxybenzoic, *p*-coumaric, ferulic, caffeic, sinapic acid) have been identified, mainly in bond forms, and only 10% of them are free acids. Benzoic acid and *p*-coumaric as well as sinapic acids [6, 7] have turned out to be most abundant. Cranberries also contain the following acids: quinic, malic and citric [8].

The fruit contains carbohydrates (glucose and fructose; 3.6–5.0 g/100 g), triterpens (oleanolic, ursolic acids, and a hydroxycinnamic derivative of ursolic acid) and vitamins, mainly vitamin C as well as salts of potassium, natrium, ferrum and calcium [9].

The fruit of *V. macrocarpon* is subject to intense phytochemical and biological research, whereas studies of *V. oxycoccos* are scarce and, so far, have concentrated on the presence of carbohydrates, flavonoids, vitamins and organic acids. According to Adamczak et al., the content of flavonoid compounds was larger by 19% in fruit dried in 35-40°C than lyophylized fruit, whereas the content of organic acids was lower in thermal drying by 15%, on average [10].

Cranberry juice has been widely used for the treatment and prophylaxis of *Escherichia coli* bacteria infections of the urinary tract.

Until recently, it has been suggested that the quinic acid caused large amounts of hippuric acid, which has an antibacterial activity, to excrete into urine [8], while organic acids in cranberry have been supposed to lower the urinary pH in which the increase of bacteria is inhibited. Cranberry does not act as a natural antibiotic, either [11].

Recent studies have shown that two types of substances are responsible for the activity of cranberry: fructose and proanthocyanidins. These compounds have been proven to inhibit adherence of *E. coli*.

The *E. coli* strains that cause UTIs have fimbriae that facilitate the adhesion of bacteria to the uroepithelial cells. The adhesion of uropathogenic *E. coli* is accomplished by binding lectins exposed on the fimbriae surface to complementary carbohydrates on the host tissue. The fimbriae produce adhesins that attach to specific carbohydrate receptors on the uroepithelial cells. The adherence of *E. coli* with type-1 fimbriae (mannose-sensitive) to cells is easily inhibited by fructose [4]. *E. coli* isolated from patients with UTIs owe their strong virulence to a different type of fimbriae – p-fimbriae which are associated with the (α-galactose (1→4) β-galactose) specific lectin and bind to the uroepithelial cell [11].

Proanthocyanidins in cranberry containing A-type linkage of flavanol units are unique and have been shown to inhibit the adherence of *E. coli* with P-type fimbrial adhesins [4].

The proanthocyanidins may also change the shape of the bacteria from rods to spheres and cause chemical changes to their surface membranes [12]. In addition, they have been shown to inhibit the adherence of P-fimbriated uropathogenic *E. coli* to the uroepithelial cells thus preventing UTIs [4, 5].

The proanthocyanidins with this A-type linkages are more effective than those with proanthocyanidins B-type which occur in other tanin-rich fruit in inhibiting adherence of bacteria to cell surfaces [12].

The results of some studies have shown that cranberry juice inhibits the growth of certain standard bacteria strains [13] and the anti-inflammatory effect of the cranberry fruit [9]. From among common fruit, that of cranberry is characterised by the greatest content of polyphenolic compounds and antioxidant activity [14].

Cranberry products may be used in prophylactic dentistry. The *in vitro* analysis of cranberry juice has proven its non-specific antiviral effects towards unrelated viral species: bacteriophages T2 and T4 of *E. coli* and the simian rotavirus SA-11 [15]. The extract from cranberry fruit inhibits the growth of *Helicobacter pylori*. Anti-*H. pylori* activity has been significantly improved by its synergistic blending with blueberry, grape seed and oregano extracts [16].

Another direction of the biological research has focussed on the anti-proliferating activity against human tumour cell line. Total cranberry extract and some fractions separated from the fruit, enriched in polyphenols, may reduce the development of human oral, colon and prostate cancer cell lines and human breast cancer [3, 17], while the fractions obtained as a result of chromatographic separation of the extract have been found to inhibit the proliferation of human tumour cell lines of multiple origins, namely from: breast, skin, colon, lung and brain [18].

Phytochemicals in cranberries, mainly flavonoids: myricetin 3-arabinoside, myricetin-3-galactoside, quercetin-3-galactoside and quercetin-3-rhamnoside, are responsible for the inhibition of LDL oxidation, the induced expression of LDL receptors and the increased uptake of cholesterol in hepatocytes. *In vitro* suppression of LDL oxidation takes place in a dose-dependent manner [13, 19].

The results of the clinical studies conducted so far have shown that cranberry products significantly reduce the incidence of UTIs, compared to *placebo* and may be recommended for the prevention of UTIs, particularly in women with symptomatic UTIs [8]. Their effectiveness in other groups such as children or elderly men and women is not clear. Current evidence suggests that it is not effective in people with a neuropathic bladder disfunction [8].

The results of many studies have shown that after the therapy the patients have been found to show a decrease in bacteriuria, pyuria and alleviation of the UTIs symptoms. In patients with frequent reccurent UTI, the number and severity of infections could be reduced. The patients treated with cranberry products have been less likely to suffer from repeated infections and need antibiotics in relatively reduced doses as compared to *placebo* group [4, 8, 10, 20, 21].

To obtain convincing evidence of the biological activity of cranberry a well-designated parallel group - large, properly randomised, *placebo*-controlled double bond blind trials of cranberry juice or cranberry products for the treatment of UTIs is needed. Recently, it has not been clear what the optimum dosage (amount and concentration) is, just as the duration of the therapy or the method of administration, e.g. juice or capsules/tablets [8, 12]. In addition, a large number of drop-outs/withdrawals from several studies have indicated that cranberry may not be acceptable over long periods [8]. Data has also shown some interaction between cranberry juice and warfarin [22].

The fruit of *V. oxycoccos* has also been used in the treatment of the urinary tract in folk medicine; its composition and activity has not been investigated thoroughly so far.

The aim of the study was to establish the content of flavonoids and proanthocyanidins by the methods recommended by Eu. Pharm. 2004 [23] and to investigate the total content of phenolic compounds by the Folin-Ciocalteu method [24, 25] in commercial samples of the fruit of *V. macrocarpon* and *V. oxycoccos.*

EXPERIMENTAL

The fruit of *V. macrocarpon* and *V. oxycoccos* were purchased in a drugstore (2007 under Zakłady Zielarskie, Kawon-Hurt, Gostyń, Poland). All materials were dried and pulverized.

Voucher specimens were deposited in the Department of Pharmacognosy, Poznan University of Medical Sciences, Poznan, Poland.

Acetone, methanol, ethyl acetate, hydrochloric acid, n-butanol and sodium carbonate were obtained from POCh (Gliwice, Poland). Gallic acid was purchased from Carl Roth GmbH Co, Germany, Folin-Ciocalteu reagent and aluminium chloride – from Merck (Darmstadt, Germany). Absorbance was measured in a spectrophotometer Specol 11 (Carl Zeiss, Jena, Germany) and a spectrophotometer Lambda 35 (Perkin-Elmer, USA).

Determination of flavonoids

The flavonoid content was determined spectrophotometrically, from 2.500 g of powdered *V. oxycoccos* fruit and 1.500 g of *V. macrocarpon,* according to Ph. Eur. 2004, for Birch leaf [23]. The absorbance of yellow flavonoid complexes in the sample with aluminium chloride was measured in the Specol 11 spectrophotometer at 425 nm. The results were calculated as hyperoside.

Determination of proanthocyanidins

Powdered fruit in the amount of 2.500 g of was collected for determination. The content of proanthocyanidins was determined according to the method recommended for the determination of proanthocyanidins in Hawthorn berries [23]. After extraction of the raw product in an acidic medium, proanthocyanidins were shaken to butanol and the absorbance of the coloured solution was measured at 545 nm. The proanthocyanidins content was expressed as cyanidin chloride.

Determination of total phenolic compounds

Standard curve for gallic acid

Stock solution: 0.0200 g of gallic acid dissolved in water in a flask of 100 mL in capacity. 0.05; 0.1; 0.15; 0.20; 0.25; 0.30 mL of stock solution and 0.5 mL of the Folin–Ciocalteu reagent was added to a flask of 10 mL in capacity containing 4 mL of distilled water. The solution was stirred and then left at room temperature for 1 min., after that 2 mL of 20% sodium carbonate solution were added and the content was supplemented with water to 10 mL. The carefully mixed contents were left for 30 min at room temperature in the dark. The absorbance of the samples was measured at λ max =760 nm, against the blank sample with distilled water instead of the extract.

Determination of phenolic compounds

Powdered fruit in the amount of 5.000 g for *V. oxycoccos* or 2.500 g for *V. macrocarpon* was extracted with 100 mL of water and moved to a flask of 100 mL in capacity, supplemented with water. 3.0 mL of this solution were collected and placed in a measuring flask of 10 mL in capacity, supplemented with water and 0.2 mL was collected to a flask of 10 mL in capacity and containing 4 mL of distilled water, to which 0.5 mL of the Folin–Ciocalteu reagent was added.

The next stages were the same as when determining the standard curve. The results were calculated as gallic acid.

RESULTS

The contents of the selected phenolic compounds from the group of flavonoids and proanthocyanidins and the total content of phenolic compounds in the fruit of *V. oxycoccos* and *V. macrocarpon* were determined. The content of flavonoids was determined according to Ph. Eur. 2004 on the basis of the measured absorbance of coloured complexes of flavonoids with aluminium chloride at 425 nm and was calculated as hyperoside [23].

The content of proanthocyanidins was determined in the cranberry fruit by the method recommended in Pharmacopoeia for the determination of procyanidins in *Hawthorn berries*. The fruit was extracted with butanol with an addition of the hydrochloride acid and the absorbance of the coloured solution was measured at 545 nm. Proanthocyanidins in the fruit were determined and calculated as cyanidin chloride (Ph. Eur. 2004).

The content of total phenolic compounds was determined by Folin-Ciocalteau reagent, using gallic acid as a standard. This method is based on the reduction of a phosphowolframate-phosphomolybdate complex by phenolics to blue reaction products. The absorbance of the coloured solutions with the Folin-Ciocalteau reagent was measured at 760 nm. The calibration curve was established as gallic acid [24, 25]. The linear course of the calibration curve corresponded to the concentration range of 1-6 μg cm^{-1}. The correlation coefficient for the regression line was r=0.9977 and a good linear of regression equation (y=ax±b) y=13.099x was found. The total content of phenolic compounds was expressed as gallic acid. The results of the six measurements of flavonoids, proanthocyanidins and phenolic compounds were statistically analysed and the statistical evaluation have been shown in Table 1. All the results have been expressed as an arithmetic mean $\bar{x}$ with a standard deviation S, average deviation S_x and the values of percentage coefficient of variance VC and the confidence interval μ.

Table 1.

Determination of flavonoids, proanthocyanidins and total phenolic compounds in *V. macrocarpon* and *V. oxycoccos* fruit (n=6; $t_{\alpha,f}$=2.571)

genus	*V. macrocarpon*			*V. oxycoccos*		
	flavonoids	cyanidins	phenols	flavonoids	cyanidins	phenols
$\bar{x}$	**0.2736**	**2.0480**	**1.8845**	**0. 0862**	**0.9240**	**0.9845**
S	0,0010	0.0000	0.0244	0.0003	0.0255	0.0060
S_x	0,0004	0.0000	0.0099	0.0001	0.0104	0.0024
VC %	0,3877	0.0000	0,0129	0.1300	0,0276	0.0061
μ	0.2736±0.0011	2.0480±0.000	1.8845±0.0256	0.0862±0.0002	0.9240±0.0267	0.9845±0.0063

$\bar{x}$ – mean (%); S – standard deviation; Sx – average deviation; VC – variation coefficient (%); μ – confidence interval

The contents of flavonoids were 0.27% and 0. 09% calculated as hyperoside, those of proanthocyanidins expressed as cyanidin chloride were 2.05% and 0.92% and the ones of the total phenolic compounds expressed as gallic acid – 1.88% and 0.98% for *V. macrocarpon* and *V. oxycoccos* fruits, respectively.

DISCUSSION

Cranberry juice has been widely used as a folk remedy for the treatment and prophylaxis of recurrent infections of *E. coli* bacteria in the urinary tract, especially in women. One in four women who develop UTIs is prone to a reccurence. The risk factors that predispose women to recurrences include contraception, antimicrobials, estrogen and genetics.

Several studies have shown that proanthocyanidins have been recognized for their anti-adherence activities to uroepithelial cells in the wall of bladder, against uropathogenic P type *E. coli* and may play a role in this activity [8]. Some authors suggest that there is a correlation between the content of polyphenols and the activity of a cranberry product against UTIs [12].

The beneficial effect on the maintenance of the urinary tract health, possible due to the A-type linkages in proanthocyanidins, has been found in cranberry, while other proanthocyanidins-rich foods, e.g. apple juice, grape juice, green tea or dark chocolate contain the B-type proanthocyanidins that exhibit minor activity or are not active [4, 5].

V. macrocarpon fruit has recently been subject to phytochemical, pharmacological and clinical studies. As to the *V. oxycoccos* species also growing in Poland in natural state, the phytochemical data are scarce, while clinical trials are practically nonexistent.

The cranberry-based preparations (tablets, capsules) and juice that are available in the Polish market are most often made of the fruit of *V. macrocarpon* and, more rarely, of the fruit of *V. oxycoccos*. Preparations are usually standardized for the amount of powdered raw products or concentrated juice, or the content of proanthocyanidins in a daily dose. Producers declare that the content of proanthocyanidins (PACs) should be 36.4 mg daily (determined by p-dimethylaminocinnamaldehyde in methanol and concentrated hydrochloric acid (DMAC method), expressed as catechin, which is equal to 89.2 mg of PAC determined by Ph. Eur. 2004 calculated as cyanidin chloride [11, 26].

The cranberry juice has usually been administered in a dose of 300 mL daily or in capsules with concentrated juice or dry extract of cranberry 400-450 mg twice day for 3–12 or 25 weeks [8, 10].

This study has reported the comparison of the contents of the phenol group compounds: flavonoids and proanthocyanidins which were determined by the method recommended by Ph. Eur., and the total content of polyphenols – determined by the well-known and often used for this purpose Folin-Ciocalteu method.

The content of flavonoids were 0.27% and 0.09% expressed as hyperoside, that of proanthocyanidins expressed as cyanidine chloride were 2.05% and 0.92%, and the content of the total phenolic compounds has been 1.88% and 0.98% expressed as gallic acid in the fruit of *V. macrocarpon* and *V. oxycoccos*, respectively. The results have shown that the fruit of *V. macrocarpon* contains almost twice as much of the phenolic compounds studied as *V. oxycoccos*. Since proanthocyanidins constitute the main group of active compounds, the content determination of these compounds should be taken into consideration during the standardization of the cranberry products.

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PORÓWNANIE ZAWARTOŚCI WYBRANYCH ZWIĄZKÓW FENOLOWYCH W OWOCACH *VACCINIUM MACROCARPON* Ait. i *VACCINIUM OXYCOCCOS* L.

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Streszczenie

W owocach *Vaccinium macrocarpon* i *V. oxycoccos* oznaczono zawartość flawonoidów, proantocyjanidyn, metodami opisanymi w Farmakopei Europejskiej V oraz całkowitą zawartość związków fenolowych, z odczynnikiem Folin-Ciocalteu. Zawartość flawonoidów wynosiła 0,27% i 0,09% w przeliczeniu na hyperozyd; proantocyjanidyn 2,05% i 0,92% w przeliczeniu na chlorek cyjanidyny; całkowita zawartość związków fenolowych 1,88% i 0,98% w przeliczeniu na kwas galusowy, w owocach *V. macrocarpon* i *V. oxycoccos*, odpowiednio.

Słowa kluczowe: *Vaccinium macrocarpon*, *Vaccinium oxycoccos*, zawartość flawonoidów, proantocyjanidyn, suma polifenoli