CONVECTIONAL EXTRACTION OF PHENOLIC COMPOUNDS FROM RED FRUITS – CHOKEBERRY (ARONIA) AND ELDERBERRY

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ABSTRACT

The aim of this study was to compare the quality of phenolic content and antioxidant capacity of Chokkeberry (Aronia) and Elderberry plants. Characterisation of phenolic compounds was carried out by Folin-Ciocalteau method. Antioxidant activity of Chokeberry (Aronia) and Elderberry products were determined using 2,2-diphenyl-2-picrylhydrazyl (DPPH). All extraction runs were carried out at constant extractive parameters chosen previously - temperature, sample particle size, extraction manner. All analyses were performed spectrophotometrically.

This study focuses on the convectional extraction - particularly conventional solid-liquid extraction (conducted via magnetic stirrer) of pre-dried Chokeberry and Elderberry. An investigation of total polyphenol contents (TPC) and total antioxidant capacity (TAC) were done. Extraction takes place at a pre-selected duration of 100 minutes. The extraction was examined with 5 solvents: aqueous solution of ethanol with concentration (20 vol. %; 50 vol. %; 80 vol. % and 96 vol. %) and distilled water. The temperature was kept constant at 40°C.

Keywords: extraction, Chokeberry, Elderbarry, polyphenols, antioxidant capacity, solvent.

INTRODUCTION

Natural products are appreciated for their wide applicability as health-beneficial foods, cosmetics ingredients, aroma and flavour compounds and colourants and in pharmaceutical preparations as well. The red fruits are a source of many bioactive compounds with a wide spectrum of health-promoting properties. They are a good source of protein, free and conjugated forms of amino acids, unsaturated fatty acids, fibre fractions, vitamins, antioxidants and minerals. Berries contain significant levels of phytochemicals that have important biological properties. The consumption of berries has been associated with health benefits, including the prevention of heart diseases, hypertension, certain forms of cancer and other degenerative or age-related diseases [1 - 3]. The health benefits of berries can be primarily attributed to their high concentration of natural antioxidants on them such as phenolic compounds, ascorbic acid and carotenoids. In a list describing the 20 foods with the highest total polyphenol concentrations, 16 berries were included [4]. Phenolic compounds that are found in the highest concentrations in berries include flavonoids (anthocyanins, flavonols and flavanols), phenolic acids (hydroxybenzoic and hydroxycinnamic acid derivatives) and tannins (proanthocyanidins, ellagitannins and gallotannins) [5].

Chokeberry (Aronia melanocarpa [Michx] Elliot, Rosaceae) is a perennial shrub native to North America, and it was introduced to Eastern Europe, Scandinavia, and Russia in early 20th century [6, 7]. Fully ripened chokeberry fruits contain different phenolic compounds such as proanthocyanidins, flavan-3-ol and flavonol glycosides, and phenolic acids [8]. Moreover, chokeberry represents one of the richest plant sources of anthocyanins exhibiting strong antioxidant activity [9]. Anthocyanins and proanthocyanidins play important role in human nutrition and the growing interest for their utilization is mainly due to their antioxidant potential and the association between their consumption and the prevention of cancer, coronary heart disease, diabetes and other degenerative disorders [10 - 12].

European elderberry (Sambucus nigra L.) is a deciduous shrub native from northern hemisphere, being nowadays present throughout the temperate and subtropical regions of Asia, North Africa and North America [13]. Its high commercial value is due to its fruits, the elderberry berries, which contain large amounts of anthocyanins and other polyphenols [13 - 14] being used as food colorants in jams and jellies, pies, yoghurts, syrups, and alcoholic beverages [15 - 18]. Due to the large amounts of phytochemicals present in the berries and to the significant antioxidant properties, berries are also used as dietary complements in the form of concentrates, juices and infusions [19], this last one being traditionally used for the treatment of constipation, diuretic and respiratory tract infections.

The aim of this study was to compare the quality of phenolic content and antioxidant capacity of Chokeberry (Aronia) and Elderberry plants. All extraction runs were carried out at constant extractive parameters chosen previously - temperature, sample particle size, extraction manner.

EXPERIMENTAL

Materials and methods

Plant materials

The red fruits of chokeberry and black elderberry were bought from herbal pharmacy in Bulgaria.

Chemical reagents

Chemical reagents 96 % ethanol was supplied by Valerus, Bulgaria. HPLC grade methanol (CH_3OH, 100 %), sodium carbonate (Na_2CO_3 > 99 %), sodium nitrate ($NaNO_3$), sodium acetate (CH3COONa), potassium chloride (KCl) and aluminium chloride hexahydrate ($Al_2Cl_3 \times 6H_2O$) were purchased by Merck, Germany. 2M solution of Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium hydroxide (NaOH), Tolox (6-hydroxy2,5,7,8-tetramethylchroman-2-carboxylic acid, 97 %), malvidin 3-glucoside (97 %), cyanidin 3-glucoside (97 %), were supplied by Sigma Aldrich, Germany. Anhydrous ammonium ferric sulfate ($NH_4Fe(SO_4)_2$) was purchased by Sharlau, Germany. Deionized water was produced by water deionizer Elix 70C Gulfstream“, trichloroacetic acid (TCA, 99 %), acetonitrile, Merck, Germany.

Extraction procedure

We selected to investigate the optimization of extraction of Aronia and Elderberry in this study following the total contents of polyphenols and antioxidant activity. The Trolox equivalent antioxidant capacity (TEAC) was also determined.

In our study, we performed convectional extraction (conducted via magnetic stirrer) using water and aqueous ethanol solutions of 20 vol. %, 50 vol. %, 80 vol. % and 96 vol. %. All by-products used (fruits of Chokeberry and Elderberry) were pre-dried. The red fruits were ground in an electric mill and sieved to separate them into different fractions. Grounded fruits of a particles fraction size from 0.5 mm - 1.0 mm and whole red fruits were used for the extraction. 5.0 g of each sample were weighed using a Sartorius Analitic balance to the nearest 0.1 mg. 100 mL of each solvent - a water and aqueous ethanol solutions of 20 vol. %, 50 vol. %, 80 vol. % and 96 vol. % are added in flask of 150 ml for an extraction time of 100 min through shaking. The temperature used for the extraction (approx. $40^{O}C$) kept constant as far as possible. Each exhausted material was carefully pressed, and the extracts were filtered through cotton and filter paper, measured and analyzed immediately after the appropriate dilution.

Total polyphenol content

The total polyphenols content was determined by the Folin-Ciocalteu (FC) colorimetric method [20, 21]. In accordance with the method, 0.02 mL aliquot of the red fruits extract was added to a tube containing 1.58 mL of deionized water. Further, 0.1 mL of Folin Ciocalteu reagent was added immediately. 30 sec later 0.3 mL of 20 % solution of Na_2CO_3 were introduced. An aliquot of 1.60 mL extract was used in this case. The samples were mixed well and kept in dark place for 2 h. Then the absorbance was measured versus a blank solution (prepared at the same time and in the same manner containing water instead of the test extract) by UV-Vis spectrophotometer „T60“(Oasis Scientific Ltd., USA) at 765 nm wavelength using 10 mm path length cuvette. The results were calculated in mg equivalents of gallic acid ($y = 0.9119x$, $R^2 = 0.9892$), pyrogallic acid (y =

1,2114x, $R^2 = 0.9907$) and tannic acid ($y = 0.4601x$, $R^2 = 0.9912$). The standard calibration curves were obtained with standard solutions containing gallic acid (0.1 - 1.0 mg mL^{-1}), pyrogallic acid (0.1 - 0.75 mg mL^{-1}) and tannic acid (0.5 - 2.0 mg mL^{-1}). The total polyphenol content of extracts of red fruits were expressed in mg equivalents of gallic acid, pyrogallic acid and tannic acid per gram of dry weight (mg GAE, PGAE, TAE gdw^{-1}) and calculated according to the following formula (Eq. 1):

$$TPC=C*Ve*F/M \quad (1)$$

where: *TPC* - total polyphenol content, mg GAE gdw^{-1}; *V* - volume of solvent used, ml; *C* - concentration of the standard used, mg ml^{-1}; *F* - dilution coefficient of the sample; *M* - mass of the sample, g.

Antioxidant activity by the method of DPPH

2,2-diphenyl-1-picrylhydrazyl (DPPH), was one of the first free radicals used to study the antioxidant activity of phenolic compounds. DPPH solution showed high absorption at 517 nm wavelength due to their dark blue colour. It allows estimating the effectiveness of the antioxidants presence [22]. The determination of the antioxidant capacity was carried out in this investigation as follows:

• Preparation of the DPPH solution: an aliquot of 0.0040 g of DPPH was dissolved in 100 mL of methanol;

• Sample preparations: an aliquot of 1 mL of the extracts (previously diluted 10 times) were added to 4 mL of DPPH solution. The samples were stored in dark for 60 min. The absorbance was measured at a wavelength of 517 nm. The free radical scavenging ability or the inhibition capacity of the tested samples was calculated using the formula (Eq. 2) [23]:

$$IC=(Ao-Aa)/Ao*100 \quad (2)$$

where: *IC* was the inhibition capacity (%); *Ao* was the average absorbance value of the blank solution used; *Aa* was the average absorbance value of the sample.

The antioxidant activity was defined by plotting a calibration curve of Trolox, concentration range (2.5 - 175 µmol L^{-1}) vs. inhibition (%), IC. The results were expressed as µmol Trolox equivalent antioxidant capacity per gram dry weight (µmol TEAC g^{-1} dw).

RESULTS AND DISCUSSION

Effect of the solvent concentration

Alcoholic solvents have been commonly employed to extract phenolics from natural sources: they give quite a high yield of total extract even though they are not highly selective for phenols. The use of ethanol (a dietary alcohol) is preferable than methanol in view of a food application of the extracts although methanol has a good effect on polyphenol extraction [24]. In fact, ethanol, a polar solvent, effectively extracts flavonoids and their glycosides, catechols, and tannins from raw plant materials [25]. Water and aqueous mixtures of acetone are commonly used to extract plant materials. The extracting solvents significantly affect extraction yield, phenolic content and biological activities of plant materials [26]. Therefore, it is not clear which solvent system is more effective in extracting the phenolic content of different materials and evaluating the antioxidant activity.

In this study, we worked with five solvents - water and aqueous ethanol solutions of 20 vol. %, 50 vol. %, 80 vol. % and 96 vol. %. We compared the solvents and determined which one of them was the better extractant. The total polyphenols content and antioxidant capacity were determined. The results obtained are presented in the tables below. In order to determine the difference in TPC in the different samples, the results are presented

Table 1. Effect of the ethanol in water solutions on the total polyphenol content of the red fruits - Chokeberry and Elderberry.

Total polyphenol content mg GAE g dw^{-1}				
Solvent	Chokeberry Whole fruits	Chokeberry (0.5 - 1.0 mm)	Elderberry Whole fruits	Elderberry (0.5 - 1.0 mm)
H_2O	1.45	16.66	8.10	8.70
Ethanol 20 %	2.46	23.39	8.08	21.29
Ethanol 50 %	7.83	42.96	13.64	31.49
Ethanol 80 %	6.98	21.37	11.14	13.41
Ethanol 96 %	1.88	4.83	3.66	4.61

Table 2. Effect of the ethanol in water solutions on the total antioxidant activity of the red fruits - Chokeberry and Elderberry.

Antioxidant activity μmol TEAC g^{-1} dw				
Solvent	Chokeberry Whole fruits	Chokeberry (0.5 - 1.0 mm)	Elderberry Whole fruits	Elderberry (0.5 - 1.0 mm)
H_2O	171	1598	1384	1400
Ethanol 20 %	987	1724	1462	1548
Ethanol 50 %	1346	2589	1681	1704
Ethanol 80 %	1297	1661	1547	1603
Ethanol 96 %	384	1130	581	757

in gallic acid equivalent.

The results presented in Table 1 and Table 2 show that the optimal results in respect to polyphenol compounds (TPC) and antioxidant activity are obtained using 50 % aqueous ethanol solution for both red fruits products. The optimal values of the total content of polyphenols when extracted with an aqueous solution of ethanol 50 %, contained in Chokeberry whole and ground fruits, as well as whole and ground fruits of black Elderberry are 7.83 mg GAE g dw^{-1}; 42.96 mg GAE g dw^{-1}, 13.64 mg GAE g dw^{-1}; 31.49 mg GAE g dw^{-1}. For extraction with ethanol solution (50 vol. %), the highest antioxidant activity is in the ground fruits of Chokeberry with a value of 2589 μmol g^{-1} trolox equivalent antioxidant capacity and the lowest in the whole fruits of Chokeberry with a value of 1346 μmol g^{-1} trolox equivalent antioxidant capacity.

The 96 % aqueous ethanol solutions appear to be the worst solvents for extracting polyphenol compounds when compared to 20 % -ethanol solution, 80 % ethanol solution and deionized water.

According to many studies, the berries of Chokeberry are among the fruits containing the highest levels of phenolic compounds and anthocyanin pigments [1, 27, 28]. Galvan D'Alessandro et al. also reported that the use of medium concentration for ethanol/water mixture (50 %) resulted in higher TP yield compared with water or other ratios of the ethanol/water mixture [29]. Our results are in accordance with previous studies which reported that binary solvent system containing hydro-organic solvents was superior than mono-component solvent system (pure water or ethanol) in the extraction of phenolic compounds [30, 31]. The extraction efficiency regarding TP and TAC contents showed improvement as the particle size decreased (Table 1 and Table 2.). Yields obtained with particle sizes 0.5 - 1.0 mm were significantly higher than those obtained with whole fruits. Our results are in accordance with previous report where the extraction from grounded Chokeberries was more efficient than the extraction from berries cut in half [29]. Smaller particle size had higher contact surface which allows the increase of mass transfer.

CONCLUSIONS

The interest in the extraction and isolation of natural products and their health beneficial uses increases. Convectional extraction of red fruits products (Chokeberry and Elderberry) was studied. The extracts were analyzed for total polyphenols content and antioxidant activity. It was found that the extraction solvent had a significant influence on extraction of polyphenolic compounds. The 50 % of ethanol-in-water solutions exhibited better efficiency on extraction of polyphenolic compounds and antioxidant activity compared to aqueous ethanol solutions of 20 vol. %, 80 vol. % and 96 vol. %. The maximum concentration of TPC and antioxidant activity with solution of ethanol 50% was found in the ground fruits of Chokeberry. The ground fruits give better results than the whole ones. We observe a higher content of polyphenols and antioxidants in Chokeberry fruit compared to black Elderberry fruit. Our results have shown high antioxidant potential and opportunities for prerequisites for application in the pharmaceutical, cosmetics and food industries.

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