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POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITIES IN INFUSION AND DECOCTION EXTRACTS OBTAINED FROM *FRAGARIA VESCA* L. LEAVES

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Abstract

Fragaria vesca L. (wild strawberry) belongs to the Rosaceae family. The leaves and roots from wild strawberry are herbal materials applied in traditional medicine. Fragaria vesca are rich source of biologically active substances like tannins, procyanidins, anthocyanidins, flavonoids and phenolic acids. The aim of this study was to compare the value of phytochemical compounds and antioxidant activities in infusion and decoction obtained from the wild strawberry leaves. The extracts were analyzed regarding their secondary metabolite content (total polyphenols, total flavonoids and total proanthocyanidins) and antioxidant activities (DPPH and CuPRAC methods). The analysis of decoction extracts from the leaves harvested in blooming period revealed the highest level of total polyphenols (46.1 mg GAE/g DW), total flavonoids (4.7 mg QE/ g DW), total proantocyanidines (22.3 mg/ g DW) and antioxidant activities – radical scavenging activity (DPPH – 325.0 mM TE/ g DW) and metal reducing ability (CuPRAC – 1257.9 mM TE/ g DW). The results showed that the water extracts from leaves of Fragaria vesca are appropriate additives for preparation of functional foods and natural cosmetic products with improved biological activity.

Key words*: Fragaria vesca L., phytochemistry, decoction, infusion, antioxidants.*

INTRODUCTION

Fragaria vesca is commonly called as wild strawberries, is a plant that grows naturally throughout the northern hemisphere (Folta and Gardiner, 2009). The leaves, roots and fruits are herbal materials used in traditional medicine. Plants material are collected during the flowering season and prepared as infusion, decoction or tincture. Different extracts from *Fragaria vesca* possess antioxidant, anti-inflammatory, antibacterial, astringentic, antidiarrheic and antidysenteric acrivities (Kiselova et al., 2006; Cheel et al., 2007; Neves et al., 2009; Sarić-Kundalić et al., 2010; Kanodia et al., 2011, Buricova et al., 2011, Borah et al., 2012, Liberal et al., 2014,). Leaves from wild strawberry are rich source of bioactive compounds such as flavonoids, ellagitannins, procyanidines and phenolic acids (Mudnic et al., 2009; Buendia et al., 2010; Buendia et al., 2010, Buricova et al., 2011, Liberal et al., 2014).

Therefore, the aim of this study is to reveal the bioactivity and polyphenols content of water extracts obtained from *Fragaria vesca* leaves collected from different areas.

MATERIALS AND METHODS

Plant material

Aerial parts (leaves) by several random chosen plants of *F. vesca* L., were collected from their natural habitats nearby "Zdravec" hut and "Vruhovruh" hut – Rhodopa mountain all in May and October 2013. The samples were dried in shade at ambient temperature for 7 days, and finely ground by homogenizer. The powder was used for different extraction.

Extraction procedure

Two aqueous extracts (infusion and decoction) were prepared according to Pistón et al., 2014.

Briefly, for decoction preparation, the dried leaves (1 g) were added to 50 mL of hot ultrapure water, than heated, kept in boiled water for 15 min and after that the mixture was removed from the heat, stood for 20 min and filtered through filter paper. Infusion was prepared by adding 50 mL of ultrapure hot water at 95°C to 1 g of dried leaves and the mixture was left to stand for 20 min to be also filtered using filter paper. Both the infusion and decoction extracts were analysed for polyphenol content and antioxidant activity

Total proanthocyanidins assay
Acid butanol was used for assaying proanthocyanidins, according to Porter et al. (1986). Six milliliters of the acid butanol reagent (950 mL of n-butanol with 50 mL concentrated HCl), 0.5 mL aliquot of the fraction, and 0.1 mL of the iron reagent (2 % ferric ammonium sulphate in 2 mol/L HCl) were added to 10 mL screw cap tube and then vortexed. The tube was capped loosely and put in a boiling water bath for 15 min. The absorbance of formed colored complex was read at 550 nm. Condensed tannins were expressed as leucocyanidin equivalent (Hagerman, 2011).

Determination of total flavonoids
Total flavonoids were determined spectrophotometrically by the method described by Kivrak et al., (2009). 0.2 ml of each obtained extract was added to test tubes containing 0.1 ml 10 % aluminium nitrate (Sigma), 0.1 ml 1M potassium acetate (Sigma) and 3.8 ml ethanol (Merck). The reaction time was 40 min at ambient temperature. The absorbance was measured at 415 nm. The results were expressed in mg equivalent of quercetin per g dry weight (DW).

Determination of total polyphenolic compounds
The total phenolic contents were measured using a Folin-Ciocalteu assay. Folin-Ciocalteu reagent (1mL) (Sigma) diluted five times was mixed with 0.2 mL of sample and 0.8 mL 7.5 % Na_2CO_3. The reaction was 20 min at room temperature in darkness. After reaction time, the absorption of sample was recorded at 765 nm against blank sample, developed the same way but without extract. The results were expressed in mg equivalent of gallic acid (GAE) per g dry weight (DW), according to calibration curve; build in range of 0.02 - 0.10 mg gallic acid used as a standard.

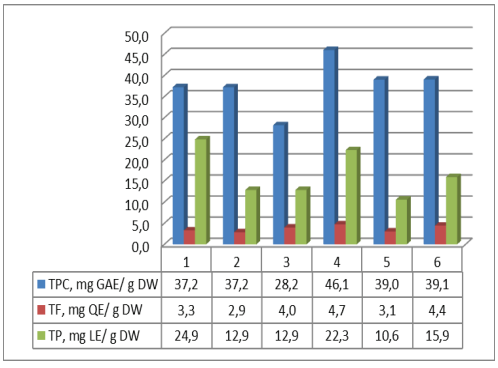
Antioxidant activity (AOA)
DPPH radical scavenging activity: Investigated extract (150 µl) were mixed with 2850 µl freshly prepared DPPH solution (0.1 mM in methanol). The mixtures were incubated for 15 min at 37 °C in darkness and the reduction of absorbance at 517 nm was measured by spectrophotometer. A standard curve was created with Trolox in concentration between 0.005 and 1.0 mM. The results are expressed in mM Trolox® equivalents (TE) per g dry weight (DW).

Cupric ion reducing antioxidant capacity: The reaction was started by mixing of 1 ml $CuCl_2xH_2O$ (10 mM in dd H_2O), 1 ml Neocuproine (7.5 mM in methanol), 1 ml ammonium acetate buffer (0.1 M; pH 7.0), 100 µl of investigated extract and 1 ml dd H_2O. The reaction time was 20 min at 50 °C. After cooling, the absorbance (450 nm) was read against a reagent blank, developed on the same way but the extract was replaced with methanol. A standard curve was created with Trolox. The results are expressed in mM Trolox® (TE) per g DW.

RESULTS AND DISCUSSIONS

Decoction extract presented the highest phenolic content (39 - 46 mg GAE /g DW), followed by the infusion (28-37 mg GAE /g DW) (Figure 1) as these content account for 10.8%, 7.3% and 6.3% of dry weight of each extract, respectively (Figure 2). The phenolic contents obtained in this study is similar to the naturally found in strawberry leaves extracts as report by Mudnic et al., (2009) and Buendia et al., (2010). Many authors detailed description and identified of phenolic compounds from strawberry leaves and other extracts (Buendia et al., 2010, Buricova et al., 2011, Liberal et al., 2014). Through HPLC–MS, GC-MS methods ware described and separated, quantificated of phenolic compounds such as flavonoids (quercetin-3-glucuronide kaempferol-3-glucoside), procyanidines (procyanidin B1, epigallocatechin, catechin, (epi)afzelechin-(epi)catechin), ellagitannins (sanguiin H-6, castalagin,

lambertianin C, galloyl-bis-HHDP-glucoseb and ellagic acid glucosides). In the present study the total amount of investigated polyphenols has been quantified. On this base the total flavonoid and total proanthocyanidins contain in water extracts has been analyzed (Figure 1) The highest content of total proanthocyanidins was obtain from leaves collected in May (blooming period) from natural habitats near "Vruhovruh" hut – 24.9 mg LE/g DW and 22.3 mg LE/g DW infusion and decoction, respectively. Similar results for the concentration of total proanthocyanidins were reported by Buendia et al., (2010) and Ivanov et al (2014). Population from "Vruhovruh" hut (24.9 mg LE/g DW) accumulated twice times more proanthocyanidins in leaves than population growth in blooming period nearby "Zdravec" hut (12.9 mg LE/g DW). High concentration of flavonoids was detected in infusion and decoction extracts obtained from natural population from "Zdravec" hut collected in October – 4.0 and 4.4 mg QE/ g DW (Figure 1).



	1	2	3	4	5	6
TPC, mg GAE/ g DW	37,2	37,2	28,2	46,1	39,0	39,1
TF, mg QE/ g DW	3,3	2,9	4,0	4,7	3,1	4,4
TP, mg LE/ g DW	24,9	12,9	12,9	22,3	10,6	15,9

Figure 1. The total polyphenolics, flavonoids and proanthocyanidins in different water extracts from *Fracaria vesca* L. leaves (from 1 to 3, infusion, and from 4 to 6 decoction); 1 and 4 from natural habitats near "Vruhovruh" hut collect in May; 2 and 5 from natural habitats near "Zdravec" hut collect in May; 3 and 6 from natural habitats near "Zdravec" hut collect in October.

In our study, we decided to evaluate antioxidant activities of water extracts of *F. vesca* by application of two methods, based on mixed hydrogen atom transfer (HAT) mechanisms (DPPH) and a method, based only on and single electron transfer SET mechanism (CUPRAC). To evaluate antioxidant activities of investigated water extracts, their abilities to scavenge DPPH radicals, as well as their power to reduce cupric (CUPRAC) ions were investigated (Figure 3). Decoction extract from Vruhovruh's hut population was the extract with the highest antioxidant activity in all used extracts (325.0 and 1257.9 mM TE/g DW for DPPH and CUPRAC methods, respectively). The extract obtained from population grown neat "Zdravec" hut collected from October was the extract with the lowest activity (164.8 and 487.5 mM TE/g DW for DPPH, and CUPRAC methods, respectively) (Figure 3).

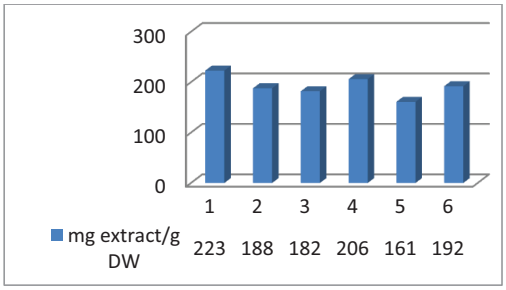
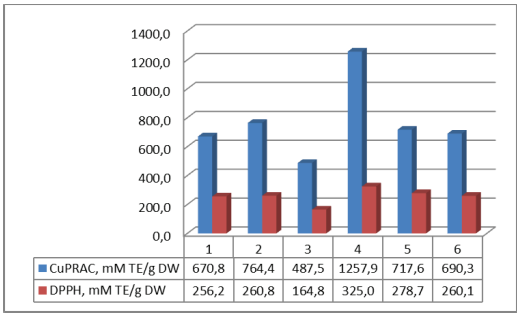


Figure 2. Total extract in different water extracts from leaves of *Fracaria vesca* L. (from 1 to 3, infusion, and from 4 to 6 decoction); 1 and 4 from natural habitats near "Vruhovruh" hut collect in May; 2 and 5 from natural habitats near "Zdravec" hut collect in May; 3 and 6 from natural habitats near hut "Zdravec" collect in October.



	1	2	3	4	5	6
CuPRAC, mM TE/g DW	670,8	764,4	487,5	1257,9	717,6	690,3
DPPH, mM TE/g DW	256,2	260,8	164,8	325,0	278,7	260,1

Figure 3 Antioxidant activity obtain from different water extracts from leaves of *Fracaria vesca* L. (from 1 to 3, infusion, and from 4 to 6 decoction); 1 and 4 from natural habitats near "Vruhovruh" hut collect in May; 2 and 5 from natural habitats near "Zdravec" hut collect in May; 3 and 6 from natural habitats near hut "Zdravec" collect in October.

The results showed the correlation between total polyphenolics in investigated extracts and their antioxidant activities 89 % and 98 % for CuPRAC and DPPH methods, respectively. Correlation between total proanthocyanidins and antioxidants activity was 45%. These results suggest that antioxidant activities were

obtained mainly from hydrolysable tannins, which were included in the total polyphenolics analysed with Folin-Ciocalteu reagent.

CONCLUSIONS

The current report detailed information for phenolic content and antioxidant activity of edible strawberry *Fragaria vesca* L leaves grown in Bulgaria. The antioxidant potential of aqueous extracts of *Fragaria vesca* L leaves, shown positively correlation with total polyphenolic contents, are important source of proanthocyanidins and tannins with potential application as radical scavengers and metal reducing activity. Therefore, this complex of biologically active substance offers many future applications in field of herbal medicine and nutrition for production of healthy food with well-pronounced healthy effect.

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