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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.

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ZWIĄZKI POLIFENOLOWE I BIOPIERWIASTKI 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 wzbudzają też zainteresowanie ze względu na zawartość związków polifenolowych. Celem pracy było oznaczenie zawartości związków polifenolowych (antocyjanów i fenoli ogółem) 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, Ważbińska 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 antioxidative 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 (1^{st} – 15^{th} October 2006, 2^{nd}-30^{th} October 2006 and 3^{rd}-15^{th} 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 prepurified 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-glucoside) 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 cm^3 of each extract and 2 cm^3 of concentrated nitric acid (HNO_3) 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 x	mean x	SD	mean x	SD	mean x	SD	mean x	SD	mean x	SD
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 2^{nd} harvest date and the lowest – on the 3^{rd} 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 2^{nd} and the lowest – on the last, 3^{rd} harvest date. The content of phenols other that 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 2^{nd} harvest date and the lowest – for the 3^{rd} one.

Fruits of Eastern teaberry collected on the 2^{nd} 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, Ważbińska et al. 2006). Collecting Eastern teaberries on different dates, the ripeness phase was taken into consideration. On the 2^{nd} 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 x	SD	mean x	SD	mean x	SD	mean x	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 2^{nd} 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.

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