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Determination of antiradical scavenging activity of some plants extracts with antidiabetes role using DPPH technique

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Abstract

The article presents the antiradical activity of aqueos and alcoholic extracts of Salvie folium. In composition, this plant, have flavonoid and phenolic compounds whith antioxidants properties, extracts obtained are used for their hypoglycemic and astringent efects.

Antiradical activity was measured using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assays and relate to activity of ascorbic acid. All the extracts present radical scavenging activity, comparative with activity of ascorbic acid.

Keywords: antioxidants, radical scavenging activity, phenolic compounds, free radical DPPH

1. Introduction

The intensity of metabolic processes is conditioned by the nature and the nutrient's quantum existing in aliments and it can be limited by different antioxidants, micronutrients from the vitamins group (vitamins A, C, E), minerals and biologically active substances (bioflavonoid, protein compounds with sulfa). [1,2]

Nowadays there are experimental and epidemiological data which indicate that the nutritional status (nutrition) plays an important role in antiradical defense. Therefore, a more moderate and a better controlled supplement of micronutrients may reduce the frequency of occurrence of certain diseases related to oxidizing aggression. [3, 4].

The oxidative stress triggered at the organism's level is involved in a great variety of degenerative processes, syndromes and diseases including: diabetes, cancer, arteriosclerosis, arthritis, Parkinson diseases; nowadays are known over 100 affections and diseases due to this.

From this point of view, it exists an increased interest towards the consume of some aliments or drinks based on plants in which we can find flavonoids, flavones, catehines, whose biological active action has been proved in preventing the accumulation of free radicals.

For the study we selected aqueous and alcoholic extracts of sage leaves, which have in their composition different percentages of compounds with antioxidant properties. Due to the presence of these compounds, the extracts that we obtained possess astringent and hypoglycemia activities. The results were reported to the activity of the ascorbic acid, which is an enshrined antioxidant.

In order to determine the antiradical activity of the extracts we analyzed, we used a quick and simple method DPPH • which utilizes the free, stable radicals DPPH • (1,1-diphenyl-2 picrilhydrazil). This radical is often used to test the ability of compounds to act as inhibitors of free radical, as a hydrogen donor, and for the evaluation of the antioxidant activity. DPPH method can be used for solid or

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liquid samples and is not specific to a particular component. [5]

The results were compared to the activity of a well known antioxidant, the ascorbic acid.

2. Materials and Method

As a determination method of the antiradical action of the analyzed extracts it was used the DPPH method, suggested by Brand-Williams [3], applied with small modifications, such as: 0.1 ml extract were added to 2.9 ml DPPH solution, the reading of the values which pursues the absorbance's diminution is made at the wave length of 571nm. In the same time, we make a witness test as well.

For the application of the DPPH method we have used:

- Ethanol (Merk)
- Bi-distilled water
- DPPH solution in methanol (MP, Biomedicals) [DPPH] =0.1mM
- Vegetal extracts obtained like this: 0.1 g plant with 25 ml solvent boiled for 2 minutes, it cools down and than it is filtered
- UV-VIS spectrophotometer Pharmacia LKB – Ultrospec III.

The plants have been harvest from the Banat area. The operational parameters, like the plant's level of mincing, the ratio plant/solvent, the extraction type and time were identical; the only difference was the used solvent's type: bi-distilled water and ethanol in different concentrations (50%, 70%, 96%).

The obtained extracts are noted as it follows:

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

The DPPH method is fast and easy and it uses stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl). This radical is often used for testing the compounds capacity for acting as inhibiters of free radicals or as donor of hydrogen, and for the evaluation of antioxidative activity. The DPPH method can be used for solids or liquids tests and it's not specific for a certain component, but it applies for the antioxidative capacity of all tests.

The principle of this analytical method is the measurement of extracts` antiradical activity comparative with the DPPH free radical. The radical's structure and its reduction by the antioxidants are presented in figure 1: [5]

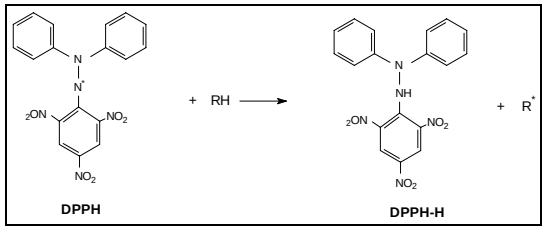

Figure 1: DPPH radical's structure

The single electron from the DPPH free radical presents the strong maximum absorption at 517 nm, violet – blue. The color is changing to yellow when the single electron catches a proton from the antioxidant, resulting the reduced form of DPPH-H.

The result of the discoloration is stoechiometric respecting the number of cached electrons. [5]

The studied tests` antiradical activity is expressed in percents pursuant to the relation:

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

Where: - A_B – the witness test absorption (t – 0 min)- A_A – the tested extracts absorption (t – 5 min). [7]

The calculus of antiradical activity was done using the extracts` absorption values from the minute 5, because after this period the absorption stays constant in time.The studied vegetal extracts` antiradical activity was compared with the one of the ascorbic acid (Asc).

3. Results and Discussion

For each extract were made five determinations, in calculus being taken their average.

The studied extracts` absorption values are presented in table 1:

Table 1: The absorption`s values for vegetal extracts

Time (min)	Extracts			
	EA	**EtOH 50%**	**EtOH 70%**	**EtOH 96%**
0	2,146	2,066	2,090	2,044
0,5	1,362	1,247	1,220	1,731
1	1,267	1,188	1,161	1,675
1,5	1,236	1,155	1,114	1,623
2	1,207	1,117	1,082	1,609
3	1,173	1,095	1,047	1,583
4	1,162	1,073	1,039	1,564
5	1,154	1,059	1,028	1,553

Table 2: The vegetal extarct`s antiradical activity

	I%				
	EA	**EtOH 50**	**EtOH70**	**EtOH96**	**Asc**
Extract	46,22	48,74	**50,81**	24,02	33,11

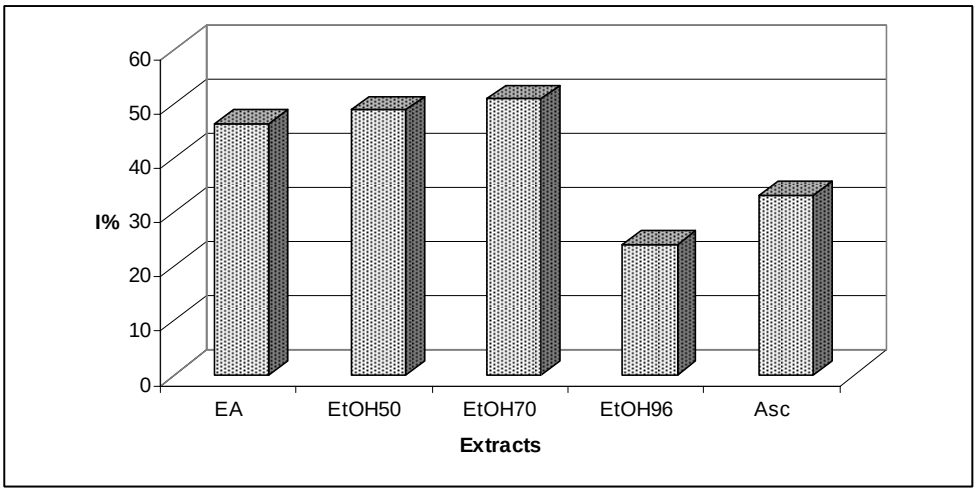

Figure 2: The antiradical activity of sage extract

4. Conclusion

The usage of DPPH method in order to determine the for antiradical activity prove that all four types of leaf extracts of sage present an antiradical activity comparable to ascorbic acid, which shows that both solvents performed an optimal extraction of components with antiradical activity.

With the exception of alcoholic extract EtOH96, all the other types of sage extract EA have a higher antiradical activity than the ascorbic acid. Compared to the antiradical activity of ascorbic acid, extract EtOH70 proved to be twice as efficient (53.5%), followed by EtOH50 (47.2%) and EA (40%).

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