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Results Of Evaluation Of Anti-Radical Properties Of Inula Grandis Schrenk (Asteraceae) Plant By DPPH Method

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Abstract

As a result of the conducted studies, it was found that alcoholic and aqueous extracts prepared from the Inula grandis plant have significant antiradical activity against DPPH free radicals. Spectrophotometric analyses based on the DPPH method showed a consistent increase in antiradical activity with increasing extract volume and incubation time. Based on the results obtained, the scientific and practical significance of the Inula grandis L. plant as a natural antioxidant source is demonstrated.

Keywords: Inula grandis, phytochemistry, flora, DPPH, antiradical.

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1. Introduction

Uzbekistan is a region naturally and geographically rich in medicinal plants, and of the approximately 4,500 species of higher plants naturally occurring in the republic, about 1,200 have medicinal properties [4,5]. The Resolution of the President of the Republic of Uzbekistan No. PQ-4670 dated April 10, 2020 “On measures for the protection, cultivation, processing and rational use of available resources of wild medicinal plants” created the legal basis for the radical development of the sector [17]

As a medicinal plant, Inula grandis Schrenk (Asteraceae) is a perennial plant. It grows on fine-grained soft slopes in the lower and middle mountain belts. The optimal resource potential is located at an altitude of 1300-1500 meters above sea level. According to modern indicators of resource potential in southern Uzbekistan, the permissible annual volume of raw material procurement is 5.16 tons [15]. Today, the phytochemistry of this species and its importance in folk medicine are being widely studied [16].

Inula grandis Schrenk is distributed in the South Chotkol region of the Fergana Valley (Namangan region, Yangikurgan district, Ungor-Tepa mountain) (Figure 1).

Human impact on the environment has increased significantly in the Fergana Valley over the past decade. The expansion of settlements, the growth of industrial zones, the reduction of pasture areas, and other factors have led to the weakening of the population of rare and endemic species of the Fergana Valley [1, 2, 3]. These indicators have increased the need to identify medicinal plant reserves in the regions and comprehensively study their phytochemistry.

In order to carry out research and analysis, the following tasks were set: 1) To comprehensively evaluate the antiradical activity of alcoholic and aqueous extracts prepared from the Inula grandis plant using the DPPH method; 2) To determine the dependence of the antiradical activity of alcoholic and aqueous extracts on time and concentration, and to calculate their IC_{50} values based on linear regression models; 3) To experimentally substantiate the fact that the aqueous extract of Inula grandis has a higher antiradical potential than the alcoholic extract.

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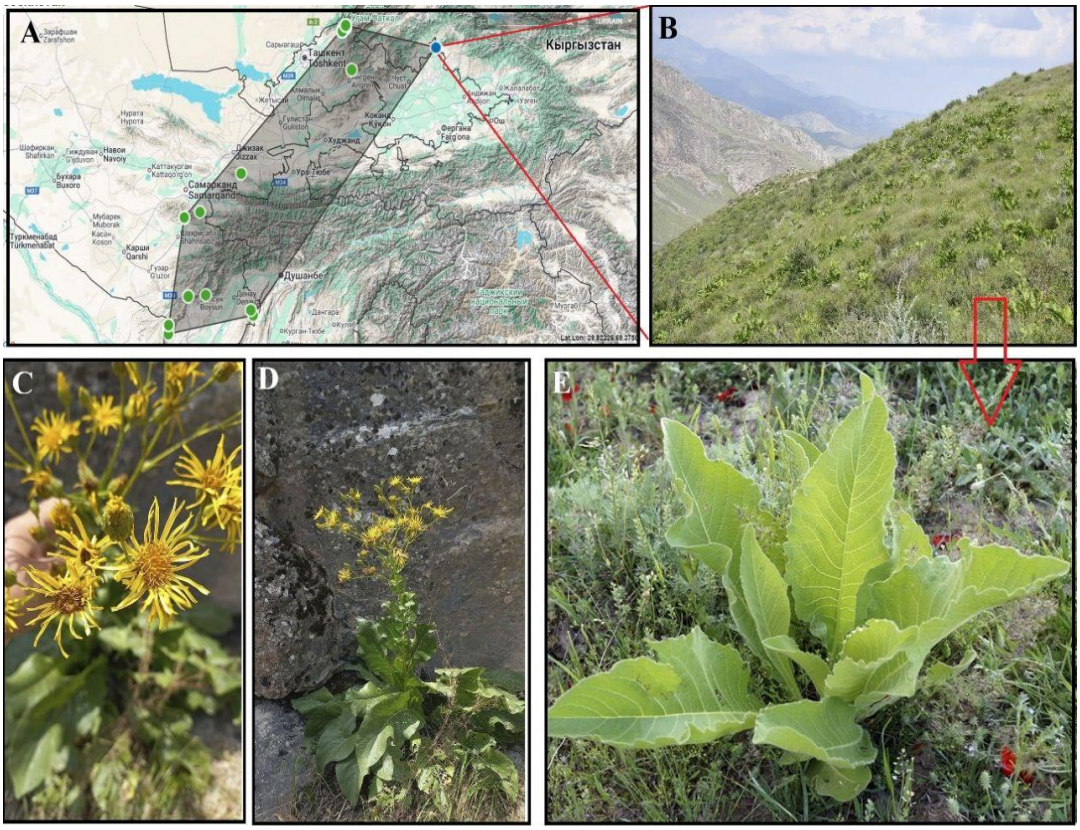

Figure 1. A) Distribution map of Inula grandis; B) Ecology; C-D-E) Images of the plant in nature.

2. Methods

Targeted and route-based field studies were conducted throughout the region during 2025 (May-August). The location coordinates of the species were determined using a GPS navigator (Garmin GPSmap 64s navigator). To obtain herbarium data, the TASH, MW (https://www.plant.depo.msu.ru) funds and the Global Biodiversity Information Facility (GBIF; https://www.gbif.org) were used. The nomenclature of taxa was given according to the international catalog Plants of the World Online (POWO; https://www.plantsoftheworldonline.org) [6, 7, 8].

Laboratory analyses were carried out at Namangan State University. The discoloration of the purple 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution allows us to determine the presence of some pure antioxidant compounds that have the properties of donating a hydrogen atom or an electron. Stable DPPH is a reagent used in spectrophotometric analysis. In this experiment, the method developed by Blois was used with minor

modifications to evaluate the inhibition of DPPH free radicals.

Preparation of DPPH working solution. A 7.92 mM DPPH solution in ethanol was prepared in a 100 ml volumetric flask, wrapped in aluminum foil, and stored in the dark at room temperature for 30 minutes [9, 10, 11].

Preparation of sample extracts. Alcoholic and aqueous extracts were prepared based on the selected sample. The preparation of sample extracts was carried out by extracting 1 g of plant sample in 25 ml of 96% ethanol and distilled water for 20 minutes in an ultrasonic bath. The obtained extract was passed through a 0.45 µm syringe filter and used for analysis. The filtered sample was diluted 10 times [12, 13, 14].

Determination of the antiradical properties of the 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 a wavelength of 517 nm was measured every 5 minutes for 30 minutes using a K7000 spectrophotometer manufactured by YOKE (China). To evaluate the antiradical properties of the sample, 25, 50, 75, 100 µl of the sample was 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 part to bring the total volume of the solution in the cuvette to 3.1 ml. The antiradical properties of the samples were calculated using the following formula:

$$\text{ARF\%} = \frac{(D_1 - D_2)}{D_1} * 100\%$$

3. Results

100 µl of alcoholic extract inhibited DPPH free radicals by 31.47% in 30 minutes, while this figure was 50.73% for the aqueous extract. The IC_{50} values calculated based on linear regression equations were 166.6 µl for the alcoholic extract and 100.1 µl for the aqueous extract, respectively. These results clearly showed that the aqueous extract had higher antiradical activity than the alcoholic extract

The results obtained suggest that the composition of Inula grandis is dominated by water-soluble phenolic and polyphenolic biologically active compounds. In general, the results of the study support the prospects for using Inula grandis as a source of natural antioxidants in the production of pharmaceutical, food and functional products (Figure 2).

Table 1. Measured light absorption and antiradical activity of blank and test Inula grandis alcoholic extract added to DPPH solution

Size, mkl	Time minutes.	Sample					
		Abs, D_2	ARF%		Time,minutes.	Abs, D_2	ARF%
25	0	1,02	0,00	75	0	1,02	0,00
	5	0,952	6.67		5	0,885	13,24
	10	0,945	5,34		10	0,861	15,59
	15	0,938	5,92		15	0,848	16,86
	20	0,931	6,41		20	0,832	18,43
	25	0,926	6,80		25	0,821	19,51
	30	0,921	**7,18**		**30**	0,814	**20,20**
50	0	1,02	0,00	100	0	1,02	0,00
	5	0,936	8,24		5	0,798	21,76
	10	0,928	9.02		10	0,771	24,41

	15	0,916	10.20		15	0,748	26,67
	20	0,904	11,37		20	0,725	28,92
	25	0,897	12.06		25	0,712	30,20
	30	0,889	**12.84**		**30**	0,669	**24,37**

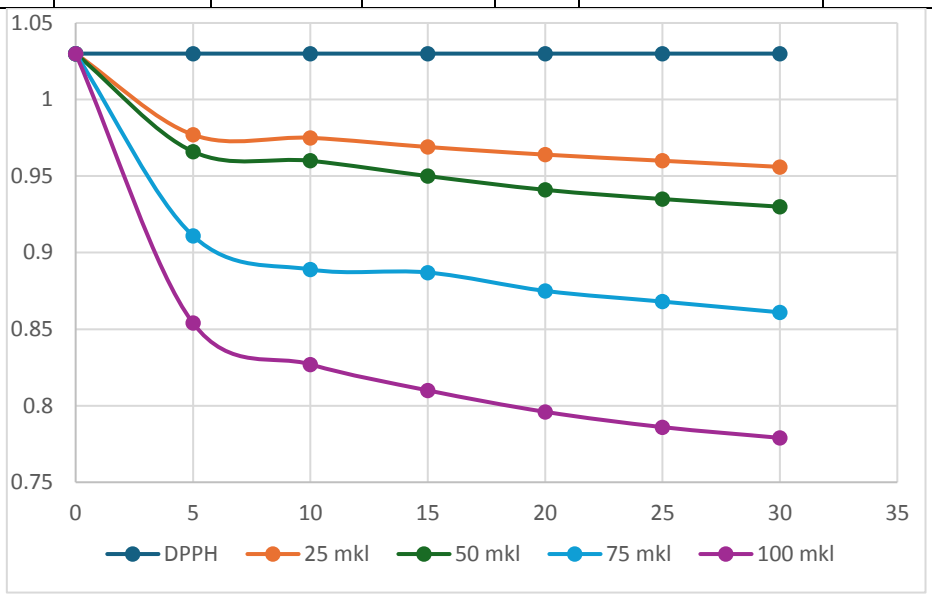

Figure 2. Graphical representation of the measured light absorption of blank and tested alcohol-extracted Inula grandis solutions spiked with DPPH solution (Inula grandis alcoholic 10x).

To calculate the Inula grandis IC50 – 50% inhibition concentration of DPPH solution, the following graph was constructed based on the 30-minute antiradical scavenging 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 3).

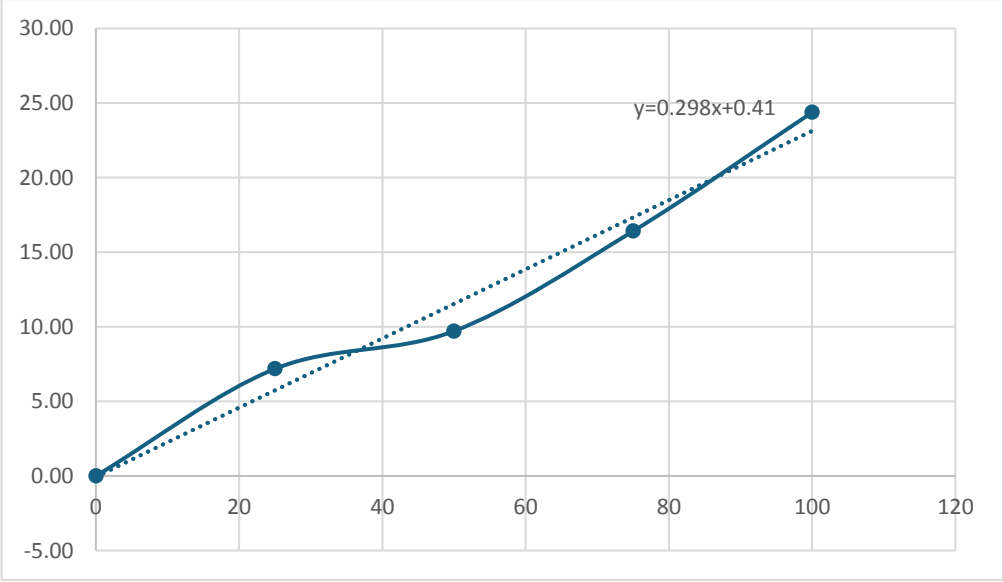

Figure 3. Relationship graph between ARF% and volumes determined at 30 minutes of alcohol-extracted Inula grandis (Inula grandis alcoholic 10x)

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

$$IC_{50} = \frac{(50 - 0.410)}{0.298} = \mathbf{166,6\ mkl}$$

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

size mkl	Time minutes.	Sample					
		Abs, D	ARF%		Time minutes.	Abs, D	ARF%
25	0	1,10	0,00	75	0	1,10	1,10
	5	0,996	9,45		5	0,932	0,996
	10	0,955	13,18		10	0,871	0,955
	15	0,924	16,00		15	0,829	0,924
	20	0,902	18,00		20	0,792	0,902
	25	0,885	19,55		25	0,764	0,885
	30	0,869	21,00		30	0,739	0,869
50	0	1,10	0,00	100	0	0	1,10
	5	0,961	12,64		5	5	0,711
	10	0,913	17,00		10	10	0,662
	15	0,878	20,18		15	15	0,621
	20	0,842	23,45		20	20	0,588
	25	0,819	25,55		25	25	0,563
	30	0,796	27,64		**30**	30	0,542

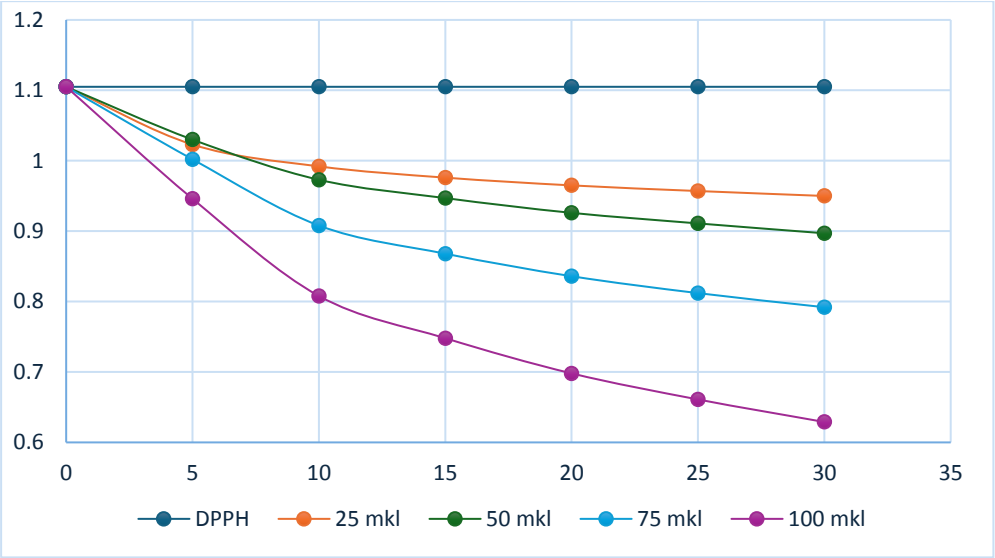

Figure 4. Graphical representation of the measured light absorption of blank and tested aqueous extracted Inula grandis solutions spiked with DPPH solution (Inula grandis watery 10x).

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

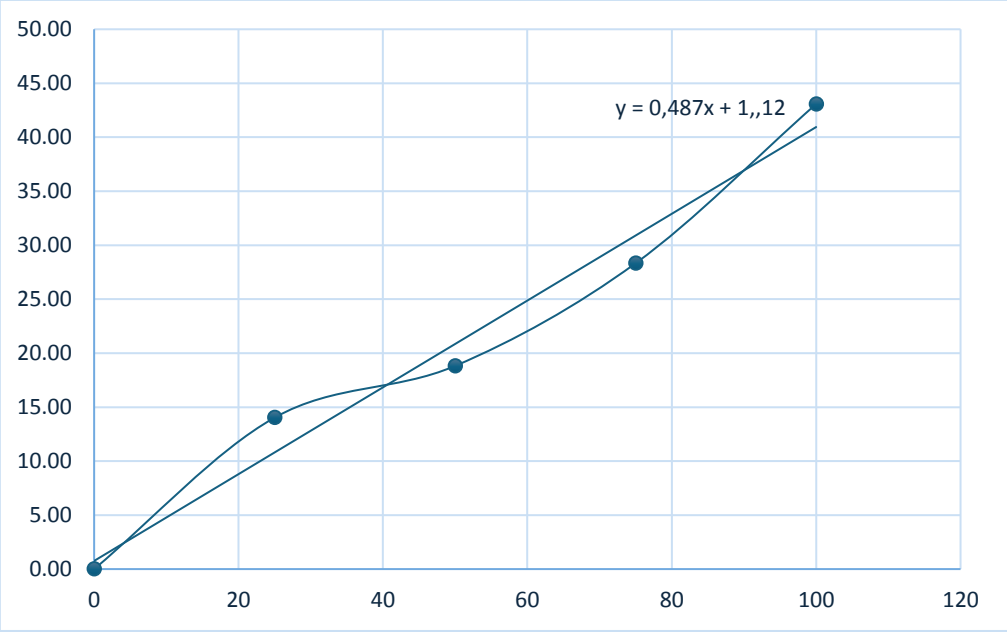

Figure 5. Relationship graph between ARF% and volume determined at 30 minutes of aqueous extracted Inula grandis.

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

$$IC_{50} = \frac{(50 - 1.12)}{0.487} = 100{,}1\text{mkl}$$

Table 3. Antiradical scavenging activity values (ARF%) of Inula grandis alcoholic extracts in 100 µl at 30 minutes

	ARF%	
	Sample is alcohol	**Sample is aqueous**
30-minutes	**31.47**	**50.73**

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

	ARF%	
	Sample is alcohol	**Sample is aqueous**
30-minutes	**166.6**	**100.1**

4. Conclusion

As a result of the conducted studies, it was found that alcoholic and aqueous extracts prepared from the Inula grandis plant have significant antiradical activity against DPPH free radicals. Spectrophotometric analyses based on the DPPH method showed a consistent increase in antiradical activity with increasing extract volume and incubation time. Based on the results obtained, the scientific and practical significance of the Inula grandis plant as a natural antioxidant source is demonstrated.

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