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Comparison of astaxanthin extraction from, red (*Gracilaria corticata*) and brown (*Sargassum polycystum*) macroalgae

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ABSTRACT

This study aimed to compare the extraction of astaxanthin under soaking conditions for 24 hours with a control solvent (ethanol), an organic solvent (a mixture of ethanol and ethyl acetate), a green solvent (a microemulsion of tributyloctylphosphonium bromide ionic liquid in water), and a vegetable oil (sunflower oil) from red (Gracilaria corticata) and brown (Sargassum polycystum) macroalgae. Solvent-to-sample ratios of 5, 12.5, and 20 were used for extraction. The amount of extracted astaxanthin, total carotenoids, astaxanthin recovery percentage, and DPPH radical scavenging activity were investigated. According to the results obtained from the extraction of astaxanthin from red (Gracilaria corticata) and brown (Sargassum polycystum) macroalgae, brown (Sargassum polycystum) macroalgae was selected as the source with the highest amount of extracted astaxanthin with a value of 86.00±2.83 mg/ml. The use of green solvent (ionic liquid microemulsion in water) in a ratio of 5 times the solvent to the sample was also selected as the best extraction conditions. The results of DPPH radical scavenging by astaxanthin extracted with the help of the mentioned solvents compared to the synthetic antioxidant BHT showed that the antioxidant activity increased with increasing astaxanthin concentration. However, this increase was always less than the antioxidant activity of BHT. In general, the results of this study showed that the use of ionic liquid-based microemulsion is a suitable alternative to conventional methods in the extraction and recovery of astaxanthin from natural biological sources. In addition, brown macroalgae (Sargassum polycystum) is also a rich source for extracting the natural astaxanthin pigment.

1-Introduction

Carotenoids are natural pigments that inactivate harmful chemical reaction initiators such as free oxygen, photosensitive triads, and free radicals, which otherwise would potentially cause cancer in biological systems. Carotenoid pigments are therefore known to contribute to the functioning of the immune system in mammals. Epidemiological studies have shown an inverse relationship between the risk of laryngeal, lung, and colon cancers and the consumption of carotenoid-containing foods. Given the importance of these pigments, their extraction from natural compounds has received much attention (1). Therefore,Marine organisms are one of the sources that can be used as a natural source for extracting these compounds due to their various pigments. Important pigments extracted from aquatic organisms include astaxanthin and phycocyanin (2). Astaxanthin is present in various microorganisms such as algae, bacteria, and yeasts. Astaxanthin is a pigment from the xanthophyll family and is one of the most famous carotenoid pigments. This compound has a polyunsaturated structure and has multiple double bonds in its structure (3). Astaxanthin is a fat-soluble pigment and its color is reddish-orange. This compound has many antioxidant properties and studies have shown that it is effective in preventing cancers, diabetes, Parkinson's and Alzheimer's diseases, eye diseases such as cataracts, cardiovascular diseases, stroke, and high cholesterol (4). Due to the aforementioned properties of astaxanthin, its extraction from natural sources is necessary for use in aquaculture and the food industry. Macroalgae or seaweeds are multicellular algae that are found in abundance on beaches with high tides, deep seas and lakes, and are considered one of the sustainable sources for extracting natural marine compounds. Due to their various applications in the pharmaceutical, food, cosmetic and health industries, as well as in aquatic nutrition to increase growth indicators, they have attracted the attention of many researchers. There is evidence that relatively low consumption of algae, due to their antioxidant properties, over a long period of time can have beneficial effects in preventing cancer and many chronic diseases, including cardiovascular diseases and type 2 diabetes, which is rapidly increasing (5). Brown algae*Sargassum polycystum*.An edible seaweed, it is rich in dietary fiber, vitamins, minerals, and polysaccharides. It is a common food source in Asian countries. Recently, various bioactive compounds in*S. polycystum* They have been identified with antioxidant, antibacterial and antitumor properties. Collectively, these properties make this seaweed very useful for a wide range of biomedical applications (6). In a study conducted by Sadati et al. (2011), the antioxidant effect of phenolic compounds from three brown macroalgae species was investigated.*Sinuous colpomenia*.*Cystoseira myrica*and*Sargassum swartzii* collected from the coasts of the Persian Gulf in the Assaluyeh region were examined and the results showed that the species*Sargassum swartzii*It is a rich source of phenolic compounds with antioxidant properties (7). On the other hand, among red algae, the genus*Gracilaria* It contains a wide variety of valuable ingredients for human nutrition and is one of the most widely cultivated and valuable seaweeds in the world. Its lipid content is low (1-5% of dry weight), but it contains docosahexaenoic acid, which is known as the most important unsaturated fatty acid for reducing the risk of cardiovascular diseases. It acts as an excellent antioxidant, strengthens cell membranes and repairs damaged cells and tissues, improves heart function and fights cancer. However, the nutritional profile of seaweeds such as*Gracilaria*It is influenced by various factors such as seaweed species, habitat, maturity stage, season, water temperature, and sampling conditions and methods used (8).*Gracilaria corticata* It is widely distributed on the coast of Iran in the

Persian Gulf and has good potential for extracting antioxidants.Many studies have been conducted to extract astaxanthin from marine organisms, including extraction using various solvents with the assistance of ultrasound, microbial fermentation, extraction with oil, and ionic liquid (9).Ionic liquids have unique physicochemical properties such as negligible vapor pressure, non-flammability, high thermal stability, and recyclability. On the other hand, low doses of ionic liquids have not resulted in cellular damage in animal and laboratory experiments. As a result, ionic liquids are potential alternatives to volatile organic solvents in the food industry (10). However, the viscosity of most ionic liquids is higher than that of organic solvents, which results in a reduced mass transfer rate. Microemulsion is a promising method that allows for the selective extraction of biomolecules in the food and chemical industries.(11). Microemulsions are a type of emulsion system that, unlike emulsions, are thermodynamically stable and the dispersed phase size in them is about 10-100 nm. To prepare a microemulsion system, four basic components are required, including water, oil, surfactant, and cosurfactant. By mixing appropriate proportions of these components, the microemulsion system forms itself (12). To date, microemulsions have been used to extract proteins, pigments, and trace elements. Gao et al. (2020) conducted a study aimed at recovering astaxanthin from shrimp waste and ultrasound-assisted extraction using an ionic liquid microemulsion in water (consisting of tributyl octylphosphonium bromide ionic liquid, Triton X100 water, and n-butanol). The results showed that compared to organic solvents (ethanol, acetone, and dimethyl sulfoxide), ionic liquid microemulsion in water significantly increased astaxanthin extraction due to stronger electrostatic interactions and hydrogen bonding interactions. In general, ionic liquid-based microemulsion is a suitable alternative to conventional methods for the extraction and recovery of astaxanthin from natural biological sources (13). Therefore, this study was conducted for the first time with the aim of comparing astaxanthin extraction under soaking conditions for 24 hours withControl solvent (ethanol), Organic solvent (combination of ethanol with ethyl acetate), green solvent (microemulsion of ionic liquid tributyloctylphosphonium bromide as hydrophobic moiety in water, where phosphonium forms the cationic moiety and bromide forms the anionic moiety) and vegetable oil (sunflower oil) from red macroalgae(*Gracilaria corticated*)and brown(*Sargassum polycystum*)It's done.

2-Materials and methods

2-1-Raw materials

Algae Sargassum polycystum and Gracilaria corticata were obtained from the Persian Gulf Research Institute with molecular identification and species confirmation. Commercial astaxanthin with a purity of >98%,[1]DPPHand[2]BHTEthanol, propanol, and ethyl acetate of chromatographic grade and tributyloctylphosphonium bromide were purchased from Sigma-Aldrich.[3](Br(4448p))and Triton X100[4]with a purity of >99% and n-butanol with a purity of 99.9% were obtained from Sigma-Aldrich. Refined sunflower oil without antioxidants was also purchased from Hayat Company. All experiments were performed at the Food and Drug Administration of Hormozgan University of Medical Sciences.

2-2-Sample preparation

Red macroalgae(*Gracilaria corticated*)and brown(*Sargassum polycystum*)After being transferred to the laboratory, in order to

1- 2, 2-Diphenyl-1- picrylhydrazyl

2- *Butylated hydroxytoluene*

3-Tributyloctylphosphonium Bromide

4-Triton X-100

remove sand or possible foreign materials, they were washed several times with ordinary water and finally once with distilled water. Then, the samples were dried without crushing for 48 hours at laboratory temperature (22°C) and away from sunlight. Finally, they were completely crushed using an electric grinder and the resulting powder was kept at 4°C after sieving until extraction.

2.3-Preparation of solvents

The green solvent used is a microemulsion of ionic liquid in water, which was prepared by stirring only and using the following method, due to the spontaneous formation of the microemulsion and the lack of need for much energy (13): Tributyloctylphosphonium bromide: Triton X 100–n-Butanol (with a ratio of 3:1 Triton X 100 to n-Butanol): water, were mixed in mass ratios of 0.13:0.25:0.62, respectively. These compositions comprise the nonpolar phase, surfactant-co-surfactant, and polar phase of the microemulsion, respectively. This solvent can be stored at room temperature. Ethanol was used as the control sample.

2-4-Extraction method

The solvents were mixed with the sample powder at room temperature in ratios of 5 times, 12.5 times and 20 times (which was determined by reviewing previous studies on astaxanthin extraction from other sources) and were soaked for 24 hours without stirring at room temperature. Then, the resulting mixture was centrifuged for 10 minutes at 8500 rpm at room temperature in a centrifuge (slotted centrifuge)Eppendorf،Germany), the upper part of the sample was separated and the sedimented part was re-extracted in the previous method (to take into account possible residues in the sedimented part) and after centrifugation, the upper part was combined with the previous amount and filtered with a 0.45 micrometer Teflon filter to prepare for analysis (14).

2-5-Exams

2-5-1-Evaluation of physical properties of microemulsion

To measure the conductivity of microemulsion using a conductivity meterinoLab Made in Germany. A densitometer was used to measure the density of microemulsions.[5]50 microliters made in Germany were used. Microemulsion diameter with dynamic light scattering device[6](DLS) (Horiba،Japan) was determined.

2-5-2-Evaluation of total carotenoid content (total astaxanthin)

Total carotenoid content was determined using spectrophotometric measurements according to the method reported by Hoch et al. (2016). To determine the total carotenoid content, the absorbance of 4 ml of the extracted solutions was measured by spectrophotometer.[7](Spectrophotometer device)UV-Vis (Shimadzu(Japan)) was measured at a wavelength of 450 nm after zeroing the absorption of the instrument for each solvent and a specific extinction coefficient of astaxanthin of 2100 was used. Based on previous studies conducted in this regard, the total carotenoid content at this wavelength was considered as total astaxanthin. Total astaxanthin content yield[8] (TAC،(micrograms per gram dry weight) was estimated using formula (1) (15).

$$TAC = \frac{V \times A \times 100}{21 \times W}$$ Formula(1)

In:Volume of solvent used(ml)

A: Absorption at 450 nm

IN:Dry weight of the material(gr)

5-Pycnometer

6-Dynamic Light Scattering (DLS)

7-Spectrophotometry

8 -total astaxanthin content

2-5-3-Analysis of astaxanthin with a spectrophotometer

The astaxanthin standard curve was drawn by preparing different dilutions of pure astaxanthin at concentrations of 0-1 µg/mL using 2-propanol as the solvent of pure astaxanthin pigment. The absorbance of astaxanthin extracts at a wavelength of 478 nm (478FROM)SpectrophotometerUV-VIS The measurements were recorded in triplicate by the absorption of alcohol containing pure astaxanthin. The astaxanthin content of the extracts was calculated using formula (2) (16).

Formula(2)$C(mg/ml) = \frac{OF_{478}}{1.97}$

C: Astaxanthin concentration in 2-propanol in terms of

and:Absorbance at 478 nm

X:Concentration in micrograms per milliliter

2-5-4-Calculating the percentage of astaxanthin recovery

For this purpose, the extracts were analyzed. The percentage of astaxanthin recovery was obtained by calculating the percentage of astaxanthin extracted under each extraction condition from the total astaxanthin content of the sample according to formula (3) (17).

Formula(3)$\%Recovery = \frac{Astaxanthin\ Extract}{Total\ astaxanthin} \times 100$

2-5-5-Radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Store the extracted astaxanthin in the refrigerator to be used to investigate the antioxidant properties of astaxanthin. Ethanol solutionDPPHIt was prepared at a concentration of 0.2 mM. 2 ml of different concentrations of extracts (0.05-0.75) were mixed with 2 ml of ethanolic solution to investigate the trend of changes in the three types of solvents used.DPPHThe absorbance of the samples was measured after 30 minutes of storage at ambient temperature and dark conditions at 517 nm and after zeroing the absorbance of the device for each sample of solvents and the radical scavenging activity was measured.DPPHIt was calculated based on formula (4). This test was performed with antioxidant capacity, synthetic antioxidantBHTIt was compared as a control at similar concentrations (18).

Formula (4)$\left[A0 - (A - \frac{Ab}{A0})\right] \times 100$= % radical scavenging activityDPPH

A_0:Solution absorptionDPPHNo sample

A:Absorption of sample mixed with solutionDPPH

A_b:Absorbing sample without solutionDPPH

2-6-Statistical analysis

Statistical analysis of data using one-way analysis of variance statistical designANOVAAt the 0.05 error level with softwareSPSS 26.0The results are averaged.±Standard deviation reported. Plotting graphs in softwareExcel.2010Done.

3-Results and Discussion

3-1-Evaluation of physical properties of microemulsion

In this study, the density of the microemulsions was determined to be in the range of 0.97151 g/cm3, their diameter was 15.8 nm, and their conductivity was 312 microsiemens at a temperature of 27.1°C.Determination of density, conductivity, and diameter were among the properties tested for microemulsion in this study. Studying the physical properties of the solvent and their temperature dependence is important for the proper design and operation of separation processes. However, the physical properties of ionic liquid-based microemulsions are scarce and have been neglected in previous studies.High conductivity of microemulsion compared to ethanol:ethyl acetate mixture (1:2) It is due to the presence of soluble ionic species that are freely present in the solution and move.

On the other hand, the small particle size indicates the stability of the solvent. High conductivity of ionic liquid microemulsions compared to ethanol: ethyl acetate mixture (1:2) It causes better interaction of the solvent with the sample and higher extraction of the pigment (13).

3.2-Evaluation of total carotenoid content

Results related to total carotenoid content extracted from red macroalgae(*Gracilaria corticated*)and brown(*Sargassum polycystum*)Using different solvents (ethanol, ethanol:ethyl acetate mixture (1:2), ionic liquid microemulsion in water, oil solvent) in different ratios (5, 12.5 and 20 times) of solvent to sample is shown in Table 1. According to the results obtained from Table 1, the total carotenoid content extracted from red macroalgae(*Gracilaria corticated*)and brown(*Sargassum polycystum*)It was significant at different solvent to sample ratios ($p < 0.05$). The results showed that among different solvent to sample ratios, the 5-fold solvent to sample ratio had higher carotenoid content. In general, brown macroalgae(*Sargassum polycystum*)In a 5-fold ratio of solvent to sample using a microemulsion of ionic liquid in water with 2.02±31.91 ml of Berggram showed the highest total carotenoid content. The reason for the high content of extracted carotenoids, especially astaxanthin, by the ionic liquid microemulsion solvent in water can be attributed to the better dissolution of pigments and the matching polarity in this solvent compared to other solvents used in the study.In the study of Liu et al. (2018), different methods such as electric field difference, ultrasound, high-pressure microfluidization, hydrochloric acid with acetone, and ionic liquids were used to extract astaxanthin. The results showed that the ionic liquids technique and the combined method of hydrochloric acid and acetone yielded the most favorable results, and the type of solvent was consistent with the present study (19).Also, the results obtained from astaxanthin extraction in the studySinget al. (2020) also showed that the ionic liquid microemulsion solvent has a high ability to extract astaxanthin, which is consistent with the results obtained in the present study. Comparison between different solvent to sample ratios showed that with increasing solvent to sample ratio, the total extracted carotenoid content decreased, whichThe reason for this can beDue to the limited dissolving capacity of the solvent.

Table 1- Total content of carotenoids (ml/g) in Gracilaria and Sargassum in different ratio of solvent to sample

Solvents	*Sargassum polycystum*			*Gracilaria corticata*		
	Solvent to sample ratio			Solvent to sample ratio		
	5	12.5	20	5	12.5	20
Ethanol	1.55^{dC}±56.89	1.48^{dB}±57.94	1.48^{and} ±59.99	1.39^{not}±57.64	0.57^{cB}±57.05	1.09^{cC}±56.28
Mixture of ethanol: ethyl acetate (1:2)	1.79^{that}±59.68	1.17^{cB}±58.67	1.45^{cC}±56.75	1.88^{dC}±54.99	3.28^{not}±57.92	1.53^{bB}±56.56
Ionic liquid microemulsion in water	2.2^{aA}±91.31	1.69^{BC}±88.09	0.57^{aB}±90.07	2.32^{aA} ±78.68	1.17^{aB}±78.45	2.32^{And}±75.18
oily solvent	1.80^{bB}±60.56	2.39^{bC}±59.44	1.09^{not} ±62.39	0.69^{that}±57.32	1.22^{dB}±56.95	0.69^{dC}±52.78

Data were reported as mean±standard deviation (n=3). Small dissimilar letters in each column indicate a significant difference between the solvents (p<0.05). Capital letters in each row indicate a significant difference (p<0.05) among ratios.

3-3-Analysis of astaxanthin with a spectrophotometer

In the present study, linear regression was used to calculate the standard curve. Coefficients of determination (R^2) showed the desired linearity in the selected range for astaxanthin. The results of extracting astaxanthin using different solvents (ethanol, ethanol:ethyl acetate mixture (1:2), ionic liquid microemulsion solvent in water and oil solvent) in 5-fold, 12.5-fold and 20-fold solvent to sample ratios are shown in Table 2. According to the results obtained, similar to the results obtained from the total carotenoid content of the brown macroalgae sample(*Sargassum polycystum*)In a 5-fold ratio of solvent to sample, using a microemulsion of ionic liquid in water with an amount 83/2±86.00 mg/ml had the highest astaxanthin content.These results were consistent with the results obtained from the total carotenoid content in the present study..Monteiro et al. (2020) in a study that evaluated four solvent systems and three extraction methods to obtain extracts of three macroalgae (*Gracilaria sp.، Fucus vesiculosus*and*Ulva rigida)* and two microalgae (*Chlorella sp*and*Nannochloropsis gaditana)* The extracts were evaluated for yield, phenolic content, and potential antioxidant activity. The results showed that a lower organic solvent to water ratio increased the extraction efficiency of macroalgae biomass. The levels of total phenols, ortho-diphenols, and flavonoids were strongly influenced by the algal material and the solvent system used (20).In a study conducted by Sadati et al. (2011), the antioxidant effect of phenolic compounds of three species of brown macroalgae was investigated.*Colpomenia sinuosa* ،*Cystoseira myrica* and*Sargassum swartzii*collected from the coasts of the Persian Gulf in the

Assaluyeh region were examined and the results showed that the species *Sargassum swartzii*It is a rich source of phenolic compounds with antioxidant properties (7).Waqmood[9]et al. (2019) in a study on the extraction of fucoxanthin and astaxanthin in macroalgae*Sargassum sp*They reported that high-performance liquid chromatographythat the methanol extract of species*Sargassum* It can be used as a good natural source of carotenoids and antioxidants, with anti-cancer properties as a dietary supplement or as an antimicrobial agent in the pharmaceutical industry. Findings showed that the use of antioxidant-rich seaweed extract can help treat diseases associated with oxidative stress (21).

Table 2- Astaxanthin (mg/ml) extracted from Gracilaria and Sargassum in different ratio of solvent to sample

Solvents	*Sargassum polycystum*			*Gracilaria corticata*		
	Solvent to sample ratio			Solvent to sample ratio		
	5	12.5	20	5	12.5	20
Ethanol	2.32^{and}±54.44	1.98^{dB}±53.34	1.28^{dC}±51.93	1.35^{that}±53.07	1.70^{cB}±52.44	1.73^{bC}±52.10
Mixture of ethanol: ethyl acetate (1:2)	2.08^{that}±54.84	1.74^{cB}±53.66	1.73^{cC}±51.70	1.50^{and}±51.63	1.27^{dB}±51.22	1.33^{dC}±50.33
Ionic liquid microemulsion in water	2.83^{aA}±86.00	2.93^{aB}±88.09	1.92^{BC}±84.07	2.42^{aA}±75.60	3.69^{aB}±74.23	2.25^{BC}±72.70
oily solvent	2.00^{bB}±55.94	2.39^{not}±59.44	2.23^{bC}±53.73	1.15^{not}±53.98	1.50^{bB}±52.78	1.17^{cC}±51.71

Data were reported as mean±standard deviation (n=3). Small dissimilar letters in each column indicate a significant difference between the solvents ($p<0.05$). Capital letters in each row indicate a significant difference ($p<0.05$) among ratios.

3-4% astaxanthin recovery

The percentage of astaxanthin recovery was calculated by calculating the percentage of astaxanthin extracted under each extraction condition from the total astaxanthin content of the sample.TAC) was obtained according to formula 3, and the results of the percentage of astaxanthin recovery from red macroalgae(*Gracilaria corticated*)and brown(*Sargassum polycystum*)Using different solvents in ratios of 5, 12.5 and 20 times solvent to sample is shown in Table 3. The results showed that the percentage of astaxanthin recovery from brown macroalgae sample(*Sargassum polycystum*)Using a microemulsion solvent of ionic liquid in water at a ratio of 5 times solvent to sample with 0.57±97.66% is the highest value. These findings were also statistically significant ($p < 0.05$). In general, under the conditions of this experiment, the higher the astaxanthin extraction rate, the higher the astaxanthin recovery percentage. These results were consistent with the results of astaxanthin analysis with a

9- Waghmode

spectrophotometer in the present study.In general, these results were predictable given the results obtained from astaxanthin extraction and total carotenoid content. In a study conducted by Feizi et al. (2024) on the extraction of astaxanthin from banana shrimp and Gammarus crustaceans using different solvents, similar results were obtained as in the present study, as they reported that the highest percentage of astaxanthin recovery from banana shrimp was obtained at a ratio of 12.5 times the solvent to the sample using ionic liquid microemulsion in water (22).

Table 3- Percentage recovery of astaxanthin from Gracilaria and Sargassum in different ratios of solvent to sample

Data were reported as mean±standard deviation (n=3). Small dissimilar letters in each column

Solvents	*Sargassum polycystum*			*Gracilaria corticata*		
	Solvent to sample ratio			Solvent to sample ratio		
	5	12.5	20	5	12.5	20
Ethanol	1.15^{and}±88.33	1.15^{dB}±87.66	1.00^{dC}±81.00	1.15^{and}±85.66	0.57^{dB}±85.33	0.88^{dC}±74.33
Mixture of ethanol: ethyl acetate (1:2)	1.05^{that}±93.00	1.00^{cB}±90.00	1.73^{cC}±85.00	1.00^{cB}±90.00	0.57^{that}±90.33	0.57^{cC}±82.00
Ionic liquid microemulsion in water	0.57^{aA}±97.66	0.57^{aB}±96.66	0.66^{BC}±93.66	0.57^{aA}±95.66	1.15^{aB}±92.66	0.00^{BC}±91.00
oily solvent	0.57^{not}±95.33	0.57^{bB}±92.33	1.00^{bC}±89.33	1.00^{not}±93.00	1.20^{bB}±91.66	1.00^{bC}±86.00

indicate a significant difference between the solvents ($p<0.05$). Capital letters in each row indicate a significant difference ($p<0.05$) among ratios.

3.5-Radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Free radical scavenging is one of the best-known mechanisms by which antioxidant compounds can inhibit lipid oxidation. Results from radical scavengingDPPHThe antioxidant activity of astaxanthin extracted by different concentrations of ethanol solvent, ethanol:ethyl acetate mixture (1:2), oil solvent and ionic liquid microemulsion solvent in water is shown in Figure 1. With increasing concentration, the antioxidant activity of astaxanthin extracted by different solvents increased. Among the solvents used for astaxanthin extraction, astaxanthin extracted by oil solvent had higher antioxidant activity than ionic liquid solvent and ethanol:ethyl acetate mixture (1:2). Although the pigment extracted by oil solvent showed antioxidant activity close toBHTHowever, the antioxidant activity was alwaysBHTIt was higher than this pigment.Antioxidants have a special place among the compounds found in natural sources, because free radicals play a major role in the development of many diseases, and the body uses different antioxidant systems to combat the oxidative damage caused by these radicals. Today, due to industrial life, there are many sources of free radical production, and humans are exposed to these destructive factors every day. The role of these free radicals in many human diseases such as cancer, diabetes, cardiovascular diseases, and neurological and mental diseases has been identified (23). In fact, numerous biochemical reactions in the body produce active oxygen, which has the ability to destroy biomolecules. This

harmful effect of free radicals can be blocked by antioxidants. These compounds trap free radicals and, as a result, cause detoxification. In addition to their role in biological systems, antioxidants also prevent the reduction of nutritional quality, safety, off-flavor, and discoloration due to the formation of toxic compounds in foods rich in unsaturated fats. Antioxidants are divided into two categories: chemical and natural (24). The chemical antioxidants that are most widely used in the food industry includeBHT, BHA, TBHQand propyl gallate, which have been shown to be carcinogenic and have negative effects on human health (25). For this reason, the use of natural antioxidants has become common today.In the present study, among the solvents used to extract astaxanthin, astaxanthin extracted by oil solvent had greater antioxidant activity than ionic liquid solvent and ethanol:ethyl acetate mixture (1:2). Although the pigment extracted by oil solvent showed antioxidant activity close toBHTHowever, the antioxidant activity was alwaysBHTIt was higher than this pigment.In general, the increased antioxidant activity of astaxanthin extracted by antioxidant-free oil solvent compared to other solvents can be attributed to better protection of the pigment in sunflower oil. In addition, due to its lipophilic nature, astaxanthin pigment is better concentrated in oil solvent and is kept away from contact with ambient oxygen and retains its antioxidant properties longer. In ionic liquid solvent, despite having the highest amount of pigment, it has lower antioxidant properties than oil solvent due to partial oxidation or possible subsequent damage during the storage period (22). According to the results of this study, it can be seen that depending on the type of solvent used to extract astaxanthin, antioxidant activity will also vary.

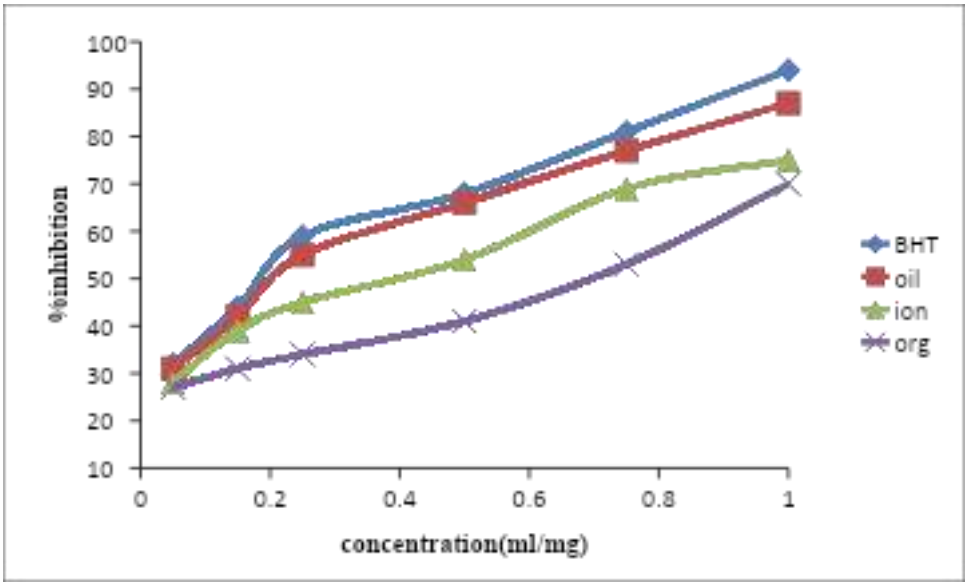

Figure 1- DPPH radical scavenging activity of astaxanthin extracted with organic, ionic and oily solvents in comparison with BHT

4- Conclusion

The results of this study showed that the density of the ionic liquid microemulsion in water was in the range of 0.97151 g/cm3, its diameter was 15.8 nm, and its conductivity was 312 microsiemens at 27.1°C. According to the results obtained from the extraction of astaxanthin fromRed macroalgae(*Gracilaria corticated*)and brown(*Sargassum polycystum*)،MicroalgaeBrown(*Sargassum polycystum*)،was selected as the source with the highest amount of extracted astaxanthin. Green solvent (ionic liquid microemulsion in water) in a ratio of 5 times solvent to sample

was selected as the appropriate solvent for extraction. The amount of astaxanthin extracted under the best conditions83/2±00.86 mg/mlwas, indicating high solubility of the pigment in the ionic liquid microemulsion. Also, the results of radical scavengingDPPHor antioxidant activity of astaxanthin extracted by the solvents used in this test compared to synthetic antioxidantBHTshowed that antioxidant activity increased with increasing astaxanthin concentration, but this increase was always less thanBHTOn the other hand, among the astaxanthin extracted by the mentioned solvents, the astaxanthin extracted by the oil solvent had the highest antioxidant activity, which can be attributed to the better protection of the pigment in sunflower oil.

5-Appreciation and thanks

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Conflict of interest

There are no items to declare.

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Authors' Contributions

All authors participated in the design, analysis, writing, and approval of the final version.

6-Resources

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مقاله علمی_پژوهشی

مقایسه استخراج آستاگزانتین از ماکروجلبک های قرمز (*Gracilaria corticata*) و قهوه ای (*Sargassum polycystum*)

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چکیده

این پژوهش با هدف مقایسه استخراج آستاگزانتین تحت شرایط خیساندن به مدت ۲۴ ساعت با حلال شاهد (اتانول)، حلال آلی (ترکیب اتانول با اتیل استات)، حلال سبز (میکروامولسیون مایع یونی تری بوتیل اکتیل فسفونیوم بروماید درآب) و روغن گیاهی (روغن آفتابگردان) از ماکروجلبک‌های قرمز (*Gracilaria corticata*) و قهوه‌ای (*Sargassum polycystum*) انجام شد. برای استخراج از نسبت های حلال به نمونه ۵ برابر، ۱۲/۵ برابر و ۲۰ برابر استفاده شد. میزان آستاگزانتین استخراجی، کاروتنوئید کل، درصد بازیافت آستاگزانتین و فعالیت مهار رادیکال DPPH مورد بررسی قرارگرفتند. با توجه به نتایج بدست آمده از استخراج آستاگزانتین از ماکروجلبک‌های قرمز (*Gracilaria corticata*) و قهوه‌ای (*Sargassum polycystum*)، ماکروجلبک قهوه‌ای (*Sargassum polycystum*)، به عنوان منبع با بالاترین میزان آستاگزانتین استخراجی با مقدار ۸۶/۲±۰۰/۸۳ میلی‌گرم بر میلی لیتر انتخاب شد. استفاده از حلال سبز(میکرو امولسیون مایع یونی در آب) در نسبت ۵ برابر حلال به نمونه نیز به عنوان بهترین شرایط استخراج انتخاب شد. نتایج حاصل از مهار رادیکال DPPH توسط آستاگزانتین استخراج شده به کمک حلال‌های ذکر شده در مقایسه با آنتی‌اکسیدان سنتتیک BHT نشان داد که با افزایش غلظت آستاگزانتین فعالیت آنتی‌اکسیدانی افزایش یافت. اما این افزایش همواره کمتر از فعالیت آنتی‌اکسیدانی BHT بود. به طور کلی نتایج حاصل ازاین پژوهش نشان داد که استفاده از میکروامولسیون مبتنی برمایع یونی جایگزین مناسبی برای روش‌های مرسوم دراستخراج و بازیابی آستاگزانتین ازمنابع زیستی طبیعی است. علاوه براین ماکروجلبک قهوه‌ای (*Sargassum polycystum*)، نیز منبع غنی برای استخراج رنگدانه طبیعی آستاگزانتین می‌باشد.