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DETERMINATION OF THE ANTIRADICAL ACTIVITY OF COMPOSITIONS OF NARROW-LEAVED OLEASTER (*ELAEAGNUS ANGUSTIFOLIA L.*) AND CHICORY (*CICHORIUM INTYBUS L.*) EXTRACTS

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Annotation. The article presents information on the causes of excessive formation of free radicals in the human body, oxidative stress, and its manifestations. The antiradical activity of extracts of narrow-leaved oleaster (*Elaeagnus angustifolia L.*) and common chicory (*Cichorium intybus L.*) was also evaluated using the DPPH method. In the study, the plant materials were mixed at mass ratios of 1:1, 3:1, and 1:3 and extracted using distilled water and 96% ethanol. According to the results obtained, among the compositions of narrow-leaved oleaster and common chicory plant materials at different ratios, the highest antiradical activity was observed in the aqueous extract at a ratio of 3:1, reaching **23.25%.**

Keywords: *Elaeagnus angustifolia*; *Cichorium intybus*; free radical; antiradical activity; extract; spectrophotometry.

How to Cite
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Introduction. It is well known that natural remedies are increasingly replacing synthetic pharmaceutical drugs used for the treatment and prevention of diseases. This issue is receiving considerable attention not only in our country but also worldwide, with extensive scientific and practical research being conducted in this field. The causes of various diseases have been identified and investigated at the molecular level. In particular, it has been established that free radicals and other reactive oxygen species (ROS) are generated in the body during cellular respiration and energy production in mitochondria, as well as under the influence of smoking, certain synthetic drugs, and toxic substances. Excessive accumulation of these reactive species beyond the capacity of the antioxidant defense system leads to oxidative stress.

Literature Review. Researchers have established that oxidative stress is accompanied by damage to DNA and RNA molecules and lipid peroxidation of biomembranes, while antioxidant defense plays an important role in the development and prevention of cardiovascular, nervous system, and oncological diseases [1–3].

Studies have emphasized that natural antioxidants present in plant raw materials, such as flavonoids, phenolic acids, vitamins C and E, and carotenoids, may serve as safer alternatives to synthetic antioxidants for neutralizing free radicals [4,5].

For evaluating the antiradical activity of such compounds, the in vitro assay based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical is one of the most widely used methods. It is characterized by simple instrumental requirements, high sensitivity, and good reproducibility [6–9].

Narrow-leaved oleaster (*Elaeagnus angustifolia L.*) is a shrub or small tree belonging to the family *Elaeagnaceae* and is widely distributed in Central Asia, Iran, India, China, and Japan [10].

The fruits, flowers, and leaves of the plant are used for food and medicinal purposes. Flavonoids, phenolic compounds, polysaccharides, saponins, and vitamins have been identified in its composition [11,12].

In traditional medicine, oleaster has long been used for blood purification, treatment of diarrhea and wounds, stimulation of appetite, as well as for diabetes, arrhythmia, and anemia [13,14].

Common chicory (*Cichorium intybus L.*), belonging to the family *Asteraceae*, is a perennial medicinal plant widely distributed in Europe and Asia. It commonly grows along roadsides, field margins, and meadows [15,16].

Its roots, stems, and leaves are rich in inulin, flavonoids, phenolic compounds, bitter sesquiterpene lactones, and vitamins. In traditional medicine, it is used as a diuretic, an anthelmintic, a hepatoprotective agent, and a remedy for diabetes and stimulation of metabolism [17–19].

Aim of the Research. The aim of this study was to comparatively evaluate the DPPH radical-scavenging activity of aqueous and ethanolic extracts of compositions prepared from oleaster (*E. angustifolia*) and chicory (*C. intybus*) raw materials at mass ratios of 1:1, 3:1, and 1:3, taking into account time- and concentration-dependent changes, and to determine the most effective ratio and extracting agent.

Materials and Methods.

Plant Materials. Leaves of narrow-leaved oleaster (*Elaeagnus angustifolia L.*) and the aerial parts of common chicory (*Cichorium intybus L.*) were used in the study. The raw materials were dried naturally in the shade at room temperature and subsequently ground using a laboratory grinder to obtain a uniform particle size. Composite mixtures of *Elaeagnus angustifolia* and *Cichorium intybus* were prepared at mass ratios of 1:1, 1:3, and 3:1 and used to evaluate their antiradical activity.

Preparation of the DPPH• Working Solution. A working solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) was prepared in ethanol at a concentration of 7.92 mM in a 100 mL volumetric flask. To minimize light-induced degradation of the solution, the flask was wrapped in aluminum foil and kept at room temperature under light-protected conditions for 30 min.

Preparation of Sample Extracts. Three types of composite mixtures of oleaster and chicory were prepared at mass ratios of 1:1, 3:1, and 1:3 using the ground raw materials. From each composition, 1.000 g was accurately weighed, and 25 mL of extracting agent (bidistilled water or 96% ethanol) was added. Extraction was carried out in an ultrasonic bath at 60 °C for 20 min. The resulting extracts were passed through a 0.45 µm syringe filter and diluted tenfold with the corresponding extracting agent [20].

Determination of the Antiradical Activity of the Samples. To prepare the control sample, 3 mL of DPPH• solution and 100 µL of ethanol were added to a 4 mL quartz cuvette and thoroughly mixed. The cuvette was placed in a spectrophotometer, and the absorbance (D_1) was measured at a wavelength of 517 nm every 5 min for 30 min using a YOKE (China) K7000 spectrophotometer.

To determine the antiradical activity of the samples, 3 mL of DPPH solution was added to 25, 50, 75, and 100 µL aliquots of the sample extracts. To ensure a total volume of 3.1 mL in the cuvette, 75, 50, 25, and 0 µL of 96% ethanol, respectively, were added. The absorbance of the mixtures at 517 nm (D_2) was measured under the same conditions described above.

The antiradical activity (ARA) was calculated using the following equation:

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

where D_1 is the optical density (absorbance) of the control sample, and D_2 is the optical density (absorbance) of the experimental sample at the corresponding time point.

Results and Discussion

Antiradical Activity of Ethanolic Extracts. The kinetics of DPPH radical scavenging by the ethanolic extracts are presented in Tables 1–3 and Figure 1. In all three compositions, the reaction proceeded

rapidly during the first 5 minutes, after which it gradually slowed down and approached a steady state at 20–30 minutes. This can be explained by the presence of a rapid initial phase followed by a slower secondary phase in the reaction between the DPPH radical and phenolic compounds.

Table 1. Optical Density and Antiradical Activity of the Ethanolic Extract Obtained from a 1:1 Composition of Oleaster and Chicory Raw Materials

Time, min.	(25 µL)		(50 µL)		(75 µL)		(100 µL)	
	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %
0	1,058	0,00	1,058	0,00	1,058	0,00	1,058	0,00
5	1,033	2,36	1,016	3,97	0,995	5,95	0,987	6,71
10	1,029	2,74	1,011	4,44	0,988	6,62	0,975	7,84
15	1,024	3,21	1,007	4,82	0,978	7,56	0,968	8,51
20	1,021	3,50	0,998	5,67	0,974	7,94	0,962	9,07
25	1,017	3,88	0,992	6,24	0,966	8,70	0,954	9,83
30	**1,014**	**4,16**	**0,988**	**6,62**	**0,961**	**9,17**	**0,945**	**10,68**

Table 2. Optical Density and Antiradical Activity of the Ethanolic Extract Obtained from a 3:1 Composition of Oleaster and Chicory Raw Materials

Time, min.	(25 µL)		(50 µL)		(75 µL)		(100 µL)	
	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %
0	1,058	0,00	1,058	0,00	1,058	0,00	1,058	0,00
5	0,998	5,67	0,955	9,74	0,933	11,81	0,886	16,26
10	0,992	6,24	0,943	10,87	0,927	12,38	0,880	16,82
15	0,985	6,90	0,934	11,72	0,921	12,95	0,872	17,58
20	0,979	7,47	0,928	12,29	0,906	14,37	0,866	18,15
25	0,974	7,94	0,922	12,85	0,898	15,12	0,861	18,62
30	**0,971**	**8,22**	**0,915**	**13,52**	**0,887**	**16,16**	**0,856**	**19,09**

Table 3. Optical Density and Antiradical Activity of the Ethanolic Extract Obtained from a 1:3 Composition of Oleaster and Chicory Raw Materials

Time, min.	(25 µL)		(50 µL)		(75 µL)		(100 µL)	
	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %
0	1,058	0,00	1,058	0,00	1,058	0,00	1,058	0,00
5	1,052	0,57	1,029	2,74	0,991	6,33	0,967	8,60
10	1,048	0,95	1,024	3,21	0,986	6,81	0,959	9,36
15	1,046	1,13	1,018	3,78	0,982	7,18	0,947	10,49
20	1,043	1,42	1,013	4,25	0,976	7,75	0,941	11,06
25	1,039	1,80	1,009	4,63	0,972	8,13	0,936	11,53
30	**1,034**	**2,27**	**1,005**	**5,01**	**0,965**	**8,79**	**0,932**	**11,91**

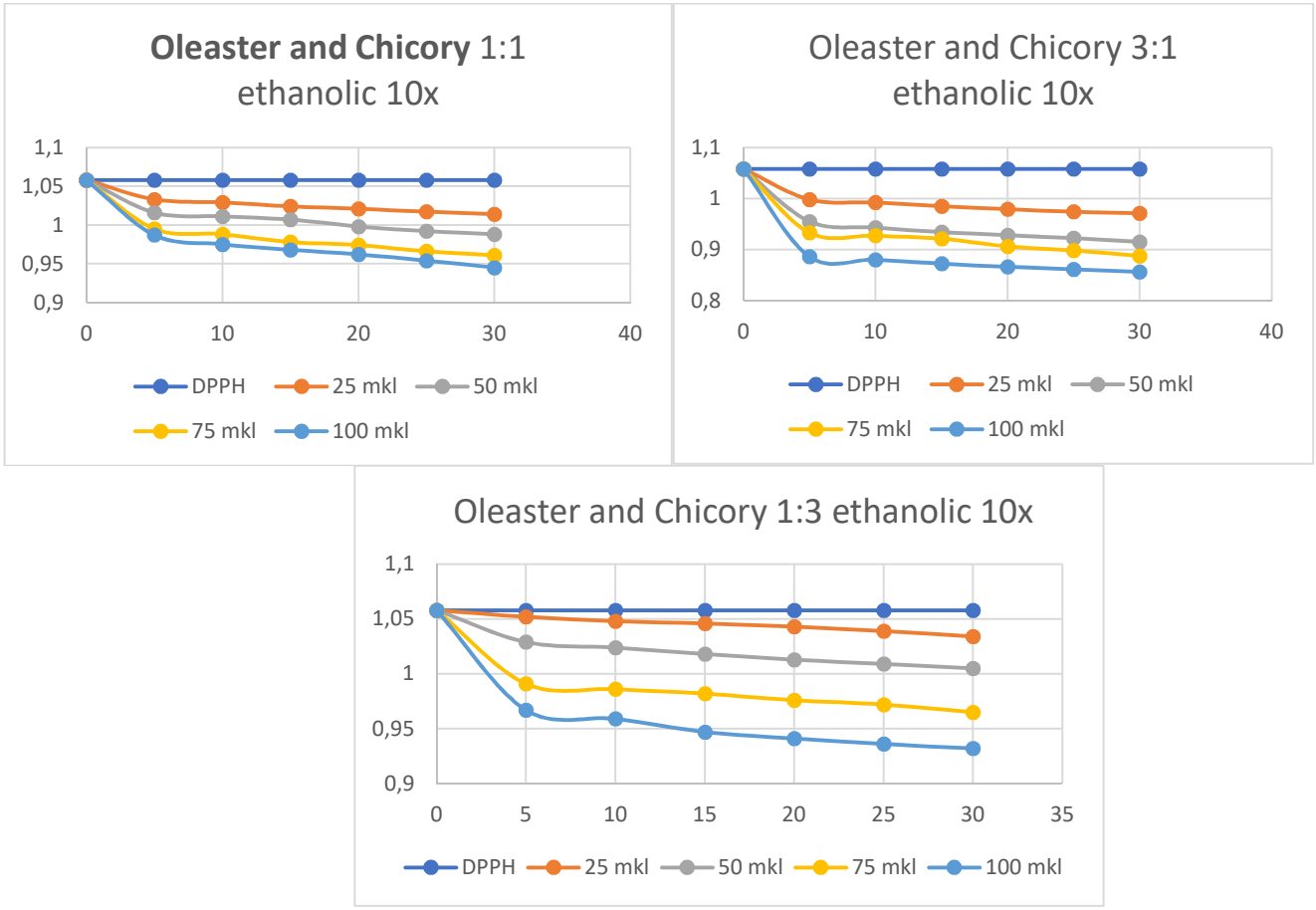


Figure 1. Graphical representation of the measured absorbance of DPPH solutions containing ethanolic extracts of composite mixtures of oleaster leaves and the aerial parts of chicory, along with the blank sample solution.

Estimation of the IC_{50} values of the Composite Extracts. IC_{50} represents the amount of extract required to inhibit 50% of DPPH free radicals. A lower IC_{50} value indicates higher antioxidant activity of the sample, whereas a higher IC_{50} value indicates relatively lower antioxidant activity. To determine the IC_{50} values, corresponding to the extract concentration required to achieve 50% inhibition of the DPPH radicals, a graph was constructed for each experiment based on the antiradical activity (ARA, %) values recorded at 30 min and the volume of the added aqueous sample. The IC_{50} values were then calculated using the trendline equation fitted to the experimental data.

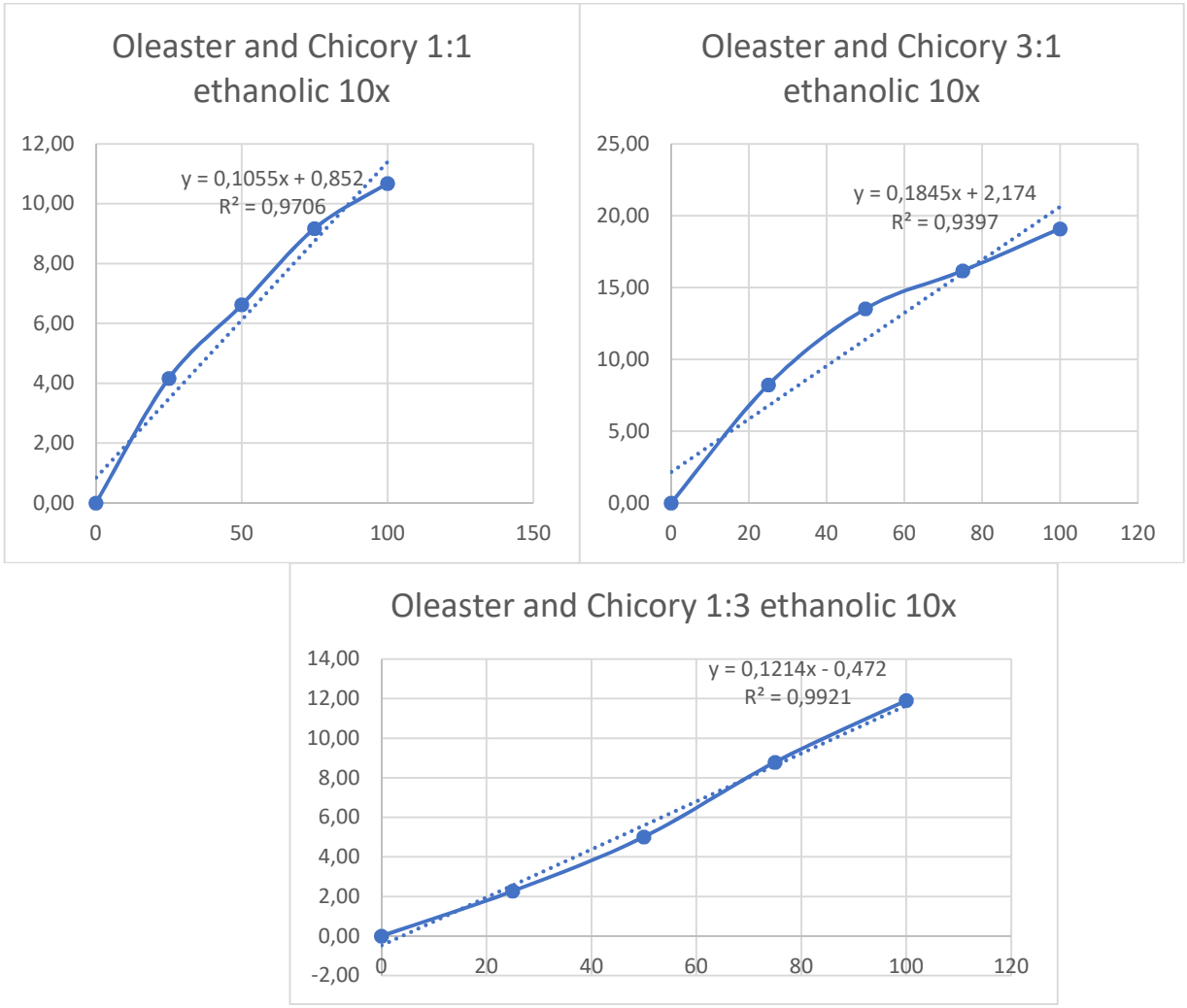


Figure 2. Relationship between the antiradical activity (ARA, %) and sample volume for the ethanolic extracts measured at 30 min.

The volume corresponding to 50% ARA (IC_{50}) was calculated using the trendline equation in the form y = mx + b, according to the formula x = (y − b)/m.

Table 4. Antiradical Activity (IC_{50}) of Composite Mixtures of Oleaster (Elaeagnus angustifolia) Leaves and Chicory (Cichorium intybus) Aerial Parts in Ethanolic Extracts

Extract	Oleaster:Chicory	Trendline (y = mx + b)	Calculation Formula	IC_{50} (µl)
Ethanolic	1:1	y = 0.1055x + 0.852	(50 − 0.852) / 0.1055	465.86
Ethanolic	3:1	y = 0.1845x + 2.174	(50 − 2.174) / 0.1845	259.22
Ethanolic	1:3	y = 0.1214x + 0.472	(50 − 0.472) / 0.1214	407.97

Antiradical Activity of Aqueous Extracts. The results obtained for the aqueous extracts are presented in Tables 5–7 and Figure 3. Although the time-dependent changes in activity were similar to those observed for the ethanolic samples, the absolute values were considerably higher at all tested ratios.

Table 5. Optical Density and Antiradical Activity of the Aqueous Extract Obtained from a 1:1 Composition of Oleaster and Chicory Raw Materials

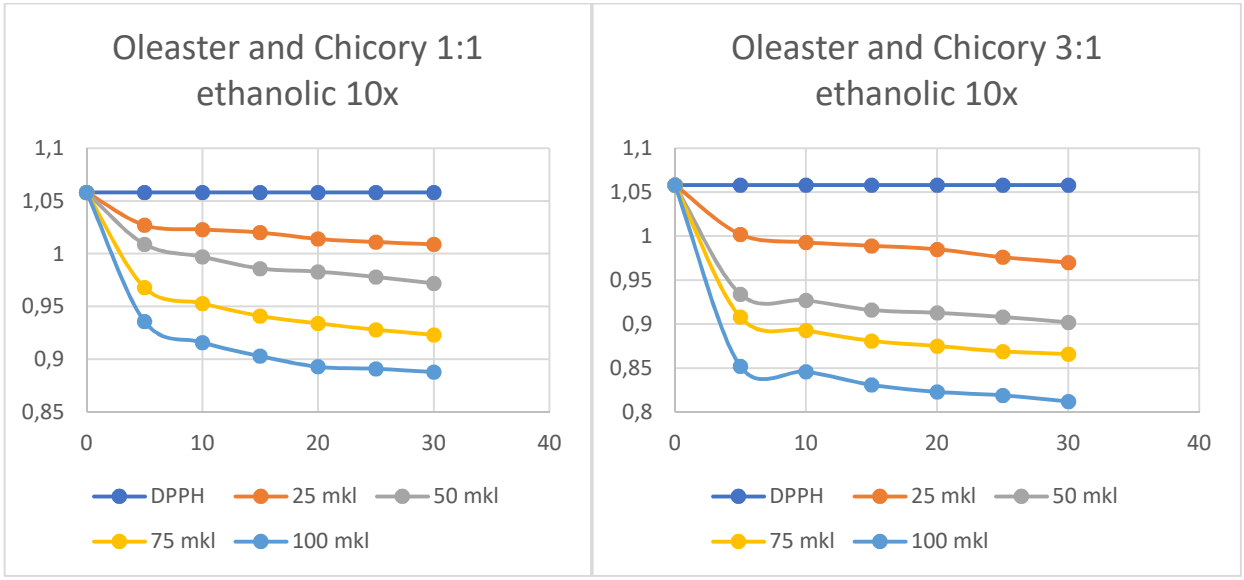
Time, min.	(25 µL)		(50 µL)		(75 µL)		(100 µL)	
	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %
0	1,058	0,00	1,058	0,00	1,058	0,00	1,058	0,00
5	1,027	2,93	1,009	4,63	0,968	8,51	0,936	11,53
10	1,023	3,31	0,997	5,77	0,953	9,92	0,916	13,42
15	1,020	3,59	0,986	6,81	0,941	11,06	0,903	14,65
20	1,014	4,16	0,983	7,09	0,934	11,72	0,893	15,60
25	1,011	4,44	0,978	7,56	0,928	12,29	0,891	15,78
30	**1,009**	**4,63**	**0,972**	**8,13**	**0,923**	**12,76**	**0,888**	**16,07**

Table 6. Optical Density and Antiradical Activity of the Aqueous Extract Obtained from a 3:1 Composition of Oleaster and Chicory Raw Materials

Time, min.	(25 µL)		(50 µL)		(75 µL)		(100 µL)	
	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %
0	1,058	0,00	1,058	0,00	1,058	0,00	1,058	0,00
5	1,002	5,29	0,934	11,72	0,908	14,18	0,852	19,47
10	0,993	6,14	0,927	12,38	0,893	15,60	0,846	20,04
15	0,989	6,52	0,916	13,42	0,881	16,73	0,831	21,46
20	0,985	6,90	0,913	13,71	0,875	17,30	0,823	22,21
25	0,976	7,75	0,908	14,18	0,869	17,86	0,819	22,59
30	**0,970**	**8,32**	**0,902**	**14,74**	**0,866**	**18,15**	**0,812**	**23,25**

Table 7. Optical Density and Antiradical Activity of the Aqueous Extract Obtained from a 1:3 Composition of Oleaster and Chicory Raw Materials

Time, min.	(25 µL)		(50 µL)		(75 µL)		(100 µL)	
	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %	D_{517}	ARA, %
0	1,058	0,00	1,058	0,00	1,058	0,00	1,058	0,00
5	1,026	3,02	0,989	6,52	0,933	11,81	0,893	15,60
10	1,022	3,40	0,987	6,71	0,927	12,38	0,886	16,26
15	1,019	3,69	0,979	7,47	0,921	12,95	0,872	17,58
20	1,015	4,06	0,973	8,03	0,916	13,42	0,863	18,43
25	1,010	4,54	0,968	8,51	0,905	14,46	0,861	18,62
30	**1,005**	**5,01**	**0,960**	**9,26**	**0,897**	**15,22**	**0,855**	**19,19**



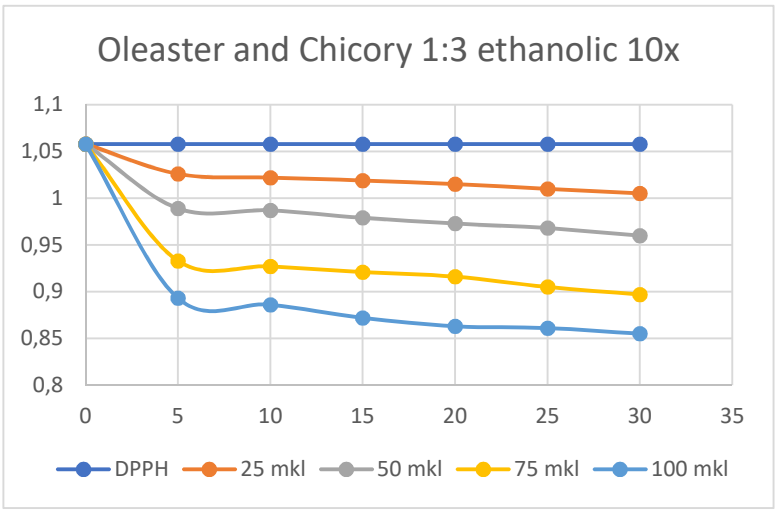


Figure 3. Absorbance profiles of DPPH solutions containing aqueous extracts of composite mixtures of oleaster leaves and chicory aerial parts, with the blank solution as a reference.

To determine the IC_{50} values, corresponding to the extract concentration required to achieve 50% inhibition of the DPPH radicals, a graph was constructed for each experiment based on the antiradical activity (ARA, %) values measured at 30 min and the volume of the added aqueous sample. The IC_{50} values were then calculated using the trendline equation fitted to the experimental data

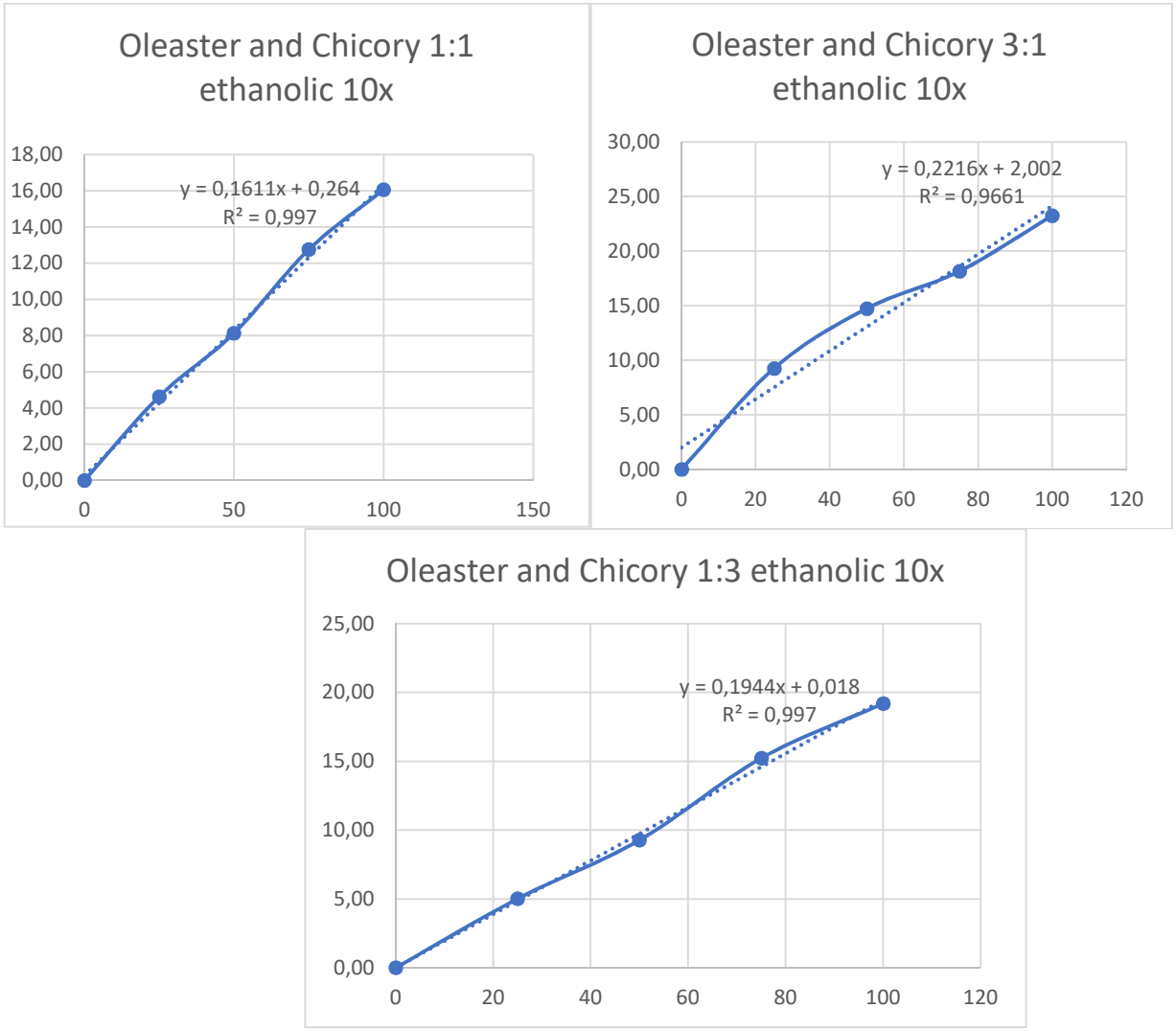


Figure 4. Relationship between the antiradical activity (ARA, %) and sample volume of the aqueous extracts measured at 30 min.

The volume corresponding to 50% ARA (IC_{50}) was calculated from the trendline equation in the form y = mx + b, using the formula x = (y − b)/m:

Table 8. Antiradical Activity (IC_{50}) of Composite Mixtures of Oleaster (Elaeagnus angustifolia) Leaves and Chicory (Cichorium intybus) Aerial Parts in Aqueous Extracts

Extract	Oleaster:Chicory	Trendline (y = mx + b)	Calculation Formula	IC_{50} (µl)
Water	1:1	y = 0.1611x + 0.264	(50 − 0.264) / 0.1611	308.73
Water	3:1	y = 0.2216x + 2.002	(50 − 2.002) / 0.2216	216.60
Water	1:3	y = 0.1944x + 0.018	(50 − 0.018) / 0.1944	257.11

Table 9. Antiradical Activity (ARA, %) at 30 min and IC_{50} Values of Oleaster and Chicory Extracts at a Sample Volume of 100 µL

Sample	ARA (%) (100 µL, 30 minute)	IC_{50} (µL, 30 minute)
Oleaster and Chicory 1:1 ethanolic	10.68	465.86
Oleaster and Chicory 3:1 ethanolic	19.09	259.22
Oleaster and Chicory 1:3 ethanolic	11.91	407.97

Oleaster and Chicory 1:1 water	16.07	308.73
Oleaster and Chicory 3:1 water	23.25	216.6
Oleaster and Chicory 1:3 water	19.19	257.11

Based on the ARA (%) values, the extracts obtained from the oleaster leaves and chicory aerial parts used in the study were ranked in the following order: **aqueous 3:1 > aqueous 1:3 > ethanolic 3:1 > aqueous 1:1 > ethanolic 1:3 > ethanolic 1:1**. Thus, in both extracting media, the **3:1 ratio**, in which the proportion of oleaster was predominant, was found to be the most effective, while aqueous extraction demonstrated higher activity than ethanolic extraction at all tested ratios.

Antioxidant activity varied significantly depending on the composition of the extracts and the extraction method. Among the ethanolic extracts, the highest antiradical activity was observed for the **3:1** oleaster-to-chicory extract, with an ARA of **19.09%** and an IC_{50} value of **259.22 µL**. Among the aqueous extracts, the **3:1** extract also demonstrated the highest activity, with an ARA of **23.25%** and an IC_{50} value of **216.6 µL**. In contrast, the ethanolic extract at a **1:1 ratio** exhibited the lowest antioxidant activity, with an ARA of **10.68%** and an IC_{50} value of **465.86 µL**.

Conclusion. The antioxidant activity of alcoholic and aqueous extracts prepared from the leaves of narrow-leaved oleaster (*Elaeagnus angustifolia L.*) and the aerial parts of common chicory (*Cichorium intybus L.*) at different ratios was evaluated using the DPPH method;

The results of the study showed that all the obtained extracts possess the ability to inhibit free radicals to a certain extent;

Antioxidant activity differed significantly depending on the composition of the extract and the extraction method. Among the alcoholic extracts, the highest antiradical activity was observed for the extract of oleaster and chicory at a ratio of 3:1, amounting to 19.09%, with an IC_{50} value of 259.22 µL. Among the aqueous extracts, the highest activity was also observed at a ratio of 3:1, with an antiradical activity of 23.25% and an IC_{50} value of 216.6 µL.

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