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EVALUATION OF THE ANTIRADICAL PROPERTIES OF ARCTIUM LAPPA OIL VIA THE DPPH ASSAY METHOD

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Abstract
The article is devoted to the evaluation of the antioxidant properties of the oil of the Arctium Lappa plant and its antiradical properties using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. In the article, the main active components of the oil of the Arctium plant, such as phytochemical compounds, essential oils and antioxidants, were studied. The radical absorption capacity of the vegetable oil and its antioxidant properties were evaluated using the DPPH method. The results of the study indicate that Arctium oil has high antiradical activity, and at the same time, it has a beneficial potential for health as a natural antioxidant.

Keywords: Antiradicality, oil, DPPH-method, fatty acids, minerals, polyphenols, spectrophotometer, purple colour, sample, research, antioxidant, extract, experiment.

Introduction

Arctium lappa oil is known for its strong anti-radical and antioxidant properties due to the active compounds it contains. Arctium lappa oil is mainly extracted from the seeds of the plant and contains various polyphenols, terpenoids and many other bioactive substances.

Elderberry seed oil is effective in neutralizing free radicals, such as superoxide anions (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). Methods such as DPPH (1,1-diphenyl-2-pyridyl) and ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)) are used to measure this effect. In addition, vegetable oil is characterized by its strong antioxidant activity, as it shows high efficiency in eliminating free radicals. The antioxidant properties of elderberry seed oil are mainly due to the phenolic compounds and terpenoids it contains.

The free radical scavenging effect of elderberry seed oil is mediated by several mechanisms. The polyphenols and terpenoids in elderberry seed oil bind to free radicals and inactivate them. The phenolic compounds in the oil extracted from the seeds of the plant reduce oxidative stress in cells,

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which in turn slows down inflammation and ageing processes [1,2,3].

It has been shown that elderberry seed oil can increase the activity of enzymes such as superoxide dismutase (SOD) and catalase. These enzymes play an important role in eliminating free radicals.

Skin protection: Elderberry oil reduces oxidative processes in the skin, thereby slowing down the ageing process of the skin. It also supports skin regeneration and softens the skin.

The phytosterols and polyphenols in the oil extracted from the plant's seeds support cardiovascular health, lower cholesterol, and improve blood vessel health.

The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is a widely used method for measuring antiradical activity, based on which substances that neutralize free radicals (DPPH) demonstrate their antioxidant properties.

Detailed information about the DPPH (2,2-diphenyl-1-picrylhydrazyl) method was presented in the 1950s by the Japanese scientist H. Blois. In 1958, the article “Antioxidant determinations by the use of a stable free radical” by H. Blois described the method for measuring antioxidant activity using the DPPH free radical. The basic principle of this method is that the DPPH free radical, which has a dark purple colour, changes colour when it reacts with an antioxidant substance (usually to orange or light green). The colour change indicates the neutralization of the free radical and the decrease in its activity. By measuring the colour change, the degree of neutralization of free radicals by the substance is determined. Currently, the DPPH method is widely used in many scientific studies due to its speed, simplicity and low cost. This method is used as a primary tool in studying the antioxidant and antiradical properties of many plant extracts, food products, pharmaceuticals, and cosmetic products [4,5,6].

We are old ladies at the laboratory of "Folk Medicine and Commodity Chemistry" of Andijan State University. We determined the antiradical properties of the oil extracted from the seeds of the plant using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

Materials and Methods

Evaluation of the antiradical properties of the sample using the DPPH method

The decolourization of a purple solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) allows the detection of the presence of some pure antioxidant compounds that have hydrogen atom or electron-donating properties. Stable DPPH is a reagent used in spectrophotometric analysis [7,8]. In this experiment, the method for evaluating the inhibition of DPPH free radicals by H. Blois was used with minor modifications [9].

A 75.12 mM DPPH solution in ethyl acetate was prepared in a 100 ml volumetric flask, wrapped in aluminium foil and kept in the dark at room temperature for 30 minutes. 3 ml of DPPH solution and 200 µl of ethyl acetate (blank sample) were added to a 4 ml quartz cuvette, placed in a spectrophotometer, and the absorbance at 517 nm was measured every 5 minutes for 30 minutes (D_1) was measured on a K7000 spectrophotometer manufactured by YOKE (China). To evaluate the antiradical activity of the sample, 100, 150, and 200 µl of the sample was mixed with 3 ml of DPPH solution and the absorbance at 517 nm was measured in the above order (D_2) was measured. Ethyl acetate was added to the remaining volume of the solution in the cuvette to bring the total volume to 3.2 ml. The antiradical activity of the samples was calculated using the following formula:

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$$ARF\% = \frac{D_1 - D_2}{D_1} \cdot 100\% \quad (1)$$

The results obtained are given in Table 1 below.

Table 1. Measured light absorption and calculated antiradical activity values of blank and test samples added to the DPPH solution

No.	Time, min.	Abs, D	ARF%
DPPH	0	0.958	
	1	0.960	
	3	0.960	
	5	0.960	
	10	0.959	
	15	0.958	
	20	0.959	
	25	0.960	
	30	0.959	
200	0	0.856	10.83
	1	0.368	61.67
	3	0.245	74.48
	5	0.176	81.67
	10	0.105	89.06
	15	0.082	91.46
	20	0.071	92.60
	25	0.067	93.02
	30	0.066	93.13
150	0	0.869	9.48
	1	0.555	42.19
	3	0.396	58.75
	5	0.294	69.38
	10	0.176	81.67
	15	0.127	86.77
	20	0.095	90.10
	25	0.078	91.88
	30	0.072	92.50
100	0	0.939	2.19
	1	0.787	18.02
	3	0.616	35.83
	5	0.517	46.15
	10	0.388	59.58
	15	0.329	65.73
	20	0.282	70.63
	25	0.250	73.96
	30	0.238	75.21

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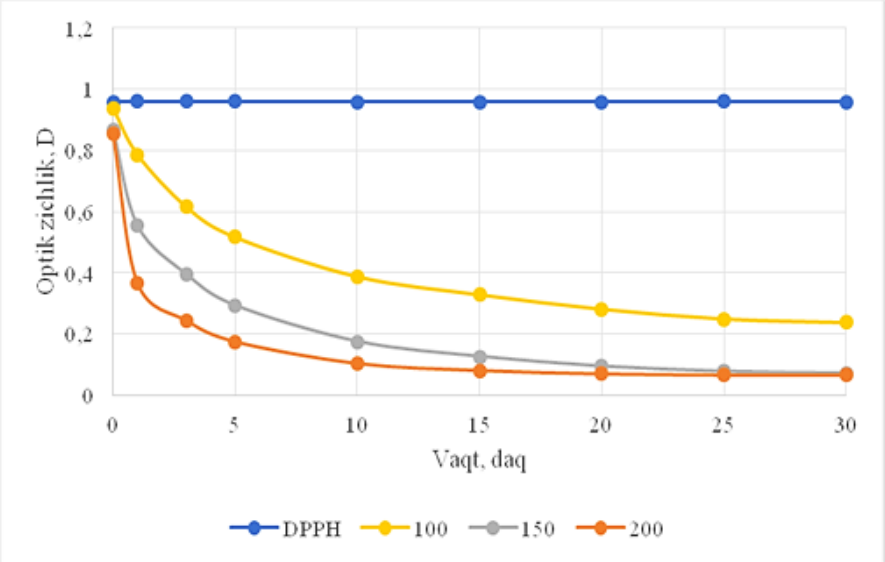

Figure 1. Graphical representation of the measured light absorption of blank and tested sample solutions added to DPPH solution.

Sample IC_{50} – To calculate the 50% inhibition concentration of the DPPH solution, the following graph was constructed based on the 30-minute antiradical scavenging activity (ARF%) values and the added sample volume in each experiment and calculated based on the trend line function applied to it.

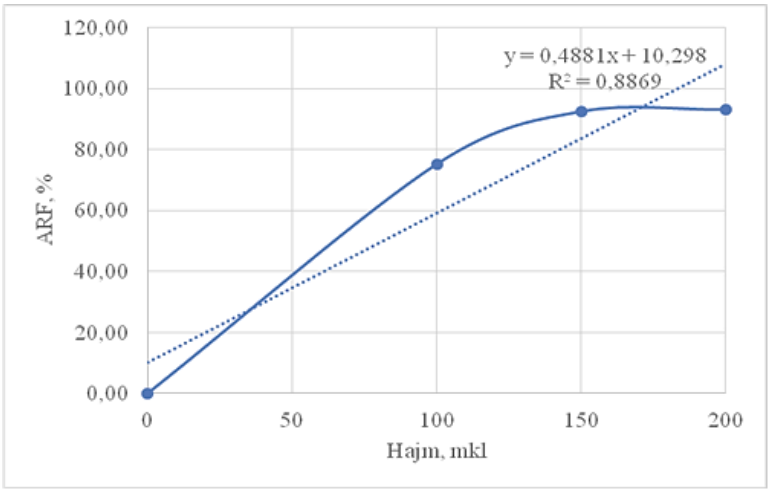

Figure 2. Graph of the relationship between ARF% and sample volumes determined at 10 minutes.

The trend line plotted on the graph was calculated from the functional formula y=mx+b, based on the formula x=(yb)/m, which represents the volume of 50% ARF% (IC_{50}):

$$IC_{50} = \frac{(50 - 10.298)}{0.4881} = 81.34 mkl$$

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Results and Discussion

The results of the above experiment show how to perform spectral analysis to assess antioxidant activity when working with a DPPH solution and how the results vary.

A solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) changes colour by accepting a hydrogen atom or electron, which helps in determining antioxidant activity. DPPH exists as a free radical, and its discolouration indicates that its radical state is being neutralized and that the antioxidant has accepted the radical. During the experiment, the absorbance of DPPH varied from 0.958 to 0.959 over time, indicating the stability of the free radical and its interaction with the antioxidant [10].

After adding the sample, the absorbance of the DPPH solution changed significantly. For example: when adding 200 µl of sample, the absorbance decreased from 0.856 to 0.066, and the ARF% reached 93.13%. This indicates a very high level of antiradical activity, that is, the 200 µl sample neutralized the DPPH free radical very effectively. In the 150 µl sample, the absorbance decreased from 0.869 to 0.072, and the ARF% reached 92.50%, indicating that the antioxidant was also effective, but slightly less active than the 200 µl sample. In the 100 µl sample, the absorbance decreased from 0.939 to 0.250, and the ARF% reached 73.96%. This means that the antioxidant activity was smaller, but it still had a certain degree of radical inhibition.

As the sample volume increases, the ARF% also increases. The 200 µl sample showed the highest antiradical activity, which means that the sample volume increases the efficiency of the radical neutralization process. The 100 µl sample showed less activity, but still significantly inhibited the DPPH free radical [10].

Conclusions

The experimental results show that the sample size and its effect are directly related to the antioxidant activity assessment with DPPH solution. After the sample was added, the absorbance of the DPPH solution decreased significantly, which reflects the effectiveness of the sample in neutralizing free radicals. The highest antiradical activity was observed when 200 µl of the sample was added, which indicates that it effectively neutralized DPPH free radicals. The 100 µl and 150 µl samples also showed significant activity, but their effect was slightly lower than that of the 200 µl sample. The results confirm that the antioxidant activity increases with increasing sample size. This study demonstrates the effectiveness of the DPPH method and its usefulness in measuring antioxidant activity. We believe that the antioxidant activity of the oil extracted from the seeds of the marigold plant can be used in folk medicine as a remedy for gastric ulcers.

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