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BIOLOGICAL ACTIVITY OF APPLE JUICE ENRICHED BY HERBAL EXTRACTS

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ABSTRACT

Herbal phytochemicals have recently become an attractive subject for scientists in many different research areas. The aim of this study was to determine antioxidant activity, total polyphenol and flavonoid content of apple juice enriched by water herbal extracts. Secondary was to evaluate sensory characteristic of enriched apple juice. It was found that applications of water herbal extracts to apple juice increase antioxidant activities, and also total polyphenol and flavonoid content with compare to pure apple juice. The highest biological activities were detected in apple juice with addition of lemon balm (14.42 mg TEAC/L; 84.38 mg TEAC/L; 50.88 mg GAE/L; 36.26 µg QE/L, oregano (14.92 mg TEAC/L; 79.97 mg TEAC/L; 50.51 mg GAE/L; 31.02 µg QE/L) and salvia (8.40 mg TEAC/L; 30.40 mg TEAC/L; 23.33 mg GAE/L; 27.67 µg QE/L) water extract. Sensorial analysis of samples showed, that enriched juices had better properties for evaluators with compared to pure juice. The aim of this study was also to mention the potential use of medicinal herbs in food industry, because plant bioactive compounds can play an important role in preventing cardiovascular diseases, cancers and reduction inflammatory action.

Keywords: Antioxidant activity, DPPH, medicinal herbs, flavonoids, polyphenols, apple juice

INTRODUCTION

Currently there has been an increased interest globally to identify antioxidant compounds that are pharmacologically potent and have low or no side effects for use in preventive medicine and the food industry. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photons and oxygen, they represent a potential source of new compounds with antioxidant activity (**Ali *et al*., 2008**). Antioxidants derived from fruits, vegetables, herbal plants and cereals are very effective and have reduced interference with the body's ability to use free radicals constructively **(Wolfe *et al*., 2003)**. Natural antioxidants mainly come from herbal plants in the form of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tocotrienols) ascorbic acid and carotenoids. The quest for natural antioxidants for dietary, food technology, cosmetic and pharmaceutical uses has become a major industrial and scientific research challenge over the last two decades. Herbal plants have been added to foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives **(Nakatani, 1994; Cutler, 1995)**. In addition to imparting characteristic flavors, certain herbs prolong the storage life of foods by preventing rancidity through their antioxidant activity or through bacteriostatic or bactericidal activity **(Shan *et al*., 2007)**. Herbs and their constituents are generally recognized to be safe, either because of their traditional use without any documented detrimental impact or because of dedicated toxicological studies **(Smid and Gorris, 1999)**. Being natural foodstuffs, herbs appeal to many consumers who question the safety of synthetic food additives. Some herbs used today are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities. The extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have gained momentum for varied pharmaceutical and food processing applications. The antimicrobial activities of plant extracts form the basis for many applications, including raw and processed food preservation, pharmaceuticals, alternative medicines and natural therapies **(Lis-Balchin and Deans, 1997).** In the food sector, there are many areas in which the food industry uses herbs and their derivatives, among them the enhancement of quality. Precooked meals are increasingly taking the place of home-cooked food, and their preparation requires the use of antioxidants, preservatives, flavor enhancers, and coloring agents, all of natural origin **(Kuete, 2013; Falawo *et al*., 2014)**.
The aim of this study was to determine antioxidant activity and total phenol and flavonoid content of apple juice enriched by herbal extracts.

MATERIAL AND METHODS

Biological material

Herbal plants (mint, lemon balm, oregano, wild thymus, salvia) were collected from nature locality Jarok (Slovak republic) in term recommended by pharmacology protocol: mint (*Mentha piperita* L.), lemon balm (*Melissa officinalis* L.) and oregano (*Origanum vulgare* L.) in June, wild thymus (*Thymus serpillum* L.) and salvia (*Salvia officinalis* L) in July. Herbal plants were dried in room temperature and homogenized to powder. Apple juice was obtained from firm Agropol, Vlčany (Slovak Republic).

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

1 g of each herbal plant was extracted with 100 mL of distilled water for 5 minute. Extraction was all carried out in triplicate. After filtration the filtrates was used for enrichment of apple juice. Several variants of apple juice enriched by herbal extract were prepared: variant 1 (60 % of apple juice + 40 % of mint water extract), variant 2 (60 % of apple juice + 40 % of lemon balm water extract), variant 3 (60 % of apple juice + 40 % of oregano water extract), variant 4 (60 % of apple juice + 40 % of wild thyme water extract), variant 5 (60 % of apple juice + 40 % of salvia water extract). The apple juice variants were used for measurement (DPPH method, phosphomolybdenum method, total phenolic content, total flavonoid content and sensory analysis).

Radical scavenging activity

Radical scavenging activity of apple juice variants was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) **(Sánchéz-Moreno *et al*., 1998)**. The sample (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). Absorbance of the reaction mixture was determined using the spectrophotometer Jenway (6405 UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10-100 $mg.L^{-1}$;

R^2=0.989) was used as the standard and the results were expressed in $mg.L^{-1}$ Trolox equivalents.

Reducing power

Reducing power of apple juice variants was determined by the phosphomolybdenum method of **Prieto *et al*. (1999)** with slight modifications. The mixture of sample (1 mL), monopotassium phosphate (2.8 mL, 0.1 M), sulfuric acid (6 mL, 1 M), ammonium heptamolybdate (0.4 mL, 0.1 M) and distilled water (0.8 mL) was incubated at 90°C for 120 min, then rapidly cooled and detected by monitoring absorbance at 700 nm using the spectrophotometer Jenway (6405 UV/Vis, England). Trolox (10-1000 $mg.L^{-1}$; R^2=0.998) was used as the standard and the results were expressed in $mg.L^{-1}$Trolox equivalents.

Total polyphenol content

Total polyphenol content of apple juice variants was measured by the method of **Singleton and Rossi, (1965)** using Folin-Ciocalteu reagent. 0.1 mL of each sample was mixed with 0.1 mL of the Folin-Ciocalteu reagent, 1 mL of 20% (w/v) sodium carbonate, and 8.8 mL of distilled water. After 30 min. in darkness the absorbance at 700 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Gallic acid (25-300 $mg.L^{-1}$; R^2=0.998) was used as the standard and the results were expressed in $mg.L^{-1}$ gallic acid equivalents.

Total flavonoid content

Total flavonoids were determined using the modified method of **Willett, (2002)**. 0.5 mL of sample was mixed with 0.1 mL of 10% (w/v) ethanolic solution of aluminium chloride, 0.1 mL of 1 M potassium acetate and 4.3 mL of distilled water. After 30 min. in darkness the absorbance at 415 nm was measured using the spectrophotometer Jenway (6405 UV/Vis, England). Quercetin (0.5-20 $mg.L^{-1}$; R^2=0.989) was used as the standard and the results were expressed in $\mu g.L^{-1}$ quercetin equivalents.

Statistical analysis

Spectrophotometric analyses were carried out in triplicate. For statistical analysis of experimental data, correlation between measured values was used (Microsoft Office – Excel).

Sensory analysis

Sensory analysis of apple juice variants was determined by method compare of sample with standard (100 % apple juice). This method was realized in Department of storage and processing plant products by eight evaluators. In prepared apple juice variants were evaluated following parameters: smell, herbal smell (presence), herbal smell (intensity), foreign smell, taste, herbal taste (presence), herbal taste (intensity), aftertaste, character of herbal taste and total effect after drinking.

RESULTS AND DISCUSSION

Radical scavenging activity

This method is based on scavenging of the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) from the antioxidants, which produces a decrease in absorbance at 515 nm. When a solution of DPPH is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of color. This delocalization is also responsible for the deep violet color, characterized by an absorption band in ethanol solution at about 515 nm. The scavenging effect of apple juice variants on DPPH radical decreased in this order: apple juice with lemon balm > apple juice with salvia > apple juice with oregano > apple juice with mint > apple juice with wild thyme > 100 % apple juice (Fig. 1). These results indicated that all variants had a noticeable effect on scavenging free radical. The higher activities were measurement in variant 2 – apple juice with lemon balm (89.72 ± 2.44 mg $TEAC.L^{-1}$) and variant 5 – apple juice with salvia (89.66 ±0.38 mg $TEAC.L^{-1}$). These results shown that pure apple juice is good source of antioxidant compounds, but herbal extracts application can increase amount of antioxidant. Enriched beverages are very popular nowadays in consumers, mainly beverage enriched by natural extracts. Is generally know, that apples and apple juice are rich source of antioxidants that may play a role in reducing the risk of various diseases originating from oxidative stress, namely coronary disease, immune system damage, asthma, and diabetes. The risk of a heart attack has been shown to decrease by 49% for apple consumers in diet at a daily amount of ≥110 g compared to those at ≤18 g. In a case study conducted in Hawai, decreased risk of lung cancer was shown for both women and men to correlate with the consumption of apples **(Boyer and Liu, 2004; Karaman *et al*., 2010)**. **Dias *et al*. (2012)** determined antioxidant activity of lemon balm by DPPH method and observed high activity and also confirm positively correlation between phenolics, flavonoids and DPPH radical scavenging activity. Many herbal also have antimicrobial activity, from this reason is potential, that application of herbal extracts to apple juice can prolong its stability. But for confirmation of this hypothesis are necessary antimicrobial assay in future.

Reducing power

For measurement of the reductive ability, the Mo^{VI} – Mo^{V+} transformation in the presence of samples was investigated. Reductive capabilities of prepared variants showed Fig. 2. Increase in absorbance of the reaction mixture indicated the reducing power of the samples. Reducing power of variants exhibited the following order: apple juice with salvia > apple juice with lemon balm > apple juice with wild thyme > apple juice with oregano > apple juice with mint > 100 % apple juice. The data presented here indicate that the marked reducing power of variants seem to be the result of their antioxidant activity. Strong reducing power was detected in variant 5 – apple juice with salvia (3127.34 ± 26.07 mg $TEAC.L^{-1}$) and variant 2 – apple juice with lemon balm (2995.60 ± 148.51 mg $TEAC.L^{-1}$). Similarly like DPPH method, higher antioxidant activity by reducing power was detected in enriched apple juice with compare to pure 100 % apple juice.

The reducing properties are generally associated with the presence of reductones **(Pin-Der, 1998)**. It is reported that the antioxidant action of reductones is based on the breaking of the free radical chain by donating a hydrogen atom, or reacting with certain precursors of peroxide to prevent peroxide formation. It is presented that the phenolic compounds in plants may act in a similar fashion as reductones by donating electrons and reacting with free radicals to convert them to more stable products and terminating the free radical chain reaction **(Liu and Yao, 2007)**. The leading phenolic compounds in apple juice are chlorogenic acid, caffeic acid, *p*-coumaric acid, ferulic acid, catechin, epicatechin, procyanidines (B1, B2, trimer C1), rutin and phloridzin. The type and amount of these phenolics show important variations with respect to the source species from which apple juice is derived **(Wu *et al*., 2007; Karaman *et al*., 2010).** Herbal plants are rich for polyphenols, mainly for phenolic acids and flavonoids. **Lu and Foo, (2001)** published that exist high positive correlation between polar polyphenols and phosphomolybdenum method (reducing power). In our study was used distilled water (polar solution) for prepare herbal extract and positive correlation was found between total polyphenol and phosphomolybdenum method ($P \leq 0.05$). Similarly, positive correlation between polyphenols and phosphomolybdenum method confirmed **Matkowski *et al*. (2008)** which evaluate biological activity of salvia water extracts.

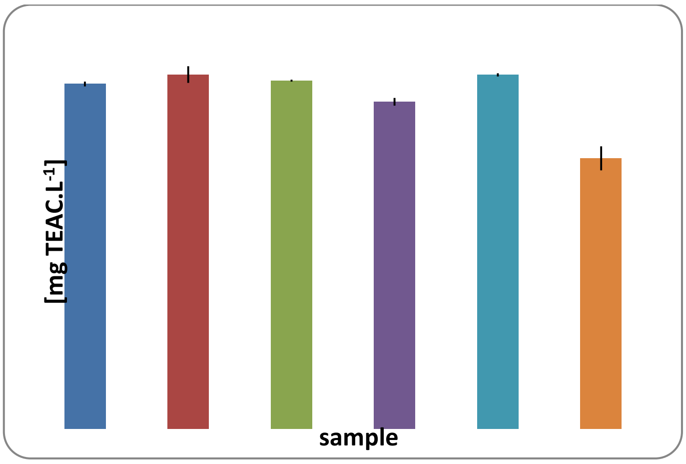

Figure 1 Radical scavenging activity of prepared variants expressed as mg Trolox equivalent antioxidant capacity per L of juice.
Variant 1 (60 % of apple juice + 40 % of mint water extract), variant 2 (60 % of apple juice + 40 % of lemon balm water extract), variant 3 (60 % of apple juice + 40 % of oregano water extract), variant 4 (60 % of apple juice + 40 % of wild thyme water extract), variant 5 (60 % of apple juice + 40 % of salvia water extract); 100 % AJ (100 % pure apple juice without herbal extract)

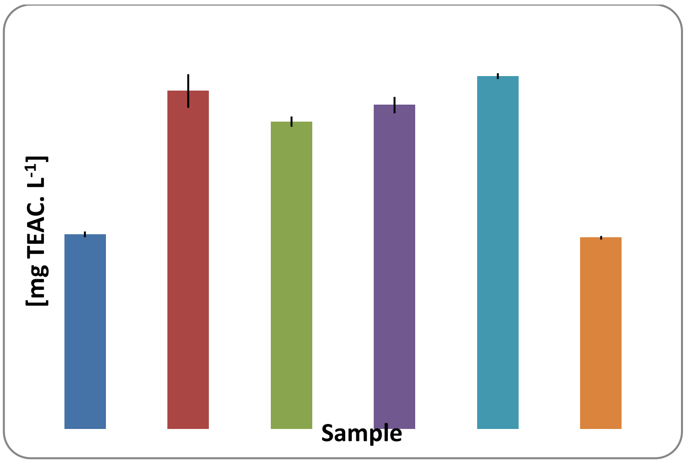

Figure 2 Reducing power of prepared variants expressed as mg Trolox equivalent antioxidant capacity per L of juice.
Variant 1 (60 % of apple juice + 40 % of mint water extract), variant 2 (60 % of apple juice + 40 % of lemon balm water extract), variant 3 (60 % of apple juice + 40 % of oregano water extract), variant 4 (60 % of apple juice + 40 % of wild thyme water extract), variant 5 (60 % of apple juice + 40 % of salvia water extract); 100 % AJ (100 % pure apple juice without herbal extract.

Table 1 The total polyphenol (TPC) and flavonoid content (TFC) of prepared variants

Sample	TPC [mg GAE.L^{-1}]	TFC [µg QE.L^{-1}]
Variant 1	268.11 ±2.22	81.12 ±1.29
Variant 2	362.56 ±5.75	112.34 ±2.05
Variant 3	500.70 ±6.70	149.08 ±0.05
Variant 4	315.89 ±3.69	108.27 ±0.04
Variant 5	342 ±5.09	185.50 ±2.05
100 % apple juice	125.52 ±5.59	63.07 ±0.02

Total polyphenol and flavonoid content

The results of total polyphenol and flavonoid content are presented in Tab. 1. Total polyphenol content of prepared variants decreased in this order: apple juice with oregano > apple juice with lemon balm > apple juice with salvia > apple juice with wild thyme > apple juice with mint > 100 % apple juice; total flavonoid content in this order: apple juice with salvia > apple juice with oregano > apple juice with lemon balm > apple juice with wild thyme > apple juice with mint > 100 % apple juice. Results showed that addition of herbal extracts to apple juice significantly increasee total polyphenol and flavonoid content. The higher content of these compounds was found in apple juice with oregano (500.70 ±6.70 mg GAE.L^{-1}; 149 ±0.05 µg QE L^{-1}), apple juice with lemon balm (362.56 ±5.75 mg GAE.L^{-1}; 112.34 ±2.05 µg QE L^{-1}) and apple juice with salvia (342 ±5.09 mg GAE.L^{-1}; 185.50 ±2.05 µg QE L^{-1}). The values of total polyphenol content were significantly and positively related with DPPH method and phosphomolybdenum method ($P \leq 0.05$).

Li *et al.* (2013) published, that for strong biological activity of salvia is responsible salvianolic acid B, tetramer of caffeic acid. Oregano is rich for caffeic, *p*-coumaric, *p*-hydroxybenzoic, ferulic, protocatechuic and rosmarinic acid and also for quercetin, catechin and epicatechin **(Vallverdú-Queralt *et al.*, 2014)**. Rosmarinic, caffeic, *m*-coumaric acid and naringin, naringenin and hesperetin were detected in lemon balm **(Dastmalchi *et al.*, 2008)**.

Results from this study showed that herbal extract, rich for biologically active compounds can be used in food industry as source of dietary antioxidant, whereas can be safer than synthetic analogues. In future is the assumption that will be an increased interest in spices, aromatic and medicinal plants as sources of natural antioxidants.

Sensory analysis

Sensory analysis of apple juice variants was determined by method compare of sample with standard (100 % apple juice). Results are demonstrated in Fig. 3. All evaluated variants reached approximately the same score. Generally we can summarize that prepared variants had better properties with compare to 100 % apple juice. The most intense, pleasurable herbal smell and taste was evaluated in variant 1 (apple juice with mint) and 5 (apple juice with salvia). The best results from total affect after drinking obtained variant 1 (apple juice with mint) and 4 (apple juice with wild thyme). Application of herbal extract into apple juice had not only positive biological effect but also positive sensorial effect.

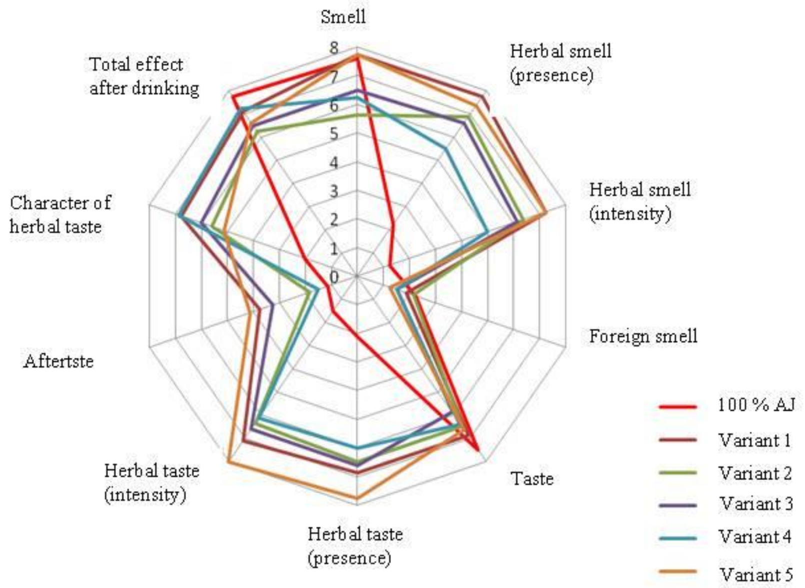

Figure 3 Sensory analysis of prepared variants
Variant 1 (60 % of apple juice + 40 % of mint water extract), variant 2 (60 % of apple juice + 40 % of lemon balm water extract), variant 3 (60 % of apple juice + 40 % of oregano water extract), variant 4 (60 % of apple juice + 40 % of wild thyme water extract), variant 5 (60 % of apple juice + 40 % of salvia water extract); 100 % AJ (100 % pure apple juice without herbal extract)

CONCLUSION

The biological data presented in this study clearly demonstrate that the herbal extracts are capable of antioxidant activity. This is of significant importance as it indicates that the extract may have the potential to prevent oxidative damage in human body. Herbal extract can be also used in food industry as potential sources of antioxidants for food preservation and nutritional quality improvement. Many herbal plants have positive sensory properties, and from this reason can be used as addition of several kinds of food (beverage, sweets). In future are necessary more studies of herbal extracts mainly antimicrobial, which can increase the possibility of use in food and pharmacology industry.

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