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COMPARISON OF POLYPHENOLIC COMPOSITION OF EXTRACTS FROM HONEYSUCKLE, CHOKEBERRIES AND GREEN TEA – A SHORT REPORT

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Key words: polyphenols, composition, extracts

The aim of the study was to compare the polyphenolic composition of extracts from different plant sources. Extracts from two new plants rich in polyphenols, *i.e.* chokeberries and honeysuckle, were compared with the well-investigated green tea polyphenols. All extracts obtained were characterised by a high content of polyphenols: green tea – 611 mg/g, honeysuckle – 633 mg/g and chokeberries – 714 mg/g. Honeysuckle and chokeberries extracts consisted mainly of anthocyanins (321.2 and 404.5 mg/g, respectively), whereas green tea extract contained mainly flavan-3-ols (587.9 mg/g).

INTRODUCTION

Polyphenolic substances of natural origin raise a high interest because of their strong biological activity. Different polyphenolics have been thought to play an important role in the prevention of cancer and heart diseases [Hollman & Arts, 2000; Higdon & Frei, 2003]. Epidemiological studies have shown a negative correlation between fruits and vegetables consumption and heart disease mortality [Hollman, 2001]. The importance of the natural polyphenolic compounds of plants origin in the maintenance of human health raises a high interest among scientists and food makers who treat these substances as important ingredients of functional food. From this point of view, it seems of outmost importance to look for polyphenol-rich plants, to develop procedures of polyphenols extraction and to improve methods of their chemical analysis. Then, the extracts can be determined for their biological activity. Our earlier experiments with the use of the tested extracts have shown their antioxidant, hypocholesterolemic and hypoglycemic activity [Frejnagel, 2007].

The most popular and well-investigated source of polyphenols in human nutrition is green tea. Leaves of this plant contain to 30% of polyphenols in dry matter mass, mainly flavanols [Balentine *et al*., 1997; Wiseman *et al*., 1997; Wang *et al*., 2000]. However, while searching for new sources of phenolics, berries, like chokeberries or honeysuckle berries, are also a good and rich source of polyphenolic compounds, especially those belonging to anthocyanins [Oszmianski *et al*., 1995; Slimestad *et al*., 2005]. Chokeberries are very popular in Poland as a source of fruits for juice production. On the other hand, honeysuckle is widely harvested in Russia, China, and Japan, but now it is also well accepted for production by Polish farmers. The content of polyphenols in these fruits riches even 2-4% of dry mass [Oszmiański *et al*., 1995; Slimestad *et al*., 2005].

The aim of the study was to compare profiles of polyphenolic compounds from new plants with the well-recognized extract of green tea.

MATERIALS AND METHODS

Chemicals. All phenolic standards were purchased from Extrasynthese company, Genay Cedex, France. Other chemicals used in the extraction procedure were purchased from POCh, Gliwice, Poland. Procyanidin standards were a gift of Professor Oszmianski, Agricultural Academy, Wroclaw, Poland.

Preparations. Honeysuckle (*Lonicera kamtschatica*) fruits (250 g), harvested in a local farm, were homogenised with 250 mL of methanol containing 200 ppm of SO_2, to prevent polyphenols oxidation, and left for 12 h. After decantation the procedure was repeated. The combined solutions were centrifuged and organic solvent was evaporated in a vacuum rotary evaporator. The residue of water suspension was transferred into a chromatographic column (85×500 mm) packed with ion-exchange resin Amberlite XAD 16 (Rohm and Haas, France) and washed with distilled water. Sugars and other water-soluble compounds present in the extract were eluted from the column with water, polyphenols with 85% methanol. Methanol was evaporated and water residue was freeze-dried.

Commercial green tea was bought in a local supermarket. Polyphenols were extracted into 75% (v/v) acetone/water

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solution at solid material to solvent ratio of 1:8 with the addition of 200 ppm of SO_2. After 4 h of extraction the procedure was repeated. After centrifugation the combined extract was evaporated in a rotary evaporator to water and freeze-dried.

A commercial extract of chokeberries fruit (*Aronia melanocarpa*) was purchased by Agrofarm company (Łódź. Poland).

Chemical analyses. The content of total polyphenols was estimated using Folin-Ciocalteu's reagent and sinapic acid as a standard. The extracts were characterized using HPLC method described by Oszmianski & Wojdylo [2005].

The results were expressed as mean value from three repetitions ± standard deviation.

RESULTS AND DISCUSSION

Extracts investigated were characterised by a high content of polyphenols (Figure 1). The content of phenolics accounted for 611 mg/g in green tea extract, 633 mg/g in honeysuckle one and 714 mg/g in chokeberries one. Due to purification of crude extracts on ion-exchange resins and using more effective solvents, the content of polyphenols in the extracts was higher than that obtained by other authors [Pearson *et al.*, 1988; Gabrielska *et al.*, 1999].

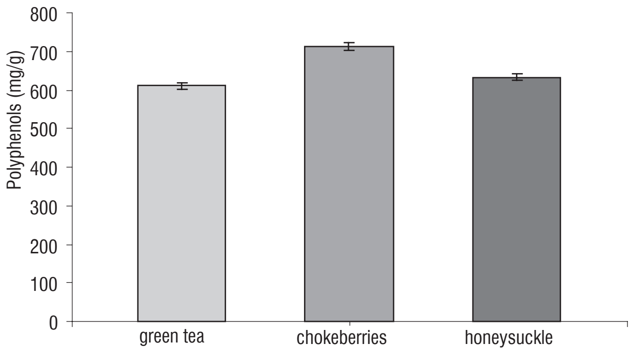

FIGURE 1. Content of total polyphenols in th extractse tested.

The green tea extract consisted mainly of flavanols (Table 1): epicatechin (262.5 mg/g) and epigallocatechin gallate (276.8 mg/g). There was also noted a small amount of epicatechin gallate (42.1 mg/g), catechin (6.5 mg/g) and some procyanidins (23.1 mg/g). The content of total polyphenols in the extract was 611 mg/g.

Green tea is known as a very rich source of polyphenols. Dry leaves of green tea contain even 30% of polyphenolic compounds and their glycosides [Balentine *et al.*, 1997; Liebert *et al.*, 1999; Yao *et al.*, 2006]. Predominantly, green tea polyphenols are composed of catechins. It is confirmed in our experiment, where flavanols were the main group of phenolics in the extract (96.22%). Like it was reported previously by other authors [Roedig-Penman & Gordon, 1997; Liebert *et al.*, 1999], polyphenols appear in green tea leaves in the forms of gallates, especially epigallocatechin gallate (EGCG) that was the dominant polyphenolic also in our extract. Contrary to other authors [Price & Spitzer, 1993], our extract was characterized by a high content of (-)epicatechin (EC) Epigallocatechin gallate and (-)epicatechin represented 88% of total polyphenols in our extract. Similarly to other green tea extracts [Apostolides *et al.*, 1996; Pearson *et al.*, 1998], only small amount of condensed forms of polyphenols was observed. Generally, in different experiments water extract of green tea was used [Apostolides *et al.*, 1996; Pearson *et al.*, 1998]. Such extracts contained about 44.8–52% of total polyphenols. Baptista *et al.* [1999] obtained water extract with higher polyphenol concentration (74.7%), however after laborious procedure. In this context, extraction technique used in our experiment seems to be more effective and faster. Roedig-Penman & Gordon [1997] and Chang *et al.* [2001] confirmed that alcohol solvents are more effective solvents (even 7-fold) than water in the case of green tea.

TABLE 1. Content of individual polyphenols in the green tea extract.

Substance	Content (mg/g)
(-)Epicatechin (EC)	262.5 ± 4.5
Epigallocatechin gallate (EGCG)	276.8 ± 4.8
Epicatechin gallate (ECG)	42.1 ± 1.4
(+)Catechin (C)	6.5 ± 0.4
Procyanidins	23.1 ± 0.8

Table 2 presents results of the chokeberries extract analysis. Anthocyanins were the dominant phenolic constituent of this extract. Together, there were 404.5 mg/g anthocyanins in the extract. The main polyphenolic was cyanidin-3-galactoside (270.2 mg/g). The second great group of polyphenols were procyanidins (146.4 mg/g). There was only a small amount of phenolic acids (105.2 mg/g). However, clearly more than quercetin and its aglycones (50.5 mg/g). The content of total polyphenols in the extract was 714 mg/g.

TABLE 2. Content of individual polyphenols in the chokeberry extract.

Substance	Content (mg/g)
Neochlorogenic acid	41.7 ± 1.1
Chlorogenic acid	63.5 ± 1.3
Cyanidin-3-galactoside	270.2 ± 2.2
Cyanidin-3-arabinoside	81.8 ± 1.4
Cyanidin-3-xyloside	40.0 ± 0.8
Cyanidin-3-glucoside	12.5 ± 0.5
Quercetin-3-glucoside	21.5 ± 0.6
Quercetin-3-rutinoside	18.3 ± 0.5
Quercetin-3-galactoside	8.9 ± 0.2
Quercetin	1.8 ± 0.04
(-)Epicatechin	7.6 ± 0.2
Procyanidins	146.4 ± 1.6

Containing even up to 2% of polyphenols in fresh fruits, chokeberries are also a good source of these substances [Wu *et al.*, 2004]. The main component of chokeberries polyphenolic fraction in our extract were anthocyanins. This is in agreement with the results of other authors who tested phenolics in chokeberries [Wu *et al.*, 2004; Wu *et al.*, 2006; Slimestad *et al.*, 2005]. Wu *et al.* [2006] have noted that American chokeberries polyphenolic fraction is composed almost of 100%

TABLE 3. Content of individual polyphenols in the honeysuckle extract.

Substance	Content (mg/g)
Neochlorogenic acid	17.8 ± 0.5
Chlorogenic acid	85.5 ± 1.8
Caffeic acid derivative	11.3 ± 0.6
Cyanidin-3-glucoside	280.8 ± 2.8
Cyanidin-3.5-diglucoside	19.8 ± 0.8
Cyanidin-3-rutinoside	20.6 ± 0.7
Peonidin-3-glucoside	5.8 ± 0.2
Quercetin-3-glucoside	6.2 ± 0.2
Quercetin-3-rutinoside	23.9 ± 0.7
Quercetin-3-galactoside	4.5 ± 0.1
Quercetin	2.6 ± 0.05
Procyanidins	154.2 ± 2.1

of anthocyanins. However, Oszmianski & Wojdylo [2005] have observed that predominant polyphenolics (50%) in *Aronia melanocarpa* are procyanidins. In the tested extract this class of polyphenols represented only 20% of total phenolics. On the other hand, in the extract reported anthocyanins represented more than 50% of polyphenols. The main compound was cyanidin-3-galactoside. This is in agreement with results reported by other researchers who observed that this cyanidin glycoside is predominant among chokeberries polyphenols [Oszmianski & Sapis, 1988; Slimestad *et al*., 2005; Gabrielska *et al*., 1999; Gasiorowski *et al*., 1997]. Similarly to results of Oszmianski & Wojdylo [2005], the concentration of flavonols in the extract tested was low. This group of polyphenols represented quercetin derivatives: 3-glucoside, 3-rutinoside and 3-galactoside. Contrary to Oszmianski & Wojdylo [2005], the extract tested contained a high quantity of chlorogenic and neochlorogenic acid whose concentration reached almost 15%.

A similar tendency was observed in the honeysuckle extract (Table 3). The greatest group of phenolics were also anthocyanins (321.2 mg/g). However, here the dominant substance was cyanidino-3-glucoside (280.8 mg/g). A higher content of procyanidins was recorded in comparison to chokeberries extract (154.2 mg/g). Two new substances were observed in the honeysuckle extract as compared to chokeberries extract. Peonidin-3-glucoside (5.8 mg/g) and caffeic acid derivative (11.3 mg/g). The content of total polyphenols was 633 mg/g.

Honeysuckle fruits are widely harvested in Russia, China and Japan. However, still this plant is little known in Poland. Its fruits contain a high level of polyphenols which exhibit high biological activity [Kahkonen *et al*., 2001]. The obtained methanol extract contained 63.3% of polyphenols. Similarly to chokeberries extract, the predominant group of polyphenols were anthocyanins whose content was 50%. Cyanidin-3-glucoside represented 44.36% of total polyphenols in the extract. This is in agreement with results of other researchers who estimated the phenolic profile of honeysuckle fruits [Oszmianski *et al*., 1995; Chaonavalikit *et al*., 2004]. Methanol extract obtained by Oszmianski *et al*. [1995] contained even 90% of cyanidin-3-glucoside in the pool of anthocyanins. Jin *et al*. [2006], in their anti-inflammatory test, used ethanol extract of honeysuckle fruits that contained 75% of anthocyanins. The reported extract contained similar to chokeberries extract amount of procyanidins and quercetin derivatives. It is necessary to emphasize the high content of neochlorogenic and chlorogenic acids that was also observed by Chaonavalikit *et al*. [2004], however it was in contrary to Oszmianski & Wojdylo [2005] who have noted only 7.5% concentration of both acids.

CONCLUSIONS

The extracts tested were characterised by high cotnents of polyphenols. Contrary to green tea extract, where the dominant phenols were flavan-3-ols, the reported extracts of chokeberries and honeysuckle are a very rich source of anthocyanins.

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REFERENCES

1. Apostolides Z., Balentine D.A., Harbowy M.E., Weisburger J.H., Inhibition of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) mutagenicity by black and green tea extracts and polyphenols. Mutation Res., 1996, 359, 159–163.
2. Balentine D.A., Wiseman S.A., Bouwens L.C.M., The chemistry of tea flavanoids. Crit. Rev. Food Nutr., 1997, 37, 693–704.
3. Baptista J.A.B., da P Tavares J.F., Carvalho R.C.B., Comparative study and partial characterization of Azorean green tea polyphenols. J. Food Comp. Anal., 1999, 12, 273–287.
4. Chang C.J., Chiu K.L., Chen Y.L., Yang P.W., Effect of ethanol content on carbon dioxide extraction of polyphenols from tea. J. Food Comp. Anal., 2001, 14, 75–82.
5. Chaovanalikit A., Thompson M.M., Wrolstad R.E., Characterization and quantification of anthocyanins and polyphenolics in bluehoneysuckle (*Lonicera caerulea L.*). J. Agric. Food Chem., 2004, 52, 848–852.
6. Frejnagel S., Functional properties of diets supplemented with different phenolic extracts. Food Res. Int., 2007 (submitted).
7. Gabrielska J., Oszmianski J., Komorowska M., Langner M., Anthocyanins extract with antioxidant and radical scavenging effect. Z. Naturforsch., 1999, 54, 319–324.
8. Gasiorowski K., Szyba K., Brokos B., Kolaczynska B., Jankowiak-Wlodarczyk M., Oszmianski J., Antimutagenic activity of anthocyanins isolated from *Aronia melanocarpa* fruits. Cancer Lett., 1997, 119, 37–46.
9. Higdon J.V., Frei B., Tea catechins and polyphenols: health effects, metabolism and antioxidant functions. Crit. Rev. Food Sci. Nutr., 2003, 43, 89–143.
10. Hollman P.C.H., Evidence for health benefits of plant phenols: local or systemic effects? J. Sci. Food Agric., 2001, 81, 842–852.
11. Hollman P.C.H., Arts I.C.W., Flavanols, flavones and flavanols – nature, occurrence and dietary burden. J. Sci. Food Agric., 2000, 80, 1081–1093.
12. Jin X-H., Ohgami K., Shiratori K., Suzuki Y., Koyama Y., Yoshida K., Ilieva I., Tanaka T., Onoe K., Ohno S., Effects of blue honeysuckle *(Lonicera caerula L.)* extract on lipopolysaccharide-induced inflammation *in vitro* and *in vivo*. Exp. Eye Res., 2006, 82, 860–867.
13. Kahkonen M.P., Hopia A.I., Heinonen M., Berry phenolics and

their antioxidant activity. J. Agric. Food Chem., 2001, 49: 4076––4082.
14. Liebert M., Licht U., Böhm V., Bitsch R., Antioxidant properties and total phenolics content of green and black tea under different brewing conditions. Z. Lebensm. Unters. Forsch., 1999, 208, 217–220.
15. Oszmianski J., Sapis J.C., Anthocyanins in fruits of *Aronia melanocarpa* (chokeberriers). J. Food Sci., 1988, 53, 1241–1242.
16. Oszmianski J., Wojdylo A., *Aronia melanocarpa* phenolics and their antioxidant activity. Eur. Food Res. Technol., 2005, 221, 809–813.
17. Oszmianski J., Souquet J-M., Moutounet M., Honeysuckle fruit anthocyanins. Zesz. Nauk. AR Wroclaw, 1995, 273, 67–72 (in Polish).
18. Pearson D.A., Frankel E.N., Aeschbach R., German J.B., Inhibition of endothelial cell mediated low-density lipoproteins oxidation by green tea extracts. J. Agric. Food Chem., 1998, 46, 1445–1449.
19. Price W.E., Spitzer J.C., Variation in the contents of individual flavonoids in a range of green teas. Fod. Chem., 1993, 47, 271–276.
20. Roedig-Penman A., Gordon M.H., Antioxidant properties of catechins and green tea extracts in model food emulsions. J. Agric. Food Chem., 1997, 45, 4267–4270.
21. Slimestad R., Torstengerpoll K., Natel H.S., Johanessen T., Giske N.H., Flavanoids from chokeberries, *Aronia melanocarpa*. J. Food Comp. Anal., 2005, 18, 61–68.
22. Wang H., Provan G.P., Helliwell K., Tea flavanoids: their functions, utilization and analysis. Trends Food Sci. Techol., 2000, 11, 152–160.
23. Wiseman S.A., Balentine D.A., Frei B., Antioxidants in tea. Crit. Rev. Food Nutr., 1997, 37, 705–718.
24. Wu X., Gu L., Prior R.L., McKay., Characterization of anthocyanins and proanthocyanidins in some cultivars of ribes, aronia and sabucus and their antioxidant capacity. J. Agric. Food Chem., 2004, 52, 7846–7456.
25. Wu X., Beecher G.R., Holden M.J., Haytowitz D.B., Gebhardt S.E., Prior R.L., Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J. Agric. Food Chem., 2006, 54, 4069–4075.
26. Yao L.H., Jiang Y.M., Caffin N., D'Arcy B., Data N., Liu X., Singanusong R., Xu Y., Phenolic compounds in tea from Australian supermarkets. Food Chem., 2006, 96, 614–620.

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PORÓWNANIE SKŁADU FENOLOWEGO EKSTRAKTÓW Z JAGODY KAMCZACKIEJ, ARONII I HERBATY ZIELONEJ – KRÓTKI KOMUNIKAT

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Celem pracy było porównanie zawartości związków polifenoli zawartych w ekstraktach uzyskanych z różnych surowców roślinnych. Porównano ekstrakty uzyskane z nowych bogatych w polifenole owoców aronii oraz jagody kamczackiej z dobrze już poznanymi polifenolami herbaty zielonej. Wszystkie uzyskane ekstrakty charakteryzowały się wysoką zawartością związków fenolowych ogółem; herbata zielona 611, jagoda kamczacka 633 i aronia 714 mg/g. Ekstrakty z aronii i jagody kamczackiej zawierały głównie antocyjany (404,5 i 321,5 mg/g, odpowiednio. Flawan-3-ole (katechiny) dominowały w składzie ekstraktu z herbaty (epikatechina 262,5 oraz galusan epigalokatechiny 276,8 mg/g).