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Rose hip extract synergistically increase antioxidant activity of fruit and herb extracts

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In the current study, we investigated the possibility to use rosehip extract for synergistic elevation of the antioxidant activity of fruit and herb extracts. The obtained rosehip extract was characteristic with very high antioxidant activity measured by the ORAC method and the addition of the extract to chokeberry, blackberry, blackcurrants or elderflower extracts increased significantly their antioxidant activity. Moreover, there was synergistic effect in ORAC antioxidant activity of the mixed extracts that reached up to 64%. The removal of rosehip polysaccharides inhibited the synergistic effect, indicating that rosehip pectic polysaccharides are at least partially involved in the observed synergism. This opens the possibility to develop rosehip based functional beverages with increased antioxidant activity.

Key words: rosehip, synergism, antioxidant activity, ORAC, fruit, herbs

INTRODUCTION

Increasing amount of evidence indicates the role of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the pathophysiology of ageing and in the development of degenerative chronic diseases [1,2]. In human body, there is a protective antioxidant system, which impairs oxidative damage and keeps balance between pro-oxidants and antioxidants. However, because of many factors related to the modern way of life the balance is often broken which lead to a physiological state known as oxidative stress. In such cases to maintain the oxidative balance, an external intake of antioxidant with the diet is required. The definition for antioxidants depends on their application and probably the most widely accepted definition is "any substance that, when present at low concentration compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" [3]. In food science, term antioxidant has a broader meaning. From one hand, antioxidants are substances that prevent rancidity of foods. On the other hand, these compounds administered with the diet decrease the detrimental effects of ROS and RNS in our bodies [4]. Diet derived antioxidants include free radical scavengers, metal ion chelators, oxidative enzymes inhibitors, and cofactors of antioxidant enzymes. Studying antioxidants in foods is an important issue because of several reasons: Endogenous or added to foods antioxidants prevent food components from oxidation; Antioxidants taken with the diet are absorbed in human body and exert their beneficial effects *in vivo.* This undoubtedly was found for vitamin E and vitamin C [5], but more and more collected evidence indicates the same for polyphenol compounds [6]. It is quite often that compound with antioxidant activity taken with the diet reveals other health benefits like antiinflammatory effect, antiischemic effect, etc., besides antioxidant activity; Since absorption of phenolic compounds is incomplete, the majority passes through the gastro-intestinal tract (GIT) and enters the colon. Their local action may nevertheless be important because the intestine is particularly exposed to oxidizing agents and may be affected by inflammation and numerous diseases such as cancer. The diet contains various prooxidants, which may induce oxidative stress in the GIT, to induce stomach ulcer and develop stomach, colon, and rectal cancers [7].

The majority of antioxidants taken with the diet are of plant origin and fruit, vegetables, and herbs are the richest sources of natural antioxidants. The most important antioxidants in plants are polyphenols including flavonoids, carotenoids, vitamin C, vitamin E, etc. [1,2,8]. There are numerous studies in the literature demonstrating the correlation between consumption of fresh fruits and vegetables with the prevention of socially significant diseases including cancer, cardiovascular diseases,

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Alzheimer disease, etc. [9,10]. Several epidemiologic studies point out that the consumption of polyphenol-rich foods and beverages is associated with lower risk of oxidative stress-related diseases [9,11]. At the laboratory of biologically active substances, Institute of Organic Chemistry with Centre of Phytochemistry, we are dedicated to the development of functional foods, rich in natural antioxidants. In searching of suitable raw materials for functional beverages, we previously investigated the antioxidant activity of 26 Bulgarian fruits [12]. Rosehip appeared to be among the fruits with the highest antioxidant activity and suitable raw material for production of functional foods with antioxidant activity. From the literature, it is known that often plant extracts consisting of several herbs or fruits exert synergistic effect in their antioxidant activity [13]. Synergism is defined as „interaction between two or more entities, factors, substances, etc. that produces an effect greater than the sum of their individual effects". In the current study, we decided to further explore the applicability of rosehip fruits for the production of mixed herb and fruit extracts with synergistic action in the antioxidant activity. Therefore, the aim of the current work was to investigate the synergism in the antioxidant activity of fruit (chokeberry, blackberry, blackcurrants) and herb extracts (elderflowers) mixed with rosehip fruit extract.

EXPERIMENTAL

Plant materials

All fruit and herb samples were collected from the region of Rhodopi mountain in the stage of full maturity in 2012. The following fruits were used: rosehip, chokeberry, blackcurrant and blackberry. Chokeberries were cultivated, while other fruits were widely collected in the nature. Fresh fruits were frozen immediately and freeze dried in a Cryodos-50 laboratory freeze drier (Telstar Industrial, Spain). Elderflowers (*Sambucus nigra)* were collected from the region of Ravnogor (Rhodopi mountain) in 2012. Fresh flowers were dried at room temperature and dry drug was stored in paper bags at ambient temperature prior to analysis.

Extraction

Plant materials were subjected to extractions under the following conditions:

Fruits: 70 g fruits (rosehip, chokeberry, blackcurrant or blackberry) were mixed with 200 ml warm water (60˚C). Mixtures were homogenized at turbulent conditions and remained at ambient temperature for overnight. After that mixtures were centrifuged (6 000 x *g)* and supernatants were collected for antioxidant activity determination.

Elderflowers: 1 g of dried elderflowers were mixed with 100 ml warm water (60˚C) Mixture was infused at room temperature for 24 hours, centrifuged (6 000 x *g)* and supernatant was collected for antioxidant activity determination.

Removal of rosehip extract polysaccharides

Rosehip extract polysaccharides were removed via the following procedure: 100 ml extract were mixed with 300 ml 96% ethanol. The mixture was left in refrigerator overnight and centrifuged (6000 x *g*). The clear supernatant without polysaccharides was collected and investigated for antioxidant activity. Precipitated polysaccharides were collected and dried in a laboratory dryer.

Antioxidant activity

Oxygen Radical Absorbance Capacity (ORAC) assay: ORAC was measured according to the method of Ou *et al.* [14] with some modifications described in details by Denev *et al.* [15]. The method measures the antioxidant scavenging activity against peroxyl radical generated by thermal decomposition of 2,2'-azobis[2-methyl-propionamidine] dihydrochloride (AAPH) at 37°C. Fluorescein (FL) was used as the fluorescent probe. The loss of fluorescence of FL was an indication of the extent of damage from its reaction with the peroxyl radical. The protective effect of an antioxidant was measured by assessing the area under the fluorescence decay curve (AUC) relative to that of a blank in which no antioxidant has been present. Solutions of AAPH, fluorescein and trolox were prepared in a phosphate buffer (75 mmol/l, pH 7.4). Samples were diluted in the phosphate buffer as well. Reaction mixture (total volume 200 μl) contained FL - (170 μl, final concentration 5.36×10^{-8} mol/l), AAPH - (20 μl, final concentration 51.51 mmol/l), and sample - 10 μl. The FL solution and sample were incubated at 37°C for 20 min directly in a microplate reader, and AAPH (dissolved in buffer at 37°C) was added. The mixture was incubated for 30 s before the initial fluorescence was measured. After that, the fluorescence readings were taken at the end of every cycle (1 min) after shaking. For the blank, 10 μl of phosphate buffer were used instead of the extract. The antioxidant activity was expressed in micromole trolox equivalents (µmol TE) per liter of extract. Trolox solutions (6.25; 12.5; 25 and 50 µmol/l) were used for defining the standard curve. ORAC analyses were carried out using a FLUOstar OPTIMA plate reader (BMG Labtech, Germany), excitation wavelength of 485 nm and emission wavelength of 520 nm were used.

Statistics

All antioxidant activity tests were repeated 4 times. The results are expressed as means ± standard deviation (SD).

RESULTS AND DISCUSSION

The Oxygen Radical Absorbance Capacity (ORAC) antioxidant activity of the investigated fruit and herb mixed extracts is presented in Table 1. It is evident from the results that the obtained rosehip extract and mixed extracts are very rich source of natural antioxidants, rendering very high ORAC values. In one of the first attempts to quantify dietary antioxidant needs of the body, Prior *et al.* [16] demonstrated that consumption of certain berries and fruits such as blueberries, mixed grape and kiwifruit was associated with increased ORAC plasma antioxidant capacity in the postprandial state. They estimated that according to the energy intake of the diet, 5000-15000 µmol TE are necessary to cover human daily antioxidant needs. This indicates that the obtained mixed extracts in our study could serve as functional beverage with high antioxidant activity. For example, only 100 ml of rosehip extract will supply the body with more than 13000 ORAC units. Furthermore, we observed a synergistic effect in the antioxidant activity of the fruit and herb extracts, when mixed with rosehip extract. The effect was concentration-dependent and was up to 64% in the case of mixed rosehip-blackberry extract. The observed synergistic effect opens new possibilities for the production of novel functional foods with increased antioxidant activity. The used fruits and elderflower contain various components with antioxidant activity [7], which could be one of the reasons for the observed synergistic effect. There are reports in the literature that compounds with antioxidant exert synergism in their action when acting together. For example, there was synergism in the common antioxidant activity of β-carotene and α-tocopherol [17]. In another study synergistic effect expressed as elevated lipid peroxidation inhibiting activity was observed for α-tocopherol and quercetin, and quercetin with rutin [18]. Significant synergistic effect was found also between the action of lycopene-lutein, lycopene-β-carotene, and α-tocopherol-β-carotene [19].

Table1. Synergism between rosehip extract and fruit and herb extracts.

Extracts Ratio, %		**ORAC, µmolTE/l (experimental)**	**ORAC, µmolTE/l (expected)**	**synergism, %**
Rosehip	**Chokeberry**			
100	0	132408.8 ± 4058.2	na	na
0	100	15429.2 ± 1405.2	na	na
75	25	121429.4 ± 6504.2	103163.9	17.7
50	50	92725.8 ± 6504.2	73919.0	25.4
25	75	51937.2 ± 2987.6	44674.1	16.3
Rosehip	**Blackberry**			
100	0	134889.6 ± 5049.6	na	na
0	100	12463.0 ± 985.2	na	na
75	25	118570.7 ± 5012.3	104283.0	13.7
50	50	91097.9 ± 3456.2	73676.3	23.6
25	75	70614.1 ± 4022.2	43069.6	64.0
Rosehip	**Blackcurrants**			
100	0	134889.6 ± 5049.6	na	na
0	100	12463.0 ± 1012.8	na	na
75	25	125698.3 ± 5069.8	103836.1	21.1
50	50	102088.1 ± 3985.6	72782.7	40.3
25	75	58014.7 ± 3046.7	41729.2	39.0
Rosehip	**Elderflower**			
100	0	126953.9 ± 5056.3	na	na
0	100	6213.8 ± 456.2	na	na
75	25	109138.4 ± 4834.4	96768.8	12.8
50	50	77050.6 ± 3695.8	66583.8	15.7
25	75	43820.8 ± 2865.7	36398.8	20.4

na – not applicable

Table2. Synergism between chokeberry extract, elderflower extract and rosehip extract with removed polysaccharide fraction.

Extracts Ratio, %		ORAC, μmolTE/l (experimental)	ORAC, μmolTE/l (expected)	synergism, %
Rosehip without polysaccharide	**Chokeberry**			
100	0	68635.6 ± 3089.9	na	na
0	100	12815.3 ± 952.1	na	na
75	25	51129.4 ± 2054.7	54680.5	-6.5
50	50	39223.3 ± 1114.2	40725.4	-3.7
25	75	26223.5 ± 2005.7	26770.4	-2.0
Rosehip without polysaccharide	**Elderflower**			
100	0	68635.6 ± 3089.9	na	na
0	100	6213.8 ± 456.2	na	na
75	25	50088.0 ± 3054.2	53030.2	-5.5
50	50	35484.2 ± 1865.2	37424.7	-5.2
25	75	20369.4 ± 1024.5	21819.2	-6.6

na – not applicable

Table3. Synergism between crude polysaccharide isolated from rosehips and chokeberry and elderflower extracts.

Extracts Ratio, %		ORAC, μmolTE/l (experimental)	ORAC, μmolTE/l (expected)	synergism, %
Crude rosehip polysaccharide (7mg/ml)	**Chokeberry**			
100	0	24508.6 ± 1256.3	na	na
0	100	12815.3 ± 957.2	na	na
75	25	23442.1 ± 1002.4	21585.3	8.6
50	50	18268.7 ± 854.2	18662.0	-2.1
25	75	15116.5 ± 802.2	15738.6	-4.0
Crude rosehip polysaccharide	**Elderflower**			
100	0	68635.6 ± 1256.3	na	na
0	100	6213.8 ± 456.2	na	na
75	25	20466.1 ± 1235.8	19934.9	2.7
50	50	21850.4 ± 1078.9	15361.2	42.2
25	75	12068.5 ± 605.2	10787.5	11.9

na – not applicable

Another example for synergism in antioxidant activity of pure compo-unds is the ability of rutin and lycopene, and rutin and luteolin to inhibit synergistically the oxidation of LDL cholesterol [20]. Yi *et al.* [21] found out that α-tocopherol and ascorbic acid act in synergism in a model of fish oil oxidation, and Maillard and Berset [22] observed synergism in the antioxidant activity of *p*-coumaric and ferulic acids. Similar effect was observed for caffeic and ascorbic acids, as well [23]. Meyer *et al.* [24] found synergism in mixtures of phenolic acids and flavonoids. Except for pure compounds, synergistic antioxidant action was observed after mixing plant extracts with pure phytochemicals. For example, Hait Darshan *et al.* [25] observed synergistic effect in the antioxidant activity of polyphenol extract from spinach and ferulic acid, caffeic acid, and epigallocatechin-3-gallate. Interes-ting is the synergistic common action of the three active components in the Chinese formula ShengMai San, which is one the most prescribed remedy in the traditional Chinese medicine [26]. Synergistic anti-oxidant effect was observed also for great burdock mixture with α-tocopherol [27] and ascorbic acid with grape seed extract [28]. The polyherbal extracts obtained with green tea also showed higher antioxidant activity compared to the separate components [13]. Chu and Hsu [29] reported 2-3 fold increment of antioxidant activity of peanut oil when using mixtures of antioxidants.

It is well known that rosehips are rich source of various classes of biologically active substances. Besides polyphenols, ascorbic acid and carotenoids that reveal antioxidant activity, fruits contain minerals and sustainable amount of pectic poly-saccharides. In order to check whether rosehip polysaccharides take part in the synergistic action we removed the polysaccharide fraction by ethanol precipitation and measured again the antioxidant activity of the obtained mixed extracts. Results are shown in Table 2. As it is evident from the results, after the removal of the pectic fraction, the synergistic effect was lost. Furthermore, to explore this effect we isolated crude pectic polysaccharide from rosehip extract and combine it with the tested chokeberry and elderflower extract (Table 3). The obtained results show that in the case of elderflower the synergistic effect was partially due to the interaction with polysaccharides.

Several authors have already observed the synergistic effect in the biological activity of polyphenols and polysaccharides. The health effects of apples, especially their cholesterol-lowering properties, were first ascribed to the content of pectin [30-32]. In more recent studies, Aprikian *et al.* [33] found that apple pectin and polyphenolic fractions lowered plasma, liver cholesterol and triglycerides, and were more effective together than were either apple pectin alone or apple phenolics alone. In the intact fruit, the fibres and the phenolic compounds are closely associated; they could reciprocally affect digestibility and possibly exert synergistic effects. The lyophilized apple diet lowered plasma and LDL cholesterol in rats and reduced triglyceride accumulation in heart and liver [34]. Many works [31-33] suggest that there is a beneficial interaction between fruit fibre and polyphenolic components and also support the benefits of eating whole fruits as opposed to dietary supplements.

CONCLUSION

Rosehips are raw material with high antioxidant activity and from the presented results, it is evident that they synergistically increase ORAC antioxidant activity of fruit (chokeberry, blackcurrant and blackberry) and herb (elderflower) extracts. The observed synergistic effect is at least partially due to the presence of pectic polysaccharides in rosehips. However, future studies are necessary to fully investigate this phenomenon. The obtained results indicate that rosehips could be used for the development of functional beverages with increased antioxidant activity. From one hand, this will increase the oxidative stability of the beverages, and from other, probably will increase the health benefits from the consumption of these beverages.

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ЕКСТРАКТ ОТ ШИПКА СИНЕРГИЧНО ПОВИШАВА АНТИОКСИДАНТНАТА АКТИВНОСТ НА ПЛОДОВИ И БИЛКОВИ ЕКСТРАКТИ

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(Резюме)

В настоящата работа е изследвана възможността за използване на екстракт от шипкови плодове за синергично повишаване на антиоксидантната активност на плодови и билкови екстракти. Екстрактът от шипки се характеризира с много висока антиоксидантна активност, измерена посредством ORAC метода, а прибавянето му към екстракти от плодове на арония, къпина, черен касис или цвят от бъз повиши значително тяхната антиоксидантна активност. Освен това бе наблюдаван и синергичен ефект в антиоксидантната активност на смесените екстракти, който достигна до 64%. Отстраняването на полизахаридите от шипковите плодове инхибира синергичния ефект, което е индикатор, че пектиновите полизахариди от шипката играят роля в синергичното действие. Наблюдаваният синергичен ефект отваря нови възможности за разработване на функционални напитки с повишена антиоксидантна активност.