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Communication

Antioxidant capacity of crude extracts containing carotenoids from the berries of various cultivars of Sea buckthorn (*Hippophae rhamnoides* L.)*

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Comparative analysis of antioxidant capacity was performed using FRAP and DPPH methods on extracts containing carotenoids acquired from fruits of Sea buckthorn. The examination included nine varieties of Sea buckthorn growing in the comparative cultivation. Conducted analysis allowed to compare the antioxidant capacity with carotenoids content measured with spectrophotometric and HPLC methods. Three of the examined cultivars indicating high antioxidant activity in both, FRAP and DPPH methods, also revealed highest ('Aromatnaya') and high ('Botanicheskaya', 'Arumnyj') total carotenoids content in HPLC analysis.

Key words: sea buckthorn, carotenoids, antioxidant capacity, FRAP, DPPH

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INTRODUCTION

In Poland, Sea buckthorn fruits and preserves obtained from them are not very well known because Sea buckthorn is encountered only occasionally as ornamental tree. However, Sea buckthorn is more known, widely eaten and cultivated in Northern Europe and Asia (Mech-Nowak *et al.,* 2011). These fruits are regarded as a valuable agent in folk medicine and nutrition (Britton & Khachik, 2009). Thanks to high content of vitamin C, carotenoids, flavonoids, tocopherols and other potentially health- beneficial components the Sea buckthorn juice and pulp are often used as food or beverages (Andersson, 2009; Suryakumar & Gupta, 2011). Recently, the amount of Sea buckthorn fruits used for production of health-supporting drugs (i.e. lotions, cosmetics and nutritional supplements) is rising (Stahl & Sies, 2003). Fruits of Sea buckthorn are one of the few exceptions among carotenoid-rich fruits and vegetables that contain high amount of lipids, which has been postulated to be significant factor of carotenoids bioavailability enhancing their absorption in humans (Ranjith *et al.*, 2006). Since carotenoids are essential in proper human diet to prevent many diseases (Canfield *et al.*, 1994; Sharoni *et al.*, 2004) and act as antioxidant agents, their source with high uptake could be valuable for medicine (Britton *et al.*, 2008). In recent years various *in vitro* and *in vivo* models were conducted to investigate the medical and pharmacological potential. There are some reports about beneficial effects of Sea buckthorn berries on gastric tissue (Suleyman *et al.*, 2001; Xing, 2002) and cholesterol concentration (Larmo *et al.*, 2009). Moreover, they have shown immunomodulatory properties (Geetha *et al.*, 2002), radioprotective, anti-stress, anti-atherogenic, and have a benign effect in treatment of coronary heart disease (Eccleston *et al.*, 2002).

In the last few years apprehension regarding importance of antioxidants and radical oxygen species in the biochemistry of living organisms (especially humans) is growing among the researchers. Nowadays many products are recognized as containing the health- beneficial antioxidants. Seeking for cultivars with highest antioxidant capacity will allow to distinguish promising varieties for future cultivation (Jones & Smirnoff, 2005; Varshneya *et al.*, 2011; Dhar *et al.*, 2012).

Comparative analysis of antioxidant capacity of crude extracts containing carotenoids obtained from fruits of Sea buckthorn harvested in 2010 was conducted using FRAP (Ferric Reducing Antioxidant Power) and DPPH (Diphenylpicrylhydrazyl). This study included nine cultivars of Sea buckthorn (of Russian origin) growing in the comparative cultivation at the Fruit Experiment Station in Brzezna near Nowy Sącz which is a part of Research Institute of Horticulture in Skierniewice.

MATERIALS AND METHODS

Sea buckthorn berries were collected from The Fruit Experiment Station located in Brzezna in 2010. Berries were harvested, weighed and then immediately frozen at –20ºC. Then, every 5 g sample of fruits was freeze-dried. After lyophilisation, carotenoids were extracted from berries using 20 ml of n-hexane/ethanol (1:1, v/v), till the point of complete exhaustion of color. The fruits extracts were kept at –20ºC for spectrophotometric and HPLC analyses. The HPLC system consisted of a Shimadzu LC-20AD model with a LiChrospher 100 RP-18, 250 mm column. The mobile phase comprised 1% water in methanol (A) — methanol (B) — 10% n-hexane in acetonitrile (C) and the column was developed in a gradient of 0–100% B at a flow rate of 2 ml/min. Detection was carried out at 450 nm and 425 nm using a Shimadzu SPD-20AD DAD detector. Quantification of total carotenoids was performed on UV/Vis Spectrophotometer JASCO V-530. The antioxidant capacity of extracts was examined by FRAP (Benzie & Strain, 1996) and DPPH (Wang *et al.,* 2010; Chaman *et al.,* 2011) methods.

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Abbreviations: DPPH, diphenylpicrylhydrazyl; FRAP, ferric reducing antioxidant power; HPLC, high performance liquid chromatography.

Table 1. FRAP value (5 min) (μM); DPPH value (%) and carotenoids content (mg/100 g) of fresh weight measured in spectrophotometer in crude extracts from berries of nine cultivars of Sea buckthorn; n=3

Cultivar	FRAP	SD	DPPH	SD	Carotenoids content	SD
Botanicheskaya	1892	35.566	35.84	1.553	43.06	2.038
Avgustinka	819	40.952	45.78	1.847	19.48	0.334
Luchistaya	676	20.212	38.13	0.208	10.94	0.056
Aromatnaya	648	30.036	45.37	2.266	25.51	0.289
Arumnyj	561	4.276	44.08	1.732	23.21	0.401
Prozrachnaya	541	22.933	39.06	1.124	14.76	0.063
Podorok Sadu	477	19.489	36.50	1.572	7.75	0.143
Moskvichka	402	19.503	31.82	1.184	24.46	0.953
Botanicheskaya Lubinteiskaya	248	12.022	37.63	1.161	8.85	0.203

RESULTS AND DISCUSSION

Investigation allowed to classify extracts of Sea buckthorn accordingly to the cultivar-depending antioxidant activity and compare them with carotenoids content acquired by spectrophotometric and HPLC methods (Ruban, 2010). In researches regarding total carotenoids content it is common that different data are acquired by spectrophotometric analysis and by HPLC. It is caused by the fact that some carotenoids with their isomers present in low concentration might be not detected as peaks and are not included in HPLC method. Generally in spectrophotometric analysis the absorbance is higher (in spectral range characteristic for carotenoids) increased by addition of absorbance other than carotenoids compounds dissolved in lipids also active in that spectral range. On the other hand it is believed that spectrophotometric methods are not reliable with mixed carotenoids samples.

Strongest response to DPPH method was shown by cultivars: 'Avgustinka', 'Aromatnaya', 'Arumnyj' and 'Prozachnaya' (Table 1). Highest values for FRAP method occurred in 'Botanicheskaya', 'Avgustinka', 'Luchistaya' and 'Aromatnaya' cultivars (Table 1). Methods of measuring antioxidant activity gave different results, which is understandable because of the specificity of reacting compounds in each method and differences observed in similar cases (Müller *et al.,* 2011). Also the lipids present in the crude extracts could affect the final results of antioxidant capacity analysis, as it was observed at material preparation phase, that some cultivars seemed to have more lipids than the others. Three cultivars that indicated high antioxidant activity in both, FRAP and DPPH method, also revealed highest ('Aromatnaya') and high ('Botanicheskaya', 'Arumnyj') total carotenoids content in HPLC (Table 2).

Responsibility for antioxidant activity might be connected with total content of carotenoids. The only exception seems to be 'Botanicheskaya' with the highest FRAP score and yet average HPLC results. However, spectrophotometric examination revealed highest absorbance of this cultivar (Table 1). This can be the result of the presence of other non-carotenoid compounds with absorbance in similar spectral range and antioxidant activity sensitive to FRAP method. Some flavonoids known as antioxidants are not only yellow in colour, but also poorly dissolve in water and may enter ethanol-hexane extracts with carotenoids. Also tocopherols and their esters, despite they are not absorbing in visible specter, may be present in fruits tissues and could be extracted in that solvents set and would significantly increase antioxidant capacity (Zadernowski, 2003).

Interesting would be examination of 'Botanicheskaya' cultivar regarding the presence of phenols. It will be also highly recommended to use longer column for HPLC allowing more precise separation of contents of the crude extracts.

It seems important to mention that some of cultivars were as berries different in colour — from yellow to deep orange. That might indicate differences in carotenoids and other compounds content causing very wide variation in all measured parameters across cultivars.

From the viewpoint of health-beneficial importance of Sea buckthorn fruits, those differences do not diminish the sense of conducted antioxidant capacity research, however it is needed to indicate that whole responsibil-

Table 2. Compounds content in berries of nine cultivars of Sea buckthorn (mg/100 g) of fresh weight measured in HPLC in crude extracts; n=3

Cultivar	Xantophylls	SD	Carotenes	SD	Total carotenoids	SD
Aromatnaya	5.67	0.086	23.3	0.391	28.97	0.31
Moskvichka	5.36	0.215	22.72	1.171	28.08	2.486
Arumnyj	4.93	0.248	16.58	0.742	21.51	1.301
Botanicheskaya	6.75	0.249	7.45	0.861	14.2	4.809
Prozrachnaya	4.16	0.111	7.58	0.26	11.74	0.337
Avgustinka	3.65	0.164	7.07	0.239	10.71	0.421
Botanicheskaya Lubinteiskaya	1.57	0.043	1	0.12	2.57	0.161
Luchistaya	1.42	0.009	0.85	0.128	2.27	0.137
Podorok Sadu	1.47	0.063	0.43	0.097	1.9	0.148

ity cannot be connected with carotenoids only. To settle this issue more accurate fractionation should be carried out, which might be a continuation of examination performed in this study.

The study allowed to distinguish several promising cultivars with high antioxidant activity or carotenoids content that would be suitable for future cultivation.

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