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EVALUATION OF ANTI-RADICAL PROPERTIES OF PRODUCED BARLEY GRAINS USING THE DPPH METHOD

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Annotation
The aim of this study is to evaluate the antiradical properties of harvested barley (Hordeum) grain using the DPPH method, compare the antioxidant activity of extracts obtained using different solvents, and evaluate the prospects of a local barley variety as a functional medicinal food product by determining its bioactive potential.

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Introduction. Currently, the number of diseases caused by the negative effects of free radicals and oxidative stress on the body is increasing. Oxidative stress plays an important role in the development of cardiovascular diseases, cancer, diabetes mellitus, Alzheimer's disease, Parkinson's disease and other degenerative diseases [5,6]. According to the World Health Organization (WHO), these diseases are among the leading causes of global death and significantly reduce the quality of life of the world's population. Although synthetic antioxidants (E319, E320, E321, etc.) are widely used in the food industry, their long-term consumption has been shown to pose a potential risk to human health [7]. Therefore, the search for natural antioxidants and the study of their properties is one of the most urgent issues in modern medicine and the food industry. Natural antioxidants are distinguished not only by their safety, but also often by the presence of additional biological activities. Bioactive compounds from plant sources, in particular phenolic compounds, flavonoids, tocopherols and carotenoids, have strong antioxidant properties and can be used for preventive and therapeutic purposes [8]. Among cereals, barley (Hordeum vulgae L.) attracts special attention due to its unique chemical composition and high bioactive potential. Barley is one of the oldest and most widespread cereals in the world, having been used in human food for more than 10,000 years. Botanically, barley belongs to the Poaceae family and is cultivated in almost all climatic regions of the world (9). Currently, barley is mainly used as feed in livestock farming and in beer production, but the many bioactive compounds it contains expand its potential as a functional food product. Barley contains valuable bioactive compounds such as β-glucan, phenolic acids (ferulic, p-coumaric, vanillic acids), flavonoids (quercetin, luteolin, apigenin), anthocyanins, tocopherols, and phytosterols [10,11]. Most of these compounds are concentrated in the grain husk and embryo, and their biological activity in

many cases exceeds that of synthetic antioxidants. Germination is the stage of resumption of the vital activity of the plant seed, during which strong biochemical changes occur in the seed. The germination process significantly changes the chemical composition of barley and increases the amount of bioactive substances several times. During milling, various enzymes (amylase, protease, lipase, etc.) are activated, breaking down proteins, carbohydrates, and fats, while new compounds with antioxidant properties are formed [12,13]. During milling, the amount of β-glucan increases by 3-5 times, the amount of phenolic compounds by 2-4 times, and the amount of vitamin E by 2-3 times [10]. Also, during milling, the amount of antinutrients such as phytic acid decreases, which improves the absorption of beneficial elements. These changes make milled barley a more valuable food product than ordinary barley.

Experimental part. There are various methods for assessing antioxidant activity, which are mainly based on the mechanism of free radical scavenging. The DPPH (2,2-diphenyl-1-picrylhydrazyl) method is one of the most common, simple and reliable methods for assessing antioxidant activity (14). This method was developed by Blois in 1958 and is based on a purple solution of a stable nitrogen radical. Under the influence of antioxidants, the DPPH radical is neutralized, and the color of the solution changes from purple to yellow, which can be measured spectrophotometrically at a wavelength of 517 nm.

Research materials. A local barley variety (Hordeum vulgae L.) was used for the study. Barley grains were obtained from farms in the Fergana region, and the germination process was carried out in laboratory conditions. Barley grains were washed with distilled water and soaked for 12 hours. Then, the moisture content was dried to 45-50% and extracted in the dark at 20-22°C for 72 hours. The extracted barley grain sample was dried at 60°C and ground.

Evaluation of the antiradical properties of the sample by the DPPH method. 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 [1]. In this experiment, the method of Blois [2] for evaluating the inhibition of DPPH• free radicals was used 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 aluminum foil and kept in the dark at room temperature for 30 minutes.

Preparation of sample extracts. Alcoholic and aqueous extracts were prepared from the harvested barley plant. The preparation of sample extracts was carried out by extracting 1 g of plant sample in 25 ml of 96% ethanol for 20 minutes in an ultrasonic bath. The obtained extract was passed through a 0.45 μm syringe filter and used for analysis. [4].

Determination of the antiradical properties of the samples. 3 ml of DPPH solution and 100 μl of ethanol (blank sample) were added to a 4 ml quartz cuvette, placed in a spectrophotometer, and the light absorption (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 activity of the sample, 25, 50, 75, 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 activity 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:

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

Volume, µl		sample					
		Abs, D	ARF%		Time, min.	Abs, D	ARF%
25	0	0,846	0,00	75	0	0,846	0,00
	5	0,818	3,31		5	0,804	4,96
	10	0,818	3,31		10	0,804	4,96
	15	0,817	3,43		15	0,802	5,20
	20	0,816	3,55		20	0,802	5,20
	25	0,816	3,55		25	0,801	5,32
	30	0,814	3,78		**30**	0,8	5,44
50	0	0,846	0,00	100	0	0,846	0,00
	5	0,807	4,61		5	0,793	6,26
	10	0,806	4,73		10	0,79	6,62
	15	0,805	4,85		15	0,787	6,97
	20	0,804	4,96		20	0,786	7,09
	25	0,804	4,96		25	0,784	7,33
	30	0,802	5,20		**30**	0,782	7,57

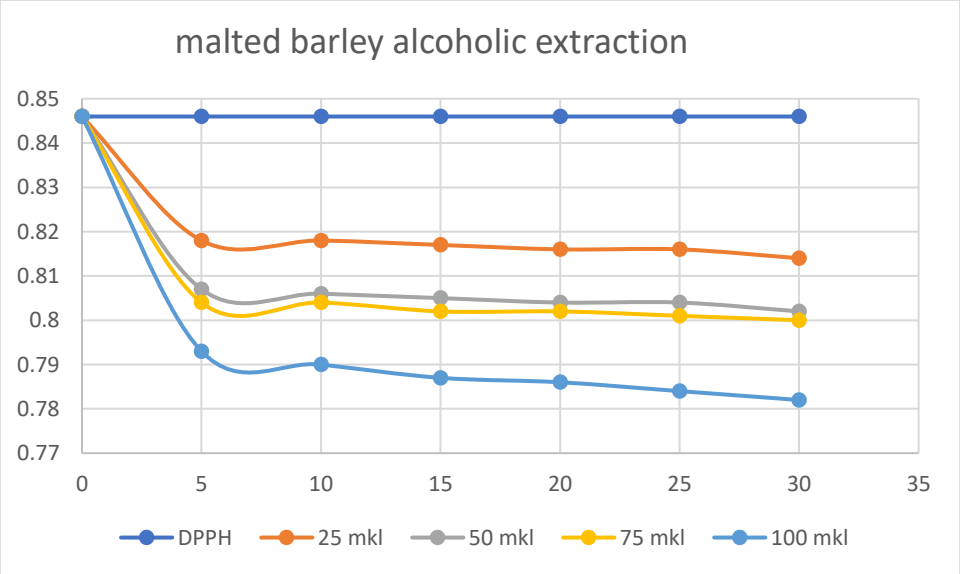


Figure 2. Graph of the relationship between ARA% and volumes determined at 10 minutes of the alcohol extracted sample.

The trend line plotted on the graph was calculated from the function formula y=mx+b, and the volume exhibiting 50% ARA% (IC_{50}) was calculated based on the formula x=(y-b)/m:

$$IC_{50} = \frac{(50 - 1{,}038)}{0{,}0672} = 728{,}60\ mkl$$

Table 2. Measured light absorption and calculated antiradical activity values of blank and test samples of water-extracted sprouted barley spiked with DPPH solution

Volume, µl	Time, min.	sample					
		Abs, D	ARA%		Time, min.	Abs, D	ARA%
25	0	0,797	0,00	75	0	0,797	0,00
	5	0,656	17,69		5	0,556	30,24
	10	0,63	20,95		10	0,507	36,39

	15	0,6	24,72		15	0,459	42,41
	20	0,574	27,98		20	0,422	47,05
	25	0,553	30,61		25	0,393	50,69
	30	0,535	32,87		**30**	0,371	53,45
50	0	0,797	0,00	100	0	0,797	0,00
	5	0,601	24,59		5	0,524	34,25
	10	0,559	29,86		10	0,471	40,90
	15	0,513	35,63		15	0,42	47,30
	20	0,478	40,03		20	0,383	51,94
	25	0,45	43,54		25	0,355	55,46
	30	0,428	46,30		**30**	0,335	57,97

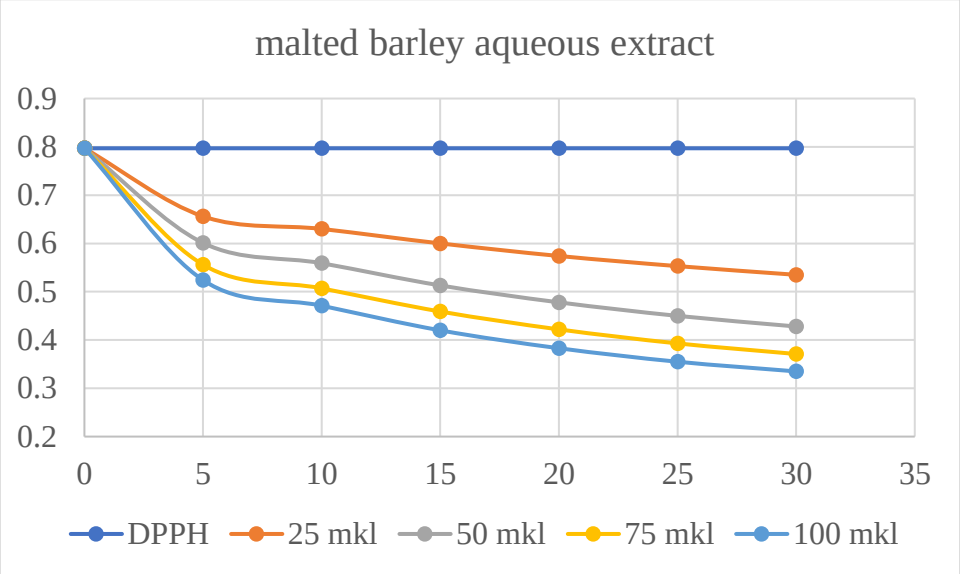


Figure 3. Graphical representation of the measured light absorption of blank and tested aqueous extracted sample solutions spiked with DPPH solution.

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

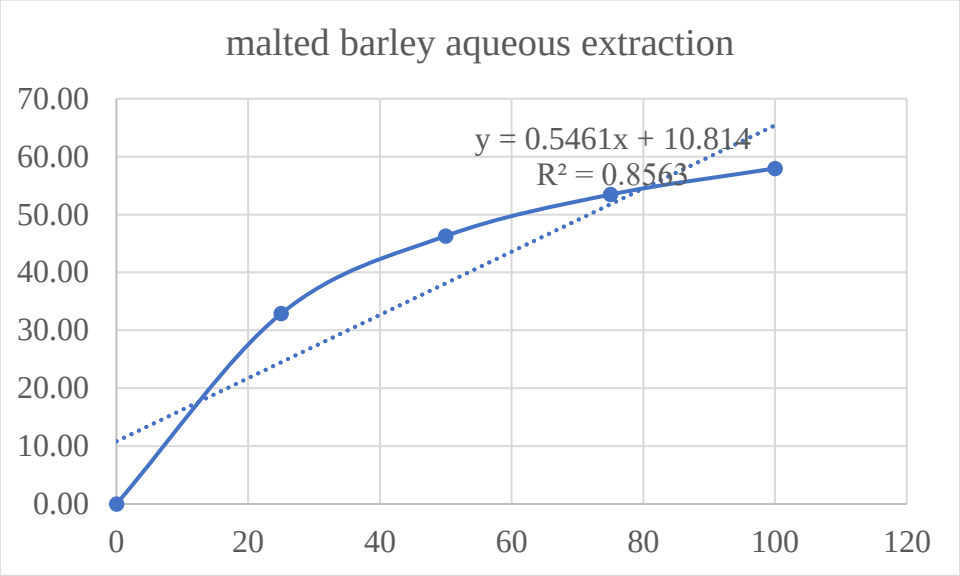


Figure 4. Graph of the relationship between ARA% and volumes determined at 10 minutes of the aqueous extracted sample.

The trend line plotted on the graph was calculated from the function formula y=mx+b, and the volume exhibiting 50% ARA% (IC_{50}) was calculated based on the formula x=(y-b)/m:

$$IC_{50} = \frac{(50 - 10{,}814)}{0{,}5461} = 71{,}756\ mkl$$

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

Time	ARA%	
	Sprouted barley, alcohol solution	***Sprouted barley, water solution***
30 minutes	**7,37**	**57,97**

Table 4. IC_{50} of samples – 50% inhibition concentration of DPPH solution (µl)

Time	ARA%	
	Germinated barley, alcohol solution раствор	Germinated barley, water solution
30 minutes	**728,6**	**71,756**

Conclusion. In conclusion, it can be noted that the alcoholic and aqueous extracts prepared from the tested samples showed antiradical activity. In particular, the aqueous extract showed the best performance, achieving 57.97% antiradical activity in 30 minutes. The IC_{50} value of this extract was 71.756 µl, indicating high antiradical activity.

The results obtained indicate that the extracted barley extracts have strong antiradical properties. The higher activity of the ethanol extract may be due to the poor solubility of phenolic

compounds in ethanol [9,10]. During the extraction process, enzymes in barley are activated, which break down the cell wall and facilitate the release of bioactive compounds [14].

Antioxidants such as phenolic compounds, flavonoids, and tocopherols are abundant in extracted barley, which effectively scavenge DPPH radicals [16,18]. Peptides formed during the extraction process may also have antioxidant properties (17).

Compared with other studies, our results confirm that extracted barley extracts have good antiradical potential [18]. These results indicate that barley can be used in the production of functional food products. These results indicate that the extracted barley plant, especially its aqueous extract, is a promising source of natural antioxidants. This research area is of great importance not only from a scientific but also from a practical point of view, as it opens up opportunities for the production of functional food products based on local raw materials.

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