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EVALUATION OF THE ANTIRADICALIC AND ANTIOXIDANT EFFECTS OF *MYRTILLI FOLIUM* EXTRACTS

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Abstract

For studies, we choose an aqueous and an alcoholic extract of Myrtilli folium plant. In its composition this plant has different compounds with antioxidant properties; the extracts obtained having also hypoglycemic and astringent effects. Antioxidant activity was measured by chemiluminescence means. Antiradical activity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assays and relate to activity of ascorbic acid. Extracts of studied plant present antioxidant activity and radical scavenging activity that is comparative with the same activity of ascorbic acid.
***Keywords**: antioxidants, radical scavenging activity, phenolic compounds, chemiluminescence technique, free radical DPPH*

Introduction

Life on Earth for both vegetal and animal regna needs two base components to exist: food and oxygen, which are involved in complex interactions. Aerobe organisms depend directly on oxygen, which is necessary to the biological oxidative processes, mostly of the energetic substrates necessary for cellular activities, tissues and organs, the organism as a whole and finally for its survival. But whether some modulators are present or not, the oxygen may have, along the benefic effects for the energetic metabolism, an evil role, which is manifested in certain situations when reactive oxygen species (ROS) are formed and which are generically called free radicals (FR). Due to the presence of oxygen not only in the atmosphere but also in all substances that make the organism the interaction of FR with oxygen is inevitable.

Formed FR are susceptible to degrade by oxidative interactions the biological molecules and their accumulation at cellular levels as well as in the entire organism triggers the oxidative stress, which is the proliferation of FR. Antioxidants are natural or synthetic substances that have the capacity to prevent free radicals formation. Antioxidant activity of natural products reflects the activity of all antioxidants that are in their composition. From the classes of compounds that have antioxidant activity, a special attention is given to bio-flavones, a cluster of compounds (flavones, catechines, phenols, iso-flavones) that are present in vegetable products and which, due to their structure, have antioxidant properties.

The oxidative stress is responsible for a whole variety of degenerative processes, syndromes and diseases like: diabetes, cancer, atherosclerosis, arthritis, Parkinson disease; currently more than 100 disorders and diseases that appear due to oxidative stress are known (Raul, 1991; Bayraktutan, 2002). The more we know about the FR the more the importance of antioxidants grew, becoming a subject discussed not only in biochemistry but also in nutrition, pharmacology and medicine. Antioxidants are natural or synthetic substances that have the capacity to prevent free radicals formation.

The intensity of metabolic processes is regulated by the nature and quantities of the nutrients present in food and can be limited by certain antioxidants: micronutrients from the vitamins group (vitamin A, C, E), minerals and biologically active substances (bio-flavones, proteins containing sulphur) (Safta, 2002).

Based on these aspects there is a high degree of interest for foods and drinks based on plants and which contain flavonols, flavones, catechins, and whom antioxidant activity is proved against the accumulation of FR (Suttajit, 1999).

In the present paperwork the huckleberry leafs were tested *(Myrtilli folium)*. The huckleberry is a shrub found generally in mountain areas. For medical purposes both leafs and fruits are used *(Myrtilli folium and Myrtilli fructus)*. Leafs contain a range of antioxidant products like flavones, tannins, a glycoside of gallic acid (neomyrtilline). Due to this leafs are found in a variety of tea mixtures and especially in those destined to the treatment of diabetes.

Experimental

The antioxidant activity was determined by chemiluminescence means (CL) and the antiradical activity was measured with the DPPH$^{\bullet}$ method that uses the DPPH$^{\bullet}$ free radical (2,2-diphenyl-1-picryhyrdazyl). Aqueous and alcoholic extracts were prepared and their antioxidant and antiradical activity was measured and compared with that of ascorbic acid. The aqueous and alcoholic extracts were obtained by the classical procedure: solid-liquid extraction followed by a 2-minute reflux boiling.

The extract obtained is coded as follows:

- Aqueous extract – EA
- Alcoholic extract with 50% ethanol – EtOH50
- Alcoholic extract with 70% ethanol – EtOH70
- Alcoholic extract with 96% ethanol – EtOH96

Antioxidant activity was measured by CL using:

- Luminol and hydro peroxide at pH = 8.6 in the presence of TRIS+HCl buffer according to the procedure described in (Dicu, 2006). The luminol was dissolved with DMSO (Merck, Germany).
- Chemiluminometer TD 20/20 (Turner Design, SUA)

CL is a natural or man-induced phenomenon and which is functioning by a physical mechanism where the absorption of the energy produced by chemical reactions determines the formation of excited substances. The appearance of chemiluminescent emissions during chemical reactions is known for a long time and is firmly connected to the formation of some intermediate free radicals.

Antioxidant activity (AA) of the vegetal extracts, as a percentage, was calculated according to the formula:

$$AA\% = \frac{I_o - I_p}{I_o} \cdot 100$$

where: I_o – initial chemiluminescence of the witness sample
I_p – chemiluminescence of the extract

For the *antiradical activity* of the studied extracts the DPPH$^{\bullet}$ method proposed by Brand-Williams. This radical is widely used to determine whether compounds are able to act as free radical inhibitors or hydrogen donors as well as for the evaluation of the antioxidant activity. The DPPH method can be used for solid or liquid samples and is not specific to a certain component but it applies for the total antioxidant capacity of the samples (Aruna, 2001). The principle of this method is the measuring of antiradical activity of the samples against the DPPH$^{\bullet}$ free radical. The structure of this radical and its reduction by the antioxidant is presented in figure 1.

The unpaired electron from the DPPH$^{\bullet}$ free radical has a maximum for the absorption at 517 nm, violet-blue color. The color modifies up to yellow when the unpaired electron captures a proton from the antioxidant and thus forming the DPPH-H, the reduced form of DPPH$^{\bullet}$. The result of discoloration is in concordance with the number of captured electrons (Aruna, 2001).

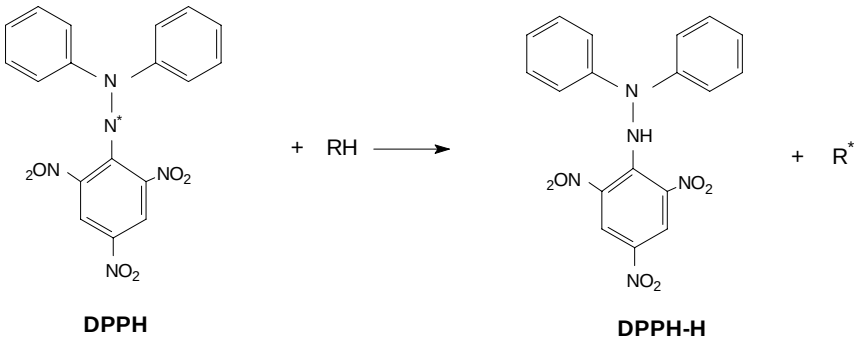

Fig. 1. Structure of DPPH$^{\bullet}$ free radical

In our study the method was applied but with slight modifications: 0.1 mL extract are added to 2.9 mL DPPH$^{\bullet}$, the reading of the values that follow the decrease of the absorbance was made at 517 nm. A witness sample is being run in parallel.

To apply the DPPH$^{\bullet}$ method the followings were used: methanol (Merck), double-distilled water, DPPH$^{\bullet}$ solution in methanol (MP, Biomedicals) [DPPH$^{\bullet}$] = 0.1 mM, vegetal extracts (obtained from 0.1 g plant with 25 mL solvent boiled for 2 minutes and then cooled and

filtered). All measurements were registered with a UV-VIS spectrophotometer Pharmacia LKB – Ultrospec III

Antiradical activity of the studied samples is expressed as percentage according to the formula:

$$\%inhibition = \left[\frac{A_B - A_A}{A_B} \right] \times 100$$

where: A_B – absorbance of the witness sample (t – 0 min)
A_A – absorbance of the tested extracts (t – 5 min).

The calculus of the antiradical activity was made using the value of the absorbance after 5 minutes due to the fact that after this period of time the values of the absorbance were constant.

The antioxidant and antiradical activity of the studied extracts were compared with those of the ascorbic acid (Asc) (Chimopar, Bucharest), freshly made solution and concentration $0.4 \cdot 10^{-3}$ M**.**

Results and Discussions

The calculated values for the antioxidant activity of each extract, compared with that of the ascorbic acid are presented in table 1.

Table 1. Values of the antioxidant activity of *Myrtilli folium* extracts

AA%				
EA	EtOH 50%	EtOH 70%	EtOH 96%	Asc
99.48	99.64	99.80	99.57	98.20

It can see that all studied extracts have a high antioxidant activity, bigger than ascorbic acid.

Out of the 4 types of extracts the alcoholic extracts come forth and having a better activity compared with the aqueous extract and also compared with the reference standard. The highest antioxidant activity is noted for the extract in 70% alcohol.

In order to determine the antiradical activity a set of five essays was made for each extract and the average of the 5 measures being used in the calculus. The values of the absorbance for the studied extracts are presented in table 2.

Table 2. Absorbance values for the studied extracts

Time (min)	Extracts			
	EA	EtOH 50%	EtOH 70%	EtOH 96%
0	2.119	2.159	1.994	2.036
0.5	1.576	1.538	1.450	1.816
1	1.365	1.324	1.224	1.653
1.5	1.251	1.184	1.202	1.584
2	1.150	1.140	1.124	1.502
3	1.111	1.077	1.079	1.430
4	1.063	1.052	1.069	1.384
5	1.050	1.037	1.046	1.335

The graphical representation of the DPPH$^{\bullet}$ decrease is presented in figure 2. By studying the graphical values it is noted that in the first 30 seconds absorbance decrease is significant, which means that most of the DPPH$^{\bullet}$ free radical was transformed in the reduced form of DPPH-H. After this period the decrease is very slow and remains constant after 5 minutes.

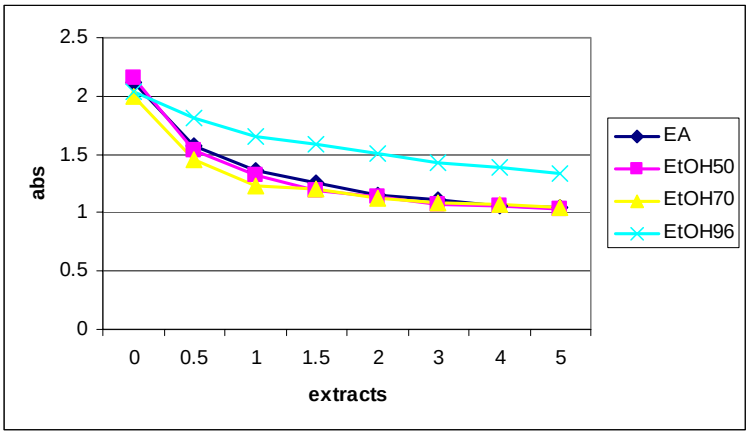

Fig. 2. DPPH$^{\bullet}$ decrease for the Myrtilli folium extracts

Antiradical activity of the vegetal extracts expressed as % inhibition and correlated with the antiradical activity of ascorbic acid is presented in table 3.

Tabel 3. Antiradical activity of the vegetal extracts

AA%				
EA	EtOH 50%	EtOH 70%	EtOH 96%	Asc
50.44	51.96	47.54	34.43	33.11

From the data presented in the table it is noted that all extracts have antiradical behavior, comparative with that of ascorbic acid but bigger than it.

From the extracts the highest antiradical activity is noted for the extract in 50% alcohol. At the same time the other types of extracts are also good radical scavengers except the extract in 96% alcohol which has a value practically equal to ascorbic acid.

The above data proves that the transfer of the antioxidant and antiradical compounds into the extracts it depends on the ration between the polar and un-polar phases and its correlation with the chemical nature of the antioxidant compounds.

Conclusions

The measuring methods for the antioxidant and antiradical activity are simple methods, easy to apply and are not component specific. The obtained extracts had antioxidant and antiradical activity comparable with that of ascorbic acid. The extraction of the antioxidant compounds form the huckleberry leafs depends on the ratio between the polar and un-polar phases. The huckleberry leafs are rich natural sources for antioxidants and this explains their positive effects in certain disorders correlated with the formation of free radicals.

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