COMPARATIVE DETERMINATIONS FOR CERTAIN PHYTOTERAPEUTICAL SUBSTANCES FROM INDIGENOUS HERBS

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This paper presents the physical-chemical analysis of the extracts from some indigenous medicinal fruits for their added value in phytotherapy for heart disease (preparation-food suplements), for improving the blood circulation. The studied fruits were: Chokeberry (Aronia melanocarpa), Rosehip (Rosa canina), Bilberry (Vaccinium myrtillus) and Hawthorn (Crateagus monogyna), with high content in antioxidant compounds. The main compounds studied compared in this paper are: flavones, phenols and anthocyanins. The fruit extracts studied are an important source of antioxidants.

Keywords: rosehip, hawthorn, biloberry, chokeberry

1. Introduction

For a long period of time the plants were considered an important source of bioactive substances which have found different uses in traditional or alternative medicine (phytotherapy) and have provided compounds with beneficial effects on the human health. In modern phytoterapy they are a major source of plant-health products and dietary supplements.

This range of natural products derived from plants, phytochemicals and vitamins helps to maintain the health and to fight against diseases [1-2].

It is no doubt that the use of herbal medicines is one of the oldest forms of health care. World Health Organization (WHO) estimates that 80% of the world population is still using botanical medication. Some of these products have been widely used in allopathic medicine.

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Their use is regulated by the Food and Drug Administration (FDA) and European Medicines Agency (EMEA), through standards of quality, safety and efficiency. In 1987, WHO has underlined the importance of scientific research on the herbal supplements, because there is insufficient evidence that these products may have beneficial effects [3-4].

There are many nutritional and non-nutritional compounds found in various plants which help the blood circulation function, which is an important process in homeostasis and various cardiovascular diseases, inflammation, and even in cancer. Various flavones have been intensively studied showing strong cardiovascular protective effects and as antioxidants they prevent and treat hypertension [5-6]. Antioxidants are a class of compounds of great interest for pharmacologists, biochemists and other health care professionals, given their role to reduce the adverse effects caused by reactive oxygen (ROS) nitrogen (SNR) or chlorine species (SCR). Antioxidants can be defined as those compounds which by their presence cause the stopping or slowing of the oxidation reaction of a substrate and they can be classified into two groups: primary or *„chain-breaking*" antioxidants and secondary or *„preventative antioxidants"*[7-9].

Untreated high levels of oxidative stress can weaken the body and contribute to the development of more than 30 diseases, especially of chronic or degenerative nature, such as cardiovascular disease, ophthalmology, Alzheimer's disease, speeding aging and cancer [7-9].

The aim of this study was the comparative determination for certain phytoterapeutical substances from indigenous herbs: flavones, phenols and anthocyanins.

2. Experimental part

2.1. Method and materials

Absorbance measurements were performed using a JASCO V530 UV-Vis spectrophotometer. All reagents used are of analytical type.

Preparation of vegetable material and the procedure

Hawthorn, rosehip, bilberry and chokeberry harvested from spontaneous flora (less polluted areas) were dried spared at the 35^0C-38^0C temperature, ground and sieved in sieve Θ=0.2mm. The obtained powders were analyzed for their compound in polyphenols, flavonols and in procyanidins by spectrophotometric methods (UV-VIS spectrometer, JASCO V530). Antioxidant activities were determined by assay: the Cuprac assay and biological assay with pig brain.

The procedure for flavones and polyphenols:

Five grams of plant material for each sample are treated with 100mL of 50% alcohol and shaked gently to mix and then 30min refluxed at 100^{o}C temperature. The samples are filtered through narrow pore filter paper (Whatman) and analyzed according to the working methods of the European Pharmacopoeia, edition in force [9].

The procedure for procianidins

Stage I: 5 grams of plant material, 30mL of 70% ethanol was added and it was refluxed for 30 minutes at 100^{o}C as temperature.

Stage II: Hydrolysis with concentrated HCl. Samples obtained after filtration were treated with 15 mL concentrated HCl, 10 mL of 70% ethanol and 10 mL distilled water. The samples were refluxed 90 minutes at a temperature of 100^{o}C. The procyanidins method corresponds to the European Pharmacopoeia, edition in force [9].

The procedure for antioxidant activity - Cuprac assay

The Cuprac asay is based on reducing Cu^{2+} to Cu^{+} in the presence of neocuproin. It forms a red-brick complex. Sample absorbances were read at 450 nm wavelength and correspond to methods of Apak R. et al. 2004 [10].

The procedure for antioxidant activity – biological assay

The antioxidant activity assay consists in the lipid peroxidation reaction with the pig brain in the presence of ascorbic acid. The results were expressed in the percentage of inhibition. The lipid peroxidation product, malondialdehyde was resulted in the reaction in which forms a colored complex with thiobarbituric acid.

It was determined by readings at 532 nm wavelength (the method corresponds to O. Rop and co. 2011 [11, 12]).

2.2. Vegetable material description

The hawthorn fruits contain natural tannins of catechesis, group of B vitamins and C vitamin, anthocyanins, flavonoids, acids (tartaric, citric, crategic, ursolic, acid, nicotinic, chlorogenic), sorbitol, choline, acetylcholine, glucose, fructose, pectin, fatty oil and mineral substance [7-9].

The chokeberry fruits contain anthocyanins, tannins substance, vitamins C, E, PP, organic acids, trace elements, carotenoids, sugars, etc. The fruits contain large amounts of vitamins and minerals (potassium, calcium, phosphorus, magnesium, iron (1.2 mg%) iodine etc. [7-9].

The bilberry fruits have high content of antioxidants compounds: anthocyanins, ellagic acid, fiber, vitamin A, B, C, E etc. tannins, flavonoids,

mirtilina, pectin, sugars, organic acids (citric, malic, oxalic, succinic, lactic) and glucokinin [7-9].

The rosehip fruits contain vitamin C, vitamin A, B1 and B2, vitamin P and vitamin K, niacin, citric and malic acid, invert sugar, tannins, flavonoids, fatty oil, volatile oil and minerals etc. Rosehip is an excellent tonic [7-9].

3. Results and discussion

The content in polyphenols, flavones, procyanidins determined by spectrophotometric methods for the mentioned fruits are presented in the Table 1, along with antioxidant activity which was measured by the two methods.

By combining these four fruits extract in certain proportions, we obtained new pharmaceutical formulas with enhanced antioxidant activity.

Table 1

The content in polyphenols, flavones, procyanidins for the mentioned fruits

No.	Vegetable material Fruits:	Total Flavones (on rutin) g%	Total poliphenols (on caffeic acid) g%	Total procianidins (on cyanidine chloride) g%	Antioxidant activity (g_{trolox}/g_{sample})	Antioxidant activity (% inhibition)
1.	Hawthorn	0.98	1.11	1.64	5.93	55.97
2.	Rosehip	0.70	1.35	0.66	5.78	63.54
3.	Bilberry	1.23	2.93	5.20	6.01	81.38
4.	Chokeberry	1.43	1.66	5.88	6.10	63.69

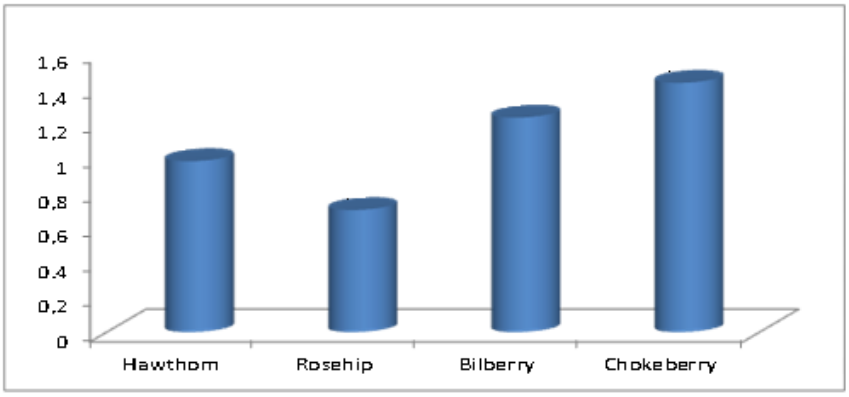

Fig. 1. Flavones variation content

The flavones' content varies between 0.7g% (rosehip) to 1.43g% (chokeberry) (fig. 1).

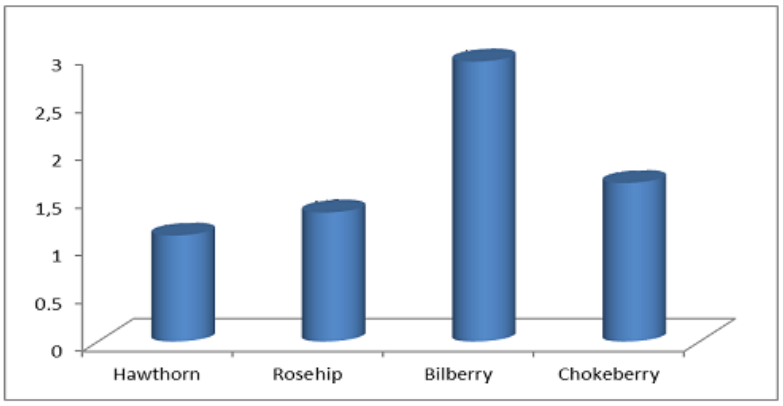

Fig. 2. Poliphenols variation content

The poliphenols' content varies, only the chokeberry has the higher content (fig.2.)

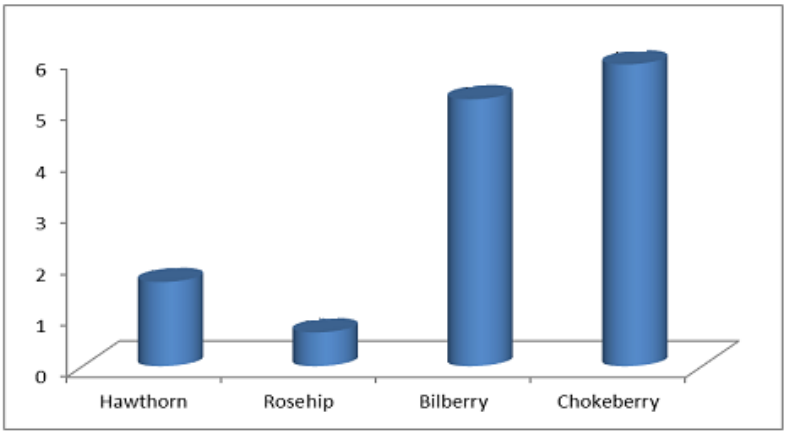

Fig. 3. Procanidins variation content

The procianidins content for the studied fruits varies in large limits between 0.66 g% to 5.88g% for the chokeberry extracts (fig. 3).

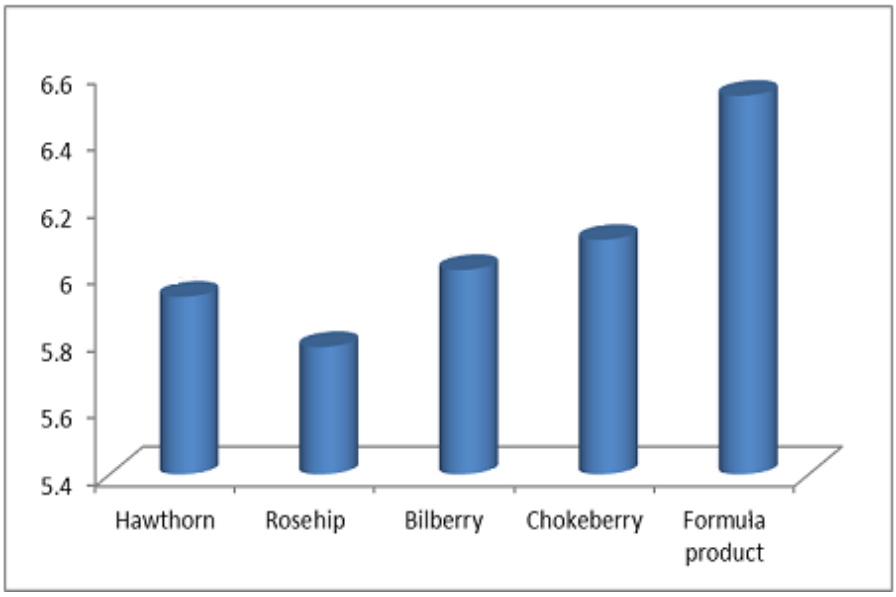

Fig. 4. Antioxidant activity variation - Cuprac assay

The rosehip extract fruits present lower antioxidant activity 5.78g_{trolox}/g_{sample} because of the higher content in vitamin C, although it shows low values for phenolic compounds.

The product formulas (S.A.) made from fruit extracts shows the highest value for the antioxidant activity (6.53 g_{trolox} / g_{sample}) (fig.4.), obtained by Cuprac assay.

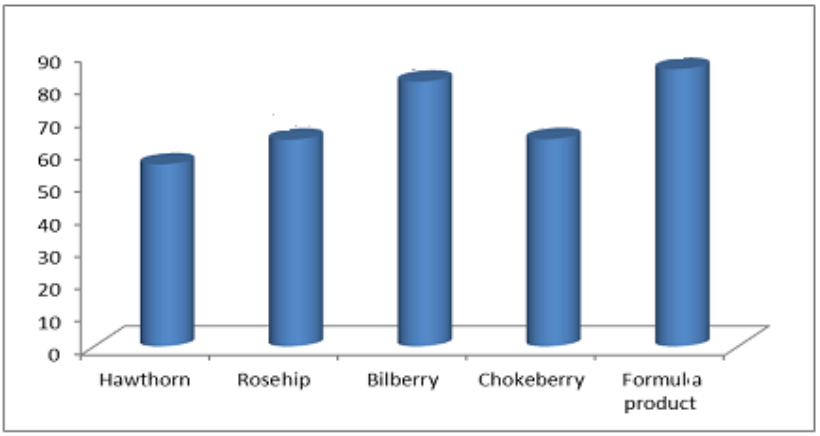

Fig. 5. Antioxidant activity variation – biological assay

The highest value for the antioxidant activity (85.23% inhibition) is observed for the product's formulas (SA) (Fig. 5)

4. Conclusions

The highest values obtained in the researched compounds (from the fruit extracts) mentioned, have conducted us to a new product formulas (SA) with complex antioxidant properties proposed as an adjuvant in the treatment of cardiovascular diseases.

The product's formula (S.A.) made through the combination of the four extracts in the optimal technological conditions, well-established, shows higher values for the antioxidant activity values that entitle further research of feasibility to complete new dietary supplement formulas with the expected effects.

The Cuprac assay put in evidence the phenolic content from the four fruit extracts and for the formula. The best results were obtained from the hawthorn extract 5.78 g_{trolox}/g_{sample} and from the formula's product extract (6.53 g_{trolox}/g_{sample})..

The biological assay underlined the lipid peroxidation in the presence of ascorbic acid. Our fruit and formula's fruit extracts contained lipids. These lipids were oxidized by the ascorbic acid, oxidation reagent to form peroxides. The best results were obtained for the bilberry (81.38% inhibition) and for the formula's product extract (85.23% inhibition).

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