

POLYPHENOLIC COMPOUNDS AND BIOELEMENTS IN FRUITS OF EASTERN TEABERRY (*GAULTHERIA PROCUMBENS* L.) HARVESTED IN DIFFERENT FRUIT MATURITY PHASES

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Abstract

Eastern teaberry (*Gaultheria procumbens* L.) is known for its high content of essential oils in leaves and fruits, which are used in the pharmaceutical, food and cosmetics industries. Recently, teaberries have been attracting more interest owing to their content of polyphenolic compounds. The purpose of our study has been to determine the content of polyphenolic compounds (anthocyanins and total phenols) as well as their antioxidative activity and the concentration of several bioelements (Ca, Mg, Fe and Zn) in extracts from fruits of Eastern teaberry harvested in three different fruit maturity stages. The content of polyphenolic compounds depended on the harvest date. The highest level of these compounds was found in extracts from teaberries collected in the full maturity phase. All teaberry extracts, independently of the harvest date, demonstrated high antioxidative activity. Among the bioelements determined, teaberry extracts contained more calcium than magnesium and more iron than zinc. The content of such bioelements as Mg, Fe and Zn (in contrast to Ca) found in teaberry extracts did not depend on the harvest date.

Key words: anthocyanins, antioxidative activity, bioelements, fruits, *Gaultheria procumbens*, phenols.

**ZWIĄZKI POLIFENOLOWE I BIORPUNKI W OWOCACH
GOLTERII ROZESŁANEJ (GAULTHERIA PROCUMBENS L.)
ZBIERANEJ W RÓŻNYCH TERMINACH DOJRZAŁOŚCI**

Abstrakt

Golteria rozesłana jest rośliną znaną z zawartości w owocach i liściach olejków eterycznych, które są wykorzystywane w przemyśle farmaceutycznym, spożywczym i perfumeryjnym. W ostatnich latach owoce golterii rozesłanej wzmacniają też zainteresowanie ze względu na zawartość związków polifenolowych. Celem pracy było oznaczenie zawartości związków polifenolowych (antocyjanów i fenoli ogólnego) i ich właściwości antyoksydacyjnych oraz zawartości biopierwiastków (Ca, Mg, Fe, Zn) w ekstraktach z owoców golterii rozesłanej zbieranej w trzech terminach dojrzałości. Zawartość związków polifenolowych w ekstraktach z owoców golterii rozesłanej zależała od terminu zbioru owoców. Najwięcej związków polifenolowych zawierały ekstrakty z owoców zbieranych w okresie pełnej dojrzałości. Niezależnie od terminu zbioru, ekstrakty z owoców golterii rozesłanej wykazywały bardzo dużą zdolność antyoksydacyjną. Spośród badanych biopierwiastków, ekstrakty z owoców golterii rozesłanej zawierały więcej wapnia niż magnezu oraz więcej żelaza niż cynku. Zawartość takich biopierwiastków, jak Mg, Fe, Zn (wyjątek Ca), w ekstraktach z owoców golterii rozesłanej nie zależała od terminu zbioru owoców.

Słowa kluczowe: antocyjany, aktywność antyoksydacyjna, biopierwiastki, fenole, golteria rozesłana, owoce.

INTRODUCTION

Ornamental cover plants, increasingly more common in gardens, parks and greens, have been drawing more and more attention in the recent years (ALEKSANDROVA 1981, MAROSZ et al. 2000, WAŻBINSKA 2000, ZARAŚ et al. 2000). Eastern teaberry, also known as checkerberry, boxberry or American wintergreen (*Gaultheira procumbens* L.), is one of such plants. It is low, evergreen shrub with creeping shoots. In July and August it is in bloom, producing white or pink bell-shaped flowers (FRAZIK 1991). Round, glistening pink, bag-shaped fruits, 8-15 mm in diameter, remain on the plant until spring the following year (CZEKALSKI 2006). Fruits and leaves of Eastern Teaberry contain essential oils, which have a medicinal use in treatment of skin and throat inflammation. In the United States, these oils are used in the food (to make chewing gums and sweets), cosmetics and pharmaceutical industries. This plant is very popular in Central and South Americas, where it is known as teaberry and used to make aromatic infusion (CLARC 1999, HUFFMAN et al. 1994, RIBNICKY et al. 2003, SENETA, DOLATOWSKI 1997). Fruits of Eastern teaberry, owing to their content of polyphenols (catechins, quercetins), have antixodative properties (ACUNA et al. 2002, MA et al. 2001).

The principal aim of the research has been to determine the content of polyphenolic compounds (anthocyanins and total phenols), their antioxida-tive activity and the concentration of bioelements (Ca, Mg, Fe and Zn) in extracts from Eastern teaberry fruits harvested on three different dates. In

addition, correlation between the total phenolic content and the level of anthocyanins as well as the correlation between the content of polyphenols and antioxidative activity in extracts from Eastern teaberry fruits were established.

MATERIAL AND METHODS

The material consisted of fruits of Eastern teaberry (*Gaultheria procumbens* L.) collected in three different time periods (1st – 15th October 2006, 2nd-30th October 2006 and 3rd-15th March 2007). Samples of Eastern teaberry fruits weighing 5 g each were prepared and stored frozen until analysis.

After defrosting, the fruits were subjected to liquid-solid extraction, for which citric acid of pH 2 was used as a solvent. The extracts were pre-purified by filtering through Whatman No 1 filter paper. The content of total phenols in raw extracts was determined according to FOLIN-CIocalteau procedure, and expressed in terms of gallic acid (SINGLETON et al. 1999). The content of anthocyanins in the extracts (re-calculated as cyanidin 3-gluco-side) was determined according to the methods designed by NIKETIĆ-ALEKSIĆ, HRAZDINA (1972) (method H) and WROLSTAD (1976) (method W). Antioxidative activity was established with an aid of the method suggested by YEN, HUNG (2000), which consisted of the determination of scavenging synthetic DPPH[•] (1,1-diphenyl-2-picrylhydrazyl) radicals in extracts from Eastern Teaberry fruits. The results were cited as inhibition percentages.

Extracts from Eastern teaberry fruits were mineralized in the so-called Teflon bomb using microwave energy. For this purpose, 1 cm³ of each extract and 2 cm³ of concentrated nitric acid (HNO₃) were used. After the mineralization, the extracts were transferred quantitatively to measuring flasks, which were filled to the full capacity with deionized water. The content of bioelements was determined using atomic absorption spectrometry (AAS) with a Unicam 939 device.

All the reagents for the above determinations were of analytical purity. The results of the chemical analyses, with three replications, underwent statistical processing for one-factor experiments, using Duncan's test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Our comparison of the content of total phenolic compounds in extracts from Eastern teaberry fruits harvested in three different times periods revealed statistically significant differences (Table 1). The content of total phe-

Table 1
Content of polyphenols (mg 100 g⁻¹ fresh matter) and antioxidative activity (%)
in *Gaultheria procumbens* extract fruits

Harvest date	Total phenols	Anthocyanins				Phenols other than anthocyanins				Antioxidative activity	
		method H		method W		method H		method W		inhibition	
		mean <i>x</i>	mean <i>x</i>	SD	mean <i>x</i>	SD	mean <i>x</i>	SD	mean <i>x</i>	SD	mean <i>x</i>
I	360.9 ^b	18.25 ^b	1.00	10.63 ^b	0.79	342.69 ^b	72.09	350.31 ^b	71.69	87.55 ^a	2.01
II	495.2 ^a	21.47 ^a	0.76	13.11 ^a	1.35	473.73 ^a	45.97	482.09 ^a	45.08	89.28 ^a	0.40
III	180.0 ^c	17.36 ^c	0.20	7.83 ^c	0.88	162.64 ^c	8.92	172.17 ^c	6.38	85.88 ^a	1.36

SD – standard deviation;

Means followed by the same letters in columns did not differ significantly at *p* < 0.05 according to Duncan test.

nols in Eastern teaberry fruit extracts was the highest on the 2nd harvest date and the lowest – on the 3rd one. Similar dependence could be observed regarding the concentration of anthocyanins in Eastern teaberry fruit extracts. Irrespective of the determination method applied, the content of anthocyanins was the highest in extracts from Eastern teaberry fruits harvested on the 2nd and the lowest – on the last, 3rd harvest date. The content of phenols other than anthocyanins in the extracts was correlated with the total phenolic concentration and the content of anthocyanins. Likewise, it was the highest for extracts from fruits collected on the 2nd harvest date and the lowest – for the 3rd one.

Fruits of Eastern teaberry collected on the 2nd harvest day had reached full maturity, which is why extracts obtained from these fruits contained the highest levels of total phenols and anthocyanins. Similar results were reached while examining fruits of black mulberry (PLISZKA et al. 2007).

A close relationship has been found in the current study between the content of total phenols and anthocyanins in fruit extracts. Positive correlation was determined between the total phenolic content and anthocyanins in extracts from Eastern teaberry determined with the different methods. High correlation was obtained for the extracts obtained by method W ($R^2 = 0.871$), whereas the correlation for the extracts produced by method H was nearly two-fold smaller ($R^2 = 0.474$).

The content of polyphenolic compounds depends on many factors, such as: species of fruit, environmental conditions, agronomic treatments and fruit maturity (ASAMI et al. 2003, KALT et al. 2001, WAŽBIŃSKA et al. 2006). Collecting Eastern teaberries on different dates, the ripeness phase was taken into consideration. On the 2nd harvest date the maturity of the fruits was optimum, therefore the extracts contained the highest levels of polyphenols (total polyphenols and anthocyanins).

Similar dependences were confirmed for black mulberries (*Morus nigra*) harvested in full maturity, in which the content of polyphenols was also the highest (PLISZKA et al. 2007). The concentration of polyphenols in fruits depends on a plant species. However, it needs to be mentioned that extracts from Eastern teaberries contain more total phenols and less anthocyanins than extracts from black mulberries, which have more anthocyanins than total phenols (PLISZKA et al. 2007).

Our analysis of polyphenolic compounds has demonstrated that extracts from Eastern teaberries were characterised by high antioxidative activity (from 85.58 to 89.28%), which did not depend on the fruit maturity (Table 1). Likewise, antioxidative properties of polyphenolic compounds in black mulberries were not dependent on a harvest date (PLISZKA et al. 2007). Polyphenolic compounds in extracts from black elberberries, tested in another study, possessed very high antioxidative properties (82 to 89%), in which they were similar to Eastern teaberries (PLISZKA et al. 2005).

Another question examined in the present study has been the relationship between the content of anthocyanins and total phenols versus their antioxidative activity in extracts from Eastern teaberries. Very strong correlation was found between the content of anthocyanins and their total antioxidative activity in extracts from Eastern teaberries obtained with H method ($R^2 = 0.761$). In contrast, the correlation between the total phenols and antioxidative activity was very low for extracts obtained from Eastern teaberry fruits with method W ($R^2 = 0.436$). KAUR, KAPOR (2002) as well as KÄHKÖNEN et al. (2001) have shown that the effect produced by polyphenolic compounds in fruit extracts on their antioxidative activity is varied (R^2 from 0.3 to 0.9). In turn, HASSIMOTO et al. (2005) claim that there is no correlation whatsoever between the content of total phenols or the content of vitamin C and antioxidative activity of extracts, thus suggesting that antioxidative properties are a product of a combination of various compounds which produce synergistic or antagonistic effects.

The antioxidative activity of polyphenols is shaped by several factors, including the species and composition of fruits, the chemical structure of phenolic compounds or the extraction method used (PLISZKA et al. 2003, 2005, RICE-EVANS et al. 1996, ZHENG, WANG 2003).

The extracts we obtained from the Eastern teaberry were subjected to chemical analyses to determine the content of some macroelements (Ca, Mg) and microelements (Zn, Fe), which are essential for human health (WIELEBA, PASTERNAK 2001).

The determinations of Mg, Fe and Zn did not reveal any statistically significant differences between their contents in the extracts of Eastern teaberry harvested on the consecutive dates (Table 2). As for calcium, significant differences were found between the levels of this macroelement in the extracts from Teaberries and the harvest date. The highest content of calcium was determined in the extracts from Teaberries collected on the 2nd

Table 2

Content of bioelements in extracts from Eastern teaberries (*Gaultheria procumbens*)
(mg 100 g⁻¹ fresh matter)

Harvest date	Ca		Mg		Fe		Zn	
	mean <i>x</i>	SD	mean <i>x</i>	SD	mean <i>x</i>	SD	mean <i>x</i>	SD
I	15.61 b	1.87	14.39 a	0.24	3.29 a	0.51	1.69 a	0.12
II	19.42 a	1.34	14.95 a	0.04	2.71 a	0.00	1.57 a	0.72
III	13.51 b	0.77	13.33 a	1.82	3.53 a	1.56	1.48 a	0.06

Explanations, see Table 1

harvest day. On the other two harvest dates, the concentrations of calcium were similar and lower than on the 2nd date.

Irrespective of the harvest date, the teaberry extracts contained more calcium than magnesium and more iron than zinc.

CONCLUSIONS

1. The content of polyphenolic compounds (total phenols, anthocyanins) in extracts from the Eastern teaberry fruits depended on the fruit harvest date. The highest content of polyphenols occurred in extracts from teaberries harvested in the full maturity phase.
2. Irrespective of the harvest date, extracts produced from Eastern teaberry fruits were characterised by very high antioxidative activity.
3. Teaberry extracts contained more calcium than magnesium and more iron than zinc.
4. The content of such bioelements as Mg, Fe or Zn (unlike Ca) in teaberry extracts did not depend on the harvest date.

REFERENCES

ACUNA U.M., ATHA D.E., MA J., NEE M.H., KENNELLY E.J. 2002. *Antioxidant capacities of ten edible North American plants*. *Phytother. Res.*, 16: 63-65.

ALEKSANDROVA M.S. 1981. *Trials on growing Gaultheria in Moscow*. *Byulleten Glavnogo Botanicheskogo Sada*, 120: 16-19.

ASAMI D.K., HONG Y.J., BARRETT D.M., MITCHELL A.E. 2003. *Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn groan using conventional, organic, and sustainable agricultural practice*. *J. Agric. Food Chem.*, 52: 1237-1241.

CLARC G.S. 1999. *An aroma – chemical profile. Methyl salicylate, or oil of wintergreen*. *Perfumer and Flavorist*, 24 (1): 5-11.

CZEKALSKI M. 2006. *Liściaste krzewy ozdobne o wszechstronnym zastosowaniu. Cz. II. [Leaf Ornamental Plants of Versatile Use. Part II]*. PWRIŁ, Warszawa, ss. 200.

FRAZIK M. 1991. *Golteria [Eastern teaberry]*. Kwiaty, 3: 27.

HASSIMOTTO N.M.A., GENOVESE M.I., LAJOLO F.M. 2005. *Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps*. J. Agric. Food Chem., 53: 2928-2935.

HUFFMAN D.W., ZASADA J.C., TAPPEINER II J.C. 1994. *Growth and morphology of rhizome cuttings and seedlings of salal (Gaultheria shallon): effects of four light intensities*. Can. J. Bot., 72(11): 1702-1708.

KALT W., RYAN D.A.J., DUY J.C., PRIOR R.K., EHLENFELDT M.K., KLOET S.P.V. 2001 *Interspecific variation in anthocyanins, phenolics, and antioxidant capacity among genotypes of highbush and lowbush blueberries (Vaccinium section cyanococcus spp.)*. J. Agric. Food Chem., 49: 4761-4767.

KAUR C., KAPOOR H.C. 2002. *Anti-oxidant activity and total phenolic content of some Asian vegetables*. Int. J. Food Sci. Tech., 37: 153-161.

KÄHKÖNEN M.P., HOPIA A.I., HEINONEN M. 2001. *Berry phenolics and their antioxidant activity*. J. Agric. Food Chem., 49: 4076-4082.

MA X.J., DU C.F., ZHENG J.H., CHEN X.Z. 2001. *Studies on chemical constituents of Gaultheria leucocarpa var. Yunnanensis (Franch.)*. Zhongguo Zhong Yao Za Zhi., 26(12): 844-845.

MAROSZ A., MATYSIAK B., BIELENIN M. 2000. *Uprawa golterii rozesłanej (Gaultheria procumbens L.) w zamkniętym systemie nawożenia [Growing Eastern teaberry (Gaultheria procumbens L.) in a closed fertilization system]*. Roczn. AR Pozn. 323, Ogrodn., 31(1): 119-122.

NIKETIĆ-ALEKSIĆ G.K., HRAZDINA G. 1972. *Quantitative analysis of the anthocyanin content in grape juices and wines*. Lebensm. Wiss. Technol., 5(5): 163-165.

PLISZKA B., MIELESZKO E., HUSZCZA-CIOŁKOWSKA G., KARCZYŃSKI F. 2003. *Skład i potencjał antyoksydacyjny antocyjanów ekstrahowanych kwasem cytrynowym z owoców aronii [Composition and antioxidative potential of anthocyanins extracted with citric acid from chokeberry fruit]*. Biul. Nauk. UWM, 22: 71-76. Mat. na X Konf. Nauk.-Prom. „Lepsza Żywność”, Olsztyn.

PLISZKA B., WAŻBIAŃSKA J., PUCZEL U., HUSZCZA-CIOŁKOWSKA G. 2005. *Biologicznie czynne związki polifenolowe zawarte w owocach różnych odmian hodowlanych i dziko rosnącego bzu czarnego [Biologically active polyphenolic compounds in fruits of different cultivars and wild varieties of European elder]*. Zesz. Probl. Post. Nauk Rol., 507: 443-449.

PLISZKA B., WAŻBIAŃSKA J., HUSZCZA-CIOŁKOWSKA G., PŁOSZAJ B. 2007. *Content of polyphenolic compounds and their antioxidative properties in black mulberry (Morus nigra L.) fruit at different ripeness phases*. Scientific Works of the Lithuanian Institute of Horticulture and Lithuanian University of Agriculture. Sodininkystė ir Darčininkystė, 26(3): 16-22.

RIBNICKY D.M., POULEV A., RASKIN I. 2003. *The determination of salicylates in Gaultheria procumbens for use as a natural aspirin alternative*. J. Nutr. Funct. Med., Foods, 4(1): 39-52.

RICE-EVANS C.A., MILLER N.J., PAGANGA G. 1996. *Structure – antioxidant activity relationships of flavonoids and phenolic acid*. Free Radic. Biol. Med. 20(7): 933-956.

SENETA B., DOLATOWSKI J. 1997. *Dendrologia [Dendrology]*. Wyd. PWN, Warszawa, 448-449.

SINGLETON V.L., ORTHOFER R., SAMUELA-RAVENTOS R.M. 1999. *Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau Reagent*. Methods in Enzymology, 299: 152-178.

WAŻBIAŃSKA J. 2000. *Rosliny alternatywne w uprawach ogrodowych i parkowych północno-wschodniej Polski [Alternative plants in gardens and parks in north-eastern Poland]*. Biul. Nauk. UWM, Olsztyn, 8: 309-315.

WAŻBIŃSKA J., PLISZKA B., PŁOSZAJ B. 2006. *Content of anthocyanins and antioxidative activity in fruit of wild elder (Sambucus nigra L.) growing on different soil objects*. Fruit Growing, 18(1): 161-164.

WIELEBA E., PASTERNAK K. 2001. *Pierwiastki śladowe w systemie antyoksydacyjnym zwierząt [Trace elements in the antioxidative system of animals]*. Med. Wet., 57(11): 788-791.

WROLSTAD R.E. 1976. *Color and pigment analyses in fruit products*. Station Bulletin 624, Agricultural Experiment Station Oregon State University, Corvallis, OR.

YEN G.-C., HUNG C.-Y. 2000. *Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tsao (Mesona procumbens Hemsl.)*. Food Res. Int., 33: 487-492.

ZARAŚ E.M., LATOCHA P., CHMIEL CH. 2000. *Liściaste krzewy okrywowe jako alternatywa dla miejskich terenów zielonych [Leaf cover plants as an alternative to urban green areas]*. Roczn. AR Pozn. 323, Ogrodn., 31(1): 195-201.

ZHENG W., WANG S.Y. 2003. *Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries and lingonberries*. J. Agric. Food Chem., 51(2): 502-509.