STUDIA UBB CHEMIA, LVII, 1, 2012 (p. 75 - 82)

ANALYSIS OF SOME NUTRITIONAL SUPPLEMENTS DERIVED FROM *SEA BUCKTHORN* AND *BLACK CURRANT*

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ABSTRACT. The interest in the consumption of fresh fruits, products and nutritional supplements made from them is due to their content of bioactive nutrients and their importance as dietary antioxidants. *Sea Buckthorn* and *Black Currant* were used in both Europe and Asia for centuries. In this context, the aims of this work are to obtain a TLC fingerprinting of some nutritional supplements derived from *Sea Buckthorn* and *Black Currant* in order to establish their origin and conditioning type and, also, to correlate their antioxidant capacity with their content of biologically active compounds.

Keywords: *Antioxidant capacity, Total polyphenolic content, Nutritional Supplements, TLC, Fingerprinting, Sea Buckthorn, Black Currant.*

INTRODUCTION

Many human diseases including different neurodegenerative disorders and cancers are considered to be due to the oxidative damages caused by the free radicals and reactive oxygen species. Antioxidants, produced by the human body or available through the diet, can protect human cells against these oxidative damages.

Studies have shown that polyphenols significantly contribute to the total antioxidant activity of many fruits and vegetables, with high flavonoid content [1]. Polyphenols are one of the most common classes of phytochemical compounds extremely important in terms of morphological and physiological characteristics [2]. The biological importance of polyphenolic compounds is closely related to their antioxidant, anti-allergic, anti-inflammatory and anti-microbial properties, as well as to their cardio-protective and vasodilator effects [3, 4]. All these led interest in discovery and identification of new such compounds, as well as on their exploitation. These results in the increased

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consumption of fruits, vegetables and products obtained from them and in the appearance on market of a high number of nutritional supplements with high contents of polyphenols [5]. Even though many plants are containing polyphenolic compounds with important health effects, their consumption is still seldom.

Sea Buckthorn (*Hippophae rhamnoides L.*) is a perennial shrub belonging to the *Elaeagnacaea* family. Over the world, there are known 6 species and 12 subspecies. Commercially available *Sea Buckthorn* is a hardy, multipurpose plant with orange, red or yellow berries with a strong ability to fix atmospheric nitrogen. Their importance is related to the prevention ability against cardiovascular diseases and cancer, as well as in treatment of skin problems, burns, digestive tract disorders, senility, inflammation, radiation and to improve the capacity of the immune system [6]. *Sea Buckthorn* fruits are used in food industry, pharmacy and cosmetics due to their high content in vitamin C (over 400-800 mg/g of fresh juice), and content of A, B1, B2, B6, B9, E, K, PP and F vitamins. *Sea Buckthorn* fruits also contain bioactive compounds such as cellulose, β- carotene, Ca, Mg, K, Na, proteins and different complex oils [7].

Black Currant (*Ribes Nigrum*) belongs to the *Grossulariaceae* family being widespread in the temperate areas of Europe and Western Asia. It grows spontaneously in shrubs, in alpine forests, but it is also cultivated for its fruits and leaves, which are used in medicine because of their content in biologically active compounds. *Black Currant*'s leaves contain tannins, vitamin C and traces of green essential oils. Their fruits contain about 150mg/100 g of vitamin C, B vitamins complex, organic acids (citric acid, malic acid), fat oils, sugars, anthocyanins, flavonoids, Ca, etc [8].

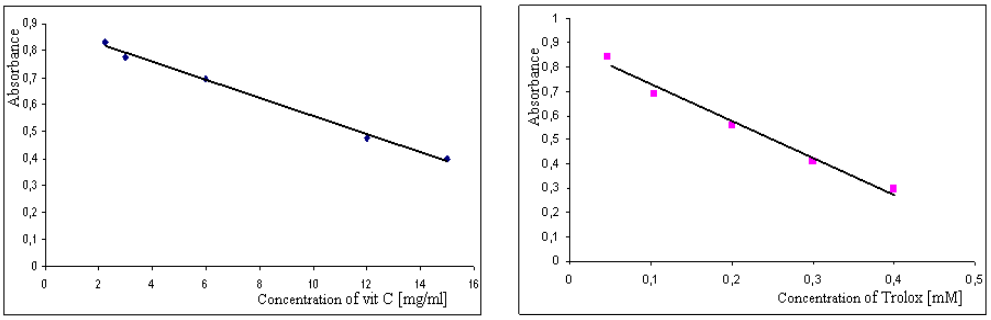
Recently, due to the modern analytical techniques more and more studies were made on different fruits and nutritional supplements obtained from them because of their high content in different biological active compounds. There is an increasing interest in the use of chromatographic methods for the analysis of chemical compounds from Sea Buckthorn and Black Currant [9], thin layer chromatography (TLC) being frequently used for the separation and the determination of natural constituents [10].

The aim of this study is the evaluation of the antioxidant activity and of the total polyphenolic content and the TLC fingerprinting of some nutritional supplements obtained from *Sea Buckthorn* and *Black Currant,* in order to establish their origin and the conditioning type.

RESULTS AND DISCUSSION

The analyzed nutritional supplements were: alcoholic tincture of *Sea Buckthorn* and *Black Currant* (15% in 30% ethanol), dried fruits, iso-maltose impregnated with juice of *Sea Buckthorn* and *Black Currant*, and *Sea Buckthorn* and *Black Currant* balsamic vinegar.

In the first step, two complementary methods, namely DPPH [11] and ABTS [12] were used for determination of the antioxidant activities of these nutritional supplements. The antioxidant capacities were expressed as vitamin C or trolox equivalents on the basis of calibration curves (figure 1). The results are presented in Table1.



a **b**

Figure 1. The calibration curves for: a - DPPH method (y=-0,0336x+0,8924; r=0,9973); b - ABTS method (y=-1,5124x+0,8784; r=0,9818).

The experimental results (Table 1) show that DPPH and ABTS methods provide similar results concluding that both methods could be used for the determination of the antioxidant activity of nutritional supplements. Also, it can be observed that in both cases the *Black Currant* nutritional supplements possess a higher antioxidant activity than those obtained from *Sea Buckthorn*. It can be remarked that all the iso-maltose impregnated with fruit juice supplements have the lowest antioxidants activity.

The total polyphenolic contents of the nutritional supplements were determined using the Folin-Ciocalteu method and were expressed in μg gallic acid/mL extract (Table 1), using the calibration curve (Figure 2). The experimental results show that the nutritional supplements derived from *Black Currant* contain the highest level of polyphenols, while the *Sea Buckthorn* nutritional supplements contain a lower quantity of polyphenols. Also, iso-maltose impregnated with fruit juice supplements contains the lowest quantity of polyphenols.

The total phenolic contents are correlated with the antioxidant activities determined both by DPPH and ABTS assays, considering each analyzed nutritional supplements.

In the second step, the TLC analysis of same nutritional supplements derived from *Sea Buckthorn* and *Black Currant* were used in order to obtain their fingerprint. The TLC fingerprints offer valuable information regarding the active compounds present in the samples and the semi-quantitative estimation of their composition.

Table 1. The antioxidant activity and total polyphenolic content of analysed nutritional supplements

No.	Samples	Antioxidant activity		Total polyphenolic content μg gallic acid /mL
		mg vit C/mL	μmol trolox/mL	
1.	Balsamic vinegar with Sea Buckthorn	135.6	1.050	681
2.	Alcoholic extract of Sea Buckthorn	110.0	0.649	590
3.	Dry Sea Buckthorn	86.6	0.281	584
4.	Lyophilized Sea Buckthorn extract	34.8	0.328	153
5.	Isomalt + Sea Buckthorn juice	3.5	0.039	28
6.	Balsamic vinegar with Black Currants	627.4	6.261	2203
7.	Alcoholic extract of Black Currants	319.8	4.578	1951
8.	Dry Black Currants	708.8	1.393	1175
9.	Dry Black Currant extract	549.0	4.549	1832
10.	Isomalt + Black Currant juice	0.9	0.010	27
11.	Isomalt + Sea Buckthorn juice + Black Currant juice	0.2	0.001	14

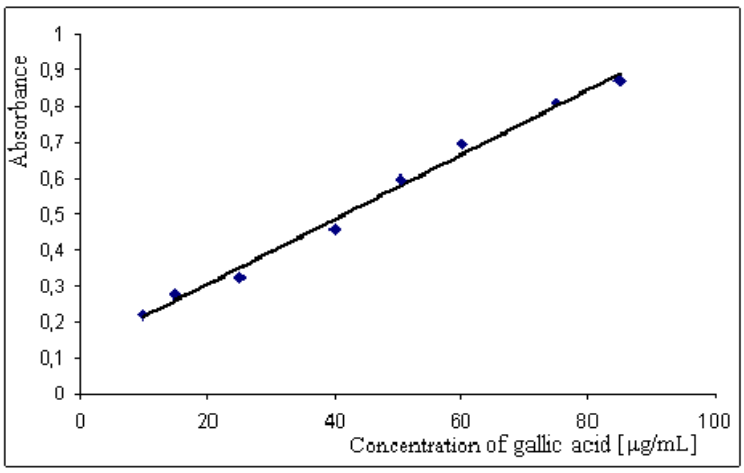


Figure 2.The calibration curve for determination of total polyphenolic content ($y=0.009x+0.1247$; $r^2=0.9854$).

The TLC separation of analyzed supplements (Figure 3) showed that even some components occurred in every sample, there are significant differences related to the composition of the samples and to the concentration of bioactive compounds.

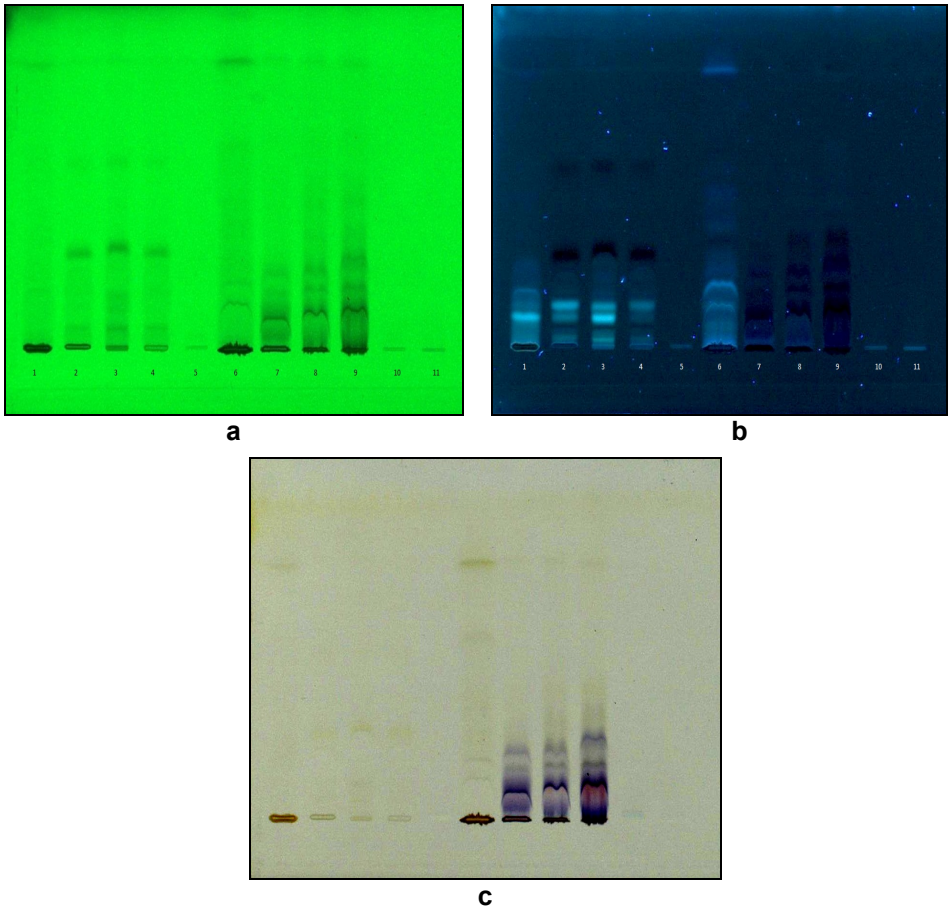


Figure 3. The fingerprint of samples (according to table1) in:
UV light at a - 254nm and b – 366nm; c- VIS light.

The comparison between the fingerprints of supplements obtained from the same fruit revealed that the composition depends on their conditioning type. TLC fingerprinting method allows the analysis of bioactive compounds in different nutritional supplements and could be applied not only to their chemotaxonomic classification but also in qualitative and semi-quantitative determinations.

CONCLUSIONS

DPPH and ABTS methods can be applied for the determination of the antioxidant activity of nutritional supplements derived from *Sea Buckthorn* and *Black Currant*, the obtained results being similar. The results showed that the antioxidant activity and the total polyphenolic content of the supplements obtained from *Black Currant* are higher than those derived from *Sea Buckthorn*. The supplements obtained by impregnation of iso-maltose with fruit juice have the lowest antioxidant activity and polyphenolic content irrespective of fruits. These results confirm that the antioxidant activity and polyphenolic content depend on conditioning type of supplements.

The chromatographic fingerprintings show differences between the nutritional supplements derived from *Sea Buckthorn* and those derived from *Black Currant.* The analyzed supplements contain different quantities of bioactive compounds and their concentration depends on their conditioning type. The nutritional supplements based on iso-maltose contain the smallest amount of active compounds, while those based on *Black Currant* contain the highest concentration of active compounds.

EXPERIMENTAL SECTION

Materials and apparatus

All chemicals and reagents were analytical grade and were purchased from Merck Germany. Chromatographic plates were purchased from Merck (Darmstadt, Germany).

Fresh fruits and nutritional supplements derived from *Sea Buckthorn* and *Black Currant* (alcoholic tincture of *Sea Buckthorn* and *Black Currant*, dried fruits, iso-maltose impregnated with juices of *Sea Buckthorn* and *Black Currant*, and *Sea Buckthorn* and *Black Currant* balsamic vinegars) were purchased from Proplanta SRL Cluj-Napoca.

All spectrophotometric measurements were performed in triplicate using a T80+ spectrophotometer (PG Instruments). Lyophilizations were performed on freeze dry system - Freezone 25 PLUS (LABCONCO).

The samples were applied on chromatographic plate using an automatic device LINOMAT 5 (Camag) and the photo-documentation of the developed plates was done using a documentation device REPROSTAR3 (Camag).

Sample preparation

Fresh fruits were dried either by drying in oven at 80^0C or by lyophilization at 0.2mbar and -80°C. Alcoholic extracts were prepared by maceration for ten days of 1g dried fruits with 10mL of ethanol-water 8:2 (v/v). The supplements

conditioned on *iso*-maltose were obtained by the impregnation of 500 g *iso*-maltose with 100 mL of fruit extract (15% in ethanol 30%) followed by solvent evaporation. 1g of each supplements were extracted with 5mL ethanol-water 8:2 (v/v) mixture by maceration for 10 days. The liquid supplements were used without any preparation.

Methods

Determination of the antioxidant activity and the total phenolic content

The DPPH and $ABTS^+$ spectrophotometric assays were used to determine the antioxidant activity of the analyzed samples.

Determination of antioxidant activity using DPPH: 0.15mL of sample were added to 3mL DPPH solution (0.09 mg/mL) and after 15 minutes the absorbance of mixtures was read at 517 nm. The blank sample was prepared from 0.15mL sample solution and 3mL distilled water. Standard solutions of vitamin C (2–15mg/mL) were used to obtain the calibration curve [11]. The results were expressed as mg of ascorbic acid/ per mL of extract.

Determination of antioxidant activity using ABTS: the $ABTS^{\bullet+}$ was obtained from the reaction of 1:1 (v/v) solution of ABTS diammonium salt (7mmol/L) with $K_2S_2O_8$ solution (2.45mmol/L). The reaction mixture was incubated for 24h at room temperature, in the dark. 0.1mL of sample were added to 3mL $ABTS^{+\bullet}$ solution and the absorbance was read at 734nm after 15 minutes against a blank solution containing 0.1 mL sample and 3 mL distilled water. Standard solutions of Trolox (0.05–0.4μmol/mL) were used to obtain the calibration curve [12]. The results were expressed as μmol of Trolox per mL of extract.

Total polyphenolic content: 0.3mL sample were mixed with 1.5mL Folin–Ciocalteu reagent (0.2N) for 5min and then 1.2mL Na_2CO_3 solution (0.7M) were added. All samples were incubated at room temperature in the dark for 2h and their absorbance was read at 760nm against blank solution containing 0.3mL of sample solution and 2.7mL distilled water. Standard solutions of gallic acid (0–100μg/mL) were used for calibration curve. The results were expressed as μg of gallic acid per mL of extract.

Chromatographic analysis

The TLC analyses of the nutritional supplements were performed on silica gel $60F_{254}$ plate (20x10cm) using a mixture of ethyl acetate: methanol: formic acid: acetic acid: water 80:10:1:1:8 (v/v/v/v/v) as mobile phase. Samples (10μL) were applied as 6 mm bands at 1.5cm from the low edge with a rate of 80nL/s. The chromatographic plate was developed on a distance of 85 mm in N chromatographic chamber pre-saturated for 30 min. The detection was done in UV light (254nm and 366nm) and in VIS light.

ACKNOWLEDGMENTS

This research is supported by National Council for Programs Management (CNMP) through the PNII- PARTENERIATE research grants 52146/2008.

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