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# ANTIOXIDANT ACTIVITY AND BIOACTIVE COMPOUNDS OF *ROSA CANINA* L. HERBAL PREPARATIONS

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***Abstract***

*The aim of the present research was to make a comparison between two Bulgarian commercial forms of Rosa canina L. for herbal preparations in terms of the possible benefits by oral intake. The plant samples were investigated for their antioxidant activity and the bioactive substances. The total phenolic content of the extracts was evaluated as well. Four reliable methods (DPPH, ABTS, FRAP and CUPRAC assays) for antioxidant activity assessment were applied. The highest results were recorded by the FRAP assay.The plant Rosa canina is known as wealthy source of carotenoids and organic acids, therefore fat soluble β-carotene, lycopene and lutein in addition to water soluble malic, citric, fumaric and ascorbic acids were evaluated by HPLC-methods. According the conducted assays the tested samples have similar composition. Lutein concentration in both tested extracts was calculated as 6.9 μg/g DW. Lycopene and β-carotene were determined to be 13.91 and 12.18 μg/g DW and 27.14 and 22.83 μg/g DW, respectively. HPLC determination of organic acids showed that the amount of citric acid in the extracts was 7343 and 6583 μg/g DW and fumaric acid content in both samples was 30 μg/g DW. The size of the plant particles used for the extraction seems to contribute significantly to the exhibited activity.*

***Key words****: herbal tea; Rosa canina; fruits; antioxidant activity; bioactive compounds.*

## INTRODUCTION

Currently there is considerable interest in new natural antioxidants to replace the synthetic ones that are used in foods and therapeutic regimens.

*Rosa canina* L. (the dog rose) is a shrub of the *Rosaceae* family, native to Europe, western Asia and north-eastern Africa. Fruits (hips) have been used in the traditional prevention and therapy of common cold and other infections, as a diuretic agent and for the treatment of various inflammatory diseases for a long time. Clinical efficacy has been demonstrated only for osteoarthritis (Chrubasik et al., 2006; Christensen et al., 2008; Chrubasik et al., 2008). Based on the results of other authors Kiliçgun and Dehen (2009) stated that the hips display an anti-inflammatory, antioxidant and anti-mutagen effect. Recently the potential of nutritional and therapeutic benefits among natural antioxidants was revealed based on traditional knowledge and western science (Aresenescu, 2008). The constituents of dog rose fruit (hips) are endowed with vitaminisant, astringent, colagogue, choleretic, diuretic, antidiarrhoea, antioxidant properties, etc. (Yi et al., 2007). In addition, Orhan et al. (2009) reported that the rose hips also have antidiabetic properties.

Several authors reported that the nutritive and therapeutical value of the mature dog rose fruit (*Cynosbati* fructus) is due to their content of sugars, organic acids, pectins, flavonoids, tannins, carotenoids (β-carotene, licopene, and isomeres of rubixanthin), vitamins (especially vitamin C, but also vitamins B1, B2, K, PP, D, and E), macro- and microelements etc. (Pârvu, 2000; Demir şi Ozcan, 2001; Tiță, 2003;

Stănescu et al., 2004; Arsenescu et al., 2008; Orhan et al., 2009). The dog rose seeds contain oil and minerals; the fatty acids within the dog rose oil are mainly represented by the linoleic, oleic, linolenic, palmitic, stearic, and arachidonic acid, (Ozcan, 2002). The varied content of the fruit (hips) confers the next properties: antiscorbutic, anti-inflammatory, even anti-mutagene; it also increases the biosynthesis of colagene, stimulates the immune system, improves the body resistance to sustained effort, (Pârvu, 2000; Tiță, 2003; Kiliçgun and Dehen, 2009; Orhan et al., 2009). Some properties of the dog rose hips are attributed to some hypothetical compounds of silicium. At the same time, *Rosa canina* L. is useful to prevent soil erosion, serves as a stock for roses, etc. (Arsenescu, 2008).

The *Rosa canina* L. fruits have constituted an important source of food and medicine for many cultures. Common food preparations using rose hips include juice, wine, tea, jelly, jam, as well as mixed with dried salmon eggs (Moerman, 2002).

The aim of the present study was to compare two typical *Rosa canina* commercial products used in the everyday life of people. Based on the results a most suitable tea form could be recommended for an oral intake.

## MATERIALS AND METHODS

### Plant material

Two different commercial available and widely used in everyday life *Rosa canina* L. forms were obtained from a local pharmacy (Plovdiv, Bulgaria). The sample A – rosehip tea in ready to use paper bags and the sample B - Rosa whole fruits, which were blended and stored at ambient temperature in the dark, until use.

### Preparation of the plant extract

For the purposes of the present study different extraction procedures were applied.

In order to evaluate the total phenolic content and the antioxidant potential of the both plant samples two extraction techniques with water were conducted as described. Water was chosen as solvent based on its simple and traditional use.

- *infusion* – extracts were obtained by allowing 2 g of the plant material to remain suspended in the boiled water for 5 min and then the solution was filtered;
- *decoction* – extracts were obtained by boiling of the 2 g plant material for 30 min with 40 ml of water; The resulting solution was then filtered.

In order to evaluate chemical composition of the two investigated rose hips forms in terms of organic acids and carotenes contents, the samples were subjected to extraction as follow:

*Extaction of organic acids* - 0.1 g plant material was extracted with 1ml of 3 % meta - phosphoric acid ($HPO_3$) as previously described by Georgieva et al. (2013a).

*Extraction of carotenes* **-** 2 ml of methanol was added to 0.1 g plant paterial (20:1) followed by addition of 5 ml of carbon tetrachloride and methanol mixture in ratio 3:1, the solution should contain 0.5 % BHT. The extraction procedure was carried out according Georgieva et al. (2013b).

### Determination of total phenolics (TPC)

A modified Kujala et al. (2000) method with Folin – Ciocalteu`s reagent was used for the determination of the total polyphenolic content (TPC). Gallic acid was employed as a calibration standard and the results were expressed as mg gallic acid equivalents (mg GAE) per gram of plant dry weight (DW).

### Determination of antioxidant activity (AOA)

*$DPPH^{\bullet}$ radical scavenging assay*

Antioxidant activity was described as having activity against the stable form of the synthetic product $DPPH^{\bullet}$ (2,2-diphenyl-1-picrylhydrazil) by the method of Brand-Williams et al. (1995) with slight modifications. A freshly prepared $4.10^{-4}$ M solution of $DPPH^{\bullet}$ (in methanol) was mixed with the sample in a ratio of 2:0.5. The unit of Trolox equivalent antioxidant capacity (TEAC) defined the concentration of Trolox having equivalent antioxidant activity expressed as μM TE/g DW.

*$ABTS^{\bullet+}$ radical scavenging assay*

The radicals scavenging activity of the ultrasound extract against radical cation ($ABTS^{\bullet+}$) was estimated according to a previously reported procedure with some modifications (Re et al., 1999). $ABTS^{\bullet+}$ was produced by reacting 7 mM of $ABTS^{\bullet+}$ solution with 2.45 mM of potassium persulphate, and the mixture was kept in the dark at room temperature for 12-16 h. At the moment of use,

the $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.7 ± 0.02 at 734 nm and equilibrated at 30 °C. 1 ml of $ABTS^{\bullet+}$ solution was added to each sample (0.01 ml) was vigorously mixed. After reacting at 30 °C temperature for 6 min, the absorbance at 734 nm was measured. The TEAC value was defined as the concentration of Trolox having equivalent antioxidant activity expressed as μM TE per gram dry weight (μM TE/g DW).

*Ferric-reducing antioxidant power (FRAP) assay*

The FRAP assay was carried out according to the procedure of Benzie and Strain (1999) with slight modification. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe (II)-tripyridyltriazine compound from colorless oxidized Fe (III) form by the action of electron donating antioxidants. Briefly, the FRAP reagent was prepared from 300 mM acetate buffer (pH 3.6), 10 mM TPTZ solution in 40 mM HCl, and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. 150 μl of plant extracts were allowed to react with 2850 μl of the FRAP reagent solution for 4 min at 37 °C and the absorbance of the reaction mixture was recorded at 593 nm. The results were expressed as μM TE/g DW.

*CUPRAC assay*

The CUPRAC assay was carried out according to the procedure of Ak and Gülcin (2008). To a test tube were added 1 ml of $CuCl_2$ solution ($1.0\times10^{-2}$ M), 1 ml of neocuproine methanolic solution ($7.5\times10^{-3}$ M), and 1 ml $NH_4Ac$ buffer solution (pH 7.0), and mixed; 0.1 ml of herbal extract (sample) followed by 1 ml of water were added (total volume = 4.1 ml), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of herbal extracts was measured as μM TE/g DW.

**Determination of organic acids and carotenes**

Analyses were performed on HPLC system Waters 2487 using dual λ absorbance detector and Waters 1525 binary pump (Waters, Milford, USA).

*Organic acids*

Chromatographic separation was accomplished with Discovery® SH C18 column (25 x 4.6 mm) RP (5 μm) (Supelco), UV detection at 244 nm and 210 nm, column temperature 30 $^0$C and sample volume injection 20 μl. For elution of the sample 6.0 mM aqueous solution of phosphoric acid (pH = 2.1) was used. The applied flow rate was as reported by Georgieva et al. (2013a).

*Carotenes*

Chromatographic separation was accomplished with Symmetry® C18 column (5μm, 15 cm x 4,6 mm), UV detection at 270 nm and 290 nm, column temperature 30 $^0$C and sample volume injection 20 μl. Elution was performed by gradient system of mobile phase A - methanol: acetonitrile in a ratio of 8:2, and mobile phase B - MTBE (methyl tert-butyl ether). HPLC analysis was conducted with a flow rate previously described by Georgieva et al. (2013b).

**Statistical analysis**

All measurements were carried out in triplicates. The results were expressed as mean ± SD using MS-Excel software.

## RESULTS AND DISCUSSIONS

**Total polyphenolic content**

The total phenolic content was determined using Folin-Ciocalteu method, reported as gallic acid equivalents by reference to a standard curve. The total phenolics in the samples ranged from 12.07 ± 0.17 to 37.17 ± 0.17 mg GAE/g DW (Table 1). The values for the good grounded tea bags in commercial form seemed to be with better phenolic content, based probably of the relatively equal plant sample particles. However, the decoction technique show better extractability compared to the infusion. The TPC for samples A and B after decoction were 37.17 ± 0.17 and 25.64 ± 5.06 mg GAE/g DW, respectively.

Dietary antioxidant capacity is related to the total polyphenol content of fruits and vegetables (Hanson et al., 2004; Leccese et al., 2007; Kubola and Siriamornpun, 2008; Beltran et al., 2009). Polyphenols clearly improve the status of different oxidative stress biomarkers

(Williamson and Manach, 2005). In this respect, it is well understood that some polyphenols, administered as supplements with food, have the ability to improve health status, and this is indicated by several biomarkers of cardiovascular risk (Keen et al., 2005).

**Antioxidant activity**

The results from the DPPH, ABTS, FRAP and CUPRAC assays are presented in Table 1. The values between the different methods varied widely. The authors therefore strongly suggested that, when analyzing the antioxidant activity of samples, it is better to use at least two methods due to the differences between the test systems (Ou et al., 2002).

The highest results were recordered by the FRAP assay (from 344.85 ± 7.25 to 771.86 ± 5.25 μM TE/g DW).

It has to be noted that the values of all conducted methods were in favor of the both decoction extracts. This statement is in agreement with the total phenol assay results.

Table 1. Total phenol content (mg GAE/g DW) and *in vitro* antioxidant activity (μM TE/g DW) of *Rosa canina* water extracts

| Samples/ Analyses | TPC | DPPH | ABTS | FRAP | CUPRAC |
|---|---|---|---|---|---|
| Sample A infusion | 18.93 ± 0.34 | 3.02 ± 0.02 | 213.38 ± 0.54 | 367.35 ± 3.23 | 115.43 ± 4.59 |
| Sample A decoction | 37.17 ± 0.17 | 6.32 ± 0.05 | 518.78 ± 0.78 | 771.86 ± 5.25 | 514.25 ± 7.19 |
| Sample B infusion | 12.07 ± 0.17 | 2.66 ± 0.03 | 283.56 ± 4.18 | 344.85 ± 7.25 | 93.15 ± 17.14 |
| Sample B decoction | 25.64 ± 5.06 | 3.66 ± 0.03 | 370.67 ± 8.34 | 571.15 ± 5.48 | 125.09 ± 20.35 |

Among the investigated extracts the decoction extract of sample A showed the higher CUPRAC value – 514.25 ± 7.19 μM TE/g DW, while the infusion of sample B the lowest - 93.15 ± 17.14 μM TE/g DW (Table 1).

The results of the antioxidant potential of the investigated samples by DPPH and ABTS assays correspond well to the already mentioned results obtained to the other methods. The both decoctions were with the highest antioxidant activity.

Noticeable the correlation among all results is high. This confirmed the better effectiveness of extraction accomplished by the decoction technique. This result confirmed the two investigated plant samples as a natural source of antioxidants. The investigated ready to use rose hip paper tea bags (sample A) revealed as more potent according all performed assays.

**HPLC**

The established amounts of the organic acids and carotenes in the investigated extracts of *R. canina* were presented in Table 2. The content of citric and fumaric acids in tested two samples was demonstrated in contrary to traces of both malic and ascorbic acids. On the other hand, Bozan et al. (1998) reported citric and ascorbic acid contents in growing in the Central Asian region, hips and described citric acid as the main organic acid in *R. canina* fruits. Pereira and co-workers (2013) investigated

Table 2. Chemical composition of *Rosa canina* extracts (μg/g DW)

| Samples | Organic acids | | | | Carotenes | | |
|---|---|---|---|---|---|---|---|
| | Malic acid | Citric acid | Ascorbic acid | Fumaric acid | Lutein | Lycopene | β-carotene |
| Sample A | Trace | 7343±24.5 | Trace | 31.25±2.5 | 6.9±0.2 | 13.91±0.7 | 27.14±2.1 |
| Sample B | Trace | 6583±20.3 | Trace | 27.8±1.3 | 6.89±0.1 | 12.18±0.1 | 22.83±3.4 |

malic, citric, ascorbic and fumaric acid content in several fruits including *R. canina* and confirmed their presence in the sample. The detected amounts of ascorbic and malic acids in the present study were outside the sensitivity of the applied HPLC assay which maybe due to

the sample preparation and probable losses of the substances.

In our research, we investigated and confirmed the presence of lutein, lycopene and β-carotene. The lutein concentration was established to be 6.9 μg/g DW for both extracts.

In general, the demonstrated results were relatively similar especially concerning lutein and lycopene contents. However, in sample A the amounts of all detected chemical components were higher compared to sample B.

Although additional research work is required in order to evaluate all potential activities of the investigated plant samples and the complete chemical composition, Rose hips could be considered as a functional food due to the reported in the literature health effects. In their review, Fan at al. (2014) reported the functional, medical, and physiological properties of *R. canina* as they confirmed the presence of lutein, lycopene and β-carotene.

## CONCLUSIONS

The present work investigated the potential beneficial effect of the commercial varieties of *Rosa canina* used in daily life for tea preparation. The evaluated antioxidant activity and total phenolic content of two water extracts revealed the capacity in favour of the rose hip sample, which was more homogeneously grounded.

The chemical composition of the tested samples show similar results in terms of several organic acids and carotenes. The predominat compounds were established to be citric acid and β-carotene. In general, sample A showed better results concerning the tested chemical compounds in accordance with the other conducted assays.

Based on the results the intake of *Rosa canina* extracts can be recommended as antioxidant supplement in addition to the other known positive effects.

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