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DETERMINATION OF THE ANTIRADICAL ACTIVITY OF GRAPE (VITIS VINIFERA) LEAVES

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Abstract
In this study, the antiradical activity of grape leaves grown in Uzbekistan was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The presence of phenolic compounds, flavonoids, and antioxidants in the chemical composition of grape leaves is one of the main factors determining its effectiveness against free radicals. In the study, an ethanol extract was obtained from grape leaves and its DPPH radical neutralisation activity was measured spectrophotometrically. The results showed that grape leaves have high antiradical activity, which further strengthens the scientific basis for their use in folk medicine.

Keywords: Grape leaf, antiradical activity, DPPH, phenolic compounds, antioxidant.

Introduction

Grapes are ancient flowering or angiosperm plants belonging to the genus Vitis of the family Vitaceae. The family Vinifera includes about 1000 species in 14 genera [1]. Grapes are native to a region stretching from northeastern Afghanistan to the southern borders of the Black Sea and Caspian Sea. They were domesticated there around 4000 BC and later spread to the Mediterranean basin, Western Europe, India, China and Japan. Grapes were introduced to the Americas by the Spanish. They are now cultivated worldwide. In some cases, they have been hybridised with local Vitis species, resulting in varieties adapted to local conditions [2, 3].

In recent years, the need for natural antioxidants has been increasing due to the increase in free radical-related diseases (cardiovascular, cancer, and ageing). Among plants, grape leaves (Vitis vinifera L.) are considered an effective antiradical agent due to their high content of phenolic compounds. In traditional medicine, grape leaves have been used to treat heart disease,

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inflammation, internal pain, high blood pressure, varicose veins, and colds. In this article, the antiradical activity of grape leaves is evaluated by the DPPH method.

Sample preparation. For the experiment, grapes of the "Kattaqurgon" variety, grown in the Andijan region of Uzbekistan, were collected. The leaves were dried in the shade and ground into a powder.

Evaluation of the antiradical properties of the sample using the DPPH method. The discolouration of a purple solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) allows the detection of the presence of certain pure antioxidant compounds that have the properties of donating a hydrogen atom or an electron. Stable DPPH• is a reagent used in spectrophotometric analysis[1]. In this experiment, Blois[2] used the method for evaluating the DPPH• free radical scavenging properties with minor modifications.[3].

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

Preparation of sample extracts. Alcoholic and aqueous extracts of grape leaves were prepared. Sample extract preparation was performed by ultrasonically extracting 1 g of plant sample in 25 ml of 96% ethanol for 20 min. The resulting extract was passed through a 0.45 μm syringe filter and used for analysis.[4].

Determination of the antiradical properties of samples. A 4-ml quartz cuvette was filled with 3 ml of DPPH solution and 100 μl of ethanol (blank sample) and placed in a spectrophotometer, and the absorbance (D1) at 517 nm was measured every 5 minutes for 30 minutes using a K7000 spectrophotometer manufactured by YOKE (China). To evaluate the antiradical property of the sample, 25, 50, 75, and 100 μl of the sample were mixed with 3 ml of DPPH solution, and the absorbance (D2) at 517 nm was measured in the above order. Ethanol was added to the remaining volume to bring the total volume of the solution in the cuvette to 3.1 ml. The antiradical property of the samples was calculated using the following formula:

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

The results obtained are given in the following table:

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Table 1. Measured light absorption and calculated antiradical activity values of blank and test grape leaf alcohol extract samples added to DPPH solution.

Volume, µl	Time, min.	sample							
		DPPH Abs, D_1	Abs, D_2	ARF%	Volume, µl	Time, min.	DPPH Abs, D1	Abs, D	ARF%
25	0	1,099	1,099	0.00	75	0	1,099	1,099	0.0
	5	1,099	1,021	7.10		5	1,099	0.81	26.3
	10	1,099	0.864	21.38		10	1,099	0.52	52.7
	15	1,099	0.771	29.85		15	1,099	0.376	65.8
	20	1,099	0.712	35.21		20	1,099	0.291	73.5
	25	1,099	0.67	39.04		25	1,099	0.24	78.2
	30	1,099	0.633	42.40		30	1,099	0.205	81.3
50	0	1,099	1,099	0.00	100	0	1,099	1,099	0.00
	5	1,099	0.971	11.65		5	1,099	0.715	34.94
	10	1,099	0.714	35.03		10	1,099	0.42	61.78
	15	1,099	0.584	46.86		15	1,099	0.288	73.79
	20	1,099	0.494	55.05		20	1,099	0.222	79.80
	25	1,099	0.431	60.78		25	1,099	0.191	82.62
	30	1,099	0.38	65.42		30	1,099	0.175	84.08

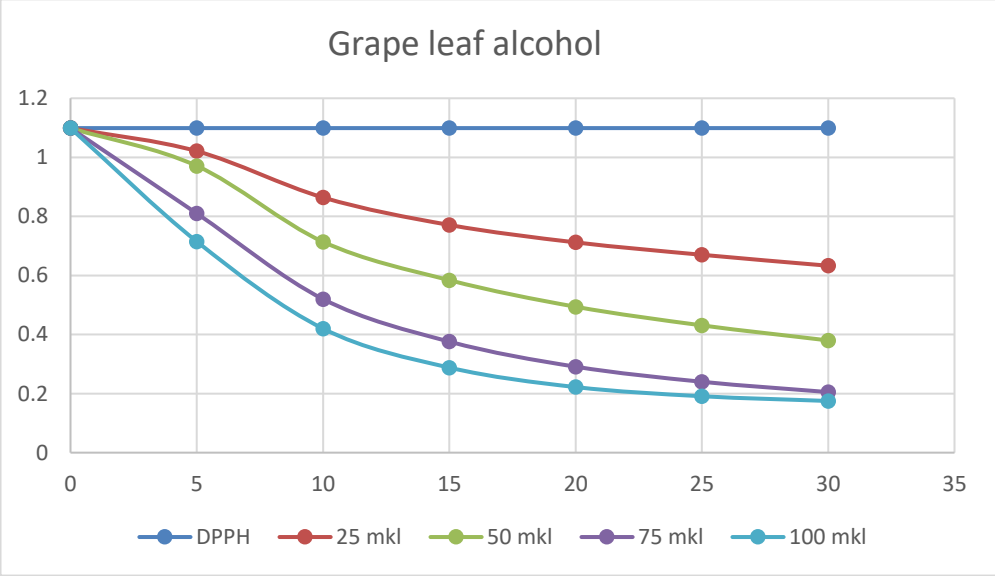

Figure 1. Graphical representation of the measured light absorption of blank and tested sample solutions added to DPPH solution.

To calculate the IC50 of the samples - the 50% inhibition concentration of the DPPH solution, the following graph was constructed based on the 30-minute antiradical activity (ARF%) values and the added sample volume in each experiment and calculated based on the trend line function applied to it.

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Web of Agriculture: Journal of Agriculture and Biological Sciences
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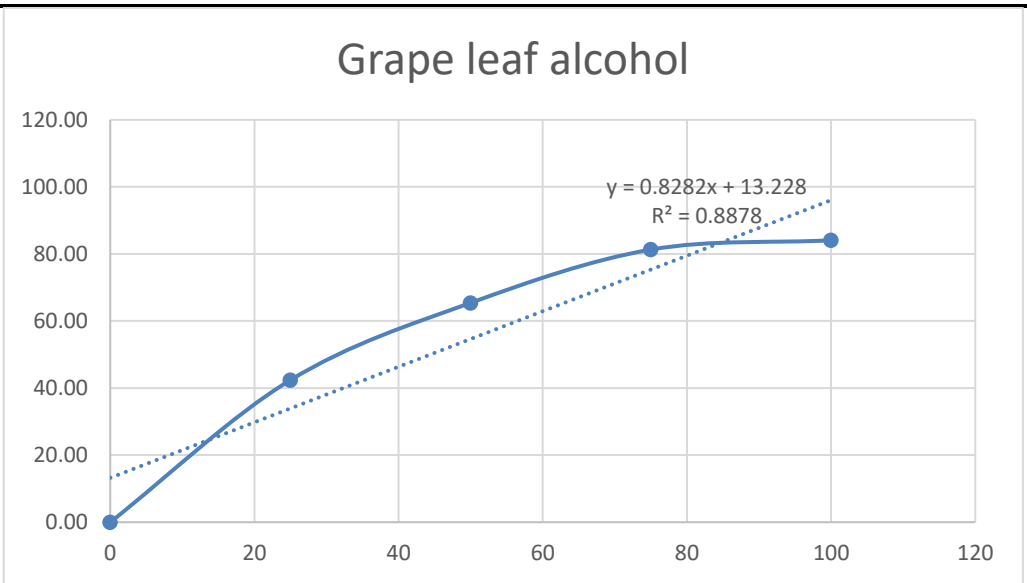

Figure 2. Graph of the relationship between ARF% and volumes determined at 10 minutes for sample 1.

The trend line plotted on the graph was calculated from the function formula y=mx+b, based on the formula x=(yb)/m, which represents the volume of 50% ARF% (IC50):

$$IC_{50} = \frac{(50 - 13.228)}{0.8282} = 36.772\ mkl$$

Table 2. Measured light absorption and calculated antiradical activity values of blank and test grape leaf aqueous extracts added to DPPH solution.

Volume, µl	Time, min.	Sample							
		DPPH Abs, D_1	Abs, D_2	ARF%	Volume, µl	Time, min.	DPPH Abs, D1	Abs, D	ARF%
25	0	1,099	1,099	0.00	75	0	1,099	1,099	0.0
	5	1,099	1,045	4.91		5	1,099	0.959	12.7
	10	1,099	0.992	9.74		10	1,099	0.829	24.6
	15	1,099	0.944	14.10		15	1,099	0.69	37.2
	20	1,099	0.931	15.29		20	1,099	0.653	40.6
	25	1,099	0.92	16.29		25	1,099	0.621	43.5
	30	1,099	0.911	17.11		30	1,099	0.593	46.0
50	0	1,099	1,099	0.00	100	0	1,099	1,099	0.00
	5	1,099	1,001	8.92		5	1,099	0.923	16.01
	10	1,099	0.906	17.56		10	1,099	0.777	29.30
	15	1,099	0.817	25.66		15	1,099	0.615	44.04
	20	1,099	0.792	27.93		20	1,099	0.569	48.23
	25	1,099	0.773	29.66		25	1,099	0.532	51.59
	30	1,099	0.756	31.21		30	1,099	0.499	54.60

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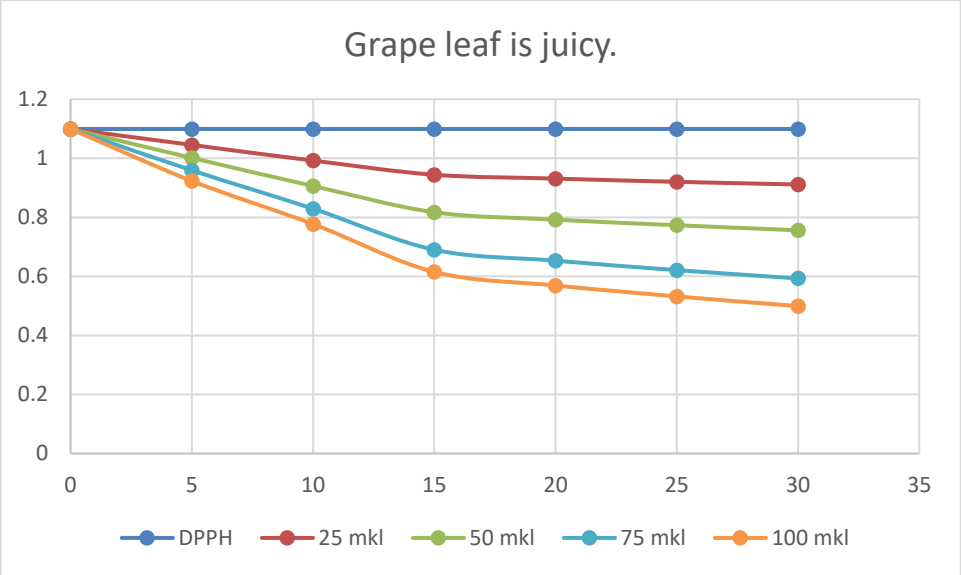

Figure 3. Graphical representation of the measured light absorption of blank and tested aqueous extracted sample solutions spiked with DPPH solution.

To calculate the IC50 of the samples - the 50% inhibition concentration of the DPPH solution - the following graph was constructed based on the 30-minute antiradical activity (ARF%) values and the volume of alcohol samples added in each experiment, and calculated based on the trend line function applied to it.

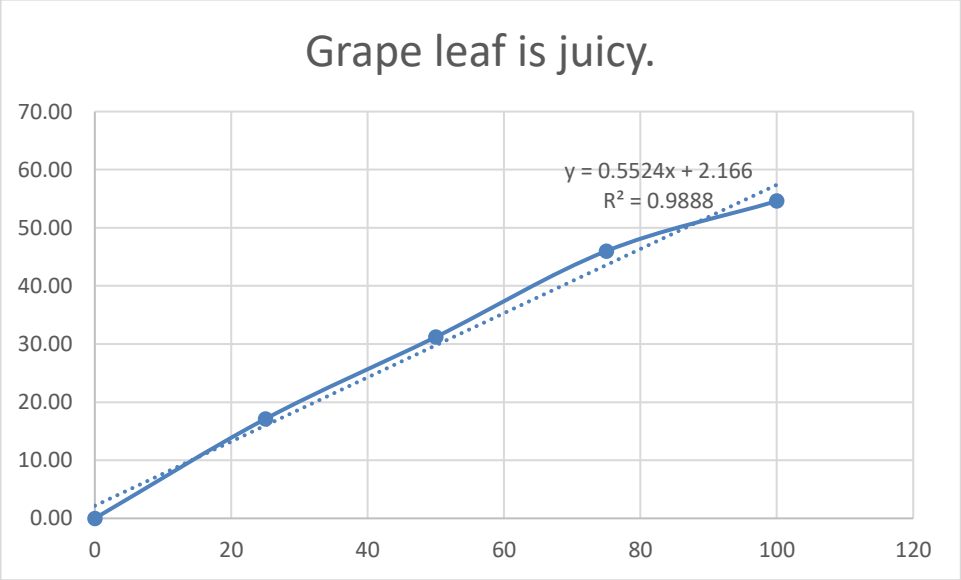

Figure 4. Relationship graph between ARF% and volume determined at 10 minutes after the aqueous extracted sample.

Volume 3, Issue 5, May - 2025
ISSN (E): 2938-3781

The trend line plotted on the graph was calculated from the function formula y=mx+b, based on the formula x=(yb)/m, which represents the volume of 50% ARF% (IC50):

$$IC_{50} = \frac{(50 - 2.166)}{0{,}5524} = 86{,}593\ mkl$$

Table 3. Antiradical activity values of 100 µl of alcoholic extracts of samples at 30 minutes(ARF%)

Time	ARF%	
	Grape leaf alcohol	Grape leaf alcohol
30th minute	84.04	54.60

Table 4. IC50 of samples – 50% inhibition concentration of DPPH solution (µl)

Time	ARF%	
	Grape leaf alcohol	Grape leaf alcohol
30th minute	36,772	86,593

Conclusions

In conclusion, it can be said that the alcoholic extracts of the tested samples exhibit antiradical activity. In particular, the best result of alcoholic extraction was the alcoholic extraction of grape leaves, which was obtained in 30 min. 84.04%. The IC50 value was 36.772 µl, demonstrating antiradical activity. The leaves of grape varieties grown in Uzbekistan have high antiradical activity and have potential for use as a drug or bioactive supplement. The results determined by the DPPH method indicate that grape leaves are an effective natural means of protecting against free radicals. In the future, it is necessary to more accurately determine the phenolic composition of grape leaf extracts and expand the analysis of biologically active substances.

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