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The survey of bioactive compounds extraction from *Spirulina platensis* algae by ultrasound-assisted ethanolic maceration

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ABSTRACT

Spirulina platensis has a wide range of uses in the food industry, pharmaceuticals, cosmetics, and healthcare, largely due of its high nutritional value and wellness-promoting attributes. Consequently, it is still difficult to extract bioactive components from it. This study aimed to compare the extraction of phenolic compounds from the microalga *Spirulina platensis* using ultrasonic and maceration methods. For the extraction processes, separate food-grade solvents (water (0% ethanol), ethanol (96% ethanol), and their mixture (50% water + 50% ethanol) were utilized. Additionally, sonication was found to produce the maximum yield of carotenoids and flavonoids when 96% ethanol was utilized as the solvent. Higher antioxidant activity were produced as a result of using ultrasound-assisted food-grade solvent extraction to extract more of the high-value added components.

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1. Introduction

Spirulina is a blue-green microalga belonging to the family of cyanobacteria (Cyanophyta), which are photosynthesizing prokaryotes with a gram-negative cell wall structure that are found in nature in single-celled forms and filaments with spring-like filaments. Spirulina is the brand name of Arthrospira which*Spirulina platensis* And*Spirulina maxima* Its most important species are Spirulina is non-toxic and has a special place due to its easy digestion due to the lack of cellulose in its cell wall (other microalgae such as Chlorella, Ankistrodesmus, Selenestrum and Sandesmus do not have this advantage). Spirulina microalgae became famous and popular in the food industry as a vitamin and protein supplement after it was successfully used by the aerospace organization as a food supplement for astronauts on space trips. The US National Aeronautics and Space Administration uses spirulina as an intensive food for space travel. In addition,*Arthrospira platensis* It is known as "super food" by the World Health Organization. Spirulina is also recognized as safe and recommended at levels of 0.5 to 3 grams per meal. The US Food and Drug Administration in 1997 based on studies conducted on rodents and long-term human use of microalgae.*Spirulina platensis*confirmed its safety and declared the maximum daily intake of this microalgae for each person to be 1.35 grams. About 1000 tons of spirulina are produced annually by the three main spirulina producing companies in America, China and Thailand. In addition to these three companies, this microalgae is cultivated in other countries such as Taiwan, Chile, India, Japan, Cuba, Spain, Argentina and Mexico and is sold as a commercial product or as a useful food with therapeutic purposes. [1].

Spirulina contains 4-7% of dry weight of fat. Linoleic acid, gamma linolenic acid, eicosa pentanoic acid and arachidonic acid are the most important fatty acids in this microalgae. This cyanobacterium contains significant amounts of fat with a composition similar to vegetable oils. According to the profile of spirulina fatty acids, neutral fats accounted for the highest amount (45%), followed by glycolipids (39%) and phospholipids (16%). One of the notable features of spirulina fats is the presence of relatively high levels of unsaturated fatty acids compared to saturated fatty acids, especially high levels of gammalinolenic acid. This microalgae has a very high concentration of vitamin A precursor and the richest source of vitamin cyanocobalamin and is very effective in treating anemia. Spirulina microalgae contains water-soluble vitamins of group B, as well as fat-soluble vitamins A, E, D and K. Spirulina is a good source of minerals such as sodium, calcium, phosphorus, iron, magnesium, manganese, zinc, copper, chromium and selenium. Iron in spirulina can be absorbed 60% better than ferrosulfate and other compounds, and as a result, this microalgae can be used as a sufficient source of iron in the diet of pregnant women suffering from anemia. This microalgae contains about 13-16% dry weight of carbohydrates, which include glucose, rhamnose, xylose, mannose and galactose. Superoxide dismutases and glutathione peroxidase are among the most important enzymes in spirulina. These enzymes have antioxidant effects and have a significant effect on delaying the cell aging process. Chlorophyll is the main pigment participating in the photosynthesis process of this microalgae. But spirulina has many pigments including chlorophyll, lutein, beta-carotene, carotenoids, xanthophyll, zeaxanthin, staxanthin, cryptoxanthin, diatoxanthine, phycobili proteins such as phycocyanin and allophycocyanin [2]. Antioxidant property*Spirulina platensis* It is mainly attributed to the phycocyanin in it. Spirulina phycocyanin inhibits free radicals and the activity of the enzyme nicotinamide adenine dinucleotide phosphate oxidase and the production of superoxide dismutase caused by the activity of this enzyme. Alcoholic extract of spirulina prevents the peroxidation of fats to a large extent compared to synthetic antioxidants. Main phenolic compounds of microalgae*Spirulina platensis*They include salicylic acid, transcinnamic acid, chlorogenic acid, caffeic acid and coimic acid, which has a direct relationship between the amount of phenolic compounds and its antioxidant potential [1].

The most common methods of extraction are based on placing the substance in a suitable solvent, and in order to increase the speed of the extraction process, stirring and heat are usually used. Among the common and conventional methods for extracting extract or active compounds, we can refer to soxhlet, distillation, soaking and percolation. Traditional methods

for extracting active compounds such as water distillation or water vapor extraction and extraction with organic solvent (soaking or immersion) have disadvantages such as loss of volatile compounds, low yield, long extraction time, destruction of unsaturated compounds and remaining toxic solvent. Therefore, nowadays, the use of extraction methods with microwave, supercritical fluid, extraction with high-speed solvent and especially ultrasound waves are used more [3]. Therefore, the purpose of this research is to investigate the percentage of ethanol consumed in the method of soaking and soaking combined with ultrasound on the content of total phenol and the antioxidant activity of the extract obtained from microalgae.*Spirulina platensis* Is.

2- Materials and methods

2-1- Raw materials

The raw materials used in this research are microalgae*Spirulina platensis* They were prepared from Ayrik Sepahan Green Gohar Company.

2-2-Methods

2-2-1- Extraction of microalgae extract*Spirulina platensis*

2-2-1- 1- Extraction by soaking method (immersion)

20 grams of microalgae powder*Spirulina platensis* It was mixed with 200 ml of solvent mixture of water, 96% ethanol and 50% ethanol and placed on a magnetic stirrer for 24 hours to separate its active ingredients. After filtering with Whatman No. 1 filter paper, the obtained extract was concentrated using a rotary evaporator under vacuum at a temperature of 40 degrees Celsius, and finally it was placed in a dry freeze dryer and kept in a refrigerator until the next tests.] 4 [.

2-2-1-2-extraction with the help of ultrasound

10 grams of microalgae powder*Spirulina platensis* It was mixed with 100 ml of solvent mixture of water, 96% ethanol and 50% ethanol and placed in an ultrasonic machine with a power of 300 watts and a temperature of 40 degrees Celsius for 45 minutes. The temperature during the extraction process was controlled by a water bath. After extraction, the extracted extract was first filtered by Whatman filter paper (No. 1) and then centrifuged at a speed of 7800 rpm for 30 minutes. Solvent removal was done using a rotary evaporator under vacuum at a temperature of 40 degrees Celsius and a pressure of 25 mmHg. Finally, after the extract was dried in a freeze dryer, the extracts were stored in dark colored glass containers at a temperature of 4 degrees Celsius [5].

2-2-2- Examining some quality characteristics of microalgae extract*Spirulina platensis*

2-2-2-1-Measurement of the total phenol content of the extract

The total amount of phenolic compounds in the extracts was investigated according to Folin-Ciocalto's method. For this purpose, 200 microliters of each obtained extract was mixed with 500 microliters of Folin Ciocalto reagent, which was diluted 1:10 with distilled water. After a period of 1 to 8 minutes, 800 microliters of sodium bicarbonate solution (7.5% weight-volume) was added to it and it was placed in a dark place for 30 minutes. Then, the absorption value of the solution was read by a spectrophotometer at a wavelength of 765 nm. The total amount of phenolic compounds was expressed as milligrams of gallic acid per gram of dry extract using the equation of the line drawn based on gallic acid [6].

2-2-2-2- Measuring the flavonoid content of the whole extract

The total flavonoid content of the extract was determined using the colorimetric method of aluminum chloride. For this purpose, 0.5 ml of each extract was mixed with 0.1 ml of 10% aluminum chloride, 0.1 ml of one molar potassium acetate and 2.8 ml of distilled water (twice distilled water). In the next step, 0.5 ml of the extract solution mixed with 1.5 ml of ethanol was added to the mixture of aluminum chloride, potassium acetate and water. The resulting final mixture was kept constant at room temperature for 30 minutes. After this time, the absorbance of the sample at 415 nm wavelength was read by a spectrophotometer. The amount of total flavonoid was expressed in terms of mg of quercetin per gram of dry extract [7].

2-2-2-3- Measurement of anthocyanin content of the whole extract

The total anthocyanin content of the extracts was measured using two buffer systems, potassium chloride buffer (0.025 M) with pH=1 and sodium acetate buffer (0.4 M) with pH=4, and based on the pH difference. For this

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purpose, 400 microliters of the solution of each extract was mixed with 3.6 milliliters of each of the buffers separately, and the absorbance of each solution was read at two wavelengths of 510 and 700 nm by a spectrophotometer. The amount of total anthocyanin in terms of mg/liter equivalent of cyanidin-3-glycoside in the extract was calculated using relations (1) and (2).

relationship (1) $= \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$

$= \frac{A \times MW \times DF \times 1000}{\varepsilon \times l}$ Total anthocyanin (mg cyanidin-3-glycoside)

$= (A510 - A700)pH1 - (A510 - A700)pH4.5$

$= (A510 - A700)pH1 - (A510 - A700)pH4.5$

relationship (2)

A= (A_{510}–A_{700}) pH1 – (A_{510}–A_{700}) pH4.5

$= (A510 - A700)pH1 - (A510 - A700)pH4.5$

In the above relation, A, MW, DF, l are, respectively, the absorption amount, the molecular weight of cyanidin (449.2 g/mol of cyanidin-3-glycoside), the dilution factor, the path length and the molar coefficient of cyanidin (26.900). 8 [.

2-2-2-4- Evaluation of antioxidant activity or inhibition of DPPH free radicals

The ability to donate hydrogen atom or electron by different compounds and extracts was investigated in this experiment with the amount of discoloration of 2 and 2 diphenyl 1-picrylhydrazyl violet solution in methanol. For this purpose, 100 µl of each extract with different concentrations (50, 100, 200, 400 and 800 µg/ml) were mixed with 1 ml of DPPH (2 and 2 diphenyl 1-picrylhydrazyl) methanolic solution and incubated for 60 minutes. The room temperature was kept in a dark place and then the absorbance of the mixture was read by a spectrophotometer at a wavelength of 517 nm. tert-butyl hydroquinone (TBHQ) was used as a positive control for comparison. The rate of inhibition of free radicals was obtained through equation (3) [9].

relationship (3)

$\times 100 = \frac{Acontrol - Asample}{Acontrol}$

$\times 100 = \frac{Acontrol - Asample}{Acontrol}$ = [($A_{control}$ _ A_{sample}) / $A_{control}$] × 100% inhibition

2-2-2-5- Check the balance of chlorophyll a and the balance of cartnoidha

Chlorophyll is the green pigment found in plants that helps absorb sunlight and convert it into energy. It is believed that this substance is very useful for the human body. Carotenoids are a group of pigments that, in addition to their role in the formation of pigments, have also been reported to have antioxidant properties [10]. To measure the amount of chlorophyll a and total carotenoids, the method of Banyan et al. [11] was used in the description. This method: Due to the vulnerability of pigments, the absorption of the obtained extract was measured by a spectrophotometer in a dark environment at wavelengths (OD) of 665.2, 652.4 and 470 nm. The corresponding equations due to the absence of chlorophyll b in*Spirulina platensis* simplified and calculated according to the following equations:

Chloriphyll a (µg/ml) = $16.72 \times OD_{665.2} - 9.16 \times OD_{652.4}$

Carotenoids (µg/ml) = $(1000 \times OD_{470} - 1.63 \times$ Chlorophyll a) /221

2-2-2-6- - Statistical analysis of data

The variables examined in this research include the method of extracting extract from microalgae*Spirulina platensis* (soaking and ultrasound) and solvent type (water, 96% ethanol and 50% ethanol) were considered. The statistical analysis of the data was done in a completely random design and in the form of a factorial test with three repetitions. The comparison of means was done based on Duncan's multiple-sentence test at the confidence level of 95% and by SPSS version 9.1 software. The graphs were drawn using Excel version 2016.

3- Results

3-1- Checking the amount of total phenol

According to the analysis of variance table 1, the independent effect of the amount of solvent ethanol as well as the interaction effect of the amount of ethanol and the extraction method have an effect on the total phenol content. According to chart 1, the amount of total phenol of spirulina algae extract in the method of soaking in ethanol solvents 0-50-96% is equal to 11.60-43.7-43 and 7.35-43 in the ultrasonic method in the same solvents respectively. It was 7.6-50.6-63.99 in terms of milligrams of gallic acid per gram of dry extract of spirulina algae. Among the results of the sample soaked in 96% ethanol solvent, it was significantly

higher compared to other samples, and Table 2 shows that the total phenol extraction values in 96% ethanol solvent are significantly higher than 50% ethanol solvent. Also, the amount of extraction in 0% ethanol solvent showed no significant difference with 96% and 50%.

Table 1 ANOVA table of total phenol content (mg of gallic acid per gram of *Spirulina platensis* dry extract)

Source	Type III Sum of Squares	df	Mean Square	F	Say.
Corrected Model	75.124	5	15.025	10.335	.001
Intercept	990.161	1	990.161	681.074	.000
Ethanol purity	40.158	2	20.079	13.811	.001
Extraction type	3.956	1	3.956	2.721	.125
Ethanol purity * Extraction type	31.010	2	15.505	10.665	.002
Error	17.446	12	1.454		
Total	1082.731	18			
Corrected Total	92.570	17			

R Squared =0.812 (Adjusted R Squared = 0.733)

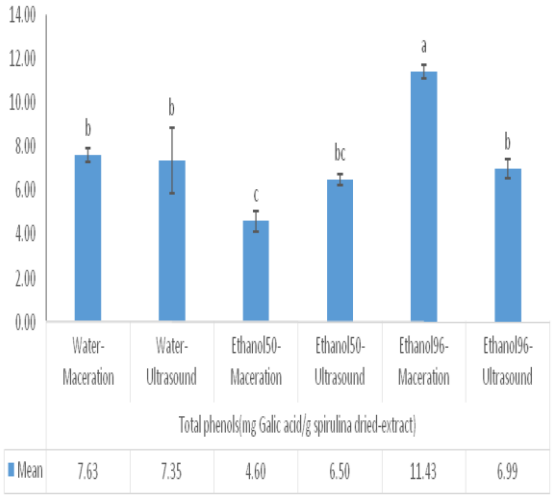

Fig 1 Investigating the amount of solvent ethanol and the extraction method of spirulina algae on the amount of total phenolic content

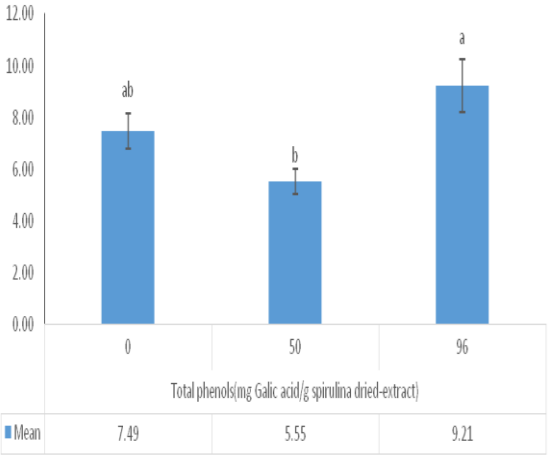

Fig 2 Investigating the effect of the ethanol content of the spirulina algae extraction solvent on the amount of total phenolic content

3-2- Checking the amount of total flavonoids

According to Table 2, the independent effect of the amount of ethanol solvent and the interaction effect of the amount of ethanol solvent and the extraction method have a significant effect on the total flavonoid values of the spirulina algae extract. Diagram 3 shows that the flavonoid values in 96% ethanol-ultrasound and water-soaking are significantly higher and lower than other treatments, respectively.

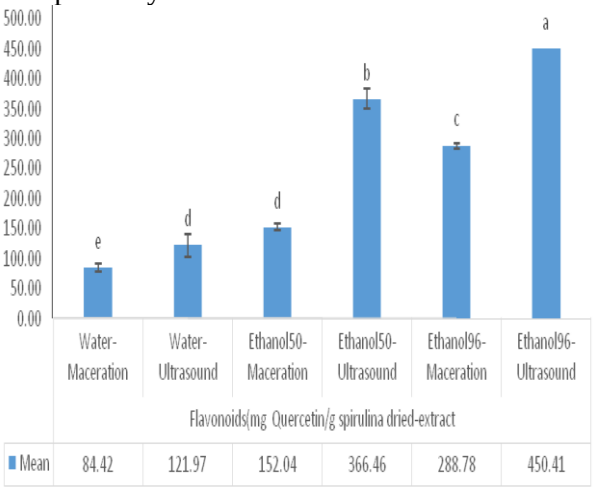

Fig 3 Investigating the effect of the amount of solvent ethanol and the extraction method of spirulina algae on the amount of total flavonoids

Also, in the amounts of 50% ethanol solvent, in the ultrasound method, the values of the above index are significantly higher than the soaking method. According to graph 4, the highest flavonoid values were observed in 96% ethanol solvent and then in 50% ethanol solvent. Also, the extraction values in 0% ethanol solvent were significantly lower than others.

Table 2 ANOVA table of flavonoid content (mg of Quercetin per gram of *Spirulina platensis* dry extract)

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Source	Type III Sum of Squares	df	Mean Square	F	Say.
Corrected Model	325256.489	5	65051.298	181.080	.000
Intercept	1071767.846	1	1071767.846	2983.430	.000
Ethanolpurity	214988.431	2	107494.215	299.227	.000
Extractiontype	85534.909	1	85534.909	238.100	.000
Ethanolpurity * Extractiontype	24733.150	2	12366.575	34.424	.000
Error	4310.882	12	359.240		
Total	1401335.218	18			
Corrected Total	329567.372	17			

R Squared = 0.987 (Adjusted R Squared = 0.981)

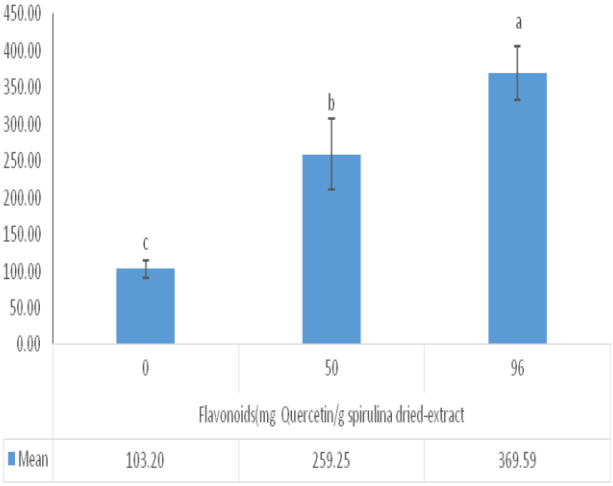

Fig 4 Investigating the effect of the solvent ethanol content of the spirulina algae extraction method on the amount of total flavonoids

3-3- Examining the amount of anthocyanin

According to Table 3, the independent effect of the solvent ethanol percentage and the interaction effect of the solvent ethanol amount and the extraction method have a significant effect on the anthocyanin values of the spirulina algae extract. Chart 5 shows that the amount of anthocyanin is the highest in 96% ethanol solvent-ultrasound and 50% ethanol solvent-soaking. Also, the values of this index are not significantly different in the treatments of 96% ethanol solvent-soaking and 50% ethanol solvent-ultrasound, as well as water-soaking and water-ultrasound. According to graph 6, the values of anthocyanin in 96% and 50% ethanol solvent with There is no significant difference between each other. Also, the lowest extraction values were observed in zero percent ethanol.

Table 3 ANOVA table of total anthocyanin content (mg cyaniding 3-glucoside per gram of *Spirulina platensis* dry extract)

Source	Type III Sum of Squares	df	Mean Square	F	Say.
Corrected Model	2.854	5	.571	83.540	.000
Intercept	5.645	1	5.645	826.096	.000
Ethanolpurity	1.660	2	.830	121.452	.000
Extractiontype	.002	1	.002	.265	.616
Ethanolpurity * Extractiontype	1.193	2	.596	87.265	.000
Error	.082	12	.007		
Total	8.581	18			
Corrected Total	2.936	17			

R Squared = 0.972 (Adjusted R Squared = 0.960)

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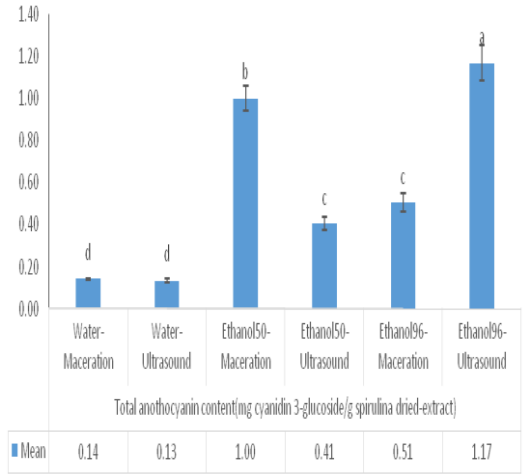

Fig 5 Investigating the effect of solvent ethanol content and spirulina algae extraction method on total anthocyanin content

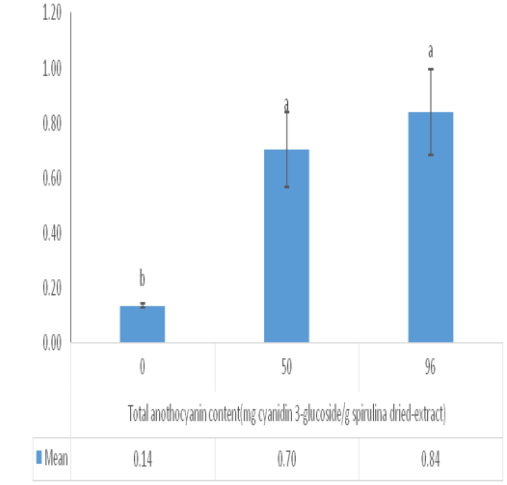

Fig 6 Investigating the effect of the ethanol content of the spirulina algae extraction solvent on the amount of total anthocyanin

3-4- Investigating the amount of DPPH radical inhibition

According to Table 4, the independent effect of ethanol solvent and extraction method, as well as the interaction effect of ethanol solvent and extraction method, solvent concentration and different concentrations of extract, as well as extraction method and extract concentration and comparison with water and TBHQ antioxidant on DPPH radical inhibition significantly It is effective. Chart 7: Investigating the effect of the amount of solvent ethanol and the extraction method of spirulina algae

DPPH free radical inhibition rate and its comparison with antioxidant

It shows TBHQ. The highest values are related to TBHQ treatment and then 50% ethanol solvent-soaking. Also, the amount of inhibition in the treatments of water-soaking and 96% soaking, as well as water-ultrasound, 50% ultrasound and 96%-ultrasound are not significantly different from each other. Figure 8 shows the effect of ethanol solvent of spirulina algae extract on DPPH free radical inhibition and its comparison with the antioxidant TBHQ. The highest value was observed in TBHQ treatment followed by 50% ethanol. Also, the values of free radical inhibition in 96% ethanol solvent and water were not significantly different from each other. Figure 9 shows the effect of spirulina algae extraction method on DPPH free radical inhibition and its comparison with TBHQ antioxidant. According to the graph, TBHQ, soaking and ultrasound treatments have the highest values. Diagram 10 shows the effect of ethanol solvent of spirulina algae extract in different concentrations on DPPH free radical inhibition and its comparison with TBHQ antioxidant. The highest values correspond to the concentration of 800 ppm TBHQ and the lowest values correspond to the concentration of 50 ppm - 96% ethanol solvent. The amount of inhibition in the treatments of 50, 100, 200 and 400 ppm TBHQ is not significantly different from each other. Diagram 11 shows the effect of extraction method and extract concentration on DPPH radical inhibition and its comparison with TBHQ antioxidant. The highest values correspond to the 800 ppm TBHQ treatment and the lowest values correspond to the 50 ppm-ultrasound concentration. The comparison between the concentrations can be explained as follows: the highest values correspond to the concentration of 50 ppm TBHQ, 100 ppm TBHQ, 200 ppm TBHQ and 400 ppm TBHQ, as well as the lowest values corresponding to the concentration 50 ppm-ultrasound, 100 ppm-ultrasound, 200 ppm-ultrasound and 400 ppm-Ultrasound and 800 ppm - ultrasound.

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Table 4 ANOVA table of antioxidant activity of *Spirulina platensis* dry extract (based on DPPH free radical inhibition)

Source	Type III Sum of Squares	df	Mean Square	F	Say.
Corrected Model	9552.527[a]	34	280.957	1203.148	.000
Intercept	566411.934	1	566411.934	2425561.048	.000
Ethanolpurity	165.720	2	82.860	354.834	.000
Extractiontype	514.443	1	514.443	2203.012	.000
Concentration	228.156	4	57.039	244.260	.000
Ethanolpurity * Extractiontype	217.862	2	108.931	466.478	.000
Ethanolpurity * Concentration	21.765	8	2.721	11.651	.000
Extractiontype * Concentration	8.399	4	2.100	8.991	.000
Ethanolpurity * Extractiontype * Concentration	19.277	8	2.410	10.319	.000
Error	16.346	70	.234		
Total	585781.485	105			
Corrected Total	9568.873	104			

R Squared = 0.998 (Adjusted R Squared = 0.997)

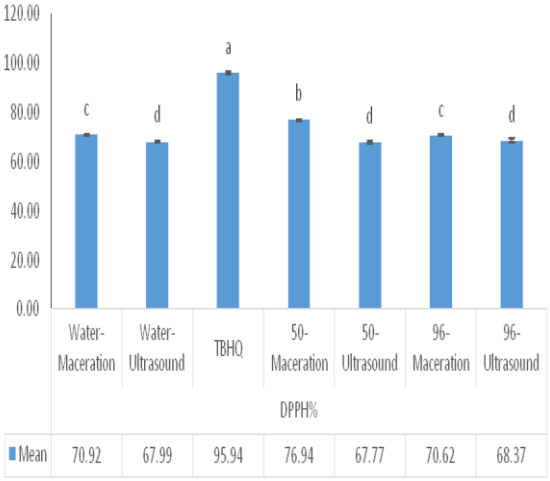

Fig 7 Investigating the effect of solvent ethanol content and the extraction method of spirulina algae on the DPPH free radical inhibition rate and comparing it with antioxidant TBHQ

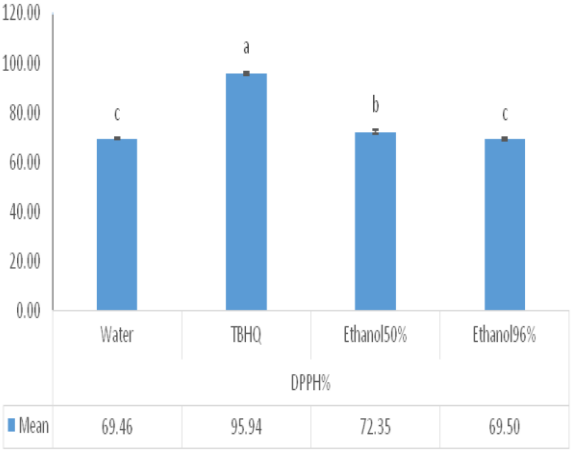

Fig 8 Investigating the effect of the ethanol content of the spirulina algae extraction solvent on DPPH free radical inhibition and comparing it with antioxidant TBHQ

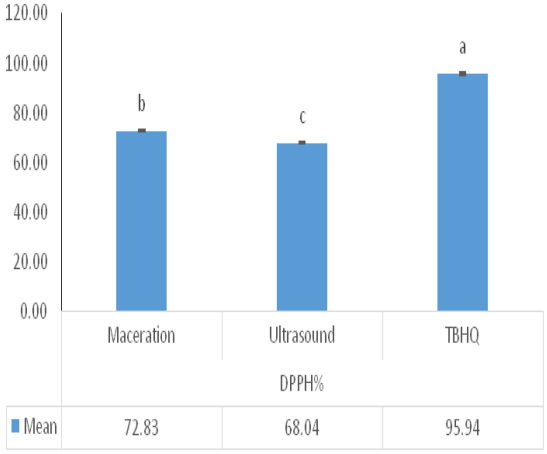

Fig 9 Investigating the effect of spirulina algae extraction type on DPPH free radical inhibition and comparing it with antioxidant TBHQ

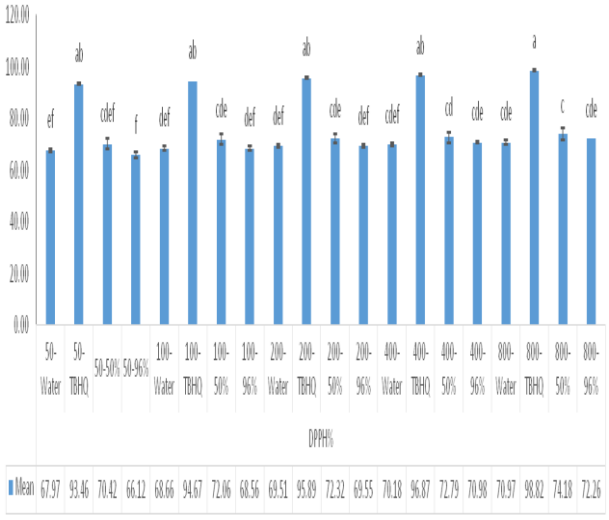

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Fig 10 Investigating the effect of different concentrations of aqueous-ethanol extracts of spirulina algae on DPPH free radical inhibition and comparing it with antioxidant TBHQ

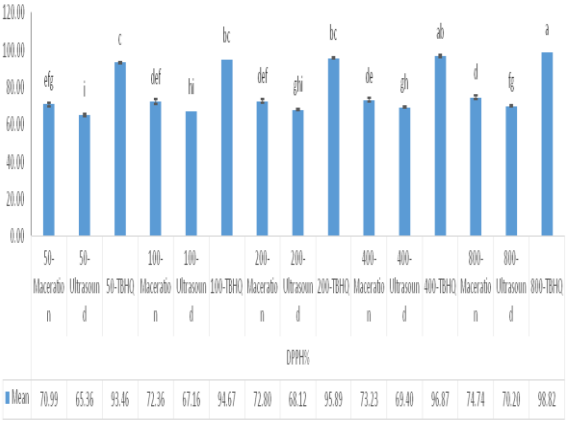

Fig 11 Investigating the effect of ectraction method in different concentrations of spirulina algae extract on DPPH free radical inhibition and comparing it with antioxidant TBHQ

5-3- Examining the amount of chlorophyll a and carotenoids

Diagram 12 shows the influence of ethanol solvent on chlorophyll and carotenoid indices. The highest values of the indicators correspond to concentrations of 96%, 50%, and the lowest values correspond to 0%, respectively. According to graph 13, the interaction effect of the extraction method and the amount of solvent ethanol on the chlorophyll index in 96% ethanol-soaking values is significantly higher than other treatments. Also, the use of water-soaking treatment significantly reduces this index. In amounts of 96% ethanol solvent, the soaking method extracts higher chlorophyll compared to ultrasound. While in amounts of 50% ethanol and using water, extraction by ultrasound has been reported to be a more successful method compared to soaking. According to chart 14, the carotenoid index in concentrations of 96%, 50% and water by ultrasound has higher values compared to similar solvents. And the method of soaking shows itself.

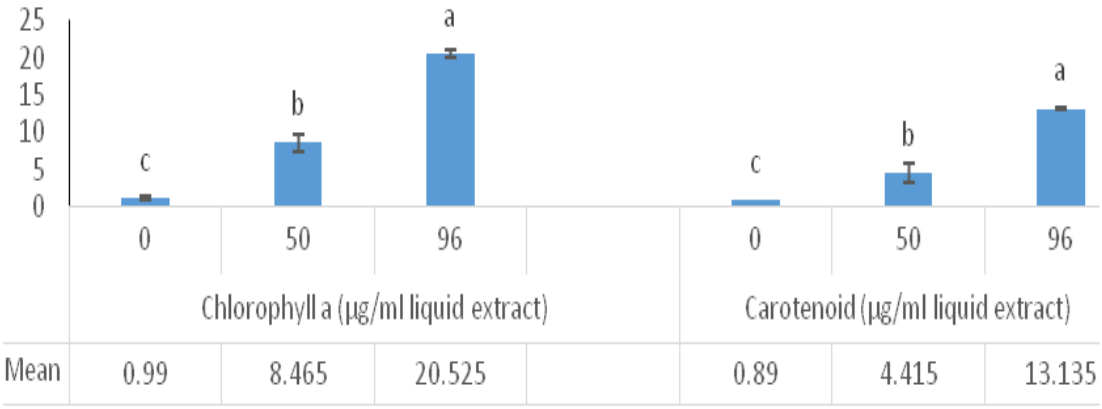

Fig 12 Investigating the influence of the solvent ethanol percentage on chlorophyll and carotenoid content

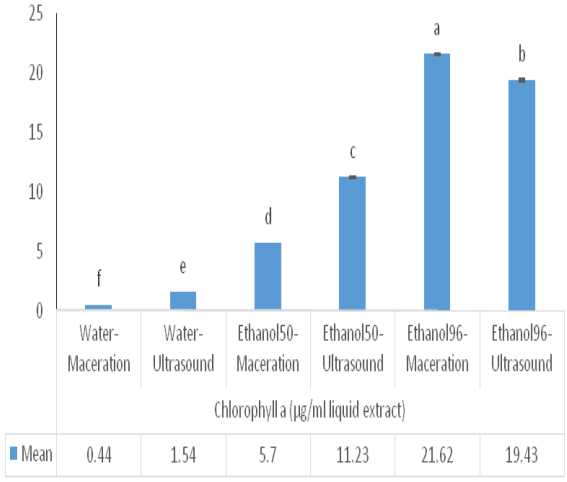

Fig 13 Investigating the effect of solvent ethanol percentage and extraction methods on chlorophyll content

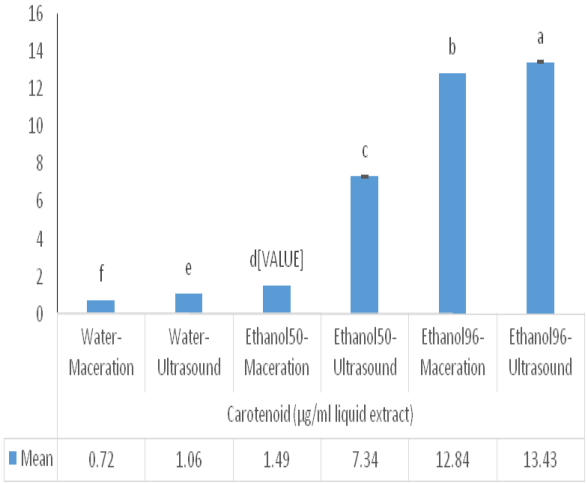

Fig 14 Investigating the effect of solvent ethanol percentage and extraction methods on carotenoid content

4 - Discussion and conclusion

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4-1- Checking the amount of total phenol

According to table and graph 1, the interaction effect of ethanol solvent amount and extraction method on total phenol values of spirulina, significantly.(P>0.01) is effective. The highest and lowest values of total phenol are observed in 96% ethanol soaking and 50% ethanol soaking treatments, respectively. Examining the independent effect of the extraction method on total phenol values showed that although the values of this index are higher in the soaking method. However, no statistically significant difference was observed between this method and the ultrasound method in terms of total phenol index. Also, according to graph 2, the use of 96% ethanol compared to 50% ethanol significantly has higher total phenol values. Also, the amount of total phenol in the 0% ethanol treatment does not show a significant difference in terms of total phenol content compared to the two treatments of 96% and 50%. Farahmand et al. [12] compared the extraction method with the help of ultrasound and the traditional methods of solvent extraction. The results showed that ethanol-soaking, water-soaking and ultrasound-water methods were not significantly different from each other in terms of the amounts of phenolic compounds. In water soaking, the extraction of phenolic compounds decreased due to the high polarity of water as a solvent. However, the extraction of phenolic compounds increased in the presence of 50% water due to the relative increase in polarity and better swelling of the pepper particles. In addition, the presence of water as a solvent leads to a decrease in the viscosity of the mixture, which leads to much greater mass transfer.Doi et al. [13] found the amount of phenolic compounds in 50% ethanol extract (soaking method, ratio of solvent to dry powder of spirulina algae 1:10 and soaking time 24 hours) to the extent of 65.33 mg of gallic acid per gram of sample. The extraction of pepper with the same solvents and ratios was done by ultrasound. In this method, the mixture was placed in an ultrasound bath at a temperature of 45 degrees for 20 minutes. The separation of extracts from pepper particles was similar to the soaking method [14]. Alavi et al reported the total phenol content of 96% methanol extract of spirulina algae (soaking method, ratio of solvent to algae powder 1:10 and soaking time 4 minutes) 67.72 mg of gallic acid per gram of spirulina [15]. Shelby et al. total phenol content of spirulina algae extract in two types of 100% methanol solvent and distilled water (soaking method, ratio of solvent to algae powder 1 to 10) were 282.76 mg and 169.15 mg per 100 g, respectively, based on gallic acid in g of the sample [16]. Ghanbari et al. total phenol content of 80% acidic methanol extract of spirulina algae (soaking method, ratio of solvent to algae powder 1:10, soaking time 24 hours) they found the total phenol content to be 2.78 mg of gallic acid calculated per gram of spirulina algae powder [17]. Golli et al. calculated the amount of total phenol in the aqueous extract of spirulina algae as 6.86 mg of tannic acid per gram of spirulina algae powder [14].

4-2- The amount of total flavonoids

According to table (3-4) the interaction effect of solvent ethanol amount and extraction method on total phenol values of spirulina, significantly(P>0.01) is effective. Total flavonoid values in 96%-ultrasound and water-soaking treatments significantly increase and decrease compared to other treatments, respectively. Also, 96% and 50% ethanol-ultrasound treatments have higher flavonoid values compared to 96% and 50%-soaking treatments.The ultrasonic method extracted 96% of the total flavonoid compounds in two solvents, distilled water and ethanol, much better than the soaking method.Although there was no statistically significant difference between the independent effect of soaking method and ultrasound method in terms of total phenol index.Alavi et al. reported the total flavonoid level to be 9.16 milligrams equivalent to quercetin per gram of spirulina algae [15]. Ghanbari et al investigated the amount of total flavonoids at 3 wavelengths of 300, 270 and 320 nm and the total results were reported as 19.95, 27.86 and 21.66 milligrams of quercetin per gram of dry weight of spirulina root [17].

3-4- Investigating the amount of total anthocyanin

According to diagram 5, the interaction effect of the amount of solvent ethanol and extraction method has a significant effect (P<0.01) on the

amount of spirulina anthocyanins. The highest amount of anthocyanin related to ultrasound-ethanol treatment is 96%. Also, the amount of anthocyanin in the soaking-ethanol 50% treatment is significantly higher compared to ultrasound-ethanol 50%. No statistically significant difference was observed between the independent effect of soaking method and ultrasound method in terms of total anthocyanin index. Farahmand et al. reported that the amount of total anthocyanin extraction of 50% water-50% ethanol by ultrasound method is more effective in comparison with other extraction methods such as 50% water-50% ethanol by soaking, ethanol or water-ultrasound and ethanol or water-soaking [12]. .

4-4- Investigation of DPPH free radical inhibition

Diagram 7 shows that the independent effect of the extraction method and the amount of ethanol solvent and their interaction, as well as the interaction and comparison with the antioxidant TBHQ, have a significant effect on the DPPH free radical inhibition. In the examination and comparison of spirulina algae extract samples with TBHQ antioxidant samples in similar concentrations, the results of industrial antioxidants were always better. The highest level of inhibition of DPPH free radical was observed in the concentration of 800 ppm of TBHQ antioxidant at the rate of 98.82% and the lowest level at the concentration of 50 ppm of the ultrasonic method was 65.36%. In the analysis of two extraction methods, the soaking method at the same concentration had higher results than the ultrasonic method. Also, when examining the type of solvent, 96% ethanol always showed higher results than the other two types of solvents. So, according to the obtained results, the highest level of free radical inhibition can be obtained in 96% ethanol solvent by soaking method. Ghanbari et al obtained the percentage of free radical activity at a concentration of 200 micrograms as 36.46% [17]. Alavi et al., the amount of IC_{50} Spirulina extract and IC_{50} The percentage of spirulina (compared to the antioxidant tert-butyl hydroquinone) was 0.364 and 0.10 mg/ml, respectively.]15[. Li et al. IC $rate_{50}$ were calculated in the inhibition of DPPH 46/72 in terms of micromol of Trolex per gram of spirulina algae aqueous extract.

4-5 - The amount of chlorophyll a and carotenoids

Diagram 12 shows that the highest and lowest chlorophyll and carotenoid values were observed in 96% ethanol and 9% ethanol treatments, respectively. As a result, increasing the concentration of ethanol solvent increases the extraction percentage of the above compounds. According to chart 13, in the concentration of 96% ethanol solvent, the amount of chlorophyll in the soaking method is significantly higher compared to ultrasound. Although, in the concentration of 50% ethanol and water, the amount of chlorophyll increased significantly when using the ultrasound method. Diagram 14 shows that carotenoid values are significantly higher in all solvent concentrations with the ultrasound method compared to the soaking method. As a result, the ultrasonic method was more suitable for the extraction of carotenoid compounds and the soaking method for chlorophyll a. Banyan et al. investigated the amount of carotenoid and chlorophyll of spirulina algae according to the variables of spirulina algae culture: volume of inoculation (turbidity), nitrogen source (urea), carbon source (date juice), duration of cultivation, light composition, exposure period and sodium chloride. . They observed the highest amount of total carotenoids 21.8 micrograms per milliliter and the lowest amount of total carotenoids 6.95 micrograms per milliliter [11].

5 - general conclusion

The use of industrial antioxidants in the food industry always shows numerous risks for human health. These antioxidants with low amount have the best results in food preservation, so it forces the producers to use such materials. In the event that natural antioxidants with numerous properties are readily available and do not endanger human health due to their lower effectiveness in food compared to industrial antioxidants, they are much less considered. Algae*Spirulina*Cyanobacteria have numerous properties that are very easy to grow and harvest.

6- Gratitude

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7- Resources

[1] Ramirez-Rodrigues, M. M., Estrada-Beristain, C., Metri-Ojeda, J., Perez-Alva, A., Baigts-Allende, D.K.(2021). Spirulina platensis Protein as sustainable Ingredient for nutritional food products development. Sustainability, 13(12): 6849.

[2] Jung, F.a., Kruger-Genge, A.b., Waldeck, P.c., Kupper, J. H. (2019). Spirulina platensis, a super food? Journal of Cellular Biotechnology, vol. 5, no. 1, pp. 43-54.

[3] Al Juhaimi, F., Ozcan, M.M., Ghafoor, K., Babiker, E.E., Hussain, S. (2018). Comparison of cold-pressing and soxhlet extraction systems for bioactive compounds, antioxidant properties, polyphenols, fatty acids and tocopherols in eight nut oils. J. Food Sci. Technol., 55: 3163-3173.

[4] Zhang, N., Li, F., Zhang, T. Li, Chun-Yang, Zhu, L. Yan, S. (2022). Isolation, identification, and molecular docking analysis of novel ACE inhibitory peptides from Spirulina platensis. Eur Food Res Technol 248, 1107-1115.

[5] Aiguo, L., Feng, J., Hu, B., Lv, J., Qi, L., Nan, F.C.Y. Chen, O., Xie, S. (2017). Arthrospira (spirulina) platensis extract improves oxidative stability and product quality of Chinese style pork sausage. Journal of Appliedy phycology, 30: 1667-1677.

[6] Ciulca, S., Roma, G., Alexa, E., Radulov, I., Cocan, I., Madosa, E., &Ciulca, A. (2021). Variation of Polyphenol Content and Antioxidant Activity in Some Bilberry (Vacciniummyrtillus L.) Populations from Romania. Agronomy, 11: 2557.

[7] Li, S., Wei, Y., Fang, Y., Zhang, W., Zhang, B. (2014). DSC study on the thermal properties of soybean protein isolates/corn starch mixture. J. Therm. Anal. Calorim., 115, 1633-1638.

[8] Rodrigues, L.R., & Jose, J. (2020). Exploring the photo protective potential of solid lipid nanoparticle-based sunscreen cream containing Aloe vera. Environmental Science and Pollution Research, 27: 20876-20888.

[9] Pathaka, J., Ahmeda, H., Rajneesha, Singhb, S.P., Häderc, D.P., & Sinha, R.P. (2019). Genetic regulation of scytonemin and mycosporine-like amino acids (MAAs) biosynthesis in cyanobacteria. Plant Gene, 17: 100172.

[10] Hasan Sultan, T., Nowrozi, M., and Amoozgar, M.A. (2015). Investigating the amount of chlorophyll a, b and total carotenoids as well as the antioxidant activity of four species of green algae isolated from the shores of Golestan, Caspian Sea. New Journal of Cell-Molecular Biotechnology, 6(24): 36-31.

[11] Banayan, S., Jahadi, M., &Fazel, M. (2019). Investigating the influencing factors on the production of chlorophyll and carotenoid pigments from Spirulina platensis using the Berman platelet design. Journal of Food Microbiology, 7(2): 70-81.

[12] Farahmand, M., Golmakani, M.T., Mesbahi, G., Farahnaky, A. (2017). Investigating the Effects of Large-Scale Processing on Phytochemicals and Antioxidant Activity of Pomegranate Juice. Journal of Food Processing and Preservation, 41(2): e12792.

[13] Dewi, N., Kurniasih, A., &Purnamayanti, L. (2018). Physical Properties of Spirulina Phycocyanin Microencapsulated with Maltodextrin and Carrageenan. Philippine Journal of Science, 147(2): 201-207.

[14] Goli, A.H., Barzegar, M., &Sahari, M.A. (2005). Antioxidant activity and total phenolic compounds of pistachio (Pistachia Vera) hull extracts. Food Chemistry, 92(3): 521-525.

[15] Alavi, N., Karamet, M., Golmakani, M., Lari, A., Shekarforosh, S. ,&Nowrozi, M. (2014). Improving the oxidation stability of virgin olive oil using spirulina microalgae as a natural antioxidant. Journal of Nutrition Sciences and Food Industries of Iran, 10(4): 63-74.

[16] Shalaby E.A., Shanabو S.M.M., Singhو V. (2010). Salt stress enhancement of antioxidant and antiviral efficiency of Spirulina platensis, J. Med. Plants Res. 4(24):2622-2632.

[17] Ghanbari, H., Sarmad, J., Ghafouri, Kh., and Zamani, H. (2014). Investigating the antioxidant activity of spirulina microalgae and measuring the antimicrobial properties of this microalgae and the phycocyanin pigment extracted from it. Gilan University, Faculty of Basic Sciences, Department of Biology (Plant Physiology Major), Scientific Information Database of Iran (Ganj), Research Thesis, Ministry of Science, Research and Technology.

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بررسی استخراج ترکیبات بیواکتیو از جلبک *اسپیرولینا پلاتنسیس* به روش خیساندن در حلال اتانلی به کمک اولتراسوند

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چکیده

اسپیرولینا پلاتنسیس **به دلیل دارا بودن مواد مغذی و ویژگی های مربوط به بهبود سلامتی، کاربردهای زیادی در کشاورزی، صنایع غذایی، دارویی و لوازم آرایشی دارد. بنابراین، استخراج ترکیبات زیست فعال آن هنوز یک چالش است. هدف از مطالعه حاضر، استخراج ترکیبات فنولیا استفاده از دو روش اولتراسوند و خیساندن و بررسی تاثیر روش‌های استخراج و غلظت عصاره بدست آمده از ریزجلبک *اسپیرولینا پلاتنسیس* بر محتوی فنول کل و فعالیت آنتی اکسیدانی بود. عصاره جلبک اسپیرولینا به دو روش خیساندن و اولتراسونیک در ۳ حلال الکل (اتانول۹۶ درصد) آب-الکل (اتانول ۵۰ درصد) و آب (اتانول صفر درصد) اندازه گیری شد. سپس میزان فنول کل، فلاونوئید کل، آنتوسیانین ها، میزان مهار رادیکال آزادDPPH، میزان کلروفیلa و کاروتنوئید آن محاسبه شد. بالاترین مقادیر فنول کل در تیمار (اتانول ۹۶ درصد-خیساندن)، فلاونوئید و آنتوسیانین در تیمار (اتانول ۹۶ درصد-اولتراسوند) مشاهده شد. در نتیجه استخراج حلال با استفاده از اولتراسوند استخراج ترکیبات فراسودمند را افزایش داده و منجر به فعالیت آنتی اکسیدانی بالاتر می‌شود.**