EVALUATION OF THE ANTIRADICAL ACTIVITY OF ALCOHOLIC EXTRACTS OF ST. JOHN'S WORT (HYPERICUM PERFORATUM L.), STINGING NETTLE (URTICA DIOICA L.), AND WORMWOOD (ARTEMISIA ABSINTHIUM L.)

DALACHOY(HYPERICUM PERFORATUM L.), GAZANDA (URTICA DIOICA L.) VA ERMON (ARTEMISIA ABSINTHIUM L.) O'SIMLIKLARINING SPIRTLI EKSTRAKSIYALARINING ANTIRADIKALLIK FAOLLIGINI BAHOLASH

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Abstract. In this study, the antiradical activity of alcoholic extracts prepared from the aerial parts of *Hypericum perforatum* L., *Urtica dioica* L., and *Artemisia absinthium* L. in different proportions was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.. The radical-scavenging capacity of the extracts was assessed by measuring the decrease in DPPH-solution absorbance. The 1:1:1 mixture exhibited the highest activity, with a radical-scavenging activity (ARF) of 23.58% after 30 min and an IC_{50} value of 218.17 μL. The extracts prepared at ratios of 3:1:1 and 1:3:1 also showed radical-scavenging activity, although the 1:1:1 mixture was the most active under the conditions tested. These findings suggest that the investigated plant extracts may be promising sources of natural antioxidants.

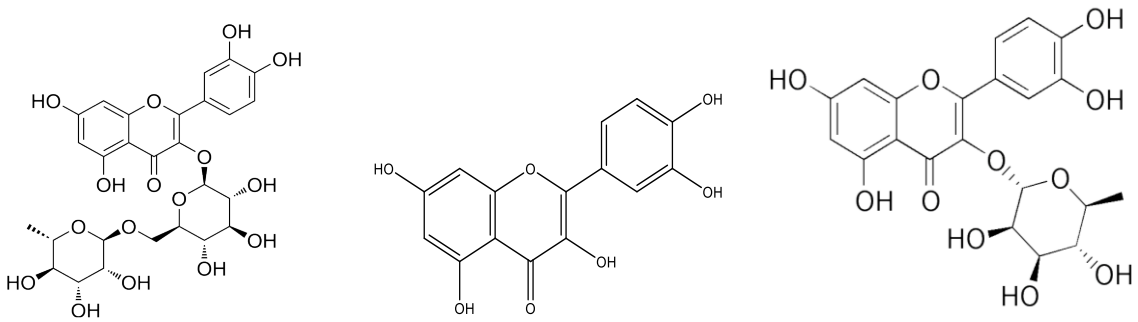
Annotatsiya. Ushbu maqolada dalachoy *(Hypericum perforatum L.)* , gazanda *(Urtica dioica L.)* va ermon *(Artemisia absinthium L.)* o`simliklarining yer ustki qismlaridan turli nisbatda olingan spirtli ekstraktlarning antiradikallik faolligi DPPH usuli yordamida baholandi.. Ekstraktlarning erkin radikallarni to'xtatish qobiliyati DPPH eritmasining yorug'lik yutilishini pasaytirishi orqali aniqlandi. Natijalarga ko'ra ekstrakti 1:1:1 nisbatda eng yuqori faollikni namoyon etdi: 30 daqiqadan so'ng ARF 23.58% ni, IC_{50} esa 218.17 mkl ni tashkil etdi. 3:1:1 va 1:3:1 nisbatlardagi ekstraktlar ham antioksidant xususiyatga ega bo'ldi, biroq 1:1:1 nisbat eng samarali ekanligi aniqlandi.Olingan natijalar ekstraktlarining tabiiy antioksidant sifatidagi salohi yatini tasdiqlaydi.

Key words: free radicals; St. John's wort (Hypericum perforatum L.); stinging nettle (Urtica dioica L.); wormwood (Artemisia absinthium L.); extract; inhibition; DPPH; IC_{50}; radical-scavenging activity ARF%.

Kalit so`zlar: erkin radikal, dalachoy *(Hypericum perforatum L.)*, gazanda *(Urtica dioica L.)*, ermon *(Artemisia absinthium L.)* , ekstrakt, ingibirlash, DPPH, IC_{50} , ARF%

INTRODUCTION. Free radicals formed in the body mainly include reactive oxygen and nitrogen species. They may arise through endogenous processes and under various physicochemical or pathophysiological conditions. Free radicals can adversely modify lipids, proteins, and DNA, and oxidative stress is associated with the development or progression of diseases such as liver and cardiovascular disorders, diabetes, and cancer. One approach to reducing oxidative processes in the body is the consumption of natural exogenous food supplements rich in beneficial plant-derived compounds [1].

St. John's wort is a perennial plant, approximately 30–100 cm tall, found throughout Uzbekistan. It has traditionally been used in preparations for liver conditions. Its constituents include flavonoids with reported radical-scavenging properties, such as rutin, quercetin, and quercitrin.

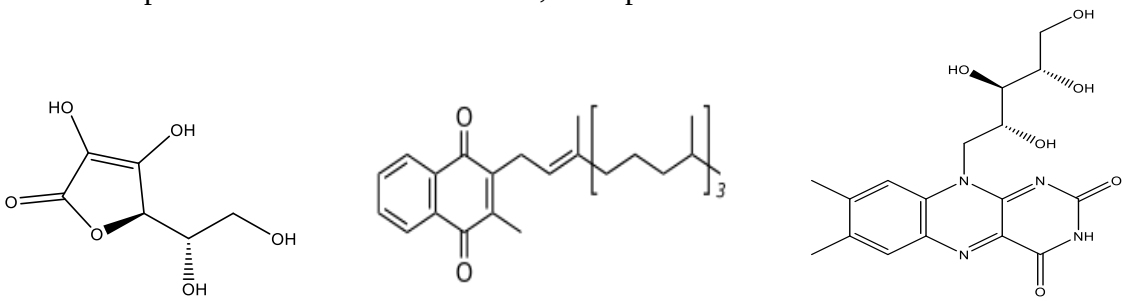


rutin quercetin quercitrin

Figure 1. Phenolic compounds found in St. John's wort.

The phenolic compounds shown in Figure 1 may contribute to the antioxidant and radical-scavenging properties attributed to St. John's wort [2].

Stinging nettle is a perennial plant, approximately 60–150 cm tall, found in many regions of Uzbekistan. Its leaves and roots have been used in traditional medicine for liver-related conditions. The plant contains several vitamins, examples of which are shown below.

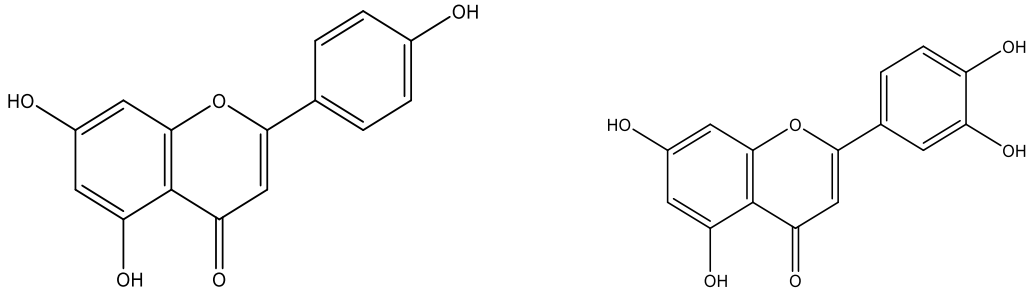


Vitamin C Vitamin K Vitamin B_2

Figure 2. Vitamins found in stinging nettle.

Figure 2 presents examples of biologically active compounds reported in the leaves of stinging nettle.

Wormwood is a perennial plant approximately 50–100 cm tall. Its aerial parts have traditionally been used for inflammatory conditions. Its stems and leaves contain flavonoids, including quercetin, apigenin, and luteolin, which are associated with antioxidant activity [1].



Apigenin Luteolin

Figure 3. Selected flavonoids found in wormwood.

The flavonoids shown in Figure 3 can scavenge free radicals in experimental systems. Some compounds found in medicinal plants have also been investigated for potential hepatoprotective effects and for their roles in cellular responses to toxic substances; such effects should not, however, be inferred from the present in vitro assay alone [3].

LITERATURE REVIEW AND METHODOLOGY

In recent years, oxidative stress associated with free radicals has been considered an important factor in the development of various diseases, including cardiovascular disorders, diabetes, inflammatory conditions, and cancer. Accordingly, identifying antioxidant compounds from natural sources and evaluating their biological activity remain important areas of research.

Plant-derived extracts may contain phenolic compounds, flavonoids, ascorbic acid,

terpenoids, and other biologically active substances capable of scavenging free radicals. Studies have reported radical-scavenging activity in alcoholic and aqueous extracts obtained from plant leaves, stems, and aerial parts.

One widely used method for evaluating radical-scavenging activity is the spectrophotometric assay based on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The method is relatively simple and rapid, and it is widely used to compare antioxidant activity. In the DPPH assay, antioxidants donate a hydrogen atom or an electron, causing the purple DPPH solution to lose colour and its absorbance to decrease.

The DPPH method introduced by Blois has subsequently been used and adapted by many researchers to assess the radical-scavenging activity of plant extracts. Published studies indicate that extraction conditions, solvent type, the ratio of plant material to solvent, and extract concentration can substantially affect measured activity. Some studies have also reported higher antioxidant activity in mixtures of plant materials than in individual extracts; this may reflect synergistic interactions among bioactive constituents, although synergy requires appropriate experimental confirmation.

The IC_{50} value is an important parameter for quantifying radical-scavenging activity. A lower IC_{50} generally indicates that a lower amount or concentration of a sample is required to achieve 50% inhibition. The DPPH assay is therefore commonly used to determine radical-scavenging activity (%) and IC_{50}.

This literature review supports the scientific and practical relevance of evaluating extracts prepared from plant materials in different proportions and investigating their potential as sources of natural antioxidants.

Natural antioxidants occur in foods, medicinal plants, and other natural products. By scavenging free radicals, they may help limit oxidative processes in experimental systems. Spices, vegetable oils, teas, seeds, grains, cocoa shells, fruits, and vegetables can contain antioxidant compounds, including ascorbic acid, tocopherols, carotenoids, flavonoids (e.g., quercetin, kaempferol, and myricetin), catechin-related compounds, polyphenols, and phenolic acids.

In the present study, the radical-scavenging activity (ARF) of extracts prepared at different plant-material ratios—X_1 (1:1:1), X_2 (3:1:1), and X_3 (1:3:1)—was evaluated against the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. The extracts contained the following plant materials:

X_1 extract (1:1:1): A mixture of the aerial parts of *Hypericum perforatum* L., *Urtica dioica* L., and *Artemisia absinthium* L. in a 1:1:1 ratio.

X_2 extract (3:1:1): A mixture of the aerial parts of *Hypericum perforatum* L., *Urtica dioica* L., and *Artemisia absinthium* L. in a 3:1:1 ratio.

X_3 extract (1:3:1): A mixture of the aerial parts of *Hypericum perforatum* L., *Urtica dioica* L., and *Artemisia absinthium* L. in a 1:3:1 ratio.

Assessment of the radical-scavenging activity of the samples using the DPPH assay

Decolourisation of the purple DPPH solution indicates the ability of a test compound to donate a hydrogen atom or an electron. The stable DPPH• radical is commonly used as a reagent in spectrophotometric assays [4]. In this study, the method described by Blois [5] was used, with minor modifications, to assess inhibition of the DPPH• radical [6].

Preparation of the DPPH• working solution. A 7.92 mM DPPH• solution was prepared in ethanol in a 100 mL volumetric flask. The flask was wrapped in aluminium foil and kept in the dark at room temperature for 30 min.

Experimental procedure. Each plant sample (1 g) was extracted with 25 mL of 96% ethanol in an ultrasonic bath for 20 min. The resulting extract was passed through a 0.45 µm syringe filter before analysis. The filtered alcoholic extracts were diluted tenfold. Extract mixtures of St. John's wort, stinging nettle, and wormwood were prepared at the reported ratios of 1:1:1, 3:1:1, and 1:3:1. The original manuscript refers to six preparations, although only three ratios are specified.

Determination of radical-scavenging activity. A quartz cuvette with a capacity of 4 mL was filled with 3 mL of DPPH solution and 100 µL of ethanol (blank) and placed in a K7000 spectrophotometer (YOKE, China). Absorbance (A_1) was measured at 517 nm every 5 min for 30 min. To evaluate the samples, 25, 50, 75, or 100 µL of each extract was mixed with 3 mL of DPPH solution, and absorbance (A_2) was measured under the same conditions at 517 nm. Ethanol was added to bring the total volume in the cuvette to 3.1 mL. Radical-scavenging activity was calculated using the formula provided in the original manuscript.

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

The results are presented in the tables below.

Table 1.

Absorbance and calculated radical-scavenging activity of the blank and alcoholic extracts of St. ***John's wort, stinging nettle, and wormwood*** the aerial parts of the plants mixed at a ratio of **1:1:1** after addition to the DPPH solution.

Volume (µL)	Time (min)	Sample					
		Absorbance (A)	ARF (%)		Time (min)	Absorbance (A)	ARF (%)
25	0	0,933	0,00	75	0	0.933	0.00
	5	0.893	4.29		5	0.839	10.08
	10	0.865	7.29		10	0.793	15.01
	15	0.861	7.72		15	0.784	15.97
	20	0.855	8.36		20	0.775	16.93
	25	0.851	8.79		25	0.769	17.58
	30	0.846	**9.32**		**30**	0.762	**18.33**
50	0	0.933	0.00	100	0	0.933	0.00
	5	0.871	6.65		5	0.803	13.93
	10	0.831	10.93		10	0.751	19.51
	15	0.826	11.47		15	0.741	20.58
	20	0.82	12.11		20	0.73	21.76
	25	0.815	12.65		25	0.723	22.51
	30	0.808	**13.40**		**30**	0.713	**23.58**

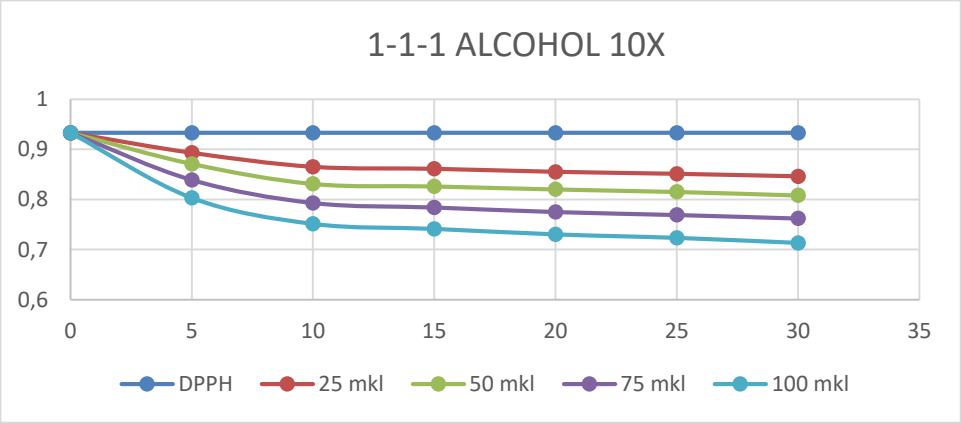


Figure 4. Absorbance profiles of the blank DPPH solution and the tested alcoholic-extract samples over time.

Figure 4 shows the absorbance values measured after adding the alcoholic extracts to the DPPH solution. To estimate the IC_{50}—the sample volume associated with 50% inhibition of the DPPH radical—the 30-min radical-scavenging activity values (%) were plotted against the volumes of extract added. The IC_{50} was estimated from the fitted trendline equation.

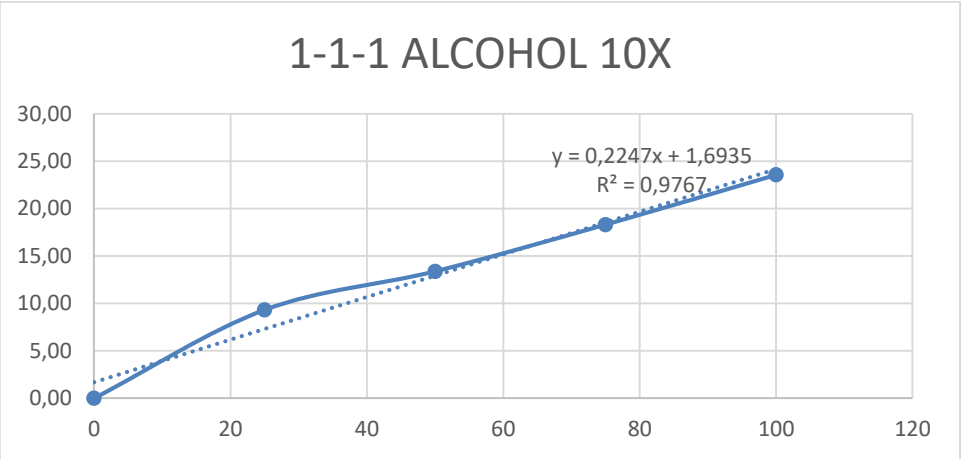


Figure 5. Relationship between radical-scavenging activity (%) and the volume of the alcoholic extract (10-fold diluted).

The trendline was expressed as y = mx + b. The extract volume corresponding to 50% radical-scavenging activity (IC_{50}) was calculated using x = (y − b)/m, with y set to 50.

$$IC_{50} = \frac{(50 - 0.9767)}{0.2247} = 218.17\ mkl$$

Table 2.

Absorbance and calculated radical-scavenging activity of the blank and alcoholic extracts of **St. *John's wort, stinging nettle, and wormwood*** the aerial parts of the plants mixed at a ratio of **1:3:1** after addition to the DPPH solution.

Volume (µL)	Time (min)	Sample					
		Absorbance (A)	ARF (%)		Time (min)	Absorbance (A)	ARF (%)
25	0	0,922	0,00	75	0	0,922	0,00
	5	0,856	7,16		5	0,835	9,44
	10	0,852	7,59		10	0,826	10,41
	15	0,848	8,03		15	0,819	11,17
	20	0,845	8,35		20	0,813	11,82
	25	0,843	8,57		25	0,808	12,36
	30	0,841	**8,79**		**30**	0,804	**12,80**
50	0	0,922	0,00	100	0	0,922	0,00
	5	0,839	9,00		5	0,818	11,28
	10	0,832	9,76		10	0,806	12,58
	15	0,827	10,30		15	0,797	13,56
	20	0,824	10,63		20	0,791	14,21
	25	0,821	10,95		25	0,785	14,86
	30	0,818	**11,28**		**30**	0,781	**15,29**

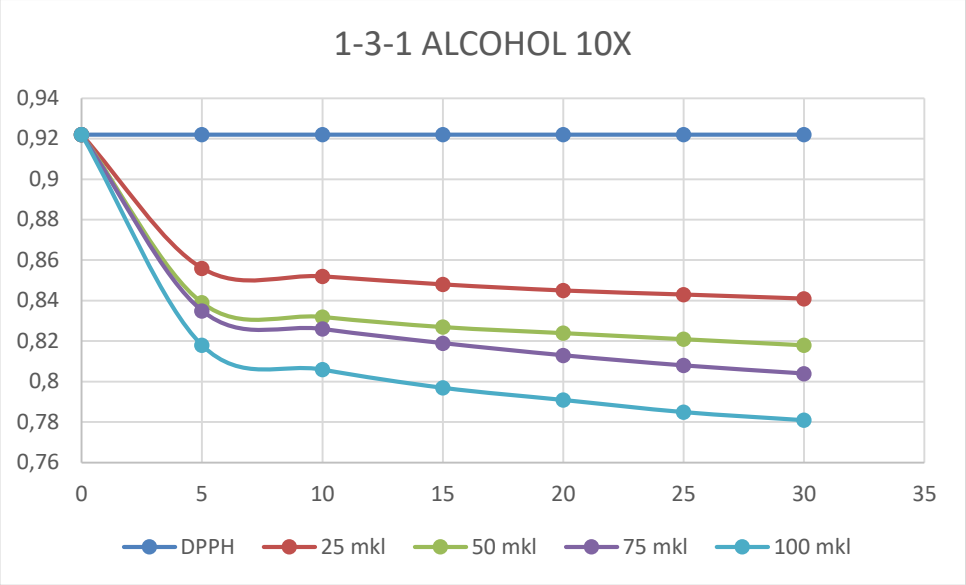


Figure 6. Absorbance profiles of the blank DPPH solution and the tested alcoholic-extract samples over time.

To estimate the IC_{50}, the radical-scavenging activity values (%) measured at 30 min were plotted against the volumes of alcoholic extract added. The IC_{50} was then estimated from the fitted trendline equation.

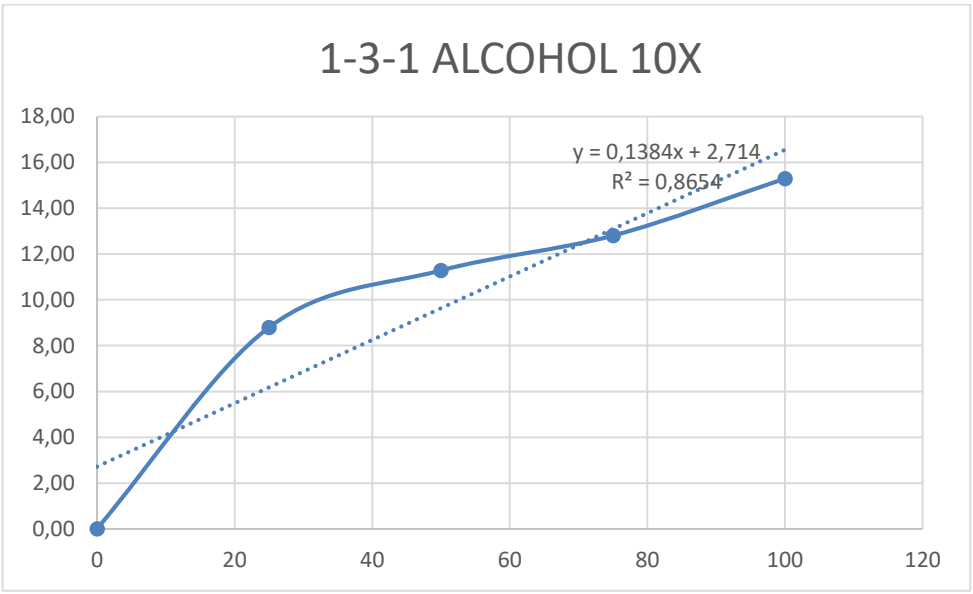


Figure 7. Relationship between radical-scavenging activity (%) and the volume of the alcoholic extract (10-fold diluted).

The trendline was expressed as y = mx + b. The extract volume corresponding to 50% radical-scavenging activity (IC_{50}) was calculated using x = (y − b)/m, with y set to 50.

$$IC_{50} = \frac{(50 - 0{,}1384)}{2.714} = 341.7\ mkl$$

Table 3.

Absorbance and calculated radical-scavenging activity of the blank and alcoholic extracts of ***St. John's wort, stinging nettle, and wormwood*** the aerial parts of the plants mixed at a ratio of **1:1:3** after addition to the DPPH solution.

Volume (µL)	Time (min)	Sample					
		Absorbance (A)	ARF (%)		Time (min)	Absorbance (A)	ARF (%)
25	0	0,912	0,00	75	0	0,912	0,00
	5	0,871	4,50		5	0,823	9,76
	10	0,865	5,15		10	0,809	11,29
	15	0,86	5,70		15	0,799	12,39
	20	0,855	6,25		20	0,789	13,49
	25	0,852	6,58		25	0,783	14,14
	30	0,848	**7,02**		**30**	0,775	**15,02**
50	0	0,912	0,00	100	0	0,912	0,00
	5	0,858	5,92		5	0,794	12,94
	10	0,849	6,91		10	0,775	15,02
	15	0,843	7,57		15	0,762	16,45
	20	0,836	8,33		20	0,746	18,20
	25	0,832	8,77		25	0,738	19,08
	30	0,827	**9,32**		**30**	0,729	**20,07**

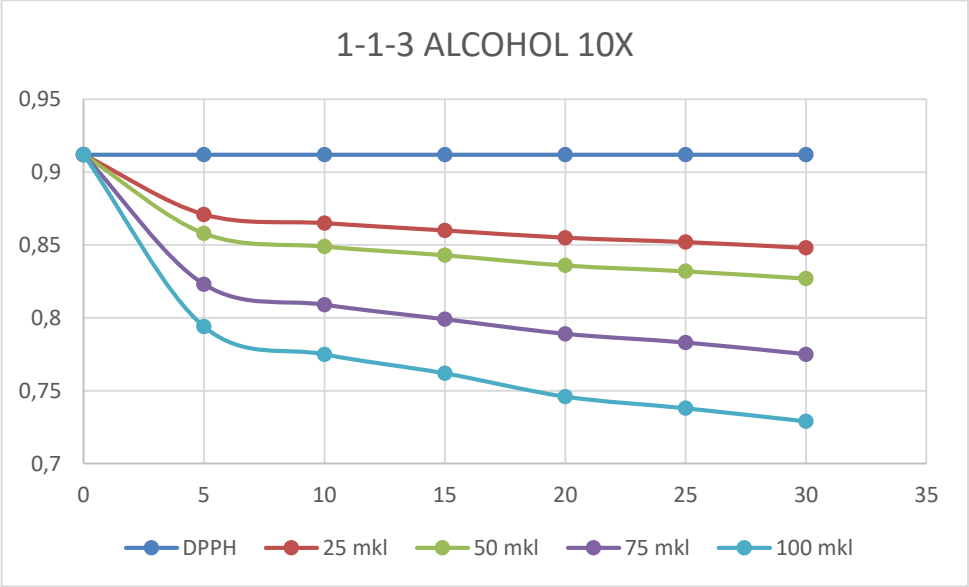


Figure 8. Absorbance profiles of the blank DPPH solution and the tested alcoholic-extract samples over time.

To estimate the IC_{50}, the radical-scavenging activity values (%) measured at 30 min were plotted against the volumes of alcoholic extract added. The IC_{50} was then estimated from the fitted trendline equation.

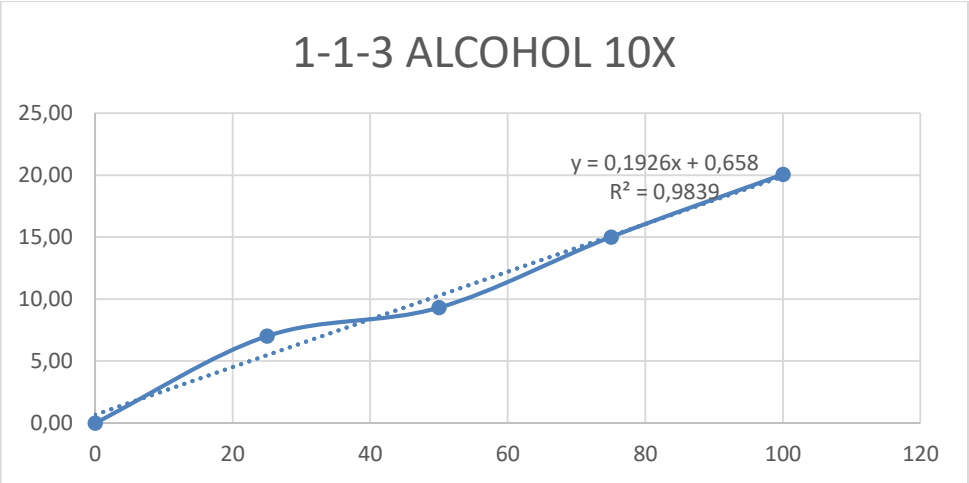


Figure 9. Relationship between radical-scavenging activity (%) and the volume of the alcoholic extract (10-fold diluted).

The trendline was expressed as y = mx + b. The extract volume corresponding to 50% radical-scavenging activity (IC_{50}) was calculated using x = (y − b)/m, with y set to 50.

$$IC_{50} = \frac{(50 - 0.658)}{0.1926} = 256.2\ mkl$$

Table 4.

Radical-scavenging activity (%) at 30 min for 100 µL of the tenfold-diluted alcoholic extracts.

Time (min)	ARF (%)		
	St. John's wort–stinging nettle–wormwood (1:1:1)	St. John's wort–stinging nettle–wormwood (1:3:1)	St. John's wort–stinging nettle–wormwood (1:1:3)
30 min	23.58	15.29	20.07

Table 4 shows the radical-scavenging activity values measured after 30 min for 100 µL portions of the tenfold-diluted alcoholic extracts. The highest value was observed for the 1:1:1 mixture of St. John's wort (Hypericum perforatum L.), stinging nettle (Urtica dioica L.), and wormwood (Artemisia absinthium L.).

Table 5.

IC_{50} values for the tenfold-diluted alcoholic extracts, expressed as the sample volume estimated to produce 50% inhibition of the DPPH radical (µL).

Time (min)	ARF (%)		
	St. John's wort–stinging nettle–wormwood (1:1:1)	St. John's wort–stinging nettle–wormwood (1:3:1)	St. John's wort–stinging nettle–wormwood (1:1:3)
30 min	218.17	341.7	256.2

As shown in Table 5, the lowest estimated IC_{50} was obtained for the 1:1:1 mixture, indicating the strongest radical-scavenging activity among the mixtures tested under the conditions used.

CONCLUSION. The alcoholic extracts investigated in this study showed measurable radical-scavenging activity. At a volume of 100 µL, the tenfold-diluted 1:1:1 mixture of St. John's wort, stinging nettle, and wormwood reduced the DPPH signal by 23.58% after 30 min. The estimated IC_{50} for this extract was 218.17 µL. These results suggest that the 1:1:1 mixture may be a promising candidate for further investigation as a potential source of natural antioxidants. Since the estimated IC_{50} exceeds the largest tested sample volume, it should be interpreted as an extrapolated estimate and confirmed using a wider concentration range.

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