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ISSN 3030-3907

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ISSN 3030-3907

EVALUATION OF ANTIOXIDANT (FREE RADICAL SCAVENGING) ACTIVITY OF MINT (MENTHA SP.) ALCOHOLIC EXTRACT BASED ON DPPH METHOD

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Annotatsiya. Mazkur tadqiqotda yalpiz (Mentha sp.) o‘simligining spirtli ekstraktining antioksidant, ya'ni erkin radikallarni neytrallash faolligi DPPH (2,2-difenil-1-pikrilgidrazil) radikali yordamida baholandi. Antioksidantlar tirik organizmlarda oksidlovchi stressni kamaytirishda muhim ahamiyatga ega bo‘lib, ularni o‘simlik manbalaridan ajratib olish va biologik faolligini o‘rganish so‘nggi yillarda keng tadqiq etilmoqda. Yalpiz namunalari 96 % etanol yordamida ultratovushli ekstraksiya usulida tayyorlandi va ekstraktning DPPH• erkin radikallarini ingibitsiyalash qobiliyati 517 nm to‘lqin uzunligida spektrofotometrik usul bilan aniqlandi. Tajribalar Blois tomonidan taklif etilgan klassik metodika asosida, ayrim modifikatsiyalar bilan olib borildi. Ekstraktning turli hajmlari (25–100 µL) DPPH eritmasi bilan reaksiyaga kiritildi va optik zichlikdagi o‘zgarishlar 30 daqiqa davomida kuzatildi. Olingan natijalar asosida radikallarni neytrallash faolligi (AF%) hamda IC_{50} qiymati hisoblandi. Yalpizning spirtli ekstrakti sezilarli darajada erkin radikallarni neytrallash xususiyatini namoyon etdi va uning IC_{50} qiymati 391,33 µL ni tashkil etdi. Ushbu natijalar yalpizning tabiiy antioksidantlar manbai sifatidagi ahamiyatini tasdiqlaydi hamda undan oziq-ovqat, farmatsevtika va xalq tabobati sohalarida foydalanish istiqbollarini ko‘rsatadi.

Kalit so‘zlar: yalpiz, antioksidant faollik, DPPH usuli, erkin radikallarni neytrallash, IC_{50}, spirtli ekstrakt, spektrofotometriya.

Abstract. This study investigated the antioxidant, i.e., free radical scavenging activity of mint (Mentha sp.) alcoholic extract using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. Antioxidants play a vital role in reducing oxidative stress in living organisms, and their extraction from plant sources has been widely studied in recent years. Mint samples were prepared using 96% ethanol via ultrasonic extraction, and the ability of the extract to inhibit DPPH• free radicals

ISSN 3030-3907

was measured spectrophotometrically at 517 nm. Experiments were performed based on the classic method proposed by Blois, with minor modifications. Different volumes of extract (25–100 µL) were reacted with DPPH solution, and changes in absorbance were monitored for 30 minutes. From these results, the radical scavenging activity (RSA%) and IC50 value were determined. The mint alcoholic extract showed significant free radical scavenging activity, with an IC50 value of 391.33 µL. These findings highlight mint as a natural source of antioxidants, with potential applications in food, pharmaceutical, and traditional medicine.

Keywords: mint, antioxidant activity, DPPH method, free radical scavenging, IC50, alcoholic extract, spectrophotometry.

Аннотация. В данном исследовании была оценена антиоксидантная, то есть способность к нейтрализации свободных радикалов, активность спиртового экстракта мяты (Mentha sp.) с использованием радикала DPPH (2,2-дифенил-1-пикрилгидразил). Антиоксиданты играют важную роль в снижении окислительного стресса у живых организмов, а их извлечение из растительных источников в последние годы широко изучается. Образцы мяты были приготовлены с использованием 96% этанола и ультразвуковой экстракции, а способность экстракта ингибировать свободные радикалы DPPH• измерялась спектрофотометрически при 517 нм. Эксперименты проводились на основе классического метода Блуа, с небольшими модификациями. Разные объемы экстракта (25–100 µL) взаимодействовали с раствором DPPH, и изменения оптической плотности отслеживались в течение 30 минут. На основании полученных результатов были рассчитаны антирадикальная активность (ARF%) и значение IC50. Спиртовой экстракт мяты продемонстрировал значительную антирадикальную активность с IC50 = 391,33 µL. Эти данные подчеркивают ценность мяты как натурального источника антиоксидантов, с перспективами применения в пищевой, фармацевтической и народной медицине.

Ключевые слова: мята, антиоксидантная активность, метод DPPH, нейтрализация свободных радикалов, IC50, спиртовой экстракт, спектрофотометрия.

INTRODUCTION.

In recent years, the importance of antioxidant compounds in maintaining human health and preventing various chronic diseases has been steadily increasing. Free radicals are generated in the body as a result of metabolic processes and can negatively affect cell membranes, proteins, lipids, and DNA structure. These processes lead to oxidative stress, which may contribute to cardiovascular diseases, oncological disorders, diabetes, and accelerated aging.

ISSN 3030-3907

Therefore, the identification of antioxidant compounds derived from natural sources and the evaluation of their activity represent an urgent scientific issue [1; 193–202].

Plants are rich in biologically active substances, particularly phenolic compounds, flavonoids, ascorbic acid, and essential oils, which possess strong antioxidant properties. Mint (*Mentha* sp.) is one of the widely used medicinal plants in folk and traditional medicine, known for its anti-inflammatory, antibacterial, antispasmodic, and sedative properties. In addition, the antioxidant potential of the biologically active compounds present in mint requires further scientific substantiation [2;].

Various chemical and biochemical methods are available for determining antioxidant activity. Among them, the spectrophotometric method based on the DPPH free radical is considered one of the simplest, fastest, and most reliable techniques. DPPH• is a stable free radical characterized by a color change from purple to yellow when reduced by antioxidant substances. This color change can be measured using a spectrophotometer, allowing a quantitative assessment of the radical scavenging activity of the sample.

The aim of this study is to determine the free radical scavenging activity of the alcoholic extract of mint using the DPPH method, to evaluate its ability to inhibit free radicals at different concentrations, and to calculate the IC_{50} value. The obtained results will contribute to the scientific validation of mint as a natural source of antioxidants.

MATERIALS AND METHODS.

The colour shift of the purple-colored 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution suggests that there are some pure antioxidant compounds having hydrogen atom/electrons donating ability. The stable DPPH• radical is widely used in spectrophotometrical analyses as a reagent. Here, the method established by Blois to measure the radical-scavenging activity of DPPH• was employed with some changes.

DPPH• solution preparation in working solution. Then a 7.92 mM solution of DPPH• was prepared in ethanol in a 100 mL volumetric flask. The solution was covered with aluminum foil and kept at room temperature in the dark for 30 min.

Preparation of plant extracts. Poplar root and willow bark mixtures (3:1, 1:1, and 1:3) were prepared in mass ratios, respectively. To extract, 1 g of plant material was added to 25 mL of solvent (distilled water or 96% ethanol) and then extracted in an ultrasonic bath for 20 min. Extracts were filtered by a 0.45 μm syringe filter for analysis. Extracts had been tested against 10-fold diluted fractions based on the high antiradical activity of extracts.

ISSN 3030-3907

Analysis: Determination of antiradical activity. For the blank samples, 3 mL of DPPH solution and 100 µL of solvent (without extract) was put into a 4 mL quartz cuvette, and absorbance D1 was recorded every 5 minutes and at 517 nm at 30 minutes on a YOKE K7000 spectrophotometer (China). For the following four doses of extract to assess the antiradical activity of 25, 50, 75 and 100 µL of extracts, and 3 mL of DPPH solution. The absorbance values (D2) were further read under similar conditions. The remaining volume was filled with solvent to the total volume of 3.1 mL in the cuvette.

$$ARF\%=\frac{D_1-D_2}{D_1}\cdot 100\%$$

RESULTS AND DISCUSSION.

The obtained results are presented in the following table:

Table 1.

Measured absorbance values and calculated radical scavenging activity (RSA%) of the blank and the tested alcoholic extract samples added to the DPPH solution.

Volume, µL	Time, min	Sample					
		Abs, D	AAF%		Time, min	Abs, D	AA%
25	0	0,967	0,00	75	0	0,967	0,0
	5	0,933	3,52		5	0,876	9,4
	10	0,935	3,31		10	0,874	9,6
	15	0,936	3,21		15	0,874	9,6
	20	0,938	3,00		20	0,875	9,5
	25	0,939	2,90		25	0,878	9,2
	30	0,938	3,00		**30**	0,874	9,6
50	0	0,967	0,00	100	0	0,967	0,00
	5	0,896	7,34		5	0,854	11,69
	10	0,895	7,45		10	0,848	12,31
	15	0,896	7,34		15	0,848	12,31
	20	0,895	7,45		20	0,846	12,51
	25	0,896	7,34		25	0,846	12,51
	30	0,896	7,34		**30**	0,845	12,62

ISSN 3030-3907

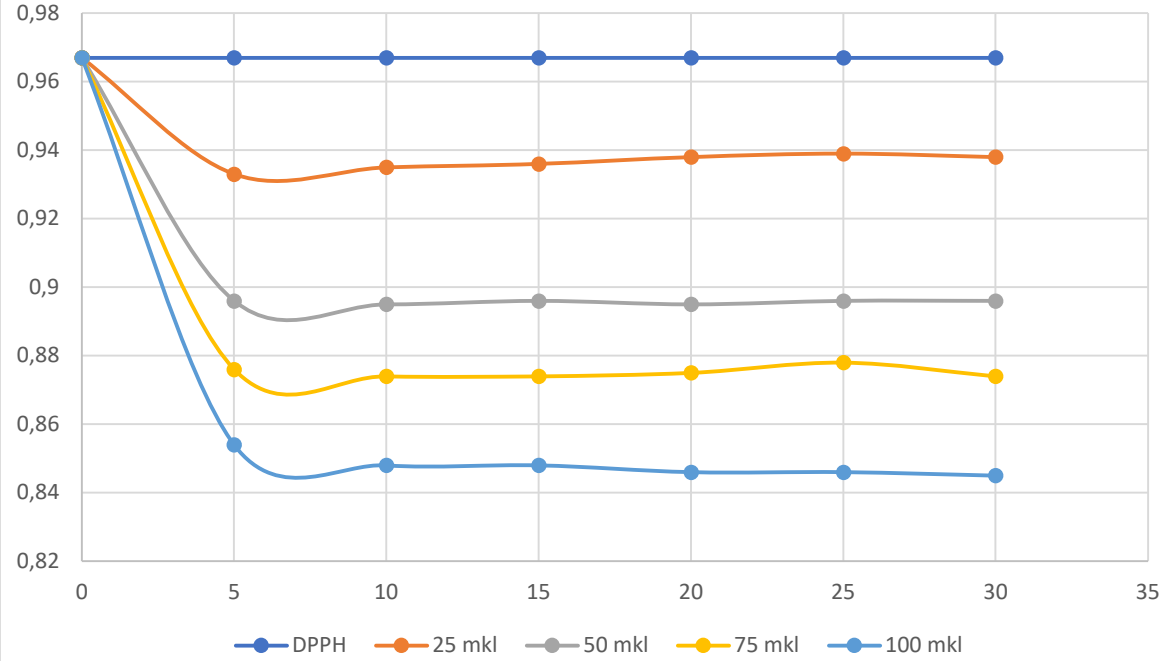


Figure 1. Graphical representation of the measured absorbance of the blank and tested alcoholic extract samples added to the DPPH solution.

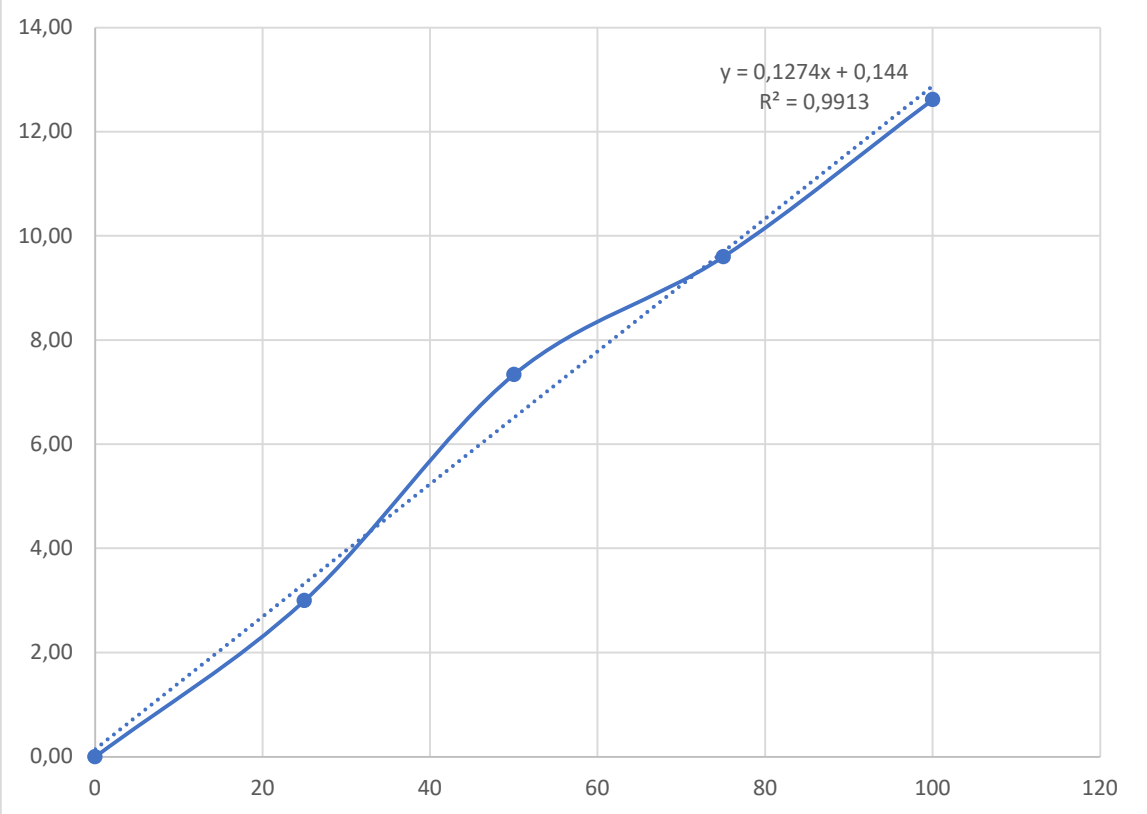


Figure 2. Graph showing the relationship between the AA% values determined at the 10th minute and the volumes of the alcoholic extract sample.

To calculate the IC_{50} value of the samples — the concentration required to inhibit 50% of the DPPH solution — a graph was constructed for each experiment

ISSN 3030-3907

based on the antiradical activity (ARA%) values measured at the 30th minute and the volume of the added alcoholic samples. The IC_{50} value was then determined using the function of the trend line fitted to the graph.

Using the equation of the trend line fitted to the graph, $y = mx + b$, the volume corresponding to 50% AA (IC_{50}) was calculated according to the formula $x = (y - b) / m$.

$$IC_{50} = \frac{(50 - 0.144)}{0.1274} = 391.33mkl$$

DISCUSSION.

In this study, the dried aerial parts of mint (*Mentha* sp.) were selected as the research object. The sample was prepared according to standard procedures, and the extraction process was carried out using 96% ethanol. To 1 g of plant material, 25 ml of ethanol was added, and the extraction was performed in an ultrasonic bath for 20 minutes. This method is advantageous as it ensures a more complete release of biologically active compounds.

The obtained extract was filtered through a 0.45 µm syringe filter, prepared for analysis, and diluted tenfold.

The DPPH• working solution was prepared in ethanol at a concentration of 7.92 mM and kept protected from light for 30 minutes. To determine antiradical activity, 3 ml of DPPH solution and different volumes (25, 50, 75, and 100 µl) of mint extract were added into a quartz cuvette. Ethanol was used as a blank sample. The total reaction mixture volume was adjusted to 3.1 ml.

Absorbance was measured using a YOKE (China) K7000 spectrophotometer at a wavelength of 517 nm at 5-minute intervals over a period of 0–30 minutes. Based on the obtained absorbance values, antiradical activity (ARA%) was calculated. The results showed that as the concentration of mint extract increased, the inhibition degree of DPPH• radicals also increased.

When 25 µl of extract was added, the ARA% value was approximately 3%, whereas at 100 µl it exceeded 12%. This confirms the presence of compounds in mint that possess electron- or hydrogen-donating properties. Based on graphical analysis, the IC_{50} value was determined to be 391.33 µl. This result indicates that the alcoholic extract of mint exhibits a moderate level of antiradical activity.

Comparison of the obtained results with literature data confirms that mint, among other medicinal plants, is an effective natural source of antioxidants.

CONCLUSION.

In conclusion, the alcoholic sample demonstrated antiradical activity. Specifically, the IC_{50} value of the alcoholic extract was 391.33 µl, indicating its antiradical potential. The conducted study revealed that the alcoholic extract of

ISSN 3030-3907

mint (*Mentha* sp.) exhibits significant antiradical activity according to the DPPH method.

During the study, it was observed that the extract's ability to inhibit DPPH• free radicals depended on time and concentration. As the sample volume increased, a decrease in absorbance values was recorded, indicating a reduction in the concentration of free radicals.

The IC_{50} value of 391.33 µl demonstrates that the alcoholic extract of mint has a certain degree of effectiveness against free radicals. These results can be explained by the presence of phenolic compounds and other biologically active substances in mint.

In summary, mint can be considered a promising natural antioxidant source for application in the food industry, pharmaceuticals, and traditional medicine. Future studies should focus on identifying individual biologically active components of mint extract, conducting their quantitative analysis, and evaluating antioxidant efficacy under in vivo conditions. The findings of this research provide a scientific basis for the development of mint-based functional products and biologically active supplements.

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ISSN 3030-3907

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