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Total phenolic content and antioxidant activity of ethanolic extracts of *Aesculus hippocastanum* L.

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Abstract: *Aesculus hippocastanum* L. (horse chestnut) belongs to the genus *Aesculus,* the most widespread genus of the Hippocastanaceae family, and it is native to the countries of the Balkan Peninsula. Different parts of the plant were used for the treatment of many diseases. Total phenolic content of ethanolic extracts of bark of twigs and fruit and fruit itself was evaluated by Folin-Ciocalteu method while antioxidant activity was tested using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging method. Preliminary investigation of chemical composition was done by TLC and aesculin was detected in samples of bark of twigs. Results for content of total phenolics were expressed as gallic acid (mg GAE/mL) and aesculin (mg AE/mL) equivalents, respectively. Among the tested samples, the highest amounts of total phenolics were detected in all bark extracts, 763.53-1048.00 mg AE/mL, while samples of pulp had the lowest content of these compounds, 43.41-66.15 mg AE/mL.

All bark extracts showed higher antioxidant activity than aesculin which was used as a standard, while extract of pulp mixed with bark showed significantly lower antioxidant potential.

INTRODUCTION

Aesculus hippocastanum (horse chestnut) is a large deciduous, rapidly-growing tree that can reach a height of 36 meters. It is native to the countries of the Balkan Peninsula, but because of its large, showy flower clusters the tree is cultivated worldwide for its beauty. Flowers are white or pink with a small red spot.

While the common name for the tree is horse chestnut, it is also known as buckeye, and like other buckeyes, is a member of the Hippocastanaceae family, rather than the chestnut family (Castanea). Historically, the seed extract was used as a treatment for many ailments, including rheumatism, rectal complaints, bladder and gastrointestinal disorders, fever (first written account in 1720), hemorrhoids (as early as 1886) and leg cramps. Currently, horse chestnut seed extract is widely used in Europe for chronic venous insufficiency, hemorrhoids, post-operative edema, and topically for clearing skin conditions.

To date, more than 210 compounds of different classes have been isolated and identified from the genus *Aesculus* (Zhang *et al.*, 2010). The primary active constituent found in horse chestnut seed extract is aescin (Sirtori, 2001, Jiang *et al*., 2011). Aescin is actually a mixture of triterpene saponins present in two forms, α and β, which are distinguished by their water solubility and melting points. Other constituents include bioflavonoids (quercetin and kaempferol), (Dudek-Makuch and Matlawska, 2011) proanthocyanidin A2 (an antioxidant), and the coumarins fraxin and aesculin (Stanić *et al.* 1999).

It is evident that the popularity of herbal medicine, as for natural or industrial formulations of nutritional supplements, is at its top, from both the points of view of consumers and researchers. With such a huge interest, the scientific role of researchers is particularly devoted to acquire more information about composition, structures and activity effects, in relation to physiological benefits and metabolic processes of these products.

A close examination of the recent literature indicates some interests in horse-chestnut products. Therefore, it has been a discover healthy benefits for human consumption of various products obtained and derived from *A. hippocastanum* L. (Baraldi *et al*, 2007, Küçükkurt *et al.,* 2010).

Except medicinal importance, *A. hippocastanum* was studied as a possible biomonitor of the heavy metal pollution (Yilmaz *et al*., 2006).

EXPERIMENTAL

Material and methods

The plant material of *A.hippocastanum* L. was collected in fall 2010 from three different locations in Sarajevo, 1-Sutjeska; 2-Ilidža; 3-Pofalići. For preparation of ethanolic extracts 10 g of bark of young twigs (BT) and fruits (BF), bark of fruit with pulp (BF+P) and a pulp itself (P) were infused into 100 mL of hot ethanol for 10 days, filtered and stored into sterile bottles.

Preliminary investigation

Preliminary investigation of extracts composition were done by thin layer chromatography in ethyl acetate:methanol:water (17:3:1) system. Detection of sample components was done using Folin-Ciocalteu and DPPH reagents, and UV light. Aesculin was used as a standard.

Total phenolic content

Total phenolic content were determined spectrophotometrically according to the Folin-Ciocalteu method, using gallic acid and aesculin as standards and expressing the results as gallic acid (mg GAE/mL) and aesculin (mg AE/mL) equivalents. Data presented are average of three measurements.

Antioxidant activity

The antioxidant activity of ethanolic extracts was evaluated using the DPPH radical-scavenging method, which is based on the reduction of stabile 1,1-diphenyl-2-picrylhydrazyl radical (Brand-Williams *et al*., 1995). DPPH• is a stable free radical compound with characteristic absorption at wavelength of 517 nm. Antioxidants upon interactions with DPPH neutralize its free radical character and the colour of the reaction mixture changes from purple to yellow.

The radical solution was freshly prepared daily, stored in a flask covered with aluminium foil, and kept in the dark at 4 C between measurements. A portion of the sample solution (100 μL) was mixed with 1 mL of 5.25 $x10^{-5}$ M DPPH• in ethanol. Decreasing of absorbance of tested samples was monitored every 60 seconds for 30 min at 517 nm on Perkin–Elmer Lambda 25 UV/Vis spectrophotometer. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH·, were calculated according to the formula (Yen and Duh, 1994),

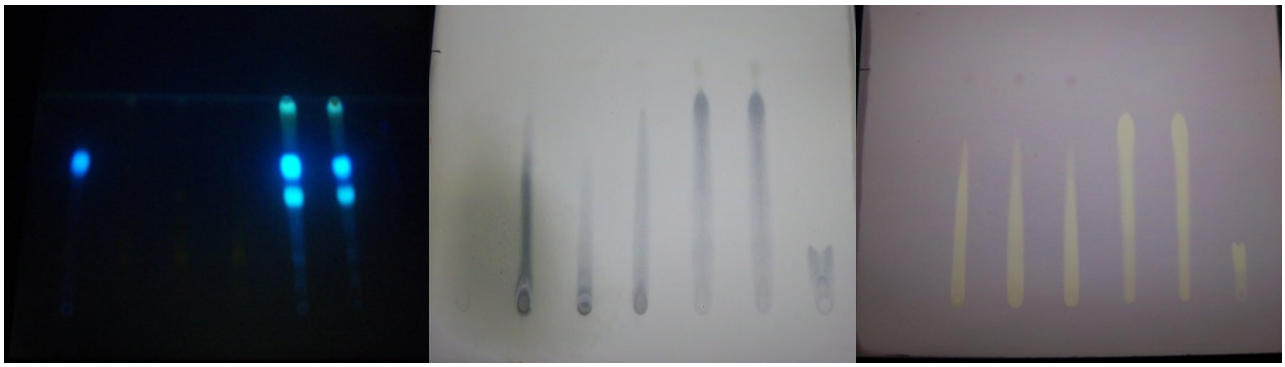
$$IC_{50}(\%) = [(A_0-A_t)/A_0] \times 100 \quad (1)$$

where A_0 and A_t are the absorbance values of the blank sample and the test sample, at particular times, respectively. Percent of inhibition after 30 min was plotted against concentration and the equation for the line was used to obtain the IC_{50} value. A lower IC_{50} value indicates greater antioxidant activity. All determinations were performed in triplicate. Aesculin was used as a positive probe.

RESULTS AND DISSCUSION

Preliminary investigation was done by thin layer chromatography (TLC) which has proved its worth as a simple, inexpensive method for the chemical and biological screening of plant extracts.

Detection of phenolic compounds was done by spraying TLC plates with Folin-Ciocalteu reagent (Stahl, 1969). Positive detections were blue spots on white background (Fig. 1). The TLC plate with samples is developed with the elution solvent and dried. It is then sprayed with a DPPH solution. The plate is examined in daylight. Active (free-radical scavenging) compounds appear as yellow-white spots against a purple background (Marston, 2011).



(a) (b) (c)

Figure 1: Thin layer chromatograms of bark extracts with different detection. (a) UV 365nm, (b) Folin-Ciocalteu reagent, (c) DPPH solution.

Presence of phenolic compounds is confirmed as a specifically blue colored spots detected with Folin-Ciocalteu reagent. Yellow spots detected with DPPH reagent indicate presence of compounds with antioxidant activity (Figure 1). Aescin (A) was used as standard which was identified in bark of twigs extracts, as well as gallic acid (GA) that is most common phenolic compound found in the plants.

Among the tested samples, the highest amounts of total phenolic were detected in all bark extracts (763.53 mg EE/mL for 2-BT to 1048.00 mg EE/mL for 3-BF), while samples of pulp had the lowest content of these compounds 43.41 mg EE/mL 1-P. Generally, data obtained as aesculin equivalents are higher than those obtained as gallic acid equivalents (Table 1).

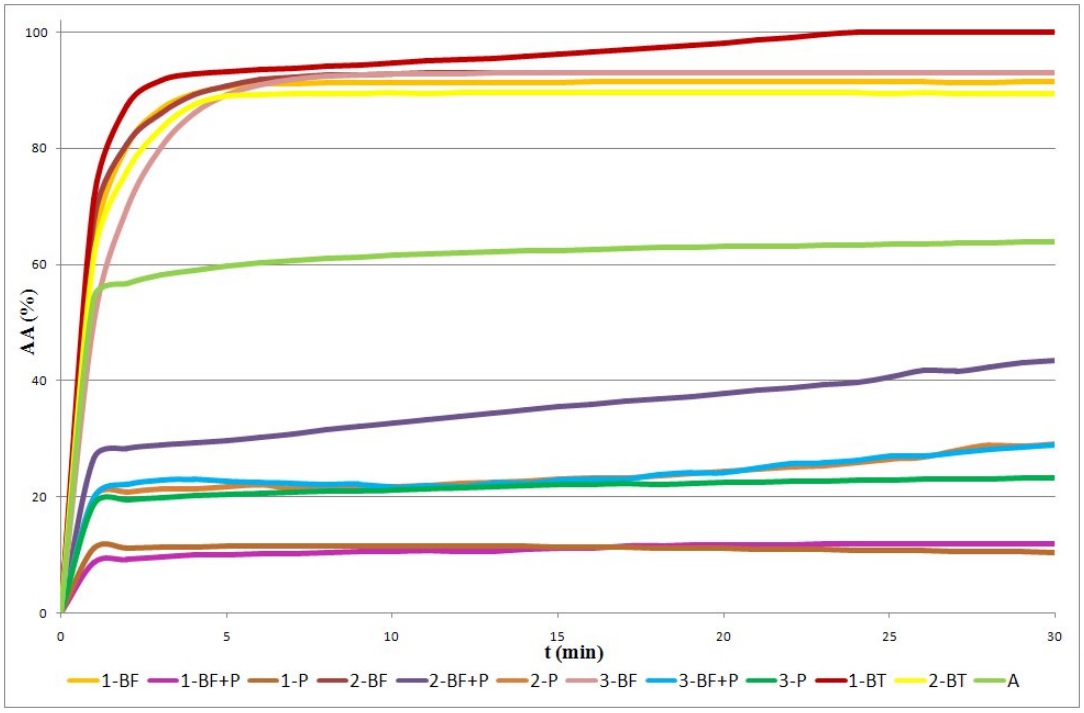


Figure 2: Reaction progress of reduction of stable DPPH radical by Horse chestnut samples.

It is well known that bark of the chestnut are rich in tannin (Barreira *et al*., 2008) and these phenolic compounds might account for the high values obtained, while in the extract of pulp the content of total phenolics was quite low.

Table 1: The total phenolic content and related antioxidant activity.

	Total phenolic		DPPH
Sample	[mg (GAE)/mL]	[mg (EE)/mL]	IC_{50} (mg/mL)
1-BF	178.45 ± 24.36	862.31 ± 117.71	**0.14 ± 0.02**
1-BT	202.71 ± 23.39	979.53 ± 113.01	0.28 ± 0.01
1-P	8.80 ± 0.58	43.41 ± 3.05	12.94 ± 0.64
1-P+BF	12.10 ± 0.17	64.87 ± 0.82	2.09 ± 0.05
2-BF	183.77 ± 20.36	888.00 ± 98.36	0.18 ± 0.01
2-BT	158.01 ± 27.76	763.53 ± 114.80	0.16 ± 0.02
2-P	12.36 ± 0.67	66.15 ± 3.60	4.38 ± 0.28
2-P+BF	14.75 ± 0.81	78.97 ± 4.33	3.87 ± 0.08
3-BF	**216.97 ± 29.37**	**1048.00 ± 141.94**	0.32 ± 0.02
3-P	10.68 ± 1.24	57.24 ± 6.66	7.38 ± 0.59
3-P+BF	14.12 ± 1.79	74.83 ± 8.26	6.26 ± 0.16
Aesculin	-	-	0.82 ± 0.06

(1) Location -1- Sutjeska; 2-Ilidža; 3- Pofalići;
Part of plant; BF- bark of fruit; BT - bark of twigs; P- pulp

DPPH radical scavenging method was used to evaluate free radical scavenging ability of ethanolic extracts of different parts of *A. hyppocastanum*. Figure 2 shows very high rate of DPPH inhibition of all bark samples (90-100%) in concentration of 1 mg/mL. Samples of pulp and bark of fruit mixed with pulp had much lower inhibition of DPPH• (10-40%), in comparison with aesculin as standard which reach the 64% in same concentration. These results are in agreement with antioxidant activity of *A. hippocastanum* from Turkey, where bark extract were rich in phenolic compounds with high correlation to its high antioxidant capacity (Celep *et al*., 2010).

CONCLUSIONS

Antioxidant activity of plant extracts are mainly attributed to the active compounds present in them. Phenolic compounds have been considered as powerful antioxidants. In general, our results showed that *A. hippocastanum* bark extracts were rich in phenolics and they possess a considerable antioxidant potential, although strong correlation is not confirmed.

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Summary/Sažetak

Aesculus hippocastanum L. (divlji kesten) pripada rodu *Aesculus,* najrasprostranjenijem rodu u familiji Hippocastanaceae i raste u zemljama Balkanskog poluostrva. Gotovo svi dijelovi biljke koriste se za tretman različitih bolesti. Sadržaj ukupnih fenola u etanolnim ekstraktima kore mladih grančica i ploda kao i u samom plodu određen je Folin-Ciocalteau metodom, dok je antioksidativna aktivnost određena DPPH metodom. Preliminarna ispitivanja hemijskog sastava vršena su hromatografijom na tankom sloju, a eskulin je detektovan u uzorcima kore grančica.

Rezultati za sadržaj ukupnih fenola izraženi su kao ekvivalenti galne kiseline (mg GAE/mL) i eskulina (mg AE/mL). Među ispitivanim uzorcima najveći sadržaj ukupnih fenola imaju uzorci kore (763.53- 1048.00 mg AE/mL), dok su uzorci ploda imali najmanji sadržaj ovih spojeva, 43.41-66.15 mg AE/mL.

Svi ekstrakti kore pokazuju bolju antioksidativnu aktivnost od eskulina koji je korišten kao standard, dok su uzorci ploda pomiješani sa korom pokazali značajno manju antioksidativnu sposobnost.