

EFFECTS OF SOLVENTS AND EXTRACTION METHODS ON THE CONTENT AND ANTIRADICAL ACTIVITY OF POLYPHENOLS FROM FRUITS *ACTINIDIA ARGUTA*, *CRATAEGUS MONOGYNA*, *GAULTHERIA PROCUMBENS* AND *SCHISANDRA CHINENSIS*

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ABSTRACT

Background. In line with the current tendency towards the production of the so-called safe foods, the use of environmentally-friendly methods for the extraction of polyphenols from fruits has been sought. Citric acid is a good solvent in the preparation of phenolic compounds for the food and pharmaceutical industries because it is a natural antioxidant and is non-toxic for the environment. Furthermore, new sources of polyphenols from fruit of orchard plants that are less known in Poland have been looked for. The aim of this study was to assess the content and antiradical activity of polyphenolic compounds in fruit extracts, depending on the extraction method employed. In addition, the yield of extraction processes was assessed.

Material and methods. The experimental materials were fruits of the following plants: *Actinidia arguta*, *Crataegus monogyna*, *Gaultheria procumbens*, *Schisandra chinensis*. For the extraction, aqueous solutions of citric acid (CAE) and methanol (ME) were used. The following were determined in fruit extracts: the content of total phenols (TP) and anthocyanins (A), and antiradical activity (DPPH and ABTS).

Results. In general, the *C. monogyna* fruit extracts were distinguished by the highest TP and A content. The TP and A content was significantly higher in fruit extracts obtained with the CAE method than in the ones produced with the ME method. The highest mean A/TP ratio was determined for *S. chinensis* fruit extracts. The antiradical activity (DPPH and ABTS) in fruit extracts did not depend on an extraction method, but on fruit species. Overall, a higher yield of extraction processes was achieved for the CAE method than for the ME method.

Conclusions. The present study indicates that the selected extraction methods are able to obtain polyphenolic extracts from fruits with a high antiradical activity and high yield. The use of citric acid in order to extract polyphenols from fruits may be an alternative to the conventional extraction method, while being eco-friendly and more effective than the latter.

Key words: ABTS, DPPH, extraction, fruits, polyphenols, solvents

INTRODUCTION

Fruits are a source of polyphenolic compounds characterized by antioxidant, anticarcinogenic and cardiologic properties (Kähkönen et al., 2001; Zheng and Wang, 2003). Nowadays, there is a tendency to

search for new sources of these compounds in fruits of orchard plants which are less popular in Poland: kiwifruit (*Actinidia* sp.), gaultheria (*Gaultheria* sp.), hawthorn (*Crataegus* sp.) and Wuweizi (*Schisandra*

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chinensis). Although these fruits have been used in traditional medicine for many years, their health-promoting properties are still being investigated, and new extraction methods are being searched in order to use this fruits in a better way in the food and pharmaceutical industries. Noteworthy are certain biological and therapeutical properties of these fruits. Consumption of kiwifruits with possess antioxidant properties, is thought to protect against cardiovascular diseases and cancers (Rush et al., 2002; Zuo et al., 2012). Hawthorn species effectively reduce the level of blood cholesterol and the risk of cardiovascular diseases (Chang et al., 2005; Zhang et al., 2002). The essential oil of *Gaultheria procumbens* contains methyl salicylate, an aspirin-like compound. This oil is marketed for external treatment of neuralgia, malign, arthralgia and other pains (Ribnicky et al., 2003; Wei-Rui et al., 2013). Recent studies have shown that *Schisandra chinensis* is known for its various biological and therapeutic properties, e.g. antioxidant, hepatoprotective (Cheng et al., 2013), immunomodulatory, anticancer (Zhao et al., 2014), and sedative-hypnotic ones (Wei et al., 2014).

The extraction of polyphenols from plant materials is the first step in the use of bioelements for the preparation of nutraceuticals, food ingredients and pharmaceutical products. For the extraction of polyphenolic compounds by conventional methods, organic solvents e.g. methanol are most often used, frequently with different proportions of water. Although these solvents are harmful to human health and also to the environment, they are effective in the extraction of polyphenols from fruits. Presently, there is a tendency for applying ecological methods of extracting polyphenolic compounds from fruit in accordance with the current trend towards the production of safe foods (Pliszka et al., 2008; 2013). In recent years citric acid has been used in a few studies on polyphenolic extractions from plant materials (Mai, 2015; Pliszka et al., 2013; Tzima et al., 2014; Wissam et al., 2012). It is worth continuing the research using citric acid for the extraction, as this is a non-toxic and environmentally-friendly solvent. In addition, citric acid is a natural antioxidant frequently added to foodstuffs in order to protect them against oxidation (Gromovaya et al., 2002).

In the present study, citric acid and methanol were used as solvents in the extraction of polyphenolic compounds from the fruits of less known orchard

plants. The aim of this study was to assess the content and antiradical activity of polyphenolic compounds in extracts from selected fruit species, depending on the extraction method employed. In addition, the yield of extraction processes was assessed.

MATERIAL AND METHODS

Plant material

The following fruits were used as the experimental material: *Actinidia arguta*, *Crataegus monogyna*, *Gaultheria procumbens* and *Schisandra chinensis*. The fruits were obtained from the Experimental Garden of the University of Warmia and Mazury in Olsztyn (Poland). Fruit samples of 10 g were weighed out and stored at -18°C for further study.

Extraction with citric acid (CAE method) and with 80% methanol (ME method)

Fruit samples were defrosted for 2 h at room temperature and then grounded in a mortar.

In the CAE method the homogenized material was poured over with 50 cm^3 of citric acid solution of $0.1\text{ mol}\cdot\text{dm}^{-3}$ concentration. The samples were left in dark for 24 h at 2°C . After that time, flasks containing samples were subject to a shaking water bath at 37°C for 15 min.

In the ME method the homogenized material was poured over with 50 cm^3 of solution 80% methanol by shaking samples in a shaker at room temperature for 30 min.

The samples were filtered through Whatman No. 1 filter paper to separate the solid phase from the extracts of both methods. New portions (30 and 20 cm^3) of the solvents (citric acid or methanol) were used for the re-shaking of samples. Extracts of the same fruit species were combined together in the final stage, respectively for the CAE and ME methods.

The extraction yield (%) was calculated as a ratio of the weight of extract to the weight of a sample used for the extraction.

Polyphenolic compounds. The content of total phenols (TP) in fruit extracts was determined by the Folin-Ciocalteu method (Singleton et al., 1999). The absorbance was measured at $\lambda = 765\text{ nm}$ on a Shimadzu UV-1800 spectrophotometer. The content of

TP was calculated from a calibration curve. Gallic acid (mg GAE·100 g⁻¹ FW) was used as the equivalent (FW – fresh weight). The content of anthocyanins (A) in fruit extracts was determined by the Giusti and Wrolstad method (2001). Quantitative determination of A was carried out by measuring the difference in extract absorbance in buffer solutions with pH 1 and pH 4.5 at λ = 525 nm and λ = 700 nm on a Shimadzu UV-1800 spectrophotometer. The content of A was expressed as cyanidin 3-glucoside equivalents (mg CGE·100 g⁻¹ FW). Moreover, the ratio of anthocyanins to total phenols in the tested extracts of fruits was calculated (A/TP ratio).

Antiradical activity. The antiradical activity (DPPH) was determined by the Yen and Hung method (2000) with slight modification. The concentration of extract was 0.8 mg·cm⁻³. The mixture was left to stand at room temperature for 30 min, in dark. Absorbance was measured after at λ = 517 nm using Shimadzu UV-1800 spectrophotometer. The results were expressed as an inhibition percentage. Inhibition of free radical DPPH in percent (I%) was calculated as follows:

$$I\% = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] 100\%$$

The antiradical activity (ABTS) was determined by the Miller et al. (1993) with slight modification. The concentration of extract was 0.2 mg·cm⁻³. The mixture was left to stand at 37°C in for 6 min. The absorbance

was measured at λ = 734 nm on a Shimadzu UV-Vis 1800 spectrophotometer. The results were expressed as inhibition [%].

Statistical analysis

The results were verified in three replications. The data regarding the content of polyphenolic compounds, A/TP ratio, the antiradical activity of fruit extracts, and yield of extraction processes were submitted to statistical analysis of variance for two-factorial experiments (t-Student test) using the Statistica 8.0 PL (StatSoft Tulsa, the USA) software package ($p < 0.05$). The calculations included the correlation between the total content of phenols and the correlation between the content of anthocyanins in fruit extracts obtained through the two methods: CAE and ME ($p < 0.05$).

RESULTS

Results on the content of polyphenols in extracts from the analysed fruit species are comprised in Table 1. Statistical calculations have demonstrated that the total phenolic (TP) content was the highest in *C. monogyna* and the lowest in *A. arguta* extracts, irrespective of the extraction method applied. The highest content of anthocyanins was detected in *C. monogyna* (CAE method) and *S. chinensis* extracts (ME method). Regardless of the extraction method, the A content in *G. procumbens* extracts was the lowest. Extracts from

Table 1. Content of polyphenolic compounds in fruit extracts

Fruit extracts	TP		A		A/TP	
	mg GAE·100 g ⁻¹ FW		mg CGE·100 g ⁻¹ FW		CAE	ME
	CAE	ME	CAE	ME	CAE	ME
<i>Actinidia arguta</i>	c 171.0 ^A ± 8.5	c 102.7 ^B ± 7.6	–*	–	–	–
<i>Crataegus monogyna</i>	a 913.0 ^A ± 41.8	a 602.7 ^B ± 17.0	a 16.2 ^A ± 0.7	b 7.3 ^B ± 0.6	c 0.018 ^A ± 0.001	c 0.012 ^B ± 0.001
<i>Gaultheria procumbens</i>	c 180.7 ^A ± 5.5	b 134.3 ^B ± 7.2	c 7.8 ^A ± 0.9	c 5.1 ^B ± 0.3	b 0.043 ^A ± 0.004	b 0.038 ^B ± 0.001
<i>Schisandra chinensis</i>	b 247.0 ^A ± 7.0	b 128.3 ^B ± 5.5	b 14.1 ^A ± 1.7	a 9.5 ^B ± 0.6	a 0.057 ^B ± 0.005	a 0.074 ^A ± 0.002

TP – total phenols. A – anthocyanins. GAE – equivalents of gallic acid. CGE – equivalents of cyanidin. FW – fresh weight. CAE – citric acid extract. ME – methanol extract. Results are mean ± SD.

*Does not contain anthocyanins.

Data in the same row denoted by capital letters are significantly different ($p < 0.05$).

For each solvent, values in the same column denoted by small letters are significantly different ($p < 0.05$).

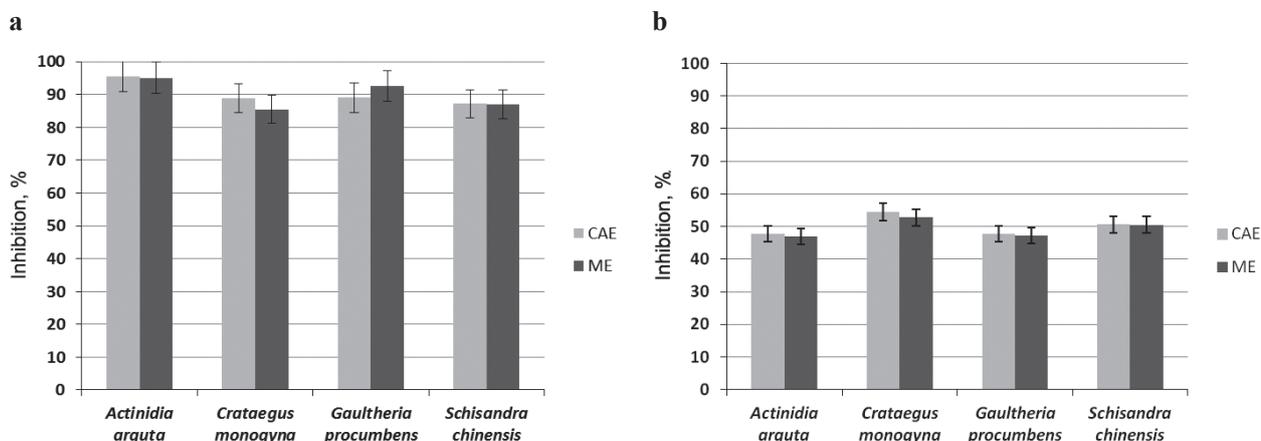


Fig. 1. Antioxidant activity of fruit extracts determined by: a – the DPPH method, b – the ABTS method. CAE – citric acid extract. ME – methanol extract

A. arguta fruits did not contain any anthocyanins. The content of TP and A was significantly higher in fruit extracts produced with the CAE method than in the ones obtained with the ME method. The correlation between the TP content in extracts obtained with the CAE method and the TP content in extracts produced with the ME method was significant. The same correlation was observed between the A content in fruit extracts obtained with the CAE and the ME method. The correlation coefficients were $r = 0.996$ and $r = 0.720$ for TP and A, respectively. The calculations proved that the A/TP ratio was the highest in *S. chinensis* fruit extracts and the lowest in *C. monogyna* fruit extracts, irrespective of the extraction method. The A/TP ratio was significantly higher in *C. monogyna* and *G. procumbens* fruit extracts obtained with the CAE method than in the ones produced with the ME method. For the *S. chinensis* extracts, the above correlation was reverse. The current experiment revealed a significant interaction between the extraction methods and a fruit species in terms of TP and A as well as the A/TP ratio.

The antiradical activity of fruit extracts determined with the two methods (DPPH and ABTS) is illustrated in Figure 1. The antiradical activity of fruit extracts did not depend on an extraction method but on a fruit species. The DPPH assay demonstrated the highest antiradical activity was observed in extracts of *A. arguta* fruit, and the lowest by *S. chinensis* and

C. monogyna fruits. In turn, the ABTS assay showed the highest antiradical activity in *C. monogyna* fruit extracts and the lowest one in *A. arguta* and *G. procumbens* extracts.

The paper compares the yield of extraction processes applied to *A. arguta*, *C. monogyna*, *G. procumbens* and *S. chinensis* fruits according to two extraction methods: CAE and ME (Table 2). Statistical analysis of the experimental results has demonstrated that significantly higher yields were achieved with the CAE method, except for *G. procumbens* fruits, for which the extraction yields obtained with either of the methods did not differ significantly. Irrespective of the extraction method, the highest extraction yield was recorded for *S. chinensis* fruits.

Table 2. Yield of extraction from selected fruit species, %

Fruit extracts	Method	
	CAE	ME
<i>Actinidia arguta</i>	b 86.32 ^A ± 0.98	c 60.94 ^B ± 1.89
<i>Crataegus monogyna</i>	bc 84.32 ^A ± 1.65	b 80.49 ^B ± 1.41
<i>Gaultheria procumbens</i>	b 86.33 ^A ± 2.12	a 83.77 ^A ± 0.49
<i>Schisandra chinensis</i>	a 89.45 ^A ± 1.91	a 85.35 ^B ± 1.57

CAE – citric acid extract. ME – methanol extract. Results are mean ± SD.

DISCUSSION

The results of the present study show that extracts from the *C. monogyna* fruits were generally distinguished by a high TP and A content, irrespective of the extraction method. Literature contains reports on the extraction of polyphenols from *A. arguta* and *S. chinensis* fruits by conventional methods using methanol, ethanol, and acetone. On the other hand, there is little information on polyphenols in *C. monogyna* and *G. procumbens* fruits. Currently, research is only carried out for other species of hawthorn and gaultheria. In different varieties of kiwifruit genotypes, the content of total phenols ranges from 41 mg to 710 mg/100 g FW (Du et al., 2009). According to Latocha et al. (2010), the content of total phenols in *A. arguta* was 79 mg/100 g FW. Wang (2013) reported that the content of TP in the fruits of *A. arguta* was 214.8 mg/100 g, whereas Zuo et al. (2012) claimed that value to be higher. Wu et al. (2011) noted that the content of total phenols in *S. chinensis* fruits was 184.7 mg/100 g, and the content of anthocyanins was 17.9 mg/100 g of fresh weight. The results obtained in the present study were similar to those provided by Wu et al. (2011). Pliszka et al. (2009), who performed extraction using citric acid, concluded that the content of total phenols in *G. procumbens* fruits ranged from 180.0 mg to 495.2 mg/100 g of fresh matter, and the content of anthocyanins varied from 7.83 mg to 13.11 mg/100 g of fresh matter, depending on the date of fruit harvest. The results obtained in the present study for *G. procumbens* (the CAE method) were in the same range as presented by Pliszka et al. (2009).

The fruit extracts obtained using the CAE method were richer in polyphenols than the extracts obtained with the ME method. The correlation coefficients indicate that two methods could be used alternately. The results of this study indicated a significant interaction between the extraction methods and the fruit species for both total phenols and anthocyanins, which supports previous studies (Pliszka et al., 2008). Kalt et al. (2001) claimed otherwise; when testing fruit extracts, they found no interaction between the extraction method and the fruit species for either total phenols or anthocyanins.

The A/TP ratio is the ratio of the content of anthocyanins (A) to the content of total phenols (TP) in the

fruits, and according to Borowska and Mazur (2008), it is typical of specific species and varieties of fruit. This study shows the A/TP ratio value was the highest for *S. chinensis*, since the share of anthocyanins in the total content of phenols was large. In extracts from the fruits analyzed here, the A/TP ratio was much lower than in fruits of black chokeberry or bilberry which are rich in red anthocyanin pigments (Pliszka et al., 2013).

The antiradical activity DPPH of the extracts from the fruits analyzed was dependent on the fruit species, and could be ranked in the following order: *A. arguta* > *G. procumbens* > *C. monogyna* = *S. chinensis*. In the ABTS assay, the sequence appeared as follows: *C. monogyna* > *S. chinensis* > *A. arguta* = *G. procumbens*. The antioxidant properties of fruit extracts are increasingly more intensive as the concentration of polyphenolic compounds increases, *albeit* only up to a certain point, which depends on both a given antioxidant and an assay applied for testing (Marinova and Yanishlieva, 1997; Pliszka et al., 2005). On the other hand, no significant difference was found between the antiradical activity of the fruit extracts obtained with the two methods. In the available literature, the antiradical activity assayed according to the DPPH and ABTS protocol was usually determined in fruit extract obtained with traditional methods. Choi et al. (2012) reported that the antiradical activity for *S. chinensis* tested with DPPH and ABTS assays ranged from 78.36% to 83.62% and from 37.97% to 42.28%, respectively. Wu et al. (2011) reported that antiradical activity (DPPH) in fruits of *S. chinensis* was 85.2%. There are also reports implicating that a DPPH-assayed antiradical activity was high for extracts from fruits of *Actinidia* sp. (Du et al., 2009; Zuo et al., 2012), and *Crataegus* sp. (Rabiei et al., 2012). The results obtained in present study for the antiradical activity of the *G. procumbens* extracts (CAE method) were similar to those obtained by Pliszka et al. (2009), who also used citric acid for extraction.

This study compared the yield of the extraction processes applied to the tested fruit received according to the CAE and ME methods. The yield of the extraction processes depended mainly on the method used, and not on the species of fruit. Higher yields were obtained with the CAE than the ME method, except *G. procumbens*, where the yields achieved with two methods were not significantly different.

CONCLUSIONS

The present study indicates that the selected extraction methods are able to obtain polyphenolic extracts from fruits with a high antiradical activity and high yield. The use citric acid in order to extract polyphenols from fruits may be an alternative to conventional methods whilst being eco-friendly and more effective than the latter. Further research into the improvement of extraction methods with citric acid is necessary before they are implemented in the food and pharmaceutical industries.

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