Biological Compounds with Antioxidant Activity Found in Hippophae rhamnoides, Ribes nigrum and Vaccinium myrtillus Juice

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Abstract. In conditions in which using plants with protection properties has almost become a "fashion" of modern society, we believe that scientific research is necessary to confirm the existence of these useful substances for health in this plants. With modern methods of scientific research were identified substances with brawl antioxidant activity in juice of Hippophae rhamnoides, Ribes nigrum and Vaccinium myrtillus. The results confirm the existence of compounds with antioxidant activity in plant products analyzed, and beneficial effects on human health that consumption of vegetable produce. However, should be avoided the approach where plants are a universal "panacea".

Keywords: food; antioxidants; juice Hippophae rhamnoides, Ribes nigrum and Vaccinium myrtillus.

INTRODUCTION

Generic and simplest definition of an antioxidant (AO) is derived from semantic name: substance that inhibits oxidation. Prevention of oxidative damage is a very topical subject. Fundamental goal of current research on antioxidants is to understand the events culminating in the incident and developing chemical and nutritional methods to mitigate or prevent the pathological condition induced by oxidative stress. This is a multidisciplinary task, involving the efforts of chemists, biologists and clinicians. (Aloisi, 2011)

Every year people spend large sums of money for the purchase of vitamins and minerals, which can be bought from pharmacies without a prescription, including well-known antioxidants such as vitamins A, C, E, beta carotene and selenium. However, the effectiveness of plant protection products was not confirmed in all cases by scientific researchers. A supplement to "promises" as coenzyme Q10 that can keep within certain limits, the LDL oxidation. This study, conducted in the doctoral training period, shows that many types of biological compounds with antioxidant found in many plant products such as berries, blackberries, underbrush, black currents, carrots and more.

Working hypothesis for this study is to establish opportunities for efficiency therapy with nutritional and non-nutritional natural antioxidants and its use as a therapeutic alternative, since the important physiological and therapeutic effects of antioxidant active substances of plant products have been proven and confirmed by therapeutic practice and medical research. The study also presents an evaluation of analytical methods for separation, detection and dosing of active substances with antioxidant properties of plant products, making short chemical characterization and quantification of levels of these substances found in different plants and different types of food. This paper aims to analyze in terms of physiological effects, pharmacological and therapeutic antioxidant several active substances (SAAO) natural, plant origin, identified in juice Hippophae rhamnoides, Ribes nigrum and
Vaccinium myrtillus, and highlight the possibility to optimize therapy with AO and its use as a natural therapeutic alternative viable and effective.

Study of antioxidant activity of plants and plant products has been and is in the concerns of many researchers around the world (Heinonen, 2007; Zadernowski, 2005, Seeram, 2008; Pisoschi and Negulescu, 2011; Antal, 2012, and others). In the food industry most of blueberry, blackberry and sea buckthorn are processed to juice. This juice can be used directly for human consumption or as a food ingredient (Shipp, 2010)

The main objectives of this study were:

A. Analysis of active substances with antioxidant properties (SAAO) in the composition of plant products from three plants (sometimes used one, two or more plant products from the same plant, such as, for example: the folium - leaf and fructus - fruit of the species *Ribes nigrum*) and their classification in two categories: nutritional and non-nutritional antioxidants. In this stage of work will quantify the antioxidant efficiency and establish the mechanism of protection provided (treatment of free radicals and prevent their formation processes of damage repair, etc.);

B. Establishment of experimental methods for determining and evaluating the quality and quantity of antioxidants review, which will be implemented in the laboratory, "in vitro";

C. Tests the effectiveness of antioxidants (AO) analyze natural AO compared to the efficiency of artificial AO (synthetic); AO will introduce natural combination in diagrams to analyze and evaluate the synergism of their action.

MATERIALS AND METHODS

The material in this case study consists of three natural products as *Hippophae rhamnoides*, *Ribes nigrum* and *Vaccinium myrtillus* juice, obtained in laboratories Hypericum Baia Mare, Maramures district. Quantitative analysis of the content of antioxidant substances above products have been completed at the University of Agricultural Sciences and Veterinary Medicine Cluj - Napoca, Department of Chemistry and Biochemistry.

The methods of antioxidant capacity evaluation, including spectrometry, chromatography and electrochemical techniques are detailed with respect to principles and analytical performances. From this research were used as research methods: titrimetric method for determination of vitamin C; spectrophotometric method for determination of total carotenoids and total anthocyanins; HPLC method for separation and identification of carotenoids; HPLC-PDA method for the separation and identification of anthocyanins, phenolic acids and flavonoids; striking and TEAC methods to determine antioxidant activity.

In the research conducted were recorded UV - Vis the wavelength range 650 - 220 nm for each sample of juice, after taking in 95% methanol acidified with 1% HCl and diluted 50 - fold concentrated juice blueberries and blackberries, 10 times that of sea buckthorn juice (dilution was done with solvent extraction). Spectrophotometer was used UV - Vis double beam, Jasco model V 530.

Total anthocyanins content of three juices (of sea buckthorn, blackberries and blueberry), obtained in laboratories Hypericum Baia Mare, was determined by spectrophotometric method. Thus, were recorded the UV - Vis spectra wavelength range 650 - 220 nm for each sample of juice in hand, after taking in 95% methanol acidified with concentrated HCl 1% dilution of 50 times for cranberry and blackberries juice (dilution was done with solvent extraction). Spectrophotometer was Jasco, model V 530. Fingerprint was calculated based on spectrophotometric total amount of anthocyanins in samples of blueberry and blackcurrant juice using the formula: \( mg / l = (A_{\text{max}} \times V \times \text{Dil}) \times 1000 / E_{\text{l cm}, \lambda_{\text{max}}} \), where: \( A_{\text{max}} \) - absorbance read at \( \lambda \) max (535 nm), \( V \) - volume of sample (ml), \( \text{Dil} \) - dilution factor, \( E_{\text{l cm}, \lambda_{\text{max}}} \) - molar extinction coefficient, which refers to the absorbance value
measured in 1 cm cell at $\lambda$ max for 1% concentration ($E_{\text{medium \ anthocyanins}} = 850$).

For extraction of vitamin C were mixed with 5 ml juice 5 ml HCl 2% of this mixture was pipetted into a vial 1 ml Erlenmeyer, over which was added distilled water, potassium iodide, hydrochloric acid and starch. It titrated with potassium iodide solution to blue. Color should persist 30 seconds. The amount of vitamin C was calculated using the formula: 

$$\text{Vitamin C (mg / l)} = (N \times 5 \times 0.352 \times \text{Dil} \times 1000) / V,$$

where: $N$ - ml potassium iodide used in titration, $V$ - ml juice used in analysis, Dil - dilution factor, 0.352 - titre solution.

**Determination of total polyphenols** from plant sources by measuring the optical density was a primary extract, by complexation with Folin - Ciocalteu reagent absorbs in the Vis wavelength $\lambda = 750$ nm. The determination of total polyphenols from plant sources by means of Folin - Ciocalteu reagents were used: ethanol, analytical grade (40%), reagent Folin - Ciocalteu (0.1 N), sodium carbonate (7.5% solution), distilled water and gallic acid - standard. Polyphenols from fruit juices were taken repeatedly in 40% ethanol. Alcoholic extracts were vortex 1 minute, sonic 15 minutes, centrifuged for 10 minutes at 3000 revolutions / minute and was then filtered through qualitative filter paper. From the filtrate obtained is 1 ml, is placed in a 100 ml flask, add 60 - 70 ml of distilled water and shake. Add 5 ml reagent Folin - Ciocalteu and mix. After one minute and eight minutes before a 15 ml 7.5% sodium carbonate solution. Record this time as time "0" and mix again. Bring everything to 100 ml with distilled water. After 2 hours the absorbance read at $\lambda = 750$ nm samples from the control (blank).

For **determination of phenolic compounds** is used HPLC method. 1 ml juice sample was taken again in 10 ml 95% methanol acidified with 1% concentrated HCl. After vortexing 1 minute, 30 minutes sonication and centrifugation 10 minutes at 3000 revolutions / min, the supernatant was filtered through filter paper and filter size milipor 0.45 mm. HPLC separation of phenolic compounds in methanolic extracts juice was made into an Agilent 1200 HPLC system with UV - Vis detector. Mobile phase gradient was used, the rate of 1 ml / min. Solvent A was methanol / acetic acid / water at a rate of 10/2/88 (V / V) and solvent B was methanol / acetic acid / water, 90/3/7. Separation was done on a Supelcosil LC 18 column size 250 mm x 4.6 mm x 5 $\mu$m, and for each injection were used 20 ml extract. Temperature was 25 °C. Chromatograms were recorded at a wavelength of 280 nm. Phenolic compounds standard of purity 95 - 99% were dissolved in HPLC - grade methanol at a concentration of 0.1 mg / ml. The solutions obtained were injected into Agilent 1200 HPLC system under the same conditions as samples. Identification of phenolic compounds was done by comparing the retention times of standards with retention times of samples.

**Separation and identification of anthocyanins** in samples blueberry juice, sea buckthorn and blackberry, was achieved by HPLC method. 1 ml juice sample was taken again in 10 ml 95% methanol acidified with 1% concentrated HCl. After vortexing for 1 minute, 30 minutes sonication and centrifugation 10 minutes at 3000 revolutions / min, the supernatant was filtered through filter paper and filter size milipor 0.45 mm. HPLC separation of methanol extracts juice anthocyanins was performed in a Shimadzu HPLC system with DAD detector column - Luna Phenomenex (C18) of dimensions 250 x 4.6 mm, 5 $\mu$m. Chromatograms (Figure 7 and 8) were recorded at a wavelength of 520 nm. Mobile phase gradient was used, flow of 0.8 ml / minute at a temperature of 35 ° C. Solvent A was 4.5% formic acid / water, solvent B was acetonitrile and peak identification was done on sites the absorption spectra recorded with the photodiode detector (PDA).

**Total carotenoids** were extracted from 10 g of sea buckthorn juice (Hippophae rhamnoides) with a mixture of 20 ml acetone / ethanol / hexane 1:1:2 (v / v / v) for 15 minutes. Extraction was carried out under stirring in reduced light. After filtering the extract, the residue was extracted again 2 times with the same mixture of solvents. To induce phase
separation the combined extracts were added to 9 ml distilled water was then agitated for 5 minutes (30 rotations / minute). After a rest of five minutes, found above the hexane was collected and then was evaporated to dryness under vacuum at 35 °C. Oleoresins obtained was dissolved in a known volume of hexane, and total carotenoids content was estimated Spectrophotometric at 450 nm wavelength (Figure 9). Recording the absorption spectrum and absorbance read at 450 nm was made with Spectrophotometer UV - VIS Jasco type V - 530.

The amount of pigment carotenoids in sea buckthorn juice was calculated using the following formula: \[ X = \left( \frac{A \times V \times 1000}{2500 \times 100} \right) \times \text{dilution} \], where: \( X \) = the amount of carotenoids in the sample (mg), \( A \) = read the sample absorbance at \( \lambda_{\text{max}} = 450 \) nm, \( V \) = sample volume (ml) 2500 = molar absorption coefficient (E1%) for carotenoids.

For determination of antioxidant activity by DPPH method, Methanol solution was used as the blank, then 2.8 ml and 400 ml DPPH sample was used for each determination, absorbance was recorded at T30 (30 minutes). The calibration curve was performed with Trolox, using different concentrations (500 \( \mu \)M, 250, 125 \( \mu \)M, 62.5 \( \mu \)M, 31.25 \( \mu \)M, 15625 \( \mu \)M, 7.812 \( \mu \)M) and then recorded the absorbance of juices under study. 1 ml sample was extracted repeatedly juice 10 ml 95% methanol acidified with 1% concentrated HCl. After vortexing 1 minute, 30 minutes sonication and centrifugation 10 minutes at 3000 revolutions / min, the supernatant was filtered and was used to determine antioxidant activity.

FRAP method (Ferric Reducing Antioxidant Power), used for determination of antioxidant activity, is based on reduction in acid medium to colorless compound formed by Fe\(^{2+}\) and TPTZ (2,4,6 - tripiridil - s - triazine) in the form of Fe\(^{2+}\) colored in blue - violet. Colored complex formed has an absorption maximum at 593 nm (Benz and Strain, 1996). Results are expressed both in \( \mu \)M Fe\(^{2+}\) and a reference substance (Ascorbic acid, Gallic acid, Uric acid). To establish the calibration curve is prepared solutions containing between 0.1 - 1.0 Fe\(^{2+}\) / ml. Use distilled water as blank. Absorption at 593 nm is determined after 10 minutes at 37 °C. Calibration curve is obtained representing graphic shock absorber according to the concentration of Fe\(^{2+}\) ions.

RESULTS AND DISCUSSIONS

The increasing incidence of diseases population worldwide has led to carry out multidisciplinary research for obtaining bioactive substances with antioxidant character, as well as products used in alternative therapies, especially in preventing the installation of these diseases. In these studies, an important place is occupied by studies of antioxidant active substances obtained by extraction from biomass plant biotechnology, plant respectively. In the generic sense, antioxidants are chemical compounds that inhibit oxidation, with different structures, because they act by different mechanisms, usually in tandem (in combination) with other substances, supplementing or regenerating one another. (Dejica, 2001)

Antioxidant capacity of fruits and vegetables is due to polyphenols (flavonoids, phenolic acids, tannins), carotenoids and vitamin C, E and pro vitamin A. When these compounds react with free radical electron relocate accepted by the phenolic antioxidant and stabilization occurs aromatic nucleus, preventing free radical chain reaction. Through our study proved that juice of *Hippophae rhamnoides*, *Ribes nigrum* and *Vaccinium myrtillus*, products in Hypericum Labs Baia Mare, Maramures district, Romania, have strong antioxidant activity and scientifically proven. The main results obtained were: (Pop, 2011)

(1) UV - Vis fingerprint (Figure 1, 2 and 3)

UV - Vis spectrum of cranberry juice and blackcurrent sample showed a peak at \( \lambda = 535 \) nm and a specific anthcyans at \( \lambda = 280 \) nm specific phenolic acids (Figure 1). UV-Vis spectra for the sample of sea buckthorn juice showed a peak at \( \lambda = 360 \) nm and a specific flavonoid at \( \lambda = 280 \) nm specific phenolic acids (Figure 3).
UV - Vis fingerprint of cranberry (*Vaccinium myrtillus*) juice

*Fig. 1.* UV - Vis fingerprint of cranberry (*Vaccinium myrtillus*) juice

UV - Vis fingerprint of blackberries (*Ribes nigrum*) juice

*Fig. 2.* UV - Vis fingerprint of blackberries (*Ribes nigrum*) juice

UV - Vis fingerprint of sea buckthorn (*Hippophae rhamnoides*) juice

*Fig. 3.* UV - Vis fingerprint of sea buckthorn (*Hippophae rhamnoides*) juice

*UV - Vis fingerprint of blueberry juice* over the methanol + 1% HCl (diluted 50 times) showed the following values: DO535 = 0.5089; DO280 = 0.6366.

*UV - Vis fingerprint blackberries juice* taken in methanol + 1% HCl (diluted 50 times) showed the following values: DO535 = 0.2042; DO280 = 0.2358.

*UV - Vis fingerprint sea buckthorn juice* over 1% HCl in methanol (diluted 10 times) showed the following results: DO360 = 0.7075; DO280 = 1.0400.
(2) **Determination of total anthocyanins**
The results were: the blueberry juice = 748.38 mg/l total amount of anthocyanins and blackberry juice = 1321.29 mg/l total amount of anthocyanins. The sea buckthorn juice was not determined the total content of anthocyanins, it has the insignificant and irrelevant.

(3) **Determination of vitamin C**
The results obtained from analyzing the three juices are presented in the Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name sample</th>
<th>Quantity of vitamin C (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blueberry (<em>Vaccinium myrtillus</em>) juice</td>
<td>2340,0</td>
</tr>
<tr>
<td>2</td>
<td>Blackberry (<em>Ribes nigrum</em>) juice</td>
<td>5947,5</td>
</tr>
<tr>
<td>3</td>
<td>Sea buckthorn (<em>Hippophae rhamnoides</em>) juice</td>
<td>3575,0</td>
</tr>
</tbody>
</table>

(4) **The amount of polyphenols** (expressed in mg/ml plant extract) was calculated using the calibration equation. The results are presented in Table 2.

<table>
<thead>
<tr>
<th>No.</th>
<th>Name sample</th>
<th>Quantity of total polyphenols (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Blueberry (<em>Vaccinium myrtillus</em>) juice</td>
<td>1.517</td>
</tr>
<tr>
<td>2</td>
<td>Blackberry (<em>Ribes nigrum</em>) juice</td>
<td>2.328</td>
</tr>
<tr>
<td>3</td>
<td>Sea buckthorn (<em>Hippophae rhamnoides</em>) juice</td>
<td>2.649</td>
</tr>
</tbody>
</table>

(5) **Separation, identification and determination of phenolic compounds** by HPLC method. In Figure 6, 5 and 6 can be seen chromatograms of three samples of juice.

![Fig. 4. Blueberry (*Vaccinium myrtillus*) juice chromatogram for separation of phenolic compounds](image-url)
Fig. 5. Blackberry (*Ribes nigrum*) juice chromatogram for separation of phenolic compounds

Fig. 6. Sea buckthorn (*Hippophae rhamnoides*) juice chromatogram for separation of phenolic compounds

In Table 3 are presented amount of phenolic compounds measured in samples of juice.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Name phenolic compound</th>
<th>Blueberry juice (<em>Vaccinium myrtillus</em>)</th>
<th>Blackberry juice (<em>Ribes nigrum</em>)</th>
<th>Sea buckthorn juice (<em>Hippophae rhamnoides</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Gallic acid</td>
<td>12,5</td>
<td>44</td>
<td>45</td>
</tr>
<tr>
<td>2.</td>
<td>Protocatecuic acid</td>
<td>25</td>
<td>17,6</td>
<td>27</td>
</tr>
<tr>
<td>3.</td>
<td>Catechin</td>
<td>46,25</td>
<td>44</td>
<td>232,6</td>
</tr>
<tr>
<td>4.</td>
<td>Chlorogenic acid</td>
<td>164</td>
<td>46,2</td>
<td>28,3</td>
</tr>
<tr>
<td>5.</td>
<td>Caffeic acid</td>
<td>15</td>
<td>22</td>
<td>6</td>
</tr>
<tr>
<td>6.</td>
<td>p-Coumaric acid</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7.</td>
<td>Ferulic acid</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>8.</td>
<td>Synapic acid</td>
<td>139</td>
<td>-</td>
<td>13,5</td>
</tr>
<tr>
<td>10.</td>
<td>Quercetin</td>
<td>-</td>
<td>99</td>
<td>225</td>
</tr>
<tr>
<td>11.</td>
<td>Kaempferol</td>
<td>-</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>12.</td>
<td>p-OH Benzoic acid</td>
<td>21,25</td>
<td>17,6</td>
<td>-</td>
</tr>
<tr>
<td>13.</td>
<td>Vanillic acid</td>
<td>-</td>
<td>-</td>
<td>18</td>
</tr>
<tr>
<td>15.</td>
<td>Salicylic acid</td>
<td>150</td>
<td>193,6</td>
<td>216</td>
</tr>
<tr>
<td>17.</td>
<td>Trans-cinnamic acid</td>
<td>-</td>
<td>-</td>
<td>7</td>
</tr>
<tr>
<td>18.</td>
<td>Ellagic acid</td>
<td>-</td>
<td>-</td>
<td>124,5</td>
</tr>
</tbody>
</table>
(6) Determination of total carotenoids

A significant amount of carotenoids was measured in sea buckthorn juice. The total amount of carotenoids in sea buckthorn juice is 34.38 mg l.

![UV-Vis fingerprint of sea buckthorn juice](image)

Fig. 7. UV-Vis fingerprint of sea buckthorn (*Hippophae rhamnoides*) juice for determination of total carotenoids

(7) Determination of antioxidant activity

a) Determination of antioxidant activity by DPPH method

Method 2,2-Diphenyl-1-picrilhidrazil (DPPH) as amended is based on measuring the complex ability of antioxidants to DPPH• stable radical related. Radical DPPH• radical nitrogen is one of the few that has a purple set which, by reducing the antioxidant fade. Reaction between DPPH and antioxidants in the extracts was monitored using a spectrophotometer at 515 nm wavelength. For juices analyzed were obtained the following values for DPPH antioxidant activity (Table 4):

<table>
<thead>
<tr>
<th>No.</th>
<th>Name sample</th>
<th>The antioxidant activity DPPH (mM Trolox / 1 ml juice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blueberry (<em>Vaccinium myrtillus</em>) juice</td>
<td>13,875</td>
</tr>
<tr>
<td>2.</td>
<td>Blackberry (<em>Ribes nigrum</em>) juice</td>
<td>20,350</td>
</tr>
<tr>
<td>3.</td>
<td>Sea buckthorn (<em>Hippophae rhamnoides</em>) juice</td>
<td>21,475</td>
</tr>
</tbody>
</table>

b) Determination of antioxidant activity by FRAP method

For juices were analyzed for antioxidant activity values obtained following shock: (Table 5)

<table>
<thead>
<tr>
<th>No.</th>
<th>Name sample</th>
<th>The antioxidant activity FRAP (mM Fe$^{2+}$ / 1 ml juice)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Blueberry (<em>Vaccinium myrtillus</em>) juice</td>
<td>0,24</td>
</tr>
<tr>
<td>2.</td>
<td>Blackberry (<em>Ribes nigrum</em>) juice</td>
<td>0,63</td>
</tr>
<tr>
<td>3.</td>
<td>Sea buckthorn (<em>Hippophae rhamnoides</em>) juice</td>
<td>0,82</td>
</tr>
</tbody>
</table>

As can be seen from the chart above, the strongest antioxidant capacity was determined (by both methods) in sea buckthorn juice. Also, this juice was determined and the
highest amount of total polyphenols (contained in fruits of sea buckthorn). Syrup of sea buckthorn (Hippophae rhamnoides) was obtained in laboratories Hypericum Baia Mare (www.hypericum-plant.ro) by cold pressing. From this plant with high content of antioxidants (AO) is also prepared natural oils, tinctures, cider, powders and other preparations.

In conclusion, the test results were:

- **in sea buckthorn juice** is the largest amount of total polyphenols (2.649 mg/l) and vitamin C (ascorbic acid), vitamin B2 (riboflavin), vitamin B3 (nicotinamide), vitamin H (biotin), alpha carotene, beta and gamma, flavonoids, potassium (K), selenium (Se), zinc (Zn), many acids (oleic, linoleic, pantothenic, palmitic, lauric, Arah, alpha-lipoic acid or thiocitic);

- **in blackberry juice** has identified the greatest amount of vitamin C (5947.5 mg/l) and anthocyanins (1321.29 mg/l) and lower amounts of polyphenols;

- **in blueberry juice** were determined amounts of anthocyanins (748.39 mg/l), vitamin C (2340 mg/l), polyphenols, flavonoids, tannins, and antioxidant potential of substances.

The increased antioxidant activity determined by DPPH method resulted in sea buckthorn juice (21.475 mM Trolox / 1 ml juice), followed by blackberry juice (20.350 mM Trolox / 1 ml juice) and blueberry (13.875 mM Trolox / 1 ml juice).

After the FRAP method for determining antioxidant activity greater value to get all the juice of sea buckthorn (0.82 mM Fe 2+ / 1 ml juice), followed by blackberry juice (0.63 mM Fe 2+ / 1 ml juice) and blueberry (0.24 mM Fe 2+ / 1 ml juice).

Antioxidant activity of various biological compounds determined in sea buckthorn juice, blueberries and blackberry was confirmed by numerous scientific research, reflected in many specialty items from around the world. For example: vitamin C content is approximately 150 to 250 mg / 100g in blackberry, 100 - 350 mg / 100 g in sea buckthorn and 100 - 200 mg / 100 g blueberries; in berries was identified phenolic acids, antochyanins and flavonoids; in blackberry, blueberries and sea buckthorn were determined amounts of anthocyanins and β-carotene (Antal, 2012; Bojor, Pop, 2010; Chaunan, Negi, Ramteke, 2007; Heinonen, 2007; Shipp, 2010; Seeram, 2008; Vlasceanu, Negru, 2009; Zadernowschi, Naczk, Nesterowicz, 2005; www.bioterapii.ro).

**CONCLUSION**

Quantitative analysis performed in the laboratory at the University of Agricultural Sciences and Veterinary Medicine Cluj - Napoca, Department of Chemistry and Biochemistry, for the three samples of juice (blueberry - *Vaccinium myrtillus*, blackberry - *Ribes nigrum* and sea buckthorn - *Hippophae rhamnoides*), obtained in the Laboratory of Micro-production of S.C. Hypericum Impex SRL, Baia Mare, Maramures County, have determined the existence of biological compounds with antioxidant effects. Analyzes findings revealed that: blueberry juice contains anthocyanins, phenolic acids, vitamin C (2340 mg/l) and polyphenols (1517 mg/l); sea buckthorn juice contains flavonoids, phenolic acids, vitamin C (3575 mg/l), polyphenols (2649 mg/l) and carotenoids; blackberry juice contains anthocyanins, phenolic acids, vitamin C (5947.5 mg/l) and polyphenols (2328 mg/l).

Antioxidants are responsible for the defence mechanism of the organism against the pathologies associated to the attack of free radicals, thus the intake of plant derived antioxidants is involved in the prevention of degenerative diseases caused by oxidative stress, such as cancer, Parkinson, Alzheimer or atherosclerosis. Antioxidants are compounds capable to either delay or inhibit the oxidation processes which occur under the influence of atmospheric oxygen or reactive oxygen species. They are used for the stabilization of polymeric products, of petrochemicals, foodstuffs, cosmetics and pharmaceuticals.
Is a fact that we can maintain or improve health by eating fresh fruits and vegetables. In these circumstances, is necessary to promoting of plant products with a high content of substances beneficial to human health. Also, need to continue research on genetic improvement of varieties of blueberry, blackberry and sea buckthorn, meaning their content enrichment in compounds with antioxidant properties.

A good solution is the combination and use of synthetic compounds and natural products as close as possible coming through dietary intake of antioxidants in concentrations to improve any deficiencies in normal diets. In the last years, has become increasingly “nutrimedicamente” (nutritional medicine) term. Therefore, the synthesis of low molecular weight compounds, soluble, which mimics the function of natural antioxidants, is an area of huge interest in the great laboratories of the world.

REFERENCES