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ANTIOXIDATIVE POTENTIAL OF EDIBLE WILD BULGARIAN FRUITS

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ABSTRACT. Aqueous and aqueous-methanolic extracts from six Bulgarian wild edible fruits have been studied for their antioxidant activity and polyphenol content. The antioxidant activity was measured by ABTS cation radical decolorization assay and presented as Uric Acid Equivalents (UAE) per gram dry weight. The content of total phenolics in the extracts was determined using Folin-Ciocalteu reagent and calculated as Quercetin Equivalents (QE) per gram dry weight. The fruits from *Sambucus ebulus* showed the highest antioxidant activity of their aqueous (1005.38 ± 18.51 µmol UAE/g) and aqueous-methanolic extracts (779.65 ± 51.54 µmol UAE/g). In both types of extracts the antioxidant activity decreased as following: *Sambucus ebulus* > *Crataegus monogyna* > *Rosa canina* > *Berberis vulgaris* > *Vaccinium myrtillus* > *Prunus spinosa.* The fruits with highest polyphenol content were *S ebulus* fruits − 73.73 ± 0.57 mg QE/g in aqueous extracts and 68.27 ± 1.93 mg QE/g in aqueous-methanolic extracts. A high degree of positive correlation was established between the polyphenol content and the antioxidant activity. The correlation coefficients were R = 0.961 for aqueous and R = 0.958 for aqueous-methanolic extracts. The excellent positive correlations indicate that polyphenols are the main factor contributing to the antioxidant potential of the fruits studied.

KEY WORDS: wild fruits, extracts, antioxidant activity, polyphenols

INTRODUCTION

Reactive oxygen species from both endogenous and exogenous sources are involved in the ethiology of diverse human diseases, such as coronary artery disease, stroke, rheumatoid arthritis, and cancer. Diets rich in fruits and vegetables are associated with reduced risk for these pathologies, and protection has often been attributed to antioxidants in these foods. Consumption of controlled diets rich in fruits and vegetables increases significantly the antioxidant capacity of plasma (Cao et al., 1998). Vitamins such as vitamin C, vitamin E, and β-carotene are some of the plant-derived antioxidants. Previous studies have established that, in general, more than 80% of the total antioxidant capacity in fruits and vegetables comes from ingredients other than vitamin C, indicating the presence of another group of potentially important antioxidants in these foods (Cao et al.,1996, Wang et al., 1996)

MATERIAL AND METHODS

Plants

We chose to study dried fruits from six wild plant species - *Crataegus monogyna* Jacq. (hawthorn), *Berberis vulgaris* L. (common barberry), *Prunus spinosa* L. (blackthorn), *Rosa canina* L. (dog rose), *Sambucus ebulus* L. (dwarf elder) and *Vaccinium myrtillus* L. (bilberry). The fruits were selected based on their wide use by Bulgarian population as food, ingredients of herbal tea combinations, and food supplements. The fruits were dried according to a standardized procedure (Petkov, 1982).

Fruit extracts

The seeds of *Berberis vulgaris, Crataegus monogyna* and *Prunus spinosa* fruits were removed, as well as the seeds and trichomes of *Rosa canina* fruits. Prior to extraction procedure dried fruits were minced into particles smaller than 0.5 mm. Two types of extracts were prepared: 1. Aqueous extracts – 100 ml boiling water was added to 0.5 g minced fruit material and after 10 min of incubation the extracts were filtered; 2. Aqueous-alcoholic extracts were prepared as described by Tang et al. (2004) with modifications – minced fruit material (250 mg) was extracted by three minutes continuous stirring at room temperature in 80% (v/v) methanol/water (fruit material:methanol = 1:20 w/v). The extraction was carried out three times. The combined supernatants were diluted to 25 ml with 80% methanol and antioxidant activity and total polyphenol content of the extracts were measured.

Antioxidant activity

Antioxidant activity was measured using the ABTS (2,2`-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)) cation radical decolorizaton assay (Re et al., 1999). The method is based on the consumption of preformed in the presence of potassium persulfate ABTS radical ($ABTS^{\bullet+}$). Addition of antioxidants to $ABTS^{\bullet+}$ reduces it to ABTS. Absorption was measured at 734 nm. Uric acid was used as a standard. The antioxidant activity is presented as Uric Acid Equivalents (UAE) per gram dry weight. The results are presented as means ± S.D. Each measurement was performed at least in triplicate.

Total polyphenol content

Total polyphenol content was measured using the Folin-Ciocalteu reagent as described by Singleton and Rossi (1965). Absorption was measured at 760 nm. Polyphenol content was expressed as Quercetin Equivalents (QE) per dry weight. Results are presented as means ± S.D. Each measurement was performed at least in triplicate.

Statistical analysis

GraphPad Prism 3.03 software was used for statistical analysis.

RESULTS

Antioxidant activity

Highest antioxidant activity was established for *Sambucus ebulus* fruits in both types of extracts – 1005,38 ± 18,51 µmol UAE/g and 779.65 ± 51.54 µmol UAE/g for aqueous and aqueous-methanolic extracts respectively. The antioxidant activity for both types of extracts decreased as following: *Sambucus ebulus* > *Crataegus monogyna* > *Rosa canina* > *Berberis vulgaris* > *Vaccinium myrtillus* > *Prunus spinosa* (Fig 1). The antioxidant activity of aqueous fruit extracts was found to be on the average 1.2 times higher than the antioxidant activity of aqueous-methanolic fruit extracts.

Total polyphenol content

S. ebulus fruits showed highest polyphenol content for both types of extracts – 73.73 ± 0.57 mg QE/g for aqueous and 68.27 ± 1.93 mg QE/g for aqueous-methanolic extracts, followed by *Rosa canina*, *Crataegus monogyna* and *Berberis vulgaris* fruits, with very high polyphenol content as well (Fig. 2).

The reduction of polyphenol content was in almost the same order as the reduction of antioxidant activity: *Sambucus ebulus* > *Rosa canina* > *Crataegus monogyna* > *Berberis vulgaris* > *Vaccinium myrtillus* > *Prunus spinosa* for aqueous and *Sambucus ebulus* > *Crataegus monogyna* > *Berberis vulgaris* > *Rosa canina* > *Vaccinium myrtillus* > *Prunus spinosa* for aqueous-methanolic extracts.

Correlation

A high degree of positive correlation between the polyphenol content and the antioxidant activity was established for both aqueous (R=0.961) and aqueous-methanolic (R=0.958) extracts. Highest polyphenol content corresponded to highest antioxidant activity.

DISCUSSION

Many types of plant foods have been studied for their antioxidant potential. Diets rich in fruits and vegetables are associated with reduced risk for various diseases (Burns Kraft et al., 2005, Goodwin et al., 1995, Gillman et al., 1995, Kohlmeier et al., 1995, Rimm et al., 1996, Steinmetz al., 1996). The protection has often been attributed to plant antioxidants – vitamins C, E, β-carotene and other phytochemicals such as polyphenols (Prior, 2003). A correlation between the polyphenol content and the antioxidant activity has been established for many herbs (Kiselova et al., 2004, Javanmardi et al., 2003). A direct correlation with total polyphenol content has been established in surveys of blueberry genotypes analyzed for antioxidant potential (Kalt et al., 2001, Moyer et al., 2002).

Different *Vaccinium* species were recognized as berries with high antioxidant activity (Bagchi et al., 2004, Halvorsen et al., 2002), for that reason *Vaccinium myrtillus* was chosen as a species to which we compared the antioxidant activity and the polyphenol content of the other fruits. There are data about a moderate antioxidant activity of *Vaccinium myrtillus* and high antioxidant activity of *Rosa canina* fresh fruits (Halvorsen et al., 2002) and *Crataegus monogyna* fresh reproductive organs (Bahorun et al.,1994), while data about the antioxidant properties of *Sambucus ebulus*, *Berberis vulgaris* and *Prunus spinosa* are limited.

Our study demonstrates highest antioxidant activity for the two types of extracts of *S. ebulus* fruits: 4.5 times higher (for aqueous) and 2.7 times higher (for aqueous-methanolic extracts) than the antioxidant activity of the *Vaccinium myrtillus* extracts. The antioxidant activities of aqueous extracts from *Crataegus monogyna*, *Rosa canina* and *Berberis vulgaris* were respectively 3.3, 3.2 and 1.7 times higher than the activity of *Vaccinium myrtillus* aqueous extract. The corresponding aqueous-methanolic extracts from the same fruits showed 2.2, 1.6 and 1.4 times higher antioxidant activity than *Vaccinium myrtillus* aqueous-methanolic extract. On the average the antioxidant activity of aqueous extracts of all studied dried fruits was 1.2 times higher than the activity of their aqueous-methanolic extracts.

Highest polyphenol content was established for *S. ebulus, C. monogyna*, *R. canina* and *B. vulgaris* fruit extracts - 3.6, 2.8, 2.9 and 2.3 times higher polyphenol content of the aqueous extracts and 2.3, 1.9, 1.6 and 1.7 times higher polyphenol content of the aqueous-methanolic extracts respectively than the corresponding *V. myrtillus* extracts.

In general the two different extraction procedures resulted in the measurement of approximately equal polyphenol concentrations, whereas the antioxidant properties of the two types of extracts differed of about 1.2 times on the average, which could be attributed to the extraction of water soluble constituents with no polyphenol nature. Besides these differences very high degree of positive correlations were established between the antioxidant activity and the total polyphenol content regardless of the methods of extraction. This indicates that polyphenols contribute to a great extent for the antioxidant properties of the wild edible fruits.

CONCLUSIONS

The excellent positive correlations indicate that polyphenols are the main factor contributing to the antioxidant potential of the fruits studied.

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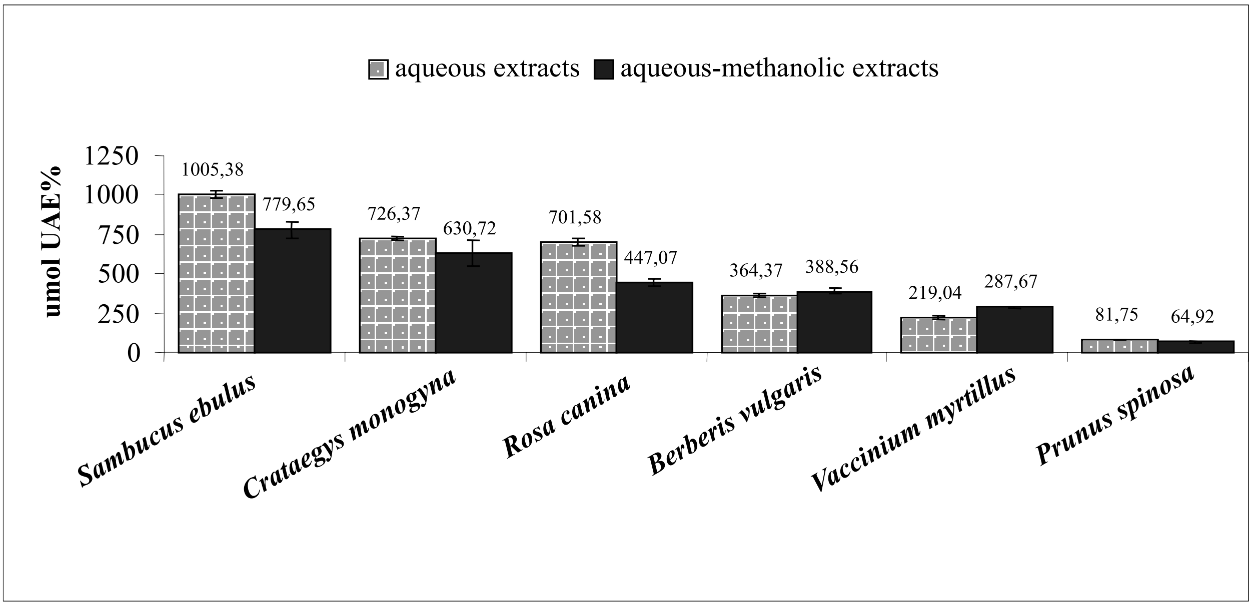

Fig 1. *Antioxidant activity of aqueous and aqueous-methanolic wild fruit extracts (μmol UAE/g).*

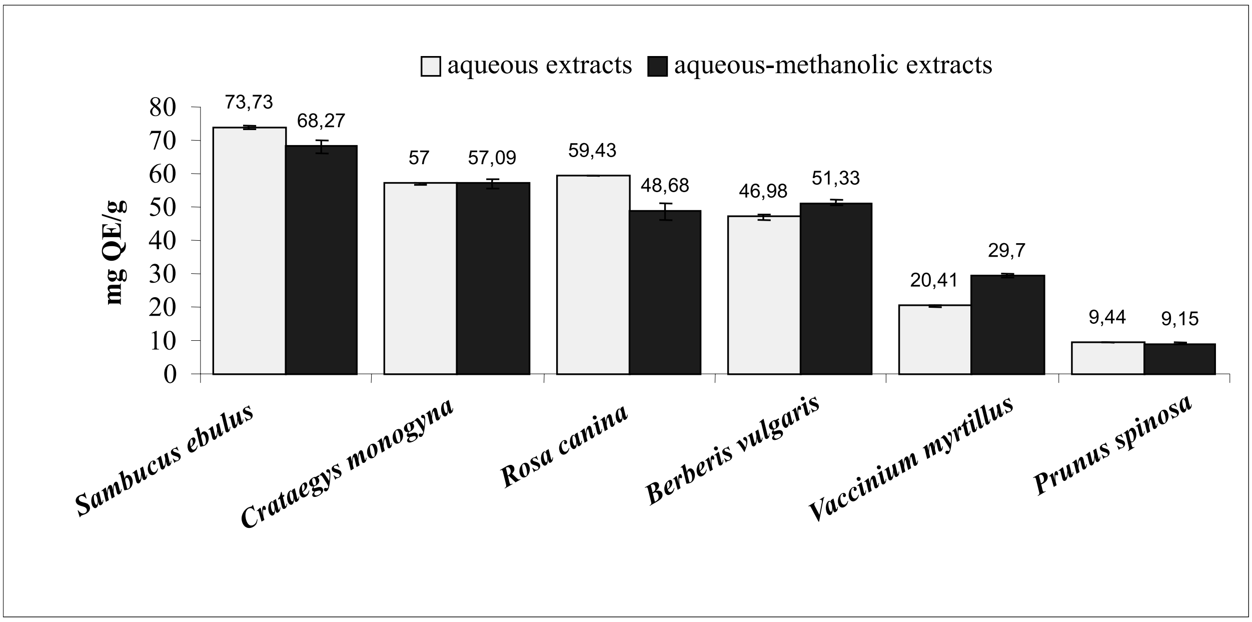

Fig. 2. *Total polyphenol content of aqueous and aqueous-methanolic wild fruit extracts (mg QE/g).*