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ANTIOXIDANT EFFECTS OF HERBAL EXTRACTS AND THEIR FOOD APPLICATION.

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ABSTRACT

Herbal extracts are considered as a good sources of antioxidant compounds. This work describes antioxidant effect of 15 kinds of herbs. Three different antioxidant assays were used, Trolox equivalent antiradical activity (TEAC), test based on deoxyribose oxidation and DPPH method. The TEAC values ranged from 0.38mM to 0.77 mM. Deoxyribose assay showed antioxidant activity of selected extracts expressed as the inhibition of formation of oxidative products of deoxyribose from 2.68 to 50.05 %. The DPPH method values ranged from 6.47 to 73.80 %.

Extracts of *Prunus spinosa* L., *Euphrasia rostkoviana Hayne* were the best antioxidants confirmed by all methods. *Scrophularia nodosa* L. extract showed the weak antioxidant effect determined by all selected methods. Phenolic content was in relation to the antioxidant effect of herbs, very high significancy between DPPH and polyphenols content was found. To improve selected properties of apple juice (taste, smell, functional properties) by addition of medicinal herbs was achieved in this work. Antioxidant effect of apple juice variants with herbs additions determined by DPPH method was high and ranged from 73 to 78.49 %. Herbal extracts can be utilised in selected combinations to improve sensory and functional properties of some kinds of beverages.

Keywords: additive, herb, antioxidant effect, TEAC, deoxyribose test, DPPH

INTRODUCTION

Dietary habits influence the risk of developing a variety of diseases, especially cancer and heart diseases. Epidemiologic observations and laboratory studies have indicated that tea consumption may have beneficial effects in reducing certain types of cancer in some populations (**Mukhtar, Ahmad, 2000**).

The traditional Chinese medicinal plants associated with anticancer demonstrated significantly stronger antioxidant activity and contained much more phenolic compounds than common vegetables and fruits. A positive and significant correlation existed between antioxidant activity and total phenolic content, revealing that phenolic compounds were the dominant antioxidant components in the tested medicinal herbs. Phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids were identified as major types of phenolic compounds. The investigation of the inter-relationship between phenolic compounds and antioxidant/anticancer activity will be a promising field to understand and elucidate possible mechanisms for the functionality of traditional Chinese medicines for cancer prevention and treatment (**Cai et al., 2004**).

Plant extracts contain polyphenols (natural antioxidants), which are believed to be effective nutrients in the prevention of oxidative stress-related diseases such as cancer and heart diseases. These extracts, possibly mainly due to their phenolic content, retard oxidative degradation of lipids (**Proestos et al., 2008**).

Herbs are important sources of compounds, that can work as antioxidat or antimicrobial agents (**Yanishilieva et al. 2006**), so herbal extracts are being used in cosmetics, food industry (**Djeridane et al, 2006**) and medicine (**Hinneburg, 2006, Yanishlieva et al., 2006**). Many of pharmaceutical products are based on substances derived from plants.

In the present, fresh fruit and vegetable juices are very popular and their improvement with natural functional (herbal) additives to increase their nutritional and sensory value is one of the trends in the soft industry.

The aims of this work were to compare antioxidant effect of water herbal extracts evaluated by different assays - TEAC (Trolox Equivalent Antiradical Capacity) and deoxyribose test (dR test), DPPH and to test their use to enrich soft drinks (apple juice).

MATERIAL AND METHODOLOGY

Herbs and their parts were analysed: acacia *(Robinia pseudo – acacia* L.*)* – flower, euphrasia *(Euphrasia rostkoviana* Hayne*)*- flowering top, silverweed (*Potentilla anserina* L.) – leaf, black thorn *(Prunus spinosa* L.) – flower, yarrow *(Achillea millefolium* L.) – flowering top, figwort *(Scrophularia nodosa* L.*)* – flowering top, gowan *(Bellis perennis* L.*)* – flower, yellow chamomile (*Anthemis tinctoria* L.) – flower, wild garlic *(Allium ursinum* L.*)* – leaf, tarragon (*Artemisia dracunculus* L.*)* – flowering top, horse-chestnut *(Aesculus hippocastanum* L.*)* – flower, betony *(Stachys officinalis* L.*)* – flowering top, germander (*Teucrium chamaedry* L.) – flowering top, bedstraw *(Galium verum* L.)- flowering top, verbena (*Verbena officinalis* L.) - flowering top.

All herbs were collected in Lieštany area (Slovakia).

Medicinal herbs of *Rubus idaeus, Tilia cordata*, *Agrimonia eupatoria*, *Centaurium erythraea*, *Mentha piperita, Calendula officinalis*, *Origanum vulgare* were used in purpose to obtain special health benefits for their application into the apple juices.

To compare the antioxidant effects were used also synthetic antioxidants- BHT (3,5 – Di-tert-butyl-4-hydroxytoluene) fy Supelco USA and ascorbic acid ($C_8H_8O_6$) Lachema, Neratovice, Czech republic.

Herbal extracts preparation

Dry and homogenized herbs (2 g) were extracted with 200 ml of boiled destilled water during 5 min of infusion time. Extracts were filtrated and used for measure.

Methods

1. Deoxyribose test

Reaction was realised in medium of 50 mM potassium phosphate buffer (PBS) pH 7.4 (55 µl) after addition 5 µl

10 mM EDTA (ethylenediamine-tetraacetic acid), 5 µl 10mM $FeCl_3$, 5 µl 10 mM ascorbic acid, 180 µl 10mM deoxyribose, 200 µl herbal extract and 50 µl 10 mM H_2O_2. Compound was mixed and incubated during 1 hour at 37 °C. Afterwards 0.5 ml 10 % TCA (trichloroacetic acid) and 0.5 ml 1 % TBA (thiobarbituric acid) were added. Mixture was incubated during 10 minutes at 90°C. Sample was cooled and measured by spectrofotometer (UV – 161 Shimadzu, Tokio, Japan) at 532 nm.

Antioxidant activity was expressed as: [1-(dRA / dR)] x 100 (%)
dR – absorbancy without antioxidant
dRA – absorbancy with antioxidant

2. TEAC/ABTS test

Radical preparation: 3.3 mg potassium persulphate ($K_2S_2O_8$) was disolved in 5 ml deionized water and added to 17.2 mg ABTS [2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)], then was mixed in the test tube and stored in the dark during 14 hours.

After that time 1 ml of the radical solution was mixed with 60 ml deionized water, 50µl of herbal extract was added and mixed rigorously. Decrease of absorbancy at 734 nm (UV – 1601 Shimadzu, Tokio, Japan) during 600 s was observed. Adjusted value of extract in 600 s was expressed by the calibration curve of Trolox concentration (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

3. DPPH

The method of BRAND – WILLIAMS et al. (1995) was modified and used. Herbal extract (0.1 ml) was added to 3.9 ml DPPH˙ ethanolic solution (25 mg. l^{-1}), mixed and absorbancy decrease was measured at 515 nm during 10 min of reaction time - spectrofotometrically.

Calculation: Efficiency of plant extracts as antioxidants was calculated:

$$\% \ inhibition = \frac{(A_C - A_{At})}{A_C} \cdot 100$$

A_C – control absorbancy of DPPH solution (time = 0 min)
A_{At} – absorbancy of solution after reaction with antioxidant (time = 10 min)

4. TOTAL POLYPHENOLS (TP)

The contents of total phenolic compunds in water extracts were estimated using the Folin- Ciocalteau reagent. Tanin was used as the standard solution.

5. APPLE JUICE CHARACTERISATION WITH MEDICINAL EXTRACTS ADDITION

Apple juice (100 %) was obtained from the small fruit producer in Slovakia and used as control sample. Selected medicinal extracts (2 g/200ml) were added to replace the apple juice in the amounts 10-20 % in these variants:
A- 10 % *Rubus idaeus* + 10 % *Tilia cordata*
B- 10 % *Agrimonia eupatoria* + 10 % *Centaurium erythraea*
C- 10 % *Mentha piperita*
D- 10 % *Calendula officinalis* + 10 % *Origanum vulgare*
E - control (100 % apple juice)
Variants of apple juices were tested for the dry matter content (refractometrically), acidity by titration (%), antioxidant effect (DPPH method) and sensory analysis.

6. STATISTICAL ANALYSIS

Statistical processing was performed with use of non-parametric methods (Kendall´s and Spearman´s correlation analysis). Datas were computed in R language with use of R software environment for statistical computing.

RESULTS AND DISCUSION

Antioxidant effects of selected herbal extracts compared to different assays

Antioxidant effect of selected water extracts from herbs which are commonly cultivated in gardens, or in nature was observed and is shown in the table 1. Water as extraction solvent was used because of polyphenols characteristics, safety and also Buřičová and Réblová

Table 1 Antioxidant effect determined by different assays and phenolics content of selected herbs

Extract	Antioxidant effect			Polyphenols ($mg.dm^{-3}$)
	dR-test (%)	ABTS test (mM)	DPPH (%)	
Euphrasia rostkoviana	23.22	0.7684	73.80	201.76
Achillea millefolium L.	18.19	0.3844	14.38	55.70
Potentilla anserina L.	26.00	0.7711	30.43	65.81
Scrophularia nodosa L.	3.97	0.3842	6.47	16.31
Bellis perennis L.	34.09	0.4017	56.07	171.43
Anthemis tinctoria L.	10.33	0.4533	58.35	128.73
Allium ursinum L.	19.90	0.3880	7.23	62.44
Artemisia dracunculus L.	21.98	0.4653	38.58	128.73
Prunus spinosa L.	50.05	0.6035	73.40	189.41
Robinia pseudo – acacia L.	20.52	0.3917	9.04	79.29
Aesculus hippocastanum L.	32.72	0.5758	58.15	233.23
Stachys officinalis L.	17.86	0.3877	11.37	28.73
Teucrium chamaedrys L.	2.68	0.4480	35.91	93.90
Galium verum L.	4.87	0.3840	11.49	25.36
Verbena officinalis L.	28.66	0.3862	9.87	51.20

Table 2 Characterisation of apple juices with herbal extracts addition

Variant	Sample	DPPH (%)	DPPH/mixture (%)	Rf (%)	Acidity (%)
A	Raspberry (10 %)	88	78.49	11	0.201
	Linden (10 %)	73.38			
B	Agrimony (10 %)	81.88	73.03	11.5	0.201
	Centaury (10 %)	14.74			
C	Mint (10 %)	78.12	76.76	12	0.241
D	Marigold (10 %)	28.24	76.21	11	0.201
	Oregano (10 %)	83.53			
E	100 % apple	76.33		14	0.241

(2008) found that the extraction of substances with the antioxidant activity was markedly more efficient using hot water than ethanol at laboratory temperatures.

Determination of antioxidant activity by ABTS radical showed the extracts of euphrasia (0.77 mM), silverweed (0.77 mM) and black thorn (0.60 mM) to be the best sources of antioxidants. Similarly use of DPPH radical showed the best antiradical effect at euphrasia (73.8 %) and black thorn (73.4 %) and the lowest at figwort (6.47 %). Assay based on oxidative damage of deoxyribose (dR) confirmed the black thorn as very strong antioxidant (50.05 %).

Figwort was confirmed as one of the weakest source of antioxidants compared to all selected antioxidant assays.

The effect of individual extracts in the activity order is different considering different components presence (table 1). The reason could be the presence of compounds with different reactivity in given reaction medium and their different ability to eliminate present type of radical. Antioxidant effect of herbal extracts is described mainly due to polyphenolic compounds, which concentration and presentation is different in individual herbs. As rich sources of phenolics were estimated euphrasia, black thorn and gowan. Shi et al. (2009) describes that chlorogenic acid isomers are the key phenolic compounds responsible for antioxidant activity of the extract from *Prunus mume* flowers.

Statistical testing of our results showed very high significancy between DPPH and polyphenols content (0.91 Spearman; 0.68 Kendal). Relationship between deoxyribose test and polyphenols was significant (0.61 Spearman; 0.47 Kendal) and between ABTS and phenolics was found middle significancy (0.53 Spearman; 0.36 Kendal).

Antioxidant effect of ascorbic acid (2 g) determined by DPPH method was high (94.6 %) even compared to euphrasia, which was classiffied as one of the best antioxidant (73.8 %) sources. BHT was tested in low amounts (0.45 g BHT) and at this sample weight was determined 52 % inhibition of DPPH radical (fig. 1). Antioxidant effect of selected fenolic acids (not shown) determined by dR- test was found to be high (54.42 %) at rosmarinic and gallic (53.48 %) acids.

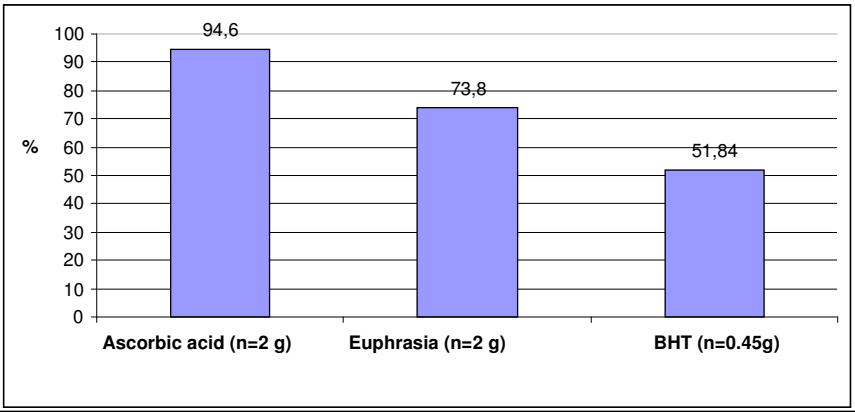
Application of selected herbal extracts in aplle juices

Sensory evaluation showed that pure (100 %) apple juice was very sweet and sensorically not very well accepted. Some authors (Buřičová, Réblová, 2008) recommend selected plants (*Rosaceae, Lamiaceae, Tiliaceae*) for consumption in various kinds of beverages or as extracts to increase the nutritional value of different foods and diets.

The aim of work was to improve properties of apple juice (taste, smell, functional properties) by addition of selected medicinal herbs in purpose of sensory and health improvements of juices.

By non-parametric testing it was found that with use of Friedman test concentrated apple juice (E variant) was

Fig. 1 Antioxidant effects (% inhib.) of different types of antioxidants determined DPPH method



statistically significant worse (p = 0.0948) compared to the rest of variants.

Method based on elimination of DPPH radical showed variant E - apple juice (100 %) as good source of antiradical compunds (76.33 %), probably because of polyphenols presence. Apple polyphenols can be divided into two big groups, group of fenolic acids including benzoic acids derivates (e.g. gallic acid) and cinnamic acid derivates (caffeic, ferulic, chlorogenic), and group of flavonoids including flavonols (quercetin), flavan-3-ols (catechin, epicatechin, procyanidins), dihydrochalcons and antocyanins **(Ondrejovič et al., 2009)**.

Regarding to the antioxidant effect of individual variants can be showed (table 2) that the best antioxidant effect was determined at variant A (raspberry and linden- 78.49 %) . Antioxidant effect of our juice variants was high and ranged from 73 to 78.49 %, probably because of use of good antioxidative extracts from herbs and good antioxidant effect of apple juice as well. Also Buřičová and Réblová (2008) state that considerable antioxidant activities were found in the extracts of plants from *Rosaceae* family (e.g. blackberry), *Lamiaceae* (oregano, mint), *Tiliaceae* (linden) and appear to be good and safe sources of antioxidants.

Dry matter content and acidity were found to be the highest at concentrated apple juice (variant E) and decreased with the additions of herbal extracts.

Mendelova et al. (2009) tested to improve sensory parameters of concentrated apple and sour cherry juices by mixing them in different proportions.

CONCLUSION

Determination of antioxidant activity by ABTS radical showed the extracts of euphrasia, silverweed and black thorn to be the best sources of antioxidants. Similarly use of DPPH radical showed the best antiradical effect at euphrasia and black thorn, assay based on oxidative damage of deoxyribose (dR) confirmed the black thorn as very strong antioxidant. Figwort was confirmed as one of the weakest source of antioxidants compared to all selected antioxidant assays. While all herbal extracts showed some antioxidant effect, achieved results in individual kinds of herbs compared to different methods indicate some differences in the activity (effect) order evaluated by different methods. The reason could be the presence of compounds with different reactivity in given reaction medium and their different ability to eliminate present type of radical.

To improve properties of apple juice (taste, smell, functional properties) by addition of selected medicinal herbs (10 %) was achieved in this work. Antioxidant effect of apple juice variants with herbs additions was high and ranged from 73 to 78.49 %. Herbal extracts can be utilised as potential food additives to avoid oxidative changes in the food products and in selected combinations to improve sensory and functional properties of some kinds of beverages.

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