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Characterization of Rose Hip (*Rosa canina* L.) Fruits Extracts and Evaluation of Their *in vitro* Antioxidant Activity

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Abstract

The aim of the current research was to determine the ascorbic acid content, total tannins and total phenolic content in three extracts (water, 50 % v/v ethanol, 70 % (v/v) ethanol) obtained from dry wild growing rose hip fruits and to evaluate their antioxidant potential by four reliable methods: DPPH, ABTS, FRAP, CUPRAC. The highest ascorbic acid content was observed in 70 % (v/v) ethanol extract – 2404 mg/100 g dw, while the total tannins dominated in the water extracts – 3.86 g/100 g dw. The 50 % ethanol extracts of rose hip demonstrated the highest total phenolic content - 6.9 g GAE /100 g and the antioxidant activity: DPPH assay – 295.0 ± 1.0; ABTS – 368.4±3.0; FRAP – 390.1±4.8; CUPRAC 1358.2±14.8 mM TE/g dw, respectively. The present study showed that the investigated wild growing *Rosa canina* L. from Bulgaria was evaluated as a rich source of antioxidants and revealed their potential application as food and herbal cosmetic preparations.

Keywords: *Rosa canina* L., antioxidant activity, phenolic content

1. Introduction

Wild growing rose hip fruits (*Rosa canina* L.) are widespread plant in Bulgaria with great importance in herbal medicine. The *Rosa canina* fruits are a valuable source for food and pharmaceutical industry. They contain a wide variety of biologically and physiologically active ingredients, such as vitamins (C, B, P, PP, E, K), flavonoids, carotenes, carbohydrates (mono- and oligosaccharides), organic acids (tartaric, citric), trace elements and others [1, 2]. These compounds play an important role in maintaining fruit quality and determining nutritive value. Rose hips are also well known to have the highest vitamin C content (300–4000 mg/100 g) among fruits and vegetables [3]. In Bulgaria rose hip fruits are typically consumed as infusion. It was found that juice and aqueous extracts from rose hip possessed exceptional antioxidant activity [3]. This makes them suitable for use both in the fresh or dry state, or in the form of extracts in food products and cosmetics [1, 2]. The quality of natural extracts and antioxidant properties depends not only on the nature of the plant source, geographical origin, weather conditions, time of harvesting and storage, but also on the method of extraction and the used solvent [4, 5].

According to some authors, the higher values of antioxidant activity (AOA) of rose hip extract is due to synergism between polysaccharides and organic acids (gallic, cinnamic, ellagic), with phenolic antioxidants: flavonoids (rutin, kaempferol, quercetin) [1, 2].

The most common antioxidants contained in fruits are ascorbic acid, carotenoids and polyphenol substances with proven antioxidant capacity [2]. Until now in our country, the wild-growing rose hip fruits were scarcely investigated about some constituents and health benefits were not completely evaluated. Therefore, the aim of the current study was to prepare and characterized the extracts from rose hip (*Rosa canina* L.) fruits and to evaluate their *in vitro* antioxidant potential and polyphenolic content of water and hydro-ethanolic extracts obtained from wild growing Bulgarian rose hip fruits.

2. Materials and methods**2.1 Chemicals**

All chemicals used in this study were of analytical grade. Folin–Ciocalteu, gallic acid, potassium ferricyanide, sodium carbonate, ferric chloride, methanol (HPLC grade), etc. were purchased from Merck (Germany) and Sigma-Aldrich (USA).

2.2 Collection of rose hip. Wild growing rose hip fruits (*Rosa canina* L.) were collected from the area of the town Kyustendil (Kyustendil Province, Southwest Bulgaria) during 2012, after full ripening, were used in the current research.

2.3. Preparation of extracts

Before extraction the rose hip fruits were washed with tap water and then air-dried. After that the dried fruits were ground in a laboratory homogenized to coarse powder with particle sizes 2 – 4 mm. The extraction process was performed with three solvents as follows: distilled water, 50 % (v/v) ethanol and 70 % (v/v) ethanol with maceration for one hour. The solid to liquid ratio were 1:20. The extraction temperature was 20 °C. The obtained extracts were filtered through filter paper and then were analyzed for the ascorbic acid, total tannins, total phenolic content and antioxidant activity.

2.4. Ascorbic acid content

The amount of ascorbic acid of the rose hips was determined according to the methods of AOAC [6].

2.5. Total tannins

The amount of total tannins in *Rosa canina* L. extracts were determined by titration methods with 0.1 N KMnO₄ with indigo carmine as indicator as previously described by Petel *et al* [7].

2.6. Total phenolic contents

Total phenolic contents were measured using a Folin-Ciocalteu reagent with some modifications. Briefly, 1 mL Folin-Ciocalteu reagent diluted five times was mixed with 0.2 mL sample and 0.8 mL 7.5% Na₂CO₃. The reaction was performed for 20 min at room temperature in darkness. Then the absorbance was measured at 765 nm against blank. The results were expressed as 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 [8].

2.7. Antioxidant activity (AOA).

The antioxidant activities of the rose hip extracts were evaluated by four methods: DPPH (1,1-diphenyl-2-picrylhydrazyl) radical and ABTS⁺ radical scavenging ability assay based on mixed hydrogen atom transfer (HAT) and FRAP (ferric reducing antioxidant power) CUPRAC assay both based only on single electron transfer mechanism.

2.7.1. The DPPH radical-scavenging ability.

Each extract of rose hip fruits (0.15 mL) was mixed with 2.85 mL freshly prepared 0.1 mM solution of DPPH in methanol. The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance at 517 nm was measured by spectrophotometer and % inhibition was calculated [9].

2.7.2. ABTS assay

ABTS radical was generated by mixing aliquot parts of water solution of 7.0 mM 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2.45 mM potassium persulfate. The reaction was performed for 16 h at ambient temperature in darkness and the generated ABTS radical was stable for several days. Before analyses, 2.0 mL of generated ABTS⁺ solution was diluted with methanol at proportions 1:30 (v/v), so the obtained final absorbance of the working solution was about 1.0 ÷ 1.1 at 734 nm. For the assay, 2.85 mL of this ABTS⁺ solution was mixed with 0.15 mL of obtained extracts. After 15 min at 37 °C in darkness the absorbance was measured at 734 nm against methanol [10].

2.7.3. Ferric reducing antioxidant power (FRAP) assay.

The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM FeCl₃ ×

6H₂O in d. H₂O. The reaction was started by mixing 3.0 mL FRAP reagent with 0.1 mL of investigated extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with methanol [8].

2.7.4. CUPRAC assay

The reaction was started by mixing of 1.0 mL CuCl₂ × 2H₂O, 1.0 ml 7.5 mM Neocuproine (Sigma) in methanol, 1.0 ml 0.1 M ammonium acetate buffer (pH 7.0), 0.1 mL of investigated extract and 1.0 ml d. H₂O. Blank sample was prepared with methanol. The reaction time was 20 min at 50 °C in darkness. After cooling the absorbance was measured at 450 nm against blank [10].

All results from the determination of antioxidant activity were performed in triplicates and expressed as mM Trolox equivalents (mM TE) by dry weight.

The presented results were average from two independent experiments carried out in triplicates. The data were expressed as mean ± SD and statistically analyzed using MS Excel software.

3. Results and discussion

3.1. The total ascorbic acid and total tannins content

The results for the total ascorbic acid and total tannins content in water, 50% (v/v) and 70% (v/v) ethanol extracts obtained from wild growing *Rosa canina* L. were presented in Table 1. Not significant difference was observed in the ascorbic acid content in three rose hip fruits extracts. Its content varied in narrow concentration range from 2189.7 mg/100 g dw for 50% (v/v) ethanol extract to 2404.0 mg/100 g dw for 70% dw (v/v) ethanol extract, respectively.

Table 1: Ascorbic acid content and total tannins in different extracts of *Rosa canina* L. dry fruits

Extraction solvent	Ascorbic acid content, mg/100 g	Total tannins, g/100 g
Distilled water	2340.2 ± 0.01	3.86 ± 0.02
50% (v/v) ethanol	2189.7 ± 0.02	3.76 ± 0.03
70% (v/v) ethanol	2404.0 ± 0.01	1.46 ± 0.02

It was established that the content of vitamin C in rose hip fruits varied and depended on numerous factors, including geographic region, species, variety, cultivation, climate, weather conditions, ripeness, region and storage time [1, 4]. The ascorbic acid content in the investigated Bulgarian wild-growing rose hip fruits was also considerably higher than the another reports for Tunisian [4], Turkish [11] and some commercial Bulgarian rose hip fruits [2]. However, our results were in agreement with previous reports of Demir *et al* [3] and Ercisli [12] - 2365 mg/100 g dry basis for rose (*Rosa canina* L.) fruits and grown wild in Turkey. It was found that the total ascorbic acid contents varied in different solvent applied in the extraction procedure. Similar to Ghazghazi *et al.* [4] we also obtained the highest values of ascorbic acid was found in ethanol extracts in our case - 70 % ethanol extract.

The total tannins content varied from 1.46 g/100 g to 3.86 g/100 g dw, and the highest value was observed for water extracts of *Rosa canina* L. fruits - 3.86/100 g dw. The correlation between the ascorbic acid content and the total tannins in water and two hydro-ethanolic extracts was established. The most proper solvent for extraction of between the ascorbic acid content and the total tannins was 70 % ethanol. The results (Fig. 1) showed that, the total tannins

content significantly depended on the used solvent during the extraction. However, the ascorbic acid content did not

influenced by the solvent type. The increase of water content in solvent system led to increase in total tannins values.

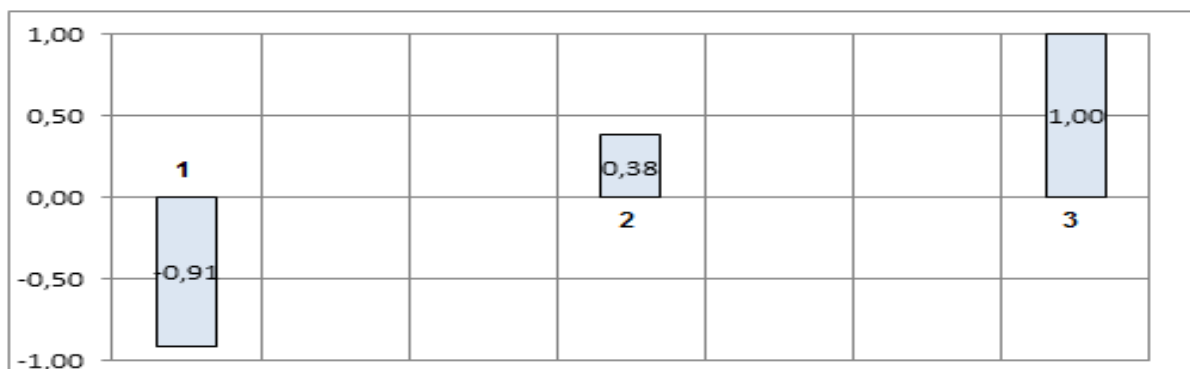


Fig 1: Correlation rate between the total ascorbic acid and tannins content, where 1. water extract; 2. 50 % v/v ethanol extract; 3. 70 % v/v ethanol extracts from *Rosa canina* L. fruits

3.2. Total phenolic content

The results for the total phenolic content in different extracts from rose hip were presented in Fig 2. Their quantity varied in the range of 55.4 to 69.4 mg GAE/g dw. The highest total phenolic content was established in 50 % (v/v) ethanol extracts from *Rosa canina* L. fruits– 69.4 mg GAE/g dw, while the lowest value was found in the water extract – 55.4 mg GAE/g dw, respectively. Therefore, from the results obtained for all investigated extracts, the most proper solvent for extraction of substances with antioxidant potential was 50 % ethanol. The results from our study were similar to the previous reports for total phenolic content in *Rosa canina* L. fruits from Turkish origin [11, 12].

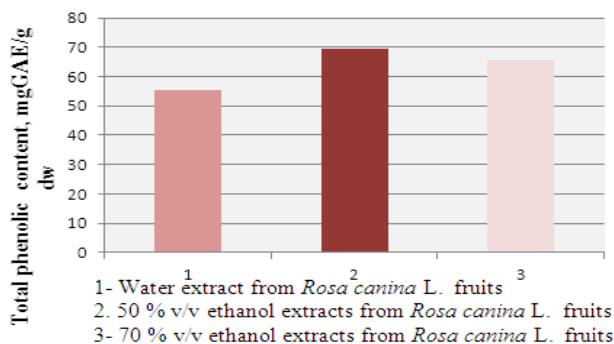


Fig 2: Total phenolic content in *Rosa canina* L. extracts mg GAE/g dw

3.3. Antioxidant activity of rose hip fruits extracts

A great variety of methods based on different mechanism for evaluation of *in vitro* antioxidant activity of herbal extracts exists. The results for antioxidant activity of the extracts from rose hip (*Rosa canina* L.) fruits was presented in Table 2.

Table 2: Antioxidant activity of rose hip fruits extracts

Extraction solvent	DPPH	ABTS	FRAP	CuPRAC
	mM TE/g dw			
Distilled water	211.5±5.5	313.1±4.5	309.5±3.0	1057.1±16.2
50% (v/v) ethanol	295.0±1.0	368.4±3.0	390.1±4.8	1358.2±14.8
70%(v/v) ethanol	286.4±2.9	358.2±5.4	357.2±6.3	1127.9±14.5

From the obtained values, 50 % ethanol extract was established to be with the highest antioxidant potential

evaluated by four assays, as CuPRAC showed the highest values – 1358.2 mM TE/g. Our results for DPPH assay were higher than the report of Mihaylova *et al* [2] and Demir *et al*. [3] for the antioxidant potential of *Rosa canina* L. fruits.

The correlation between the antioxidant activity evaluated by four methods and the total phenolic content was established (Table 3).

Table 3: Correlation coefficient (r^2) between antioxidant activity and total phenolic content (TPC)

TPC Solvents	DPPH	ABTS	FRAP	CuPRAC
Distilled water	0.2848	0.5204	0.8532	0.8564
50% (v/v) ethanol	0.9965	0.9975	0.9948	0.9995
70%(v/v) ethanol	0.4586	0.5992	0.8684	0.8756

The positive linear correlation between the total phenolic content and the antioxidant activity evaluated by DPPH, ABTS, FRAP and CuPRAC assays was observed. The r^2 values varied from 0.2848 to 0.9995 that showed significant importance of presence of the total phenols in analyzed extracts from *Rosa canina* L. fruits. The antioxidant methods DPPH radical and ABTS+ radical scavenging ability based on mixed hydrogen atom transfer (HAT) showed low correlation with the total phenol content for water and 70 % (v/v) ethanol extracts. The high radical scavenging ability established by these assays probably could be explained with the high content of ascorbic acid and tannins (Table 1). The antioxidant activity of 50 % ethanol extracts was mainly due to the high level of total phenolic content. CUPRAC assay based only on single electron transfer mechanism were considered as the more relevant method for evaluation of antioxidant potential of extracts, because the reaction conditions is close to the pH of human blood serum.

4. Conclusion

The water and hydro-ethanolic extracts obtained from wild growing rose hip fruits were evaluated as a rich source of biological active substance and demonstrated well-pronounced antioxidant activity. The significant high correlation ($r^2 > 0.90$) between the total phenolic content and the antioxidant activity in 50 and 70% (v/v) rose hip extracts was established. The obtained results revealed the possible application of investigated extracts as a natural source of antioxidant compounds with health benefits. The incorporation of rose hip

fruits extracts will be perspective in design and formulation of food and cosmetic products with improved added value.

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