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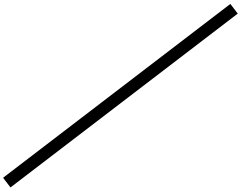

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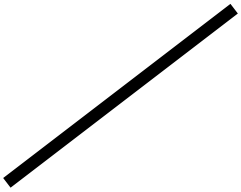

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EVALUATION OF THE ANTIRADICAL PROPERTIES OF BAY LEAF

ОЦЕНКА АНТИРАДИКАЛЬНЫХ СВОЙСТВ ЛАВРОВОГО ЛИСТА

RAYHON BARGINING ANTIRADIKAL XUSUSIYATLARINI BAHOLASH

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Abstract

During normal metabolic processes in the body, free radicals are generated, and their excessive accumulation leads to oxidative stress. Oxidative stress damages cell membranes, proteins, and nucleic acids, and plays an important role in the pathogenesis of many chronic diseases. Antioxidants are considered natural or synthetic substances that neutralize this process and protect cells. One of the methods for evaluating antioxidant activity is the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, which is widely used to analyze the antiradical properties of various bioactive compounds, including bay leaf. In this article, the antiradical activity of bay leaf was determined using the DPPH method, and the results were analyzed.

Аннотация

В ходе нормальных метаболических процессов в организме образуются свободные радикалы, и их чрезмерное накопление приводит к окислительному стрессу. Окислительный стресс повреждает клеточные мембраны, белки и нуклеиновые кислоты, играя важную роль в патогенезе многих хронических заболеваний. Антиоксиданты рассматриваются как природные или синтетические вещества, которые нейтрализуют этот процесс и защищают клетки. Одним из методов оценки антиоксидантной активности является тест DPPH (2,2-дифенил-1-пикрилгидразил), который широко используется для анализа антирадикальных свойств различных биологически активных соединений, включая лавровый лист. В данной статье антирадикальная активность лаврового листа определялась с использованием метода DPPH, и результаты были проанализированы.

Annotatsiya

Tana ichida kechadigan normal metabolik jarayonlar davomida erkin radikallar hosil bo‘ladi va ularning ortiqcha to‘planishi oksidlovchi stressga olib keladi. Oksidlovchi stress hujayra membranalari, oqsillar va nuklein kislotalarga zarar yetkazadi hamda ko‘plab surunkali kasalliklarning patogenezida muhim rol o‘ynaydi. Antioksidantlar ushbu jarayonni neytrallashtiruvchi va hujayralarni himoya qiluvchi tabiiy yoki sun’iy moddalar sifatida qaraladi. Antioksidant faollikni baholash usullaridan biri DPPH (2,2-difenil-1-pikrilgidrazil) testi bo‘lib, u turli bioaktiv birikmalarning, jumladan rayhon bargining antiradikal xususiyatlarini tahlil qilishda keng qo‘llaniladi. Ushbu maqolada rayhon bargining antiradikal faolligi DPPH usuli yordamida aniqlanib, natijalar tahlil qilindi.

Key words: *bay leaf, antiradical properties, DPPH method, biological activity, essential oil*

Ключевые слова: *лавровый лист, антирадикальные свойства, метод DPPH, биологическая активность, эфирное масло*

Kalit so‘zlar: *rayhon bargi, antiradikal xususiyatlar, DPPH usuli, biologik faollik, efir moyi*

INTRODUCTION

Bay laurel (*Laurus nobilis* L.) is a perennial evergreen species from the Lauraceae family, which consists of about 2,500–3,500 taxa worldwide. This tree may reach 8–10 meters in height and is easily recognized by its tough, glossy, and elongated leaves, usually positioned alternately

on the stem. The margins of the leaves are slightly undulated, and their size typically does not exceed 10 cm in length and 3 cm in width. The species is dioecious, meaning that male and female flowers develop on separate plants, and reproduction is usually carried out by vegetative propagation [1].

The natural range of *L. nobilis* is concentrated in the Mediterranean region, where it has been cultivated since ancient times as a spice and culturally significant plant. At present, it is not only an economically valuable crop but also widely grown as an ornamental tree in various European and American countries. In addition, the species has become naturalized in certain parts of Eastern Europe, including Bulgaria and the Black Sea coastal zone [2].

Ethnopharmacological records indicate that aqueous infusions prepared from bay leaves and fruits have been used for centuries to alleviate disorders of the nervous system, dermatological problems, and urological diseases. Nowadays, essential oil obtained from *L. nobilis* still retains its importance in traditional medicine and is applied for reducing inflammation, allergic reactions, digestive discomforts, rheumatism, and skin irritations such as dermatitis [3,4].

Phytochemical studies demonstrate that bay laurel accumulates a broad spectrum of secondary metabolites. These include essential oils and fatty acids, flavonoids such as quercetin, kaempferol, apigenin, and luteolin, both hydrolyzable and condensed tannins, as well as sesquiterpene alcohols like caryophyllene alcohol and germacrene-D-ol. Furthermore, alkaloids (e.g., lauritin and boldine), minerals (calcium, magnesium, iron, zinc, potassium), and vitamins (A, C, B-complex) have also been reported [5,6]. The leaf essential oil is particularly rich in volatile constituents, with 1,8-cineole, eugenol, linalool, methyleugenol, sabinene, α-pinene, β-pinene, and caryophyllene being the dominant compounds [7].

Moreover, bay leaf essential oil and extracts possess antimicrobial, anti-inflammatory, spasmolytic, and antioxidant properties. Therefore, they are widely applied in folk medicine, as well as in the pharmaceutical and cosmetic industries [8].

To further evaluate the biological activity of bay leaf, its antiradical activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

MATERIALS AND METHODS

The reduction in color intensity of the purple 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution is an indicator of the presence of antioxidant molecules capable of donating hydrogen atoms or electrons. Due to its stability and reactivity, DPPH is one of the most commonly employed reagents in spectrophotometric assays for evaluating free radical scavenging activity [9]. In the present study, the procedure originally described by Blois [10] was used with slight modifications in order to determine the radical-neutralizing capacity of the bay leaf extract [11]

Preparation of the DPPH working solution A stock solution of DPPH (7.92 mM) was prepared in ethanol using a 100 mL volumetric flask. The solution was protected from light by covering the flask with aluminum foil and was maintained at room temperature in darkness for 30 minutes prior to use.

Preparation of the sample extract.

An alcoholic extract of bay leaf was used as the sample: 1 g of plant material was extracted with 25 mL of 96% ethanol in an ultrasonic bath for 20 minutes. The obtained extract was filtered through a 0.45 μm syringe filter and used for analysis [12].

Determination of the antiradical activity of the samples.

For the blank control, 3 mL of DPPH solution was combined with 100 μL of ethanol in a 4 mL quartz cuvette. The cuvette was then placed into a YOKE K7000 spectrophotometer (China), and the absorbance (D_1) was recorded at 517 nm at 5-minute intervals over 30 minutes.

To determine the radical scavenging capacity of the extracts, aliquots of 25, 50, 75, and 100 μL were each mixed with 3 mL of DPPH solution. The absorbance (D_2) was measured at 517 nm under the same experimental conditions, and the final volume in the cuvette was adjusted to 3.1 mL with ethanol.

The antiradical activity (AR%) of the samples was calculated using the following equation:

$$ARA\% = \frac{D_1 - D_2}{D_1} \times 100$$

where D_1 represents the absorbance of the blank control, and D_2 corresponds to the absorbance of the tested extract.

The results obtained from these calculations are summarized in the table below.

Table 1.Measured absorbance values and calculated antiradical activity of blank and alcoholic extract samples of bay leaf added to the DPPH solution.

Volume, mkl	Time, min	Sample					
		Abs, D	ARA%		Time, min	Abs, D	ARA%
25	0	1,034	0,00	75	0	1,034	0,00
	5	0,92	11,03		5	0,863	16,54
	10	0,896	13,35		10	0,713	31,04
	15	0,865	16,34		15	0,615	40,52
	20	0,853	17,50		20	0,588	43,13
	25	0,842	18,57		25	0,564	45,45
	30	0,832	**19,54**		**30**	0,542	**47,58**
50	0	1,034	0,00	100	0	1,034	0,00
	5	0,892	13,73		5	0,764	26,11
	10	0,819	20,79		10	0,667	35,49
	15	0,763	26,21		15	0,606	41,39
	20	0,742	28,24		20	0,561	45,74
	25	0,725	29,88		25	0,525	49,23
	30	0,71	**31,33**		**30**	0,496	**52,03**

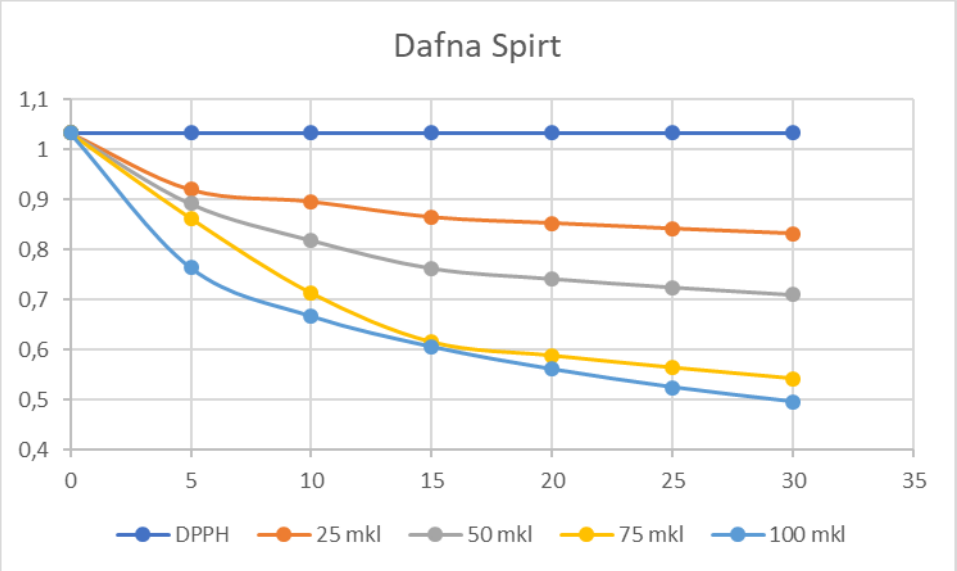

Figure 1. Graphical representation of the measured absorbance of blank and tested alcoholic extract samples added to the DPPH solution.

To calculate the IC_{50} — the concentration of the extract required to inhibit 50% of the DPPH solution — a graph was constructed in each experiment based on the antiradical activity (ARF%) values at the 30th minute and the volume of the added alcoholic samples. The IC_{50} value was determined using the trend line function applied to the plotted data.

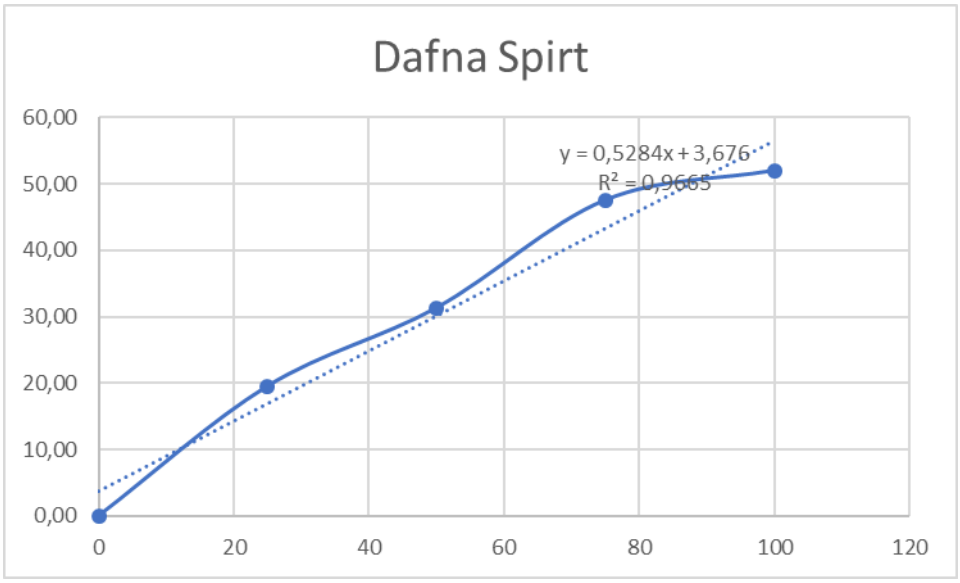

Figure 2. Graph showing the relationship between ARF% values determined at the 10th minute and the volumes of the alcoholic extract samples.

Based on the trend line function applied to the graph, using the formula $y = mx + b$, the volume corresponding to 50% ARF% (IC_{50}) was calculated according to the formula:

$$IC_{50} = \frac{(50 - 3{,}676)}{0{,}5284} = 87{,}668\ mkl$$

DISCUSSION OF RESULTS

Due to the presence of phenolic compounds, flavonoids, tocopherols, and other biologically active substances, bay leaf extract exhibits high antioxidant activity. The study enables a comparison of the efficiency of bay leaf extract in neutralizing free radicals with that of extracts from other medicinal plants. Previous studies have shown that the antioxidant activity of bay leaf extract is comparable to that of traditional antioxidants, including vitamin E (α-tocopherol) and other plant extracts rich in phenolic compounds.

CONCLUSION

In summary, the ethanol extract of bay leaf exhibited notable antiradical potential. The most pronounced effect was observed at the 30-minute interval, where the extract achieved an activity level of 52.03%. The IC_{50} value was 87.668 µL, confirming its antiradical potential. This experiment suggests that, due to the richness of its composition and antioxidant properties, bay leaf can be recommended for the development of biologically active supplements applicable in medicine, cosmetology, and the food industry.

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