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ANTIRADICAL ACTIVITY OF COMPOSITIONS OF LEMON BALM AND LICORICE MEDICINAL PLANTS

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Annotation: This article presents an evaluation of the antiradical activity of aqueous and alcoholic extracts of compositions of medicinal plants—lemon balm (Melissa officinalis L.) and licorice (Glycyrrhiza glabra L.)—used in scientific medicine and combined in different ratios. The antiradical activity of the extracts was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging method. The studied compositions were found to possess high antiradical activity. The minimum volumes of aqueous and ethanolic extracts of mixtures at ratios of 1:1, 1:3, and 3:1 required to scavenge 50% of DPPH free radicals under standard conditions were investigated.

The obtained results indicate that mixtures of lemon balm and licorice, particularly the composition at a ratio of 3:1, may be considered promising therapeutic agents for the prevention of oxidative stress and may have potential applications in traditional medicine.

Keywords: Melissa officinalis L., Glycyrrhiza glabra L., extraction, antiradical activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), spectrophotometry, IC50, food additive.

How to Cite

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Introduction

Medicinal Lemon balm (*Melissa officinalis L.*) belongs to the higher plants, division *Magnoliophyta,* class *Magnoliopsida,* subclass *Lamidae*, and family *Lamiaceae* [1]. Medicinal Melissa officinalis L. is a perennial, densely pubescent herbaceous plant, 30–60 cm in height, with a characteristic lemon scent. The stem is quadrangular, either single or highly branched, with opposite branching. The leaves are ovate, pointed, with large serrated margins, pubescent on the upper surface, short-petioled, and oppositely arranged on the stem. The white, pubescent flowers are borne on pedicels in the axils of the leaves, forming ring-shaped inflorescences. The fruit consists of four nutlets. The plant flowers from June to August, and the fruits ripen in July–August [2, 3].

Licorice (Glycyrrhiza glabra L.), also known as sweet licorice, is a perennial rhizomatous medicinal plant belonging to the family Fabaceae. In Uzbekistan, licorice grows naturally and has long been used in

traditional medicine. The stem is highly branched, coarse, erect, and 40–150 cm tall. The leaves are compound, imparipinnate, and oblong. The flowers are violet and arranged in racemose inflorescences. The fruit is an elongated legume. The plant flowers and bears fruit from April to June. It reproduces by seeds and vegetatively through rhizomes. The root system is strongly developed and penetrates the soil to a depth of up to 3 m. Licorice is a well-known medicinal plant with a rich chemical composition.

In our studies, the antioxidant activity of Melissa officinalis L. and licorice plants was investigated. According to the results obtained, it was established that their compositions also exhibited high antioxidant activity. This article presents the results of studies conducted to determine the antiradical activity of mixtures prepared from the vegetative parts of Melissa officinalis L. and licorice in ratios of 1:1, 1:3, and 3:1, with the aim of developing a food additive with standardized levels of biologically active substances.

Materials and Methods

A stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH•), was employed to assess the antiradical activity of the investigated samples. The gradual fading of the intense violet color of the DPPH• solution, including its transition to a pale or nearly colorless state, was considered an indication of the presence of biologically active compounds with antioxidant properties in the tested samples. This color change occurs as a result of the reduction of the DPPH• radical following the transfer of an electron or hydrogen atom from antioxidant constituents. Accordingly, the extent of DPPH• radical neutralization was considered an important parameter reflecting the free-radical-scavenging capacity of the investigated extracts.

The DPPH• assay is widely used for the preliminary assessment of the antioxidant properties of plant materials and their extracts due to its relative simplicity, rapid execution, and compatibility with spectrophotometric measurements. The analytical principle of the method is based on the interaction of the DPPH• radical with antioxidant compounds, which alters the electronic state of the radical and consequently decreases its optical absorbance. The reduction in absorbance observed during the reaction provides a quantitative basis for evaluating the radical-scavenging efficiency of the tested samples.

In the present study, a modified version of the spectrophotometric procedure originally proposed by Blois was used to determine the ability of the samples to inhibit the DPPH• free radical [4]. Several technical adjustments were introduced according to the experimental conditions and the specific characteristics of the investigated plant extracts [5]. The change in optical density resulting from the interaction between DPPH• radicals and antioxidant constituents of the samples was used as the principal analytical parameter.

The antiradical activity of the samples was evaluated from the difference between the initial and final absorbance values of the DPPH• reaction system. Thus, the decrease in color intensity and spectrophotometric absorbance of the DPPH• solution was used as an indicator of the ability of the investigated compositions to neutralize free radicals [3–5].

Preparation of the DPPH• Solution

The working DPPH• solution was prepared in a 100 mL volumetric flask using ethyl alcohol as the solvent to obtain a concentration of 7.92 mM. To protect the solution from light exposure, the flask was wrapped in aluminum foil and kept at room temperature in the dark for 30 min.

Preparation of Sample Extracts

Samples were prepared to evaluate the antioxidant properties of alcoholic and aqueous extracts obtained from lemon balm and licorice raw materials. For extraction, 1 g of finely ground plant material was treated with 25 mL of solvent, either distilled water or 96% ethanol, and subjected to ultrasonic treatment for 20 min. The resulting extracts were clarified by filtration through a 0.45 μm pore-size syringe filter and subsequently prepared for analysis [4]. Prior to analysis, the obtained filtrates were diluted tenfold.

Determination of Antiradical Activity

The antiradical activity of the investigated extracts was determined using a spectrophotometric assay based on their ability to interact with the DPPH• free radical. Initially, a control solution was prepared by transferring 3.0 mL of the working DPPH• solution and 100 μL of ethanol into a quartz cuvette with a capacity of 4 mL. The optical density of the prepared solution was recorded at a wavelength of 517 nm using

a K7000 spectrophotometer manufactured by YOKE (China). Absorbance measurements were performed over a 30-min reaction period at 5-min intervals [6,7].

To assess the radical-scavenging efficiency of the investigated samples, aliquots of the extracts (25, 50, 75, and 100 µL) were separately introduced into 3.0 mL of the DPPH• solution. The optical density of the resulting reaction mixtures was monitored spectrophotometrically at the specified time intervals. The antiradical activity of the samples was characterized according to the degree of DPPH• radical neutralization.

The absorbance of the reaction mixtures was recorded at 517 nm (D_2) under the experimental conditions described above. The volume of ethanol corresponding to the volume of the added sample was used to adjust the total volume of the reaction mixture in the cuvette to 3.1 mL. The DPPH• radical inhibition rate, expressed as antiradical activity (ARA, %), was calculated from the measured optical density values using the following equation:

Results and Discussion

Analysis of the experimental results presented in the following tables demonstrated that the lemon balm–licorice compositions prepared at different ratios exhibited pronounced antiradical activity. At a constant extract concentration, an initial rapid increase in ARA was observed for all tested extract volumes. After approximately 25–30 min of reaction, the ARA values showed only minor changes and approached a relatively stable level.

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

Therefore, the ARA values recorded at the 30-min time point were selected for determining the IC_{50} parameter. This parameter represents the minimum volume of the extract required to achieve 50% neutralization of the DPPH• free radicals under the specified experimental conditions. For each experiment, a graphical relationship was established between the extract volume of the investigated food-supplement composition and the corresponding ARA (%) value measured after 30 min of reaction.

The experimental data were processed using the least-squares method [8], and a mathematical equation describing the linear function that provided the best fit to the experimental points was determined (Figures 1–12). The resulting quantitative parameters obtained from these calculations are presented in the following tables.

Table 1 Measured absorbance values and calculated antiradical activity values of the blank and tested samples extracted with alcohol at a Melissa officinalis L.–licorice ratio of 1:1, added to the DPPH solution.

Volume, µL	Time, min	Sample					
		Abs, D	ARF%		Time, min	Abs, D	ARF%
25	0	1,024	0,00	75	0	1,024	0,00
	5	0,971	5,18		5	0,839	18,07
	10	0,964	5,86		10	0,823	19,63
	15	0,959	6,35		15	0,814	20,51
	20	0,952	7,03		20	0,802	21,68
	25	0,948	7,42		25	0,794	22,46
	30	0,943	7,91		30	0,789	22,95
50	0	1,024	0,00	100	0	1,024	0,00
	5	0,912	10,94		5	0,778	24,02
	10	0,905	11,62		10	0,767	25,10
	15	0,892	12,89		15	0,755	26,27
	20	0,886	13,48		20	0,741	27,64

	25	0,875	14,55		25	0,733	28,42
	30	0,869	15,14		30	0,727	29,00

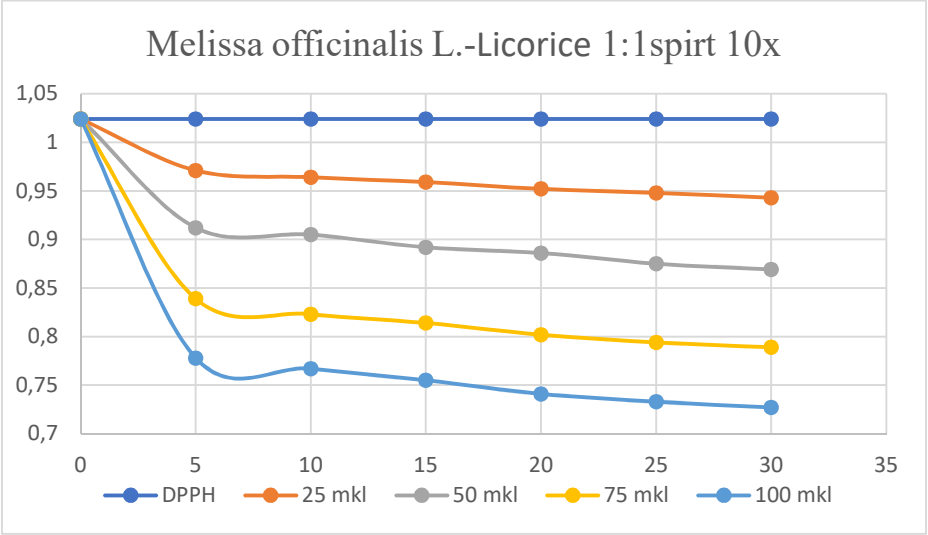
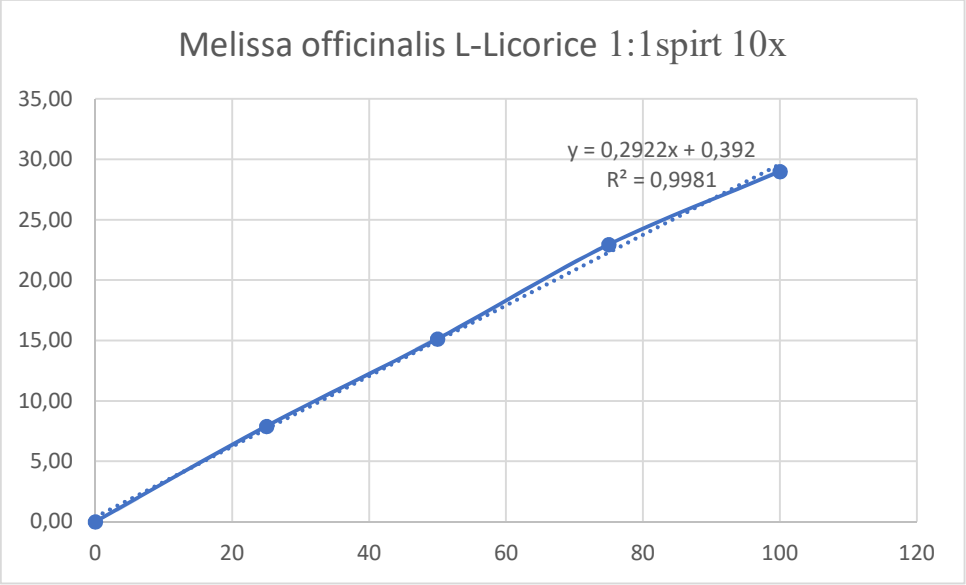


Figure 1. Graphical representation of the measured absorbance of the blank and tested alcoholic extract sample solutions added to the DPPH solution.

To determine the IC50 values of the samples, i.e., the concentration required to achieve 50% inhibition of the DPPH radical, the antiradical activity (ARF%) values recorded at 30 minutes in each experiment and the volumes of the aqueous extracts added to the reaction mixture were analyzed in relation to each other. Based on the obtained data, a graphical relationship was established, and the IC50 values were calculated using the equation of the trendline.



2-Figure. Spirtli estraksiya qilingan namunaning 30-daqiqada определенная ARF% lari va hajmlari o‘rastidagi relationship grafigi.

The volume corresponding to 50% antiradical activity (ARA, %) was calculated from the trendline equation fitted to the graph, y = mx + b, using the following formula:

[

$$IC_{50} = \frac{(50 - 0{,}392)}{0.2922} = 169.77 \text{ mkl}$$

Table 2. Measured absorbance values and calculated antiradical activity values of the blank and tested alcoholic extracts at a Melissa officinalis L.–licorice ratio of 3:1, added to the DPPH solution

Volume, µL	Time, min	Sample					
		Abs, D	ARF%		Time, min	Abs, D	ARF%
25	0	1,024	0,00	75	0	1,024	0,00
	5	1,004	1,95		5	0,955	6,74
	10	0,998	2,54		10	0,95	7,23
	15	0,992	3,13		15	0,946	7,62
	20	0,987	3,61		20	0,941	8,11
	25	0,981	4,20		25	0,937	8,50
	30	0,976	4,69		30	0,934	8,79
50	0	1,024	0,00	100	0	1,024	0,00
	5	0,969	5,37		5	0,937	8,50
	10	0,966	5,66		10	0,931	9,08
	15	0,963	5,96		15	0,924	9,77
	20	0,96	6,25		20	0,918	10,35
	25	0,958	6,45		25	0,913	10,84
	30	0,956	6,64		30	0,908	11,33

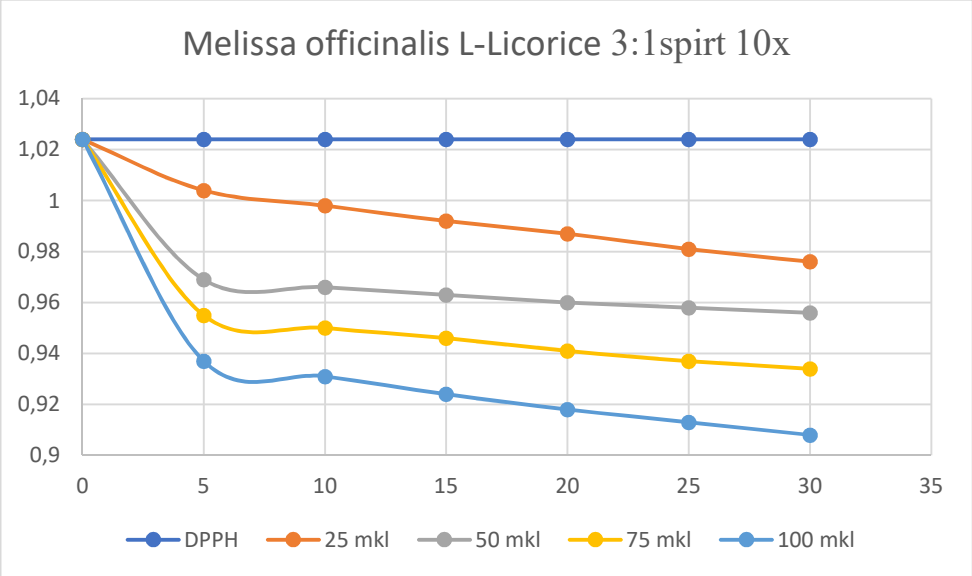


Figure 3. Graphical representation of the measured absorbance of the blank and tested alcoholic extract sample solutions added to the DPPH solution.

To determine the IC50 values of the samples, i.e., the concentration required to achieve 50% inhibition of the DPPH radical, the antiradical activity (ARF%) values recorded at 30 minutes in each experiment and the volumes of the aqueous extracts added to the reaction mixture were analyzed in relation to each other. Based on the obtained data, a graphical relationship was established, and the IC50 values were calculated using the equation of the trendline.

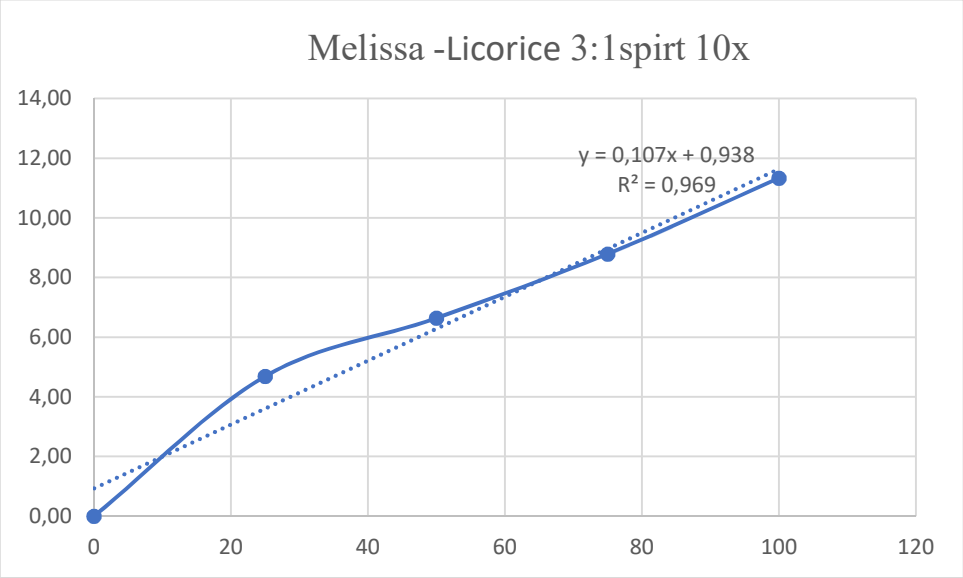


Figure 4. Graph showing the relationship between the ARA (%) values determined at 30 minutes and the volume of the alcoholic extract sample.

The volume corresponding to 50% ARA (IC_{50}) was calculated from the trendline equation y = mx + b using the formula:

$$IC_{50} = \frac{(50 - 0{,}938)}{0{,}107} = 458{,}52 \text{ mkl}$$

Table 3. Measured absorbance values and calculated antiradical activity values of the blank and tested alcoholic extracts at a Melissa officinalis L.–licorice ratio of 1:3, added to the DPPH solution.

Volume, µL	Time, min	Sample					
		Abs, D	ARF%		Time, min	Abs, D	ARF%
25	0	1,024	0,00	75	0	1,024	0,00
	5	0,996	2,73		5	0,976	4,69
	10	0,995	2,83		10	0,972	5,08
	15	0,994	2,93		15	0,968	5,47
	20	0,993	3,03		20	0,964	5,86
	25	0,992	3,13		25	0,96	6,25
	30	0,991	3,22		30	0,956	6,64
50	0	1,024	0,00	100	0	1,024	0,00
	5	0,99	3,32		5	0,955	6,74
	10	0,988	3,52		10	0,95	7,23
	15	0,986	3,71		15	0,945	7,71
	20	0,984	3,91		20	0,94	8,20
	25	0,982	4,10		25	0,935	8,69
	30	0,98	4,30		30	0,93	9,18

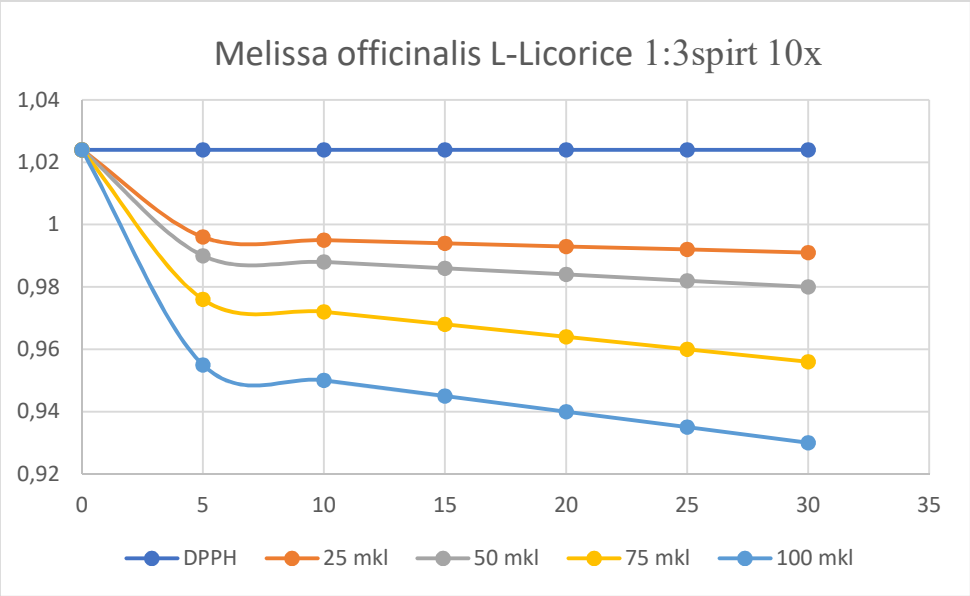


Figure 5. Graphical representation of the measured absorbance of the blank and tested alcoholic extract sample solutions added to the DPPH solution.

To determine the IC50 values corresponding to 50% inhibition of the DPPH solution, the ARF% values obtained at the end of the 30-minute experimental period were used as the primary parameter. A graph was constructed based on the volumes of the aqueous samples and their corresponding antiradical activity values. The IC50 values were determined mathematically using the equation of the trendline fitted to the experimental data points.

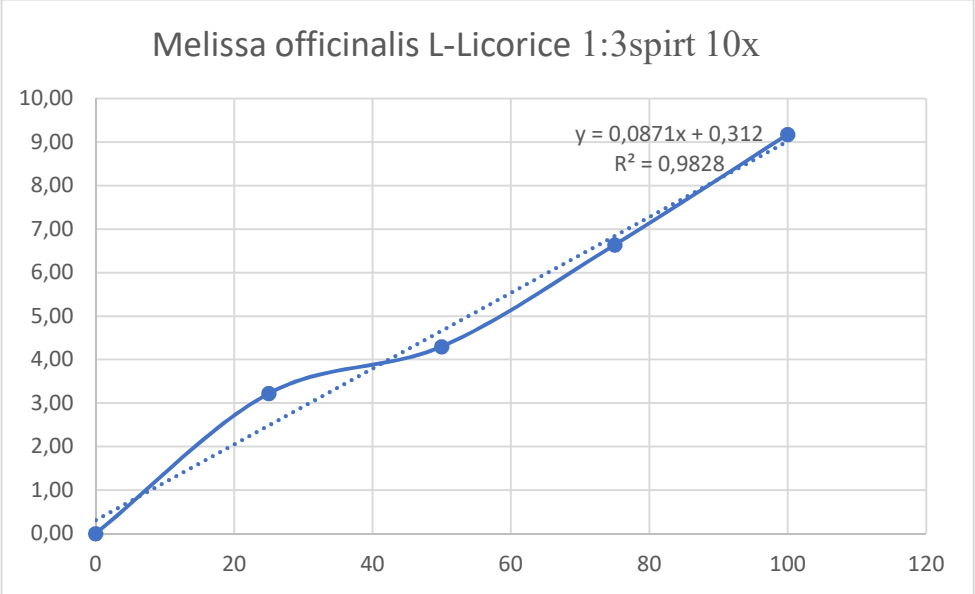


Figure 6. Graph showing the relationship between the ARA (%) values determined at 30 minutes and the volume of the alcoholic extract sample.

The volume corresponding to 50% ARA (IC_{50}) was calculated from the trendline equation y = mx + b using the formula:

$$IC_{50} = \frac{(50 - 0.312)}{0.871} = 570.47 \text{ mkl}$$

Table 4. Measured absorbance values and calculated antiradical activity values of the blank and tested aqueous extracts at a Melissa officinalis L.–licorice ratio of 1:1, added to the DPPH solution.

Volume, µL	Time, min	Sample					
		Abs, D	ARF%		Time, min	Abs, D	ARF%
25	0	1,024	0,00	75	0	1,024	0,00
	5	0,975	4,79		5	0,739	27,83
	10	0,962	6,05		10	0,711	30,57
	15	0,949	7,32		15	0,694	32,23
	20	0,931	9,08		20	0,672	34,38
	25	0,907	11,43		25	0,657	35,84
	30	0,889	13,18		30	0,634	38,09
50	0	1,024	0,00	100	0	1,024	0,00
	5	0,832	18,75		5	0,522	49,02
	10	0,819	20,02		10	0,513	49,90
	15	0,802	21,68		15	0,504	50,78
	20	0,791	22,75		20	0,495	51,66
	25	0,779	23,93		25	0,486	52,54
	30	0,767	25,10		30	0,478	53,32

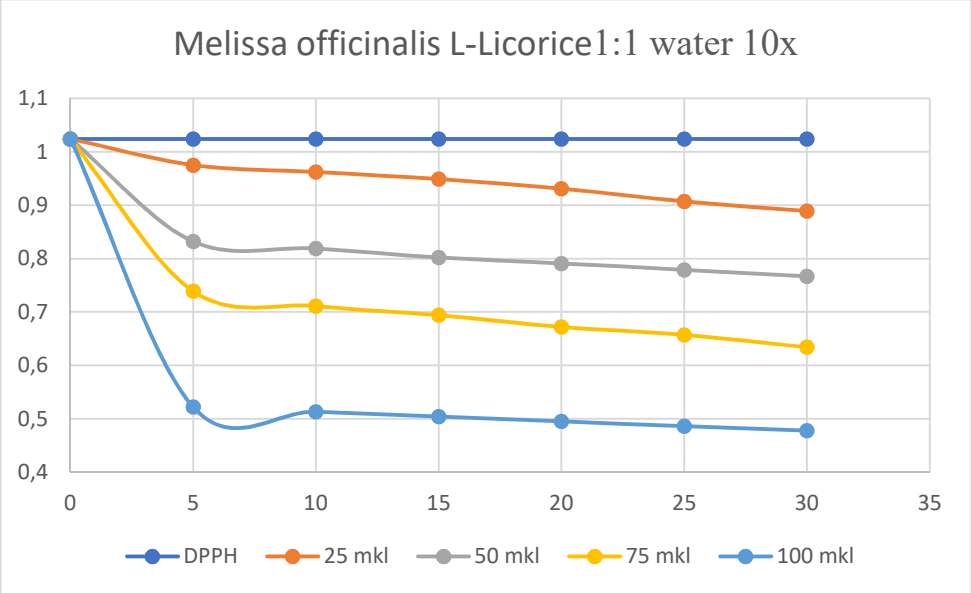


Figure 7. Graphical representation of the measured absorbance of the blank and tested aqueous extract sample solutions added to the DPPH solution.

To determine the IC50 values corresponding to 50% inhibition of the DPPH solution, the ARF% values obtained at the end of the 30-minute experimental period were used as the primary parameter. A graph was constructed based on the volumes of the aqueous samples and their corresponding antiradical activity values. The IC50 values were determined mathematically using the equation of the trendline fitted to the experimental data points

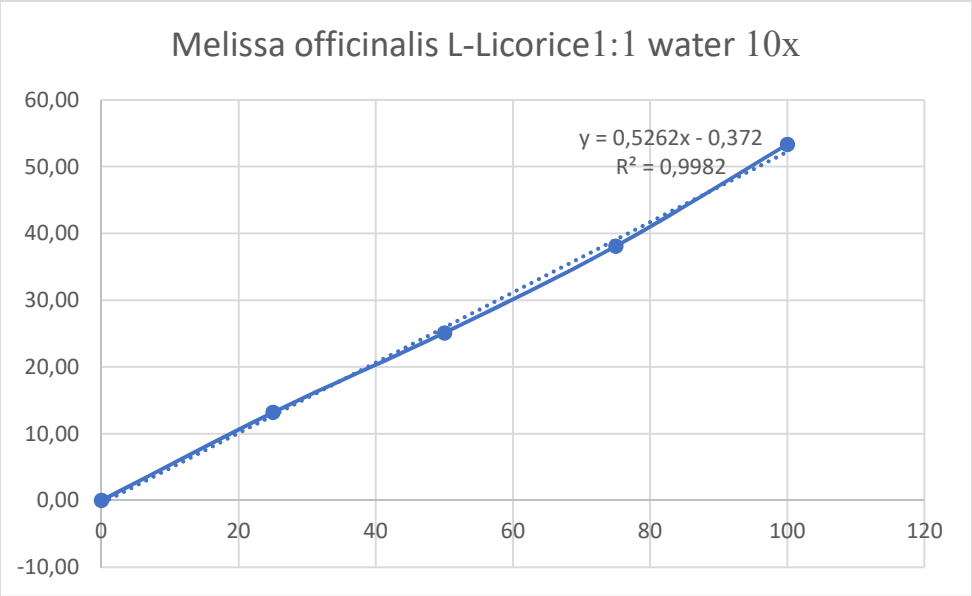


Figure 8. Graph showing the relationship between the ARA (%) values determined at 30 minutes and the volume of the aqueous extract sample.

The volume corresponding to 50% ARA (IC_{50}) was calculated from the trendline equation y = mx + b using the formula:

$$IC_{50} = \frac{(50 - 0.372)}{0.5262} = \mathbf{94.314}\ \text{mkl}$$

Table 5. Measured absorbance values and calculated antiradical activity values of the blank and tested aqueous extracts at a Melissa officinalis L.–licorice ratio of 3:1, added to the DPPH solution.

Volume, µL	Time, min	Sample					
		Abs, D	ARF%		Time, min	Abs, D	ARF%
25	0	1,024	0,00	75	0	1,024	0,00
	5	0,945	7,71		5	0,578	43,55
	10	0,903	11,82		10	0,551	46,19
	15	0,901	12,01		15	0,527	48,54
	20	0,883	13,77		20	0,506	50,59
	25	0,871	14,94		25	0,489	52,25
	30	0,85	16,99		30	0,466	54,49
50	0	1,024	0,00	100	0	1,024	0,00
	5	0,755	26,27		5	0,481	53,03
	10	0,719	29,79		10	0,447	56,35
	15	0,689	32,71		15	0,403	60,64
	20	0,668	34,77		20	0,378	63,09
	25	0,647	36,82		25	0,352	65,63
	30	0,638	37,70		30	0,324	68,36

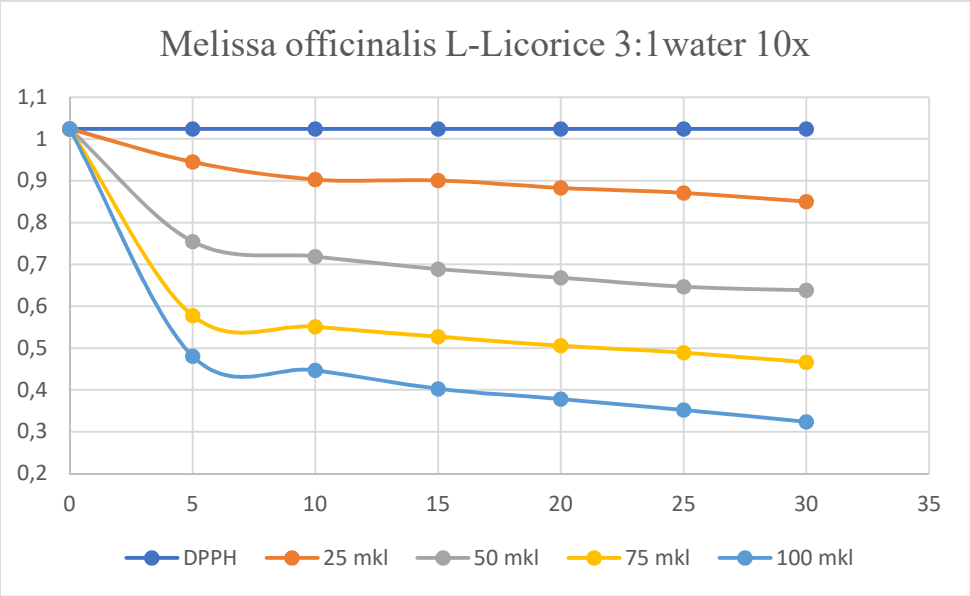


Figure 9. Graphical representation of the measured absorbance of the blank and tested aqueous extract sample solutions added to the DPPH solution.

To determine the IC50 values corresponding to 50% inhibition of the DPPH solution, the ARF% values obtained at the end of the 30-minute experimental period were used as the primary parameter. A graph was constructed based on the volumes of the aqueous samples and their corresponding antiradical activity values. The IC50 values were determined mathematically using the equation of the trendline fitted to the experimental data points

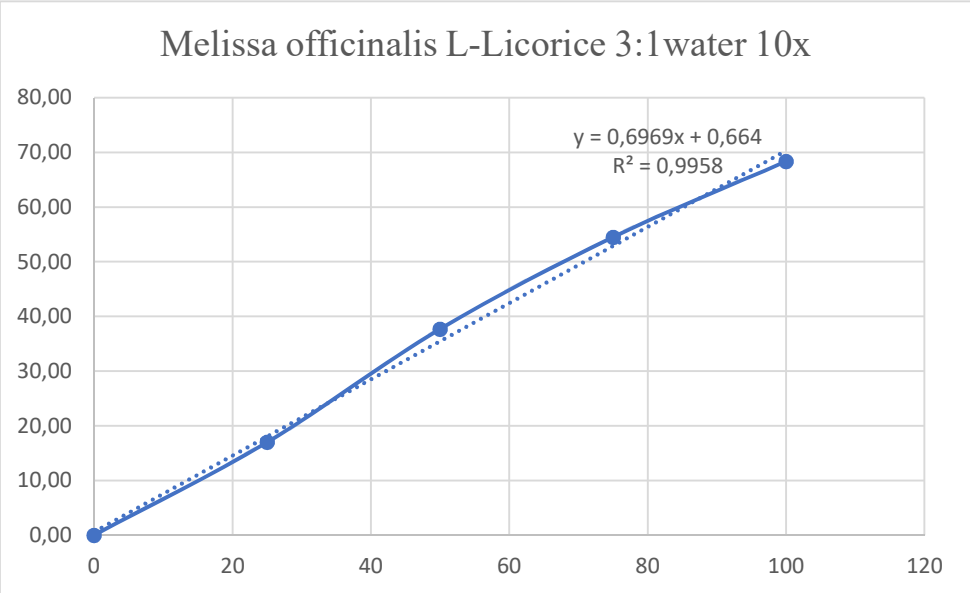


Figure 10. Graph showing the relationship between the ARA (%) values determined at 30 minutes and the volume of the aqueous extract sample.

The volume corresponding to 50% ARA (IC_{50}) was calculated from the trendline equation y = mx + b using the formula:

$$IC_{50} = \frac{(50 - 0.664)}{0.6969} = 70.794 \text{ mkl}$$

Table 6. Measured absorbance values and calculated antiradical activity values of the blank and tested aqueous extracts at a Melissa officinalis L.–licorice ratio of 1:3, added to the DPPH solution.

Volume, µL	Time, min	Sample					
		Abs, D	ARF%		Time, min	Abs, D	ARF%
25	0	1,024	0,00	75	0	1,024	0,00
	5	0,989	3,42		5	0,817	20,21
	10	0,972	5,08		10	0,795	22,36
	15	0,959	6,35		15	0,787	23,14
	20	0,947	7,52		20	0,775	24,32
	25	0,938	8,40		25	0,762	25,59
	30	0,932	8,98		30	0,754	26,37
50	0	1,024	0,00	100	0	1,024	0,00
	5	0,907	11,43		5	0,693	32,32
	10	0,895	12,60		10	0,684	33,20
	15	0,887	13,38		15	0,677	33,89
	20	0,875	14,55		20	0,671	34,47
	25	0,862	15,82		25	0,664	35,16
	30	0,854	16,60		30	0,659	35,64

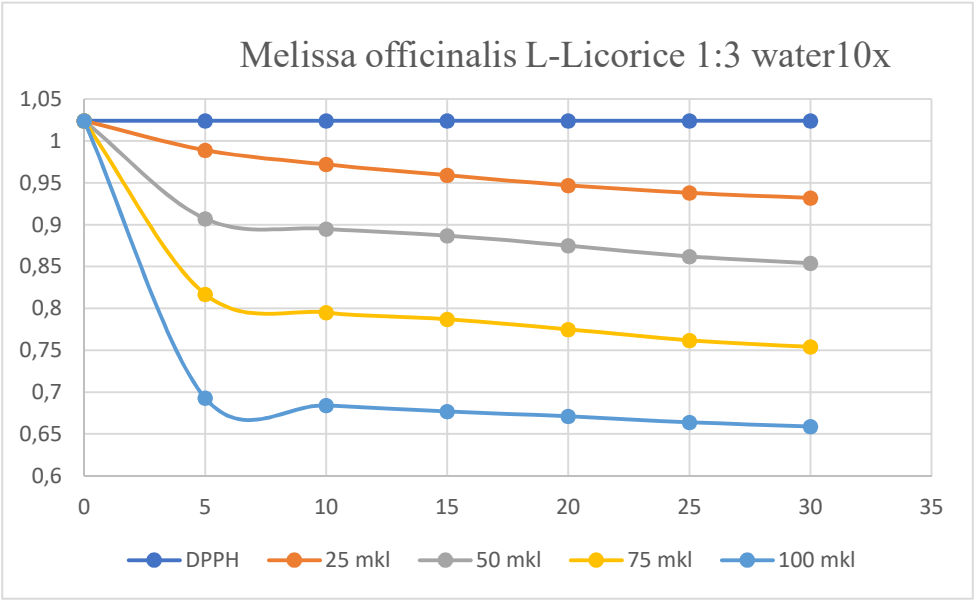


Figure 11. Graphical representation of the measured absorbance of the blank and tested aqueous extract sample solutions added to the DPPH solution.

To determine the IC50 values corresponding to 50% inhibition of the DPPH solution, the ARF% values obtained at the end of the 30-minute experimental period were used as the primary parameter. A graph was constructed based on the volumes of the aqueous samples and their corresponding antiradical activity values. The IC50 values were determined mathematically using the equation of the trendline fitted to the experimental data points

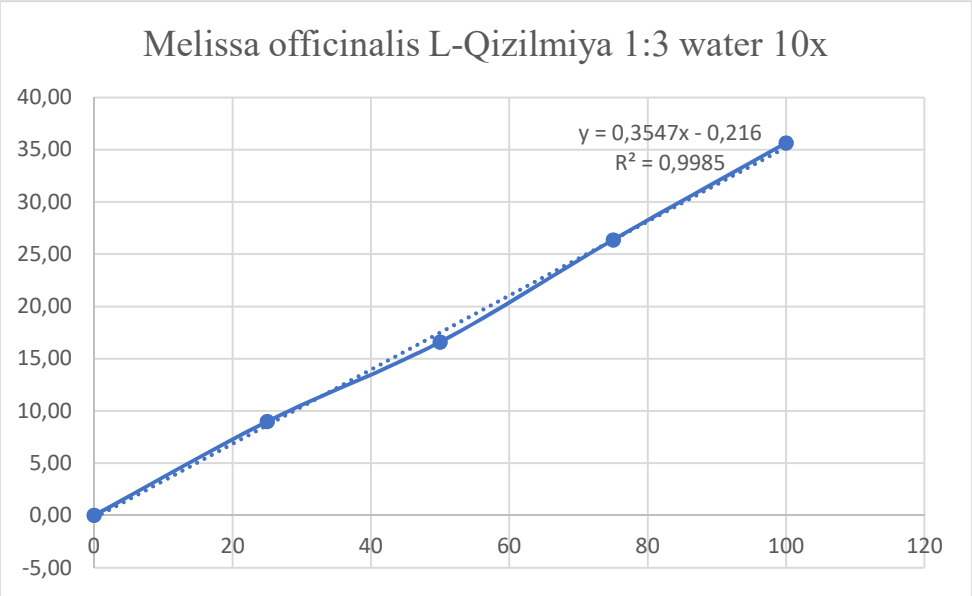


Figure 12. Graph showing the relationship between the ARA (%) values determined at 30 minutes and the volume of the aqueous extract sample.

The volume corresponding to 50% ARA (IC_{50}) was calculated from the trendline equation y = mx + b using the formula:

$$IC_{50} = \frac{(50 - 0.216)}{0.3547} = 140.36 \text{ mkl}$$

Table 7. Antiradical activity (ARA, %) values of the alcoholic and aqueous extracts of the samples at a volume of 100 µL after 30 minutes.

Time	ARA%					
	Melissa officinalis L.-Licorice 1:1 alcoholic	Melissa officinalis L.-Licorice 3:1 alcoholic	Melissa officinalis L.-Licorice 1:3 alcoholic	Melissa officinalis L.-Licorice 1:1 aqueous	Melissa officinalis L.-Licorice 3:1 s aqueous	Melissa officinalis L.-Licorice 1:3 aqueous
30-min.	29.00	11.33	9.18	53.32	68.36	35.64

Table 8. IC_{50} values of the samples—the concentrations (μL) required to inhibit 50% of the radicals in the DPPH solution.

Time	IC50					
	Melissa officinalis L.-Licorice 1:1 alcoholic	Melissa officinalis L.-Licorice 3:1 alcoholic	Melissa officinalis L.-Licorice 1:3 alcoholic	Melissa officinalis L.-Licorice 1:1 aqueous	Melissa officinalis L.-Licorice 3:1 s aqueous	Melissa officinalis L.-Licorice 1:3 aqueous
30-min.	169.77	458.52	570.47	94.314	70.794	140.36

Conclusion

In conclusion, it can be stated that the alcoholic and aqueous extracts based on the investigated Melissa officinalis L. and licorice exhibit antiradical activity. In particular, among the alcoholic extracts, the best result was obtained for the Melissa officinalis L.–licorice composition at a ratio of 1:1, with an antiradical activity of 29.00% after 30 minutes. The IC_{50} value was 169.77 μL, indicating antiradical activity.

Among the aqueous extracts, the best result was obtained for the Melissa officinalis L.–licorice composition at a ratio of 3:1, with an antiradical activity of 68.36% after 30 minutes. The IC_{50} value was 70.794 μL, demonstrating antiradical activity.

When the results measured after tenfold dilution were recalculated, it was determined that the ARA (%) values of the extracts in their original state were 10 times higher, while the IC_{50} values were 10 times lower. Thus, their effectiveness as natural antioxidants became more evident.

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