Acta Poloniae Pharmaceutica – Drug Research, Vol. 69 No. 3 pp. 381–387, 2012 ISSN 0001-6837
Polish Pharmaceutical Society

ANALYSIS

EVALUATION OF POLYPHENOLS AND ANTHOCYANINS CONTENTS IN BLACK CHOCKEBERRY – *PHOTINIA MELANOCARPA* (MICHX.) FRUITS EXTRACT

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Abstract: An evaluation of total polyphenols and anthocyanins contents in dietary supplements is important analysis in medical aspect of human and animal diets. The content of the mentioned compounds should be higher in 100 g of solid extracts than in 100 g of fruits. Thus, the presented work concerns the evaluation of total polyphenols and anthocyanins contents in black chockeberry – *Photinia melanocarpa* (Michx.) extract – dietary supplement (DS) available on market. The spectrophotometric analysis of DS were performed. The usage of certain conditions of measurements such as dilution factor, storage conditions and filtration, has the significance in the determination of the analyzed compounds in the extract.

Keywords: anthocyanins, polyphenols, black chockeberry – *Photinia melanocarpa* (Michx.)

Polyphenols are one of the most numerous and ubiquitous compounds of plants metabolites and constitute an integral part of both human and animal diets. The compounds are involved in growth and reproduction and provide plants with resistance to pathogens and predators, they protect crops from plague and preharvest seed germination (1). Their industrial applications can be the production of paints, paper, cosmetics, tanning agents and additives in food industry as natural colorants and preservatives. Polyphenolic groups possess a lot of significant abilities such as binding and precipitating macromolecules (dietary protein, carbohydrate, digestive enzymes), thereby reduce food digestibility, and antioxidant and free radical-scavenging.

Currently more than 8000 phenolic structures are known (2). They may be classified into different groups according to function of the number of phenol rings that they contain and to structural elements that bind rings to one another.

Therefore, polyphenols are categorized into the groups shown in Figure 1 (3).

The increased interest in plant polyphenols, in particular proanthocyanidins and anthocyanins as the most active groups of the compounds, has been caused by epidemiological studies performed in USA and France. Testing the causes of the low mortality from coronary heart disease of the inhabitants of Toulouse in France in comparison with the inhabitants of the USA shown that the consumption of fat and the other components of diet was similar in both countries with the exception of wine consumption. The phenomenon was described as “the French paradox”. Polyphenols - anthocyanins and proanthocyanidins were responsible for therapeutic properties of wine. Polyphenols of wine exhibited the ability to decrease blood platelets aggregation through the specific oxygen enzyme.

Anthocyanins (in Greek *anthos* means flower, and *kyons* means blue) are the more important plant

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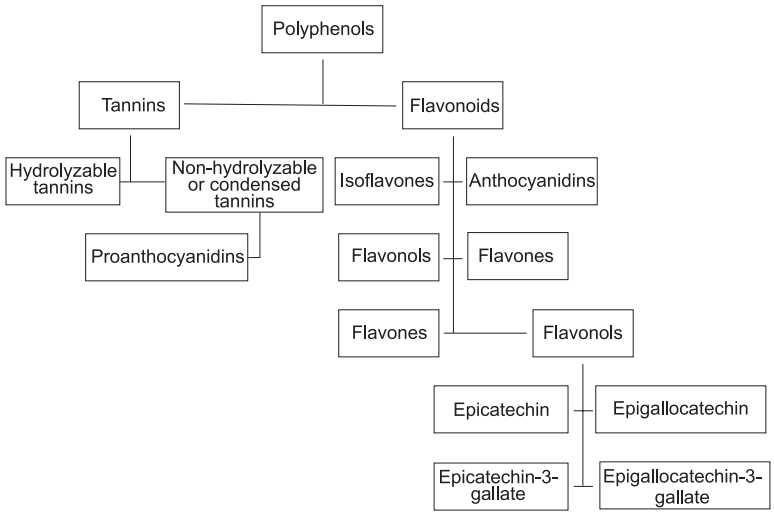

Figure 1. Classification scheme of polyphenols

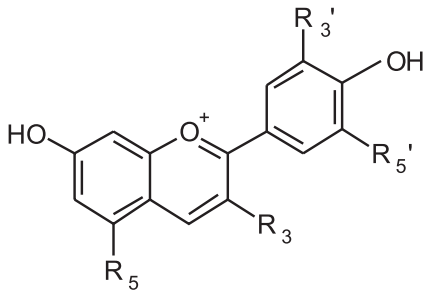

Figure 2. The structure of flavylium cation

pigments visible to the human eye. They are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium or flavylium salts. The basic structure of anthocyanins is the flavylium cation (Fig. 2).

Anthocyanins are water-soluble compounds and their color and color intensity in solution is greatly influenced by pH. Color variations are connected with the pH dependent structural changes of anthocyanins.

Anthocyanins play an important biological role as antioxidants. Their antiatherosclerotic and anticancer properties have been exploited in medicine. The mechanism responsible for the antioxidant activity of anthocyanins appears to be dual. The most important function of anthocyanins is their ability to impart color to the plants or plant products in which they occur. They play a definite role in the attraction of animals for pollination and seed dispersal, they are of considerable value in the co-evolution of these plant-animal interactions. They can act as antioxidants or as antibacterial agents (5). Anthocyanins are flavonoids present in a variety of pigmented food and like other flavonoids, seem to play a role in preventing human pathologies related to oxidative stress. In fact, anthocyanins have been shown to exert antiproliferative effects in cell cultures and exhibit antiinflammatory and vasoprotective activities in animal models and these biological activities have been related to their antioxidant properties. Anthocyanins possess pharmacological properties and are used by humans for therapeutic purposes (6–9).

In addition to their functions in plants, anthocyanins have many other applications. Many dietary supplements are containing the anthocyanins due to their positive influence on human health.

EXPERIMENTAL

Material

DS - the solid extract from Black chockeberry – *Photinia melanocarpa* (Michx.) fruits purchased from the Polish pharmaceutical company.

Chemicals and reagents

(+)-Catechin hydrate was purchased from Fluka (Buchs, Switzerland); Folin-Ciocalteau reagent was purchased from Chempur (Piekary

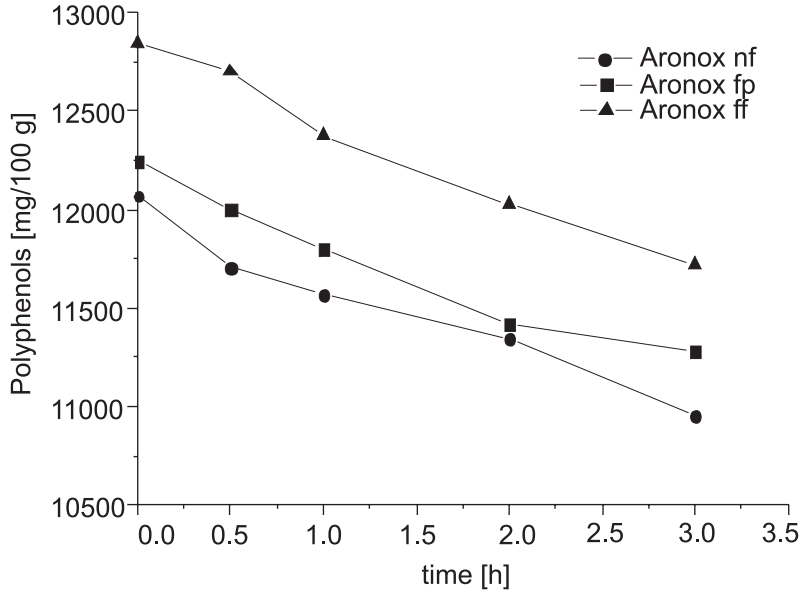

Figure 3. The influence of the filtration mode and time on the changes in polyphenols contents

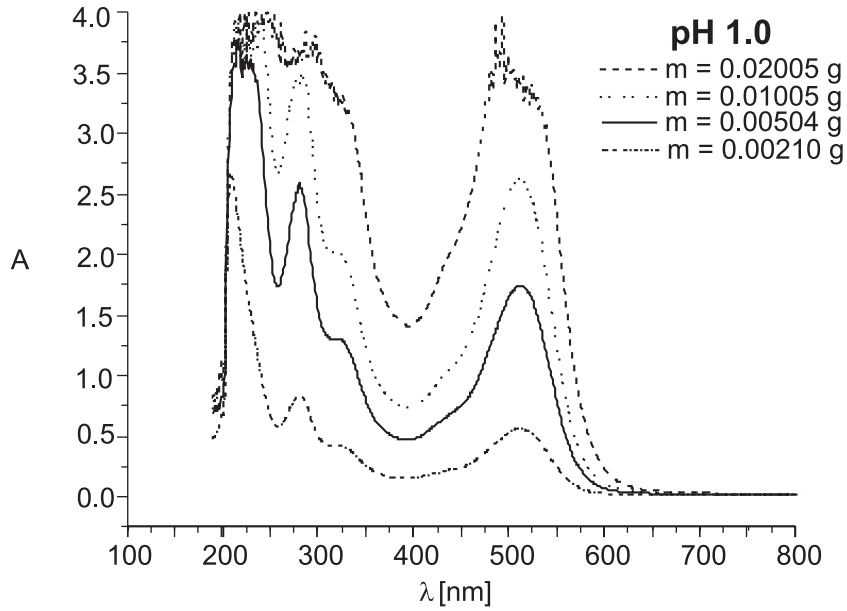

Figure 4. The influence of different mass of the sample on the absorbance at pH 1.0

Śląskie, Poland); KCl, $CH_3CO_2Na \times 3H_2O$ and Na_2CO_3 were purchased from Stanlab (Lublin, Poland). All reagents were weighed with an accuracy of ± 0.0001 g. Other chemicals and reagents were of analytical grade.

Determination of total polyphenols content

Total polyphenols content was determined using the Folin-Ciocalteau method described by Emmons et al. (10) with some modifications. In order to prepare calibration curve, the following volumes of (+)-cate-

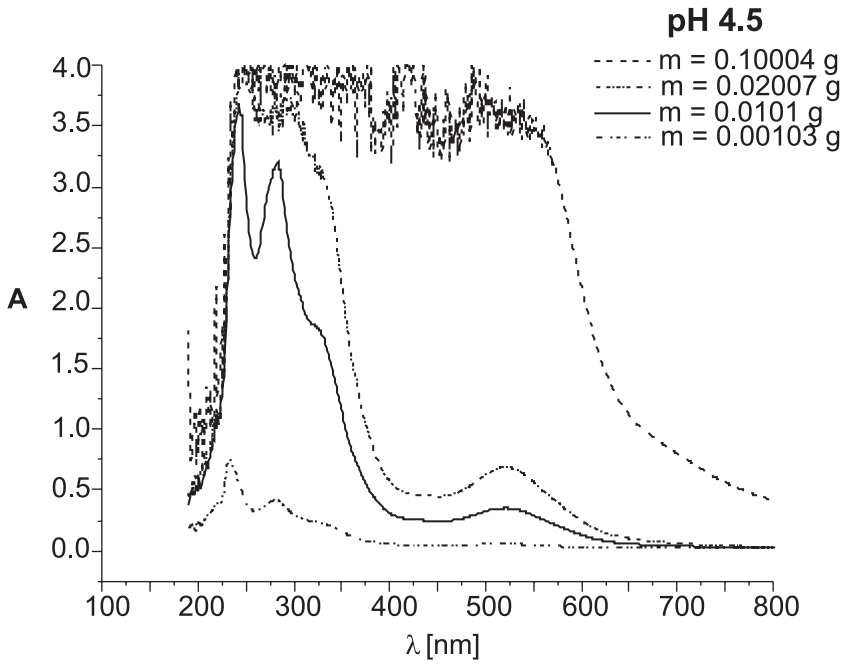

Figure 5. The influence of the different mass of the sample on the absorbance at pH 4.5

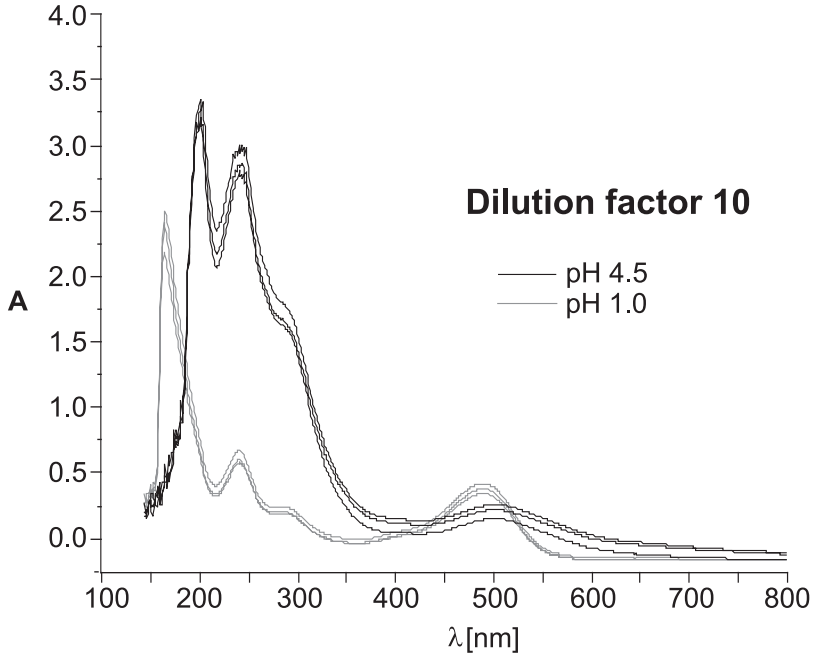

Figure 6. The comparison of absorbance spectra of DS solutions with dilution factor 10

chin standard solution in 5% ethanol: 0.0, 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 13 and 15 mL were placed in 50 mL flasks. Afterwards, 20 mL of distilled water, 0.5 mL of Folin-Ciocalteau reagent and 5 mL of 20% solution of Na_2CO_3 were added to each flask. At the end, the distilled water was added to the mark of flasks. The samples were mixed thoroughly. Then, the solutions were left in the dark room for 20 min. The determination of total polyphenols of standards was performed by using an UV-visible spectrophotometer

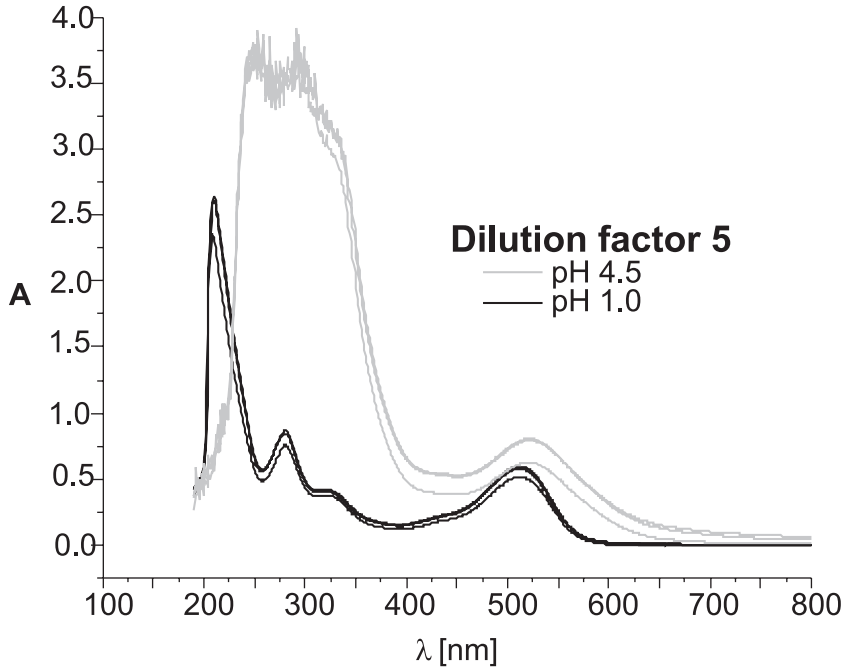

Fig. 7. The comparison of absorbance spectra of DS solutions with dilution factor 5

Hewlett Packard 8453, monitoring at 776 nm. The linear calibration curve was drawn as the function of absorption in different concentration of (+)-catechin solution in 5% ethanol [mg/mL]. The equation of the calibration curve was y = 1.45374 x + 0.02222 and the correlation coefficient of the calibration curve was $R^2 = 0.98474$.

The sample (0.2 ± 0,0001 g) was dissolved in 50 mL of 5% ethanol solution and left for 1 min. The prepared solution (1 mL) was placed into 50 mL flask, then 20 mL of distilled water, 0.5 mL of Folin-Ciocalteau reagent and 5 mL of 20% solution of Na_2CO_3 were added. At the end, the distilled water was added to the mark of flasks. The samples were mixed thoroughly. Then, the solution was left in the dark room for 20 min. The measurement of the absorbance prepared solution was performed at λ = 700 nm relatively to the control sample (the first solution from calibration curve). The total phenolic content in the sample was calculated on the basis of the calibration curve and the formula:

$$\text{Polyphenols} = \frac{K}{V}\ [\frac{\text{mg}}{\text{mL}}]$$

where: K = catechin concentration in samples from calibration curve [mg] and V = volume [1 mL].

Determination of total anthocyanins content

Anthocyanins were determinated by spectrophotometric method according to the procedure described by Fuleki and Francis (11) with some modifications. The principle of this method is based on the fact that monomeric anthocyanin pigments reversibly change color with a change in the pH. The colored oxonium form exists at pH 1.0, and the colorless hemiketal form predominates at pH 4.5. The difference in the absorbance of the pigments at 520 nm is proportional to the pigment concentration (12). The samples were diluted with the potassium chloride buffer (KCl, 0.025 M, pH 1.0) and sodium acetate buffer ($CH_3CO_2Na \times 3H_2O$, 0.4 M, pH 4.5), mixed and left in dark place for one hour. The measurements of the absorbance of the samples were performed at the wavelength λ = 510 nm. The buffers of pH 1.0 and pH 4.5 were used as the reference solutions for the measurement.

Data analysis

All experiments were performed in triplicate (n = 3). The mean values of each treatment were obtained by using Statistica 9 software. The results represented the means ± standard error (SE) of three replicated determinations.

RESULTS

Contents of total polyphenols

The determined samples were measured in three series: non-filtered (nf), filtered using paper

Table 1. The contents of total polyphenols in the samples of DS non-filtered (nf), filtered with paper (fp) and Buchner funnel (ff).

Name	Mass of sample [g]	Absorbance [776 nm]	Concentration [mg/mL]	Percent of total [%]
DS (nf)	0.20006	0.98705	0.61337	61.46 ± 0.23
DS (fp)	0.20005	0.91177	0.56730	61.23 ± 0.21
DS (ff)	0.20006	1.0315	0.64203	64.23 ± 0.25

Table 2. Influence of dilution factor, filtration and light protection conditions on total amount of anthocyanins.

Sample	Dilution factor	Absorbance pH 1.0	Absorbance pH 4.5	TAcy [mg/100 g]	TAcy [%]
DS before leaving in dark	10*	0.57247	0.39682	1598.11	15.98 ± 0.89
DS (nf) after leaving in dark		0.59765	0.34224	1699.53	16.99 ± 0.59
DS (fp) after leaving in dark		0.59955	0.35009	1700.58	17.00 ± 0.73
DS before leaving in dark	5**	0.58393	0.77085	1613.58	16.13 ± 0.80
DS (nf) after leaving in dark		0.58671	0.78879	1611.93	16.11 ± 0.75
DS (fp) after leaving in dark		0.52025	0.66348	1442.22	14.42 ± 0.74

*(1 mL sample and 9 mL buffer solution); ** (2 mL sample and 8 mL buffer solution)

(fp) and Buchner filter funnel (ff) (Table 1). The filtration of the samples was performed because the flowing particles in the solution were observed. The value of the DS (ff) was higher than the DS (nf). The higher value of polyphenols in the DS (ff) sample appeared because the filtration with Buchner filter funnel could have smashed the particles of the supplement. Thus, the concentration of DS (ff) was about 3% higher than the DS (nf).

The analysis concerning the influence of the filtration and time on the changes in contents of polyphenols was performed. The contents of polyphenols reduced in time both in filtered and non-filtered samples are presented in Figure 3.

Contents of total anthocyanins (TAcy)

In order to determine the contents of TAcy, the direct usage of samples without their dilution was made. Thus, four masses of sample were used to choose the appropriate one. The mass of the sample was fixed at pH 1.0 and 4.5. The influence of different masses of the sample on the absorbance is shown in the Figures 4 and 5. The masses of 0.00210 g at pH 1.0 and 0.0101 g at pH 4.5 were chosen due to the good image of the curves.

On the other hand, the dilution factors were used because the application only of adequate mass did not solve the relationship between the sample at pH 4.5 and 1.0. The analysis concerning the usage of two dilution factors (10 or 5), filtration and with light protection on total amount of anthocyanins were applied (Table 2). The values of anthocyanins at the dilution factor 10 and at 5 and of the usage the appropriate mass were almost the same. The incubation conditions did not have the considerable influence on the mass of anthocyanins in 100 g of the product.

The pictures of the relationship of DS solutions (non-filtered, filtered using paper and Buchner filter funnel) in corresponding pH are presented in Figures 6 and 7.

DISCUSSION AND CONCLUSION

In the present study, polyphenols and anthocyanins in DS were determined. The total polyphenols content of nf, fp and ff DS samples were determined to be 61.46, 61.23 and 64.23% of mass of preparation, respectively. The influence of the filtration and time on the changes in contents of polyphe-

nols showed that they were reduced in time both in filtered and non-filtered samples. By measuring the content of total anthocyanins in DS samples, the incubation conditions did not have considerable influence on it. Dilution factor of samples by using the appropriate masses of DS confirmed that the correlation between the mass of the sample used in analysis and the total anthocyanins content exists. It is worth dealing with the study because the supplement is present on market.

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Received: 15. 03. 2011