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Original scientific paper

CRANBERRY – A GOOD SOURCE OF NATURAL ANTIOXIDANTS

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The influence of extracts of cranberry fruit and mixed tea (containing 40% cranberry) on stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals has been investigated by electron spin resonance (ESR) spectroscopy. All investigated extracts possess very high antioxidant activity, which increased dose-dependently at mass concentrations ranging from 0.5 to 3.5 mg/ml. The high contents of phenolics (3.60-4.52 mg/g), anthocyanins (0.23-1.52 mg/g), flavan-3-ols (1.25-3.05 mg/g) and vitamin C (0.07-0.15 mg/g) in investigated extracts indicated that these compounds significantly contributed to the antioxidant activity. All these results show that the extracts of cranberry fruit and mixed tea can be used as easily accessible source of natural antioxidants and as a possible food supplement.

KEYWORDS: Cranberry; phenolics; flavan-3-ols; anthocyanins; vitamin C; antioxidant acivity; ESR

INTRODUCTION

Recent epidemiological studies have indicated that diets rich in fruits and vegetables are associated with lower incidences of oxidation-linked disseases such as cancer, CVD, and diabetes. These protective effects of fruits and vegetables are now linked to the presence of vitamins and phenolic phytochemicals having antioxidant activity, which support the body's antioxidant defense system. Phenolic phytochemicals, due to their phenolic ring and hydroxyl substituents, can function as effective antioxidants capable of quenching free electrons.

Berries, like many other fruits, are rich in vitamin C and phenolic compunds, which include biphenyls, flavonoids and phenolic acids. Many cultivars and native species of berries exist, some with substantially higher antioxidant levels than others (1).

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Cranberry (*Vaccinium macrocarpon, Ericaceae*), with an attractive bright red apperance and distinctive flavor, is recognized as a concentrated source of dietary flavonoids, including anthocyanins, flavonol glycosides and proanthocyanidins (condensed tannins), as well as various phenolic acids (2, 3). The pigments of cranberry, anthocyanins, are mainly peonidin and cyanidin galactosides. Quercetin and myricetin glycosides are the most abundant flavonol glycosides in cranberry extracts (4), while the most abundant cranberry phenolic acids are *p*-coumaric, sinapic, caffeic and ferulic acid (5). Also, cranberry is known to be a good source of vitamin C (6).

Cranberry and their products have been associated historically with many positive benefits on human health. For many decades, cranberry juice has been widely used as a folk remedy to treat urinary tract infections in women and other gastrointestinal disorders. Cranberry juice extracts have also been suggested to exhibit anticancer effects and to inhibit the oxidation of low-density lipoprotein *in vitro*, potentially preventing the development of heart diseases (7).

To provide a better understanding of the antioxidant properties of cranberry extract, the total phenol, anthocyanin, flavan-3-ol, and vitamin C content were evaluated by the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay.

EXPERIMENTAL

Chemicals

Methanol, acetone and acetic acid were obtained from ”Zorka” Šabac (Serbia). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, gallic acid and (±)-catechin were purchased from Sigma Chemical Co. (USA). Other used chemicals and solvents were of the highest analytical grade.

Plant material

Cranberry fruit and mixed tea containing 40% cranberry fruit, 40% apple and 20% hibiscus flower, were purched from the local herbal drug store.

Methods

Extraction. Dried cranberry fruit and mixed tea (10 g) were extracted with 100 ml of 80% (v/v) methanol containing 0.5% (v/v) acetic acid or 80% (v/v) acetone containing 0.5% (v/v) acetic acid at room temperature for 3x24 hours. The obtained extracts of cranberry (acetone extract – CAE and methanol extract – CME), and of mixed tea (acetone extract – MTAE and methanol extract – MTME) were evaporated to dryness under reduced pressure. The yields, average of triplicate analysis, of extracts were: $m_{CAE} = 5.82 \pm 0.28$ g; $m_{CME} = 6.11 \pm 0.30$ g; $m_{MTAE} = 5.58 \pm 0.26$ g; $m_{MTME} = 6.40 \pm 0.31$ g.

Total phenolics. Total phenolics in extracts were determined spectrophotometrically using the Folin-Ciocalteu reagent and the results are expressed as gallic acid equivalents per g dry weight (8).

Total flavan-3-ols. Content of total flavan-3-ols in extracts was determined spectrophotometrically using the vanillin assay and the results are expressed as catechin equivalents per g dry weight (9).

Total anthocyanins. Anthocyanin quantitation was perfomed spectrophotometrically using the pH differential method (10). Antocyanins were quantified as cyanidin-3-glucoside using an extinction coefficient of 26 900 and resultant values were expresed in terms of anthocyanin per g dry weight.

Vitamin C. Vitamin C content in extracts was analyzed volumetrically using Tilmans method and the results are expressed as mg per g dry weight (11).

DPPH radical assay. Blank probe was obtained by mixing 400 µl 0.4 mM methanolic solution of DPPH and 200 µl of acetone. A volume of x µl of 10 mg/ml acetone solution of extract was added to a mixture of (200-x) µl of acetone and 400 µl of 0.4 mM methanolic solution of DPPH radical (probe). The range of the investigated extract concentrations was 0.5-3.5 mg/ml. After that the mixture was stirred for 2 min and transferred to a quartz flat cell ER-160FT. The ESR spectra were recorded on an ESR spectrometer Bruker 300E (Rheinstetten, Germany) under the following conditions: field modulation 100 kHz, modulation amplitude 0.256 G, receiver gain $2 \cdot 10^4$, time constant 40.96 ms, conversion time 327.68 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23°C.

The antioxidant activity (AA_{DPPH}) of the extract on DPPH was defined as:

$$AA_{DPPH} = 100 \times (h_o - h_x)/h_o \, [\%]$$

where h_o and h_x are the height of the second peak in the ESR spectrum of DPPH radicals of the blank and the probe, respectively.

RESULTS AND DISCUSSION

The total contents of phenolics, flavan-3-ols, and anthocyanins, as well as the content of vitamin C in CAE, CME, MTAE and MTME are shown in Fig. 1.

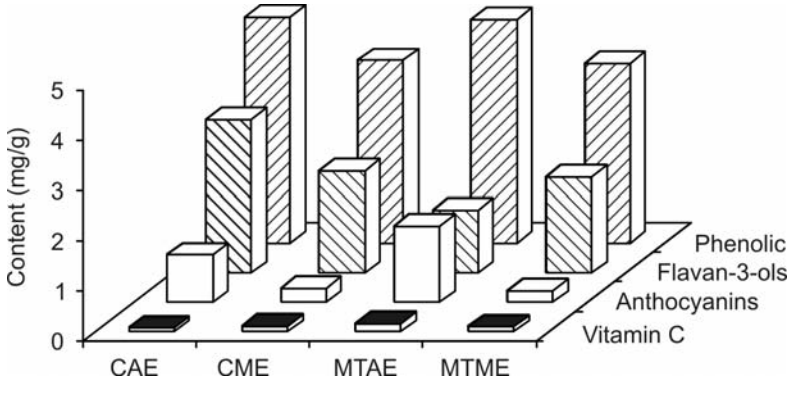
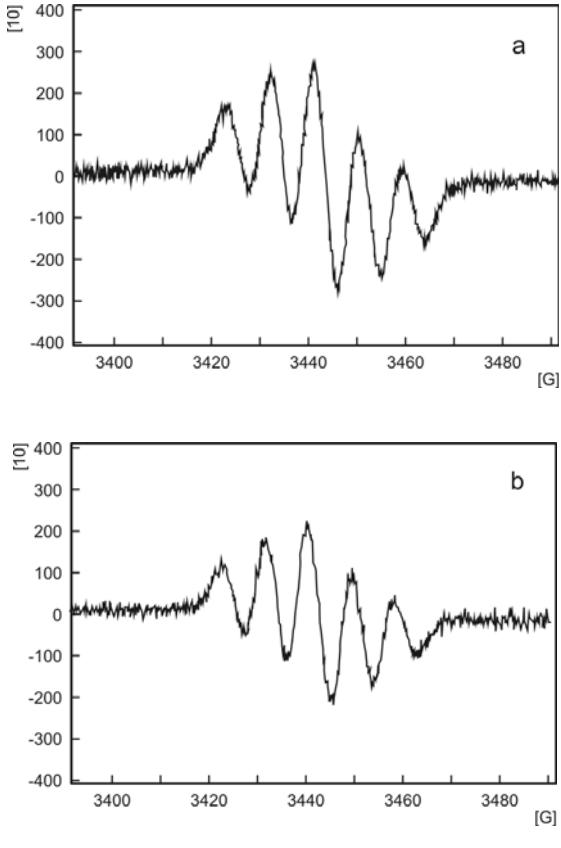


Fig. 1. Total contents of phenolics, flavan-3-ols, anthocyanins, and vitamin C in CAE, CME, MTAE and MTME

The contents of total anthocyanins were 0.27±0.012 mg/g in CME, 0.23±0.009 mg/g in MTME, 0.95±0.037 mg/g in CAE, and 1.52±0.080 mg/g in MTAE. The levels of flavan-3-ols in CAE (3.05±0.195 mg/g) were higher than in MTAE (1.25±0.083 mg/g), and these differences were statistically significant (p=0.001). In the case of CME and MTME, the flavan-3-ols were present in the similar concentartion (2.03±0.149 mg/g for CME and 1.91±0.095 mg/g for MTME, p=0.065). There were no statistically significant differences between the contents of phenolics in CAE and MTAE (4.52±0.234 and 4.48±0.201 mg/g, respectively; p=0.137), and also, in CME and MTME (3.66±0.256 and 3.60±0.163 mg/g, respectively; p=0.380). Also, there were no discernible differences (p=0.139) in concentration of vitamin C in CME (0.12±0.014 mg/g) and in MTME (0.10±0.003 mg/g), while the content of vitamin C was twice higher in MTAE (0.15±0.008 mg/g) than in CAE (0.07±0.006 mg/g).

In this study, the stable DPPH radicals have been used to investigate cranberry antioxidant activity. The DPPH radical has been widely used to evaluate the free radical scavenging activity of various polyphenolic antioxidants commonly found in food (12), plant extracts (13-15) and beverages (16). The reduction of DPPH radical is followed by monitoring the decrease in its absorbance at characteristic wavelenght during the reaction (17, 18). However, in our work, ESR spectroscopy was used to identify DPPH radical.



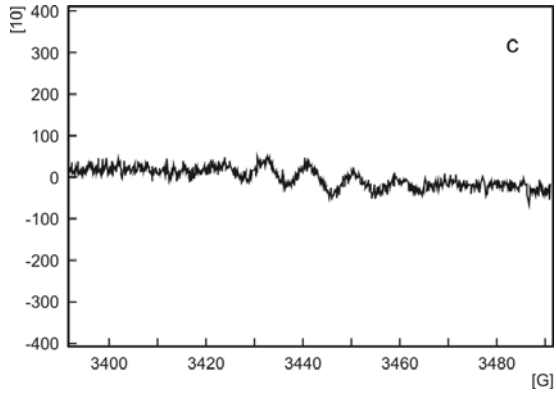


Fig. 2. ESR spectra of the DPPH radicals: a) in the absence of extracts (blank); b) in the presence of 0.5 mg/ml of CAE; c) in the presence of 2.0 mg/ml of MTME

The ESR spectra of DPPH radical in the blank and in probe with 0.5 mg/ml CAE and 2.0 mg/ml MTME are characterized by their five lines of relative intensities 1:2:3:2:1 and hyperfine splitting constant a_N=9.03 G (Fig. 2). No change in the hyperfine structure of ESR spectra was detected, but the intensities of the line corresponding to the concentration of DPPH radicals, decreased in the presence of extracts.

Antioxidant activity (AA_{DPPH}) of different concentrations of CAE, CME, MTAE and MTAE is evident from the data given in Fig. 3.

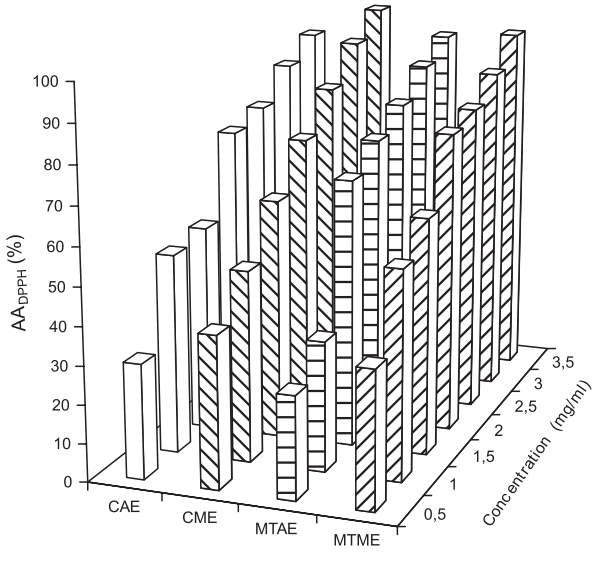


Fig. 3. Antoxidant activity of different concentracions of CAE, CME, MTAE and MTME on DPPH radicals

The extracts obtained from cranberry and mixed tea exhibited significant antioxidant activity on stable DPPH radical. The DPPH radical antioxidant activity (AA_{DPPH}) of investigated extracts increased dose-dependently at mass concentrations ranging from 0.5 to 3.5 mg/ml (Fig. 3). With increasing concentrations, AA_{DPPH} of extracts increased from 30% to 100%. It is quite realistic to assume that antioxidant compounds present in apple and hibiscus flower contributed to good antioxidant activity of mixed tea extracts.

The total content of phenolics, flavan-3-ols, and anthocyanins, as well as content of vitamin C in extracts were correlated with AA_{DPPH}. In the cases of anthocyanins and vitamin C the coefficients were very good (r^2=0.89 and r^2=0.71, respectively; $p<0.05$). On the other hand, in the cases of phenolics and flavan-3-ols, linear regression analysis showed that the correlation was rather poor ($r^2 < 0.5$, $p<0.05$). It appears, therefore, that the differences between the correlation of phenolics, flavan-3-ols, anthocyanins, and content of vitamin C with AA_{DPPH} suggested that the free radical scavenging activity of extracts is probably a consequence of the synergistic action of the different constituents. Indeed, the interaction of different species may promote changes in the overall antioxidant capacity, which is difficult to predict on the basis of their individual antioxidant capacity.

CONCLUSION

- The highest content of phenolics (4.52 mg/g) and flavan-3-ols (3.05 mg/g) were detected in CAE, while MTAE possessed the highest content of anthocyanins (1.52 mg/g) and vitamin C (0.15 mg/g).
- Employing ESR spectroscopy, the antioxidant activity of obtained extracts on stable DPPH radicals was established;
- The investigated extracts possess very high antioxidant activity (AA_{DPPH}=30%-100%) which increased dose-dependently at mass concentrations ranging from 0.5 to 3.5 mg/ml.
- Results of linear regression analysis suggest that the free radical scavenging activity of extracts is probably a consequence of the synergistic action of phenolics, flavan-3-ols, anthocyanins and vitamin C.

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БРУСНИЦА – ЗНАЧАЈАН ИЗВОР ПРИРОДНИХ АНТИОКСИДАНАТА

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Утицај ектраката бруснице и чајне мешавине (40% брусница) на стабилне 1,1-дифенил-2-пикрилхидразил (DPPH) радикале испитан је електрон спин резонантном (ESR) спектроскопијом. Сви испитивани екстракти показали су високи проценат антиоксидативне активности на DPPH радикале. Антиоксидатвна активност екстраката расте са порастом концентрације у испитиваном опсегу 0,5-3,5 mg/ml. С обзиром на висок садржај фенола (3,60-4,52 mg/g), антоцијана (0,23-1,52 mg/g), флаван-3-ола (1,25-3,05 mg/g) и витамина Ц (0,07-0,15 mg/g) у екстрактима, може се претпоставити да ова једињења значајно доприносе антиоксидативној активности. Добијени резултати указују на то да су екстракти бруснице и чајне мешавине добар извор антиоксиданата, те да се могу користити као додатак хране.

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