Iranian Journal of Fisheries Sciences 20(5) 1354-1370 2021
DOI: 10.22092/ijfs.2021.124956

Research Article

Ultrasound-assisted extraction of phenolic compounds from *Azolla filiculoides* using Taguchi method: Antioxidant and antibacterial capabilities

Naghdi Sh.[1]; Babakhani Lashkan A.[2*]; Rashidian Gh.[3]

Received: July 2020 Accepted: February 2021

Abstract

In the present study, ultrasonication was applied to extract phenolic compounds from *Azolla filiculoides*. This may due to its ability to combine ultrasonication and the commonly solvent extraction to improve the output and degree of selectivity and quality extract. Three factors consisting of sonication time, solvent and ratio of dry powder to solvent were optimized by the Taguchi experimental design. However, the optimized conditions by the highest extraction yield were an extraction time of 10 min, solvent extraction of water, and dry powder to the solvent ratio (1:10). Moreover, the parametric evaluation showed that sonication time had no significant effect on TPC ($p>0.05$), while the dry powder to solvent ratio showed a significant effect on TPC ($p<0.05$). The highest degree of DPPH, ABTS and metal chelating was 14.68±2.05, 74.40±8.50 and 56.74±1.01, respectively. The results of the antibacterial properties of all treatments against two Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella enterica*) showed a significant effect on all treatments ($p<0.05$). These results indicated that the ultrasound method could be used to extract phenolic compounds from *A. Filiculoides*. Also, *A. filiculoides* can be applied as a source of antioxidants and antibacterial compounds.

Keywords: Ultrasound-assisted extraction, Phenolic compounds, *Azolla filiculoides*, biological activities

1-Fisheries Department, Faculty of Marine Science, Tarbiat Modares University, Noor, Iran.
2-Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, Iran.
3-Aquaculture Department, Faculty of Marine Science, Tarbiat Modares University, Noor, Iran.
*Corresponding author: Babakhani@guilan.ac.ir

Introduction

Nowadays, due to human living conditions, many factors such as cigarette smoking, radiation and environmental pollutants, can be effective in the progression of oxidative stress (Medina-Torres *et al.*, 2017). Oxidative stress is one of the risk factors for development of diseases and cancers worldwide, which has attracted much attention from the researchers. The balance between the antioxidant defenses and produced reactive oxygen species (ROS) is defined as oxidative stress. ROS can dramatically increase in bad conditions, and are primary factors in disruptioning biological macromolecules, such as DNA, proteins, and lipids (Espada-Bellido *et al.*, 2017 ; Drevelegka and Goula 2020). Researchers recently reported many solutions to oxidative stress problems, consuming antioxidant compounds in daily life (Jovanović *et al.*, 2017; Alboofetileh *et al.*, 2019). Antioxidants are compounds found in many sources, such as plants, animals, seaweeds, capable of terminating the chain reactions created by ROSs (Ciric *et al.*, 2020). In this case, antioxidant sources of aquatic origin have drawn scientists' attention recently. Marine organisms possess great taxonomic diversity and synthesize biologically active metabolites in various structures with possible applications in food industry, cosmetics, biotechnology, and pharmacy (Balboa *et al.*, 2013). Similarly, macrophytes species require a strong antioxidant system to cope with drastic changes in their immediate environment (Alboofetileh *et al.*, 2019; Yuan *et al.*, 2019).

Azolla filiculoides, known as water fern, can grow very fast in the optimum conditions and reach double size in the period between 2-5 days (Shemami *et al*., 2018). These species are among the well-known economical floating aquatic macrophytes in the world, which can grow in ponds, wetlands and slow-moving streams of temperate and tropical regions (Pouil *et al.*, 2020). There is limited information on antioxidants and antioxidative effects of *A. filiculoides*.

Various methods are used to extraction bioactive compounds from a different source such as vegetables, seaweeds, algae, and fruits, consisting of conventional methods and innovative green methods (Babakhani *et al.*, 2012; Ciric *et al.*, 2020; Drevelegka and Goula 2020). The conventional methods, like solvent extraction, have a huge number of problems, including duration of the time, the toxicity of solvent, environmental pollutants and low output, several methods are developed to solve these defects and ultrasound-assisted is considered as one of the methods (Drevelegka and Goula 2020; Oroian *et al*., 2020). The phenomenon of cavitation created by the ultrasound system is a factor to be superior over the other methods. Cavitation involving the implosion of bubbles formed in the liquid medium generating fast adiabatic compression of gases and vapors inside the bubbles that favors solvent penetration and increases the transport between the

solid and the liquid phases (Chemat *et al.*, 2017; Drevelegka and Goula 2020). In this method, several factors include sonication time, dry powder to solvent ratio, solvent type and concentration, power, temperature, and pH, which can affect the extraction process (Ciric *et al.*, 2020). Therefore, the optimization of these parameters is necessary to reach the maximum output of the obtained compound, which researchers used Response Surface Methodology and Taguchi method to do that. The Taguchi design method is used to optimize the extraction process employed in industrial-scale to good manufacture (Moghbeli *et al.*, 2020). This method is very useful and has applied instead of full factorial design.It decreases the number of experiments required and is more outspoken to use, faster and simultaneously precise, and reliable, saves time and reduces costs (Moghbeli *et al.*, 2020).

In the present study, the extraction of phenolic compounds from *A. filiculoides* was optimized by the Taguchi method, and also antibacterial properties of extracts were evaluated. Therefore, this study aimed to investigate the effects of sonication time, dry powder to solvent ratio and the type of solvents on antioxidant and antibacterial properties of obtained compounds.

Materials and Methods

Materials

Plant materials of *A. filiculoides* were collected from the Anzali wetland, Iran. The obtained water fern was washed and dried at 40°C for 5 days. Then, the samples were crushed by a blender with 0.5 mm sieve and kept in sealed packaging until use. ABTS, DPPH, and ferozin were obtained from Merck. Ethanol was purchased from Taghtir Khorasan Company. Other chemicals used in the experiment were of analytical grade.

Methods

Taguchi design

To design the experiments, we used Taguchi L9 optimization. In this method, the time and cost of the solvent may be reduced. A L9 orthogonal array scheme was adapted, requiring 9 experiments to complete the optimization process (Mandal *et al.*, 2008). The condition of each extraction process under orthogonal design is shown in Table 1.

Extraction process

A high-power ultrasonic probe system with a titanium horn (frequency 15 kHz, max power 400 W) was used for UAE. As shown in Table1 for each extract, we used 5 grams of dry powder in a 100 mL Erlenmeyer flask, and mixed it with the relating solvent mixture. The mixture was ultrasonicated at the designed solvents (ethanol, water, ethanol/water 50/50), time (5, 10 and 15 min), and solid-to-water ratios (1:10, 1:15 and 1:20 g/mL). After the sonication time the mixtures were filtered through Whatman paper No. 4.

Table 1: Ultrasonic extraction conditions for recovery of phenolic compounds from *A. filiculoides* extracts using Taguchi L9 optimization.

Runs	Solvents	Sonication time	Rate of dry powder to solvent (w/v)
1	Ethanol	5	1/10
2	Ethanol/Water	5	1/15
3	Water	5	1/20
4	Ethanol	10	1/15
5	Ethanol/Water	10	1/20
6	Water	10	1/10
7	Ethanol	15	1/20
8	Ethanol/Water	15	1/10
9	Water	15	1/15

The yield of extraction

Yield determination was determined following the method of Nipornram *et al.* (2018). A 100 mL volume of the filtered liquid extract was concentrated by rotary evaporator at 65°C for 10 min and dried by freeze dryer. The dried extracts were stored in an amber glass bottle at −24°C until used. The yields of extractions of each treatment were calculated by comparing the weight of dried sample with the weight of originally dried ground samples.

Yield of extract (%) = $(W_O/W_P) \times 100$

Wo is the weight of freeze dried sample extracts (g)

W_P is the weight of original samples (g)

Determination of antioxidant activities

Total Phenolic Compounds

After mixing the extracts with 2 mL of 10% Folin Ciocalteau reagent, a 1.6 mL of 7.5 % Na2CO3 was added and kept at room temperature for 30 min. The absorbance of the mixture was measured at 765 nm by the Ultraviolet-Visible Spectrophotometer (UV-VIS Shimadzu Model UV100) and reported the total phenolic compound content as Gallic acid equivalent (GAE) (Alonso-Carrillo *et al.*, 2017).

DPPH scavenging activity

This experiment was performed in 1 mg/mL concentration of samples by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method (Kumar *et al.*, 2008). To make the DPPH solution, 1 mg DPPH powder was first mixed with 25 mL of absolute ethanol. Then, about 1 mL of extract was mixed with 1 mL of DPPH solution and kept for 30 min. Finally, the ELISA microplate reader at 515 nm was used to measure the absorbance. DPPH radical scavenging activity was calculated using the following equation:

DPPH scavenging activity %=[Ac–As/Ac]×100

where Ac is the absorbance of the control (100 µL of ethanol mixed with 100 µL of the DPPH solution) and As is the absorbance of sample solution.

ABTS scavenging activity

To measure the ABTS assay of various experiments, we used the method developed by Alonso-Carrillo *et al*. (2017) with slight modifications. 50 mL of 1 mg/mL concentration of extract from each sample was mixed with 150 µL of ABTS solution. Then, the absorbance of sample solutions was

measured at 734 nm using ELISA microplate reader after keeping for 20 min in the dark. To make an ABTS solution, we mixed 7 mM of ABTS solution with 2.45 mM potassium persulfate and kept for 16 h at darkroom temperature. Finally, the absorbance of the ABTS solution was moved to 0.7 at 734 nm with ethanol. The ABTS radical scavenging activity was calculated using the following equation:

ABTS scavenging activity %= [Ac– As/Ac] ×100

Where Ac is the absorbance of the control (50 µL of ethanol missed with 150 µL of the ABTS solution) and As is the absorbance of extract sample.

Reducing power

In this phase, we used the method Morales-Medina et al. (2016) developed to measure the frozin reducing power of different extracts. 100 µL of obtained sample in 1 mg/mL concentration was first mixed with 150 µL of distilled water and 50 µL of 2 mM $FeCl_2$ was added. Then, after 5 min of incubation at room temperature, 200 µL of ferrozine solution (5 mM) was added to the mixtures. Finally, the mixture was vigorously vibrated and kept at room temperature for 10 min. EDTA was used as a positive control.

The absorbance of solutions was measured at 562 nm, and the chelating activity (%) was calculated as follow:

Metal chelating activity (%)= [(ODC+ODB−ODS)/ODC]×100,

where ODC, ODB, and ODS represent the absorbance of the control, the blank (positive control of EDTA) and the sample reaction tubes, respectively. The experiments were done in triplicate.

Antibacterial activity

Antibacterial activity of extracts was evaluated by agar diffusion method, and antibacterial activity against two Gram-positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and two Gram-negative (*Escherichia coli* and *Salmonella enterica*) were measured (Gofar *et al*., 2018). After passaging bacteria in Tryptic soy broth, 200 µL of the culture suspensions was spread on nutrient agar, when their absorbance at 600 nm was 0.08-0.1. Then, 50 µL of extracts in 30 mg/mL concentration was loaded into the cleaned punched wells (6 mm in diameter). Finally, the petri dishes stored in the incubator and incubated for 24 h at 37°C. The inhibition halo around the wells was used to evaluate the antimicrobial activity. Dimethyl sulfoxide (DMSO) was tested as negative control and Gentamycin and Tetracycline (10 µg/disc) was taken as positive control (standard).

Statistical analysis

All experiments were repeated at least three times. MINITAB® (v14.1) software was used to design the experiments based on Taguchi L9 design. All statistical analysis was performed using SPSS version 16.0. The results were presented as mean ± standard deviation.

Results

Optimization of Extraction Conditions by Taguchi L9 Design

In this study, three factors, including sonication time, solvent and dry powder to solvent ratio, each using three levels were applied to the extraction of phenolic compounds from *A. filiculoides*, using Taguchi L9 (3^3) orthogonal design. All treatments including nine experimental groups were evaluated. The results of data analysis showed that the treatment group 6 had the optimum conditions (such as extraction time of 10 min, extraction solvent of water, and dry powder to solvent ratio (1:10)) for TPC. However, in other antioxidant tests containing DPPH, ABTS and metal chelating, the highest optimum performance varied in different treatments. For DPPH, group 4 had the optimum achievement rate (an extraction time of 10 min, extraction solvent of ethanol, and dry powder to solvent ratio (1:15)), and also the optimum performance of ABTS and metal chelating was obtained in treatment 3 (extraction time of 5 min, extraction solvent of water, and dry powder to solvent ratio (1:20)).

The yield of extraction

The results of yields of extraction showed a significant difference in all samples. As shown in Table 2, the run of 6 presented the highest yield of extraction.

Amounts of total phenolic compounds (TPC)

The influence of each parameter on total phenolic compounds (TPC) was assessed and the results are shown in Fig. 1. We used several times (5, 10 and 15 min) to evaluate the effects of sonication time on TPC and the results revealed that sonication time had no significant effect on TPC (Fig.1A). Increasing sonication time from 5 to 10 min led to increased TPC, while a slight decrease in TPC was observed in sonication time from 10 to 15 min. However, the results of studies conducted on the effects of solvent types (ethanol, water and ethanol/water (50/50)) on total phenol compounds indicated that water is more effective than the others. The effect of the dry powder to solvent ratio on TPC was evaluated in the present study and results are shown in Fig. 1. The results showed significant difference between treatments ($p<0.05$).

Table 2: The experiment data of percent of extraction, TPC, DPPH, ABTS and metal chelating using orthogonal design L9 (33).

Runs	Yield of extraction (%)	TPC (mg GAE/g dw)	DPPH scavenging activity (%)	Ferrous ion-chelating activity (%)	ABTS chelating activity (%)
1	26.22±1.32	1.78±0.82	9.92±1.96	49.57±1.24	43.72±8.36
2	25.46±0.96	2.32±1.48	13.44±2.03	55.97±1.07	62.26±5.54
3	29.16±3.73	2.20±1.54	14.53±2.81	56.74±1.01	74.40±8.50
4	33.76±2.00	0.49±0.81	14.68±2.05	55.20±3.31	53.05±15.53
5	34.36±2.20	2.86±0.50	12.15±2.28	54.09±3.46	60.92±10.90
6	36.23±2.22	2.95±0.87	11.07±1.03	52.99±3.59	66.40±15.77
7	27.06±1.16	2.72±1.48	12.51±1.39	54.19±2.66	56.50±17.98
8	28.10±0.43	1.53±1. 31	13.29±2.97	54.19±3.55	64.70±16.18
9	31.93±1.41	1.12±1. 11	12.08±3.47	53.90±4.34	59.18±9.25

All treatments are performed in triplicate and the data represent mean ± SD.

DPPH scavenging activity (%)

The results of this test showed that time had no significant effects on DPPH scavenging activity, but the percent of DPPH scavenging activity was increased by increasing sonication time. Fig. 2 shows the effects of dry powder to solvent ratio (g/mL). As shown in Fig. 2, the highest DPPH scavenging activity was observed in the ratio of 1 g dry powder to 15 mL solvent, although it had no significant effect on other treatments (p>0.05).

ABTS radical scavenging activity

As shown in Fig. 3, as sonication time increased, ABTS radical scavenging activity increased, and the highest degree was attached to 15 min (p<0.05).

Ferrous ion-chelating activity

Ferrous ion-chelating activity between different sonication times, solvents and ratio of dry powder to solvent were 15 min, ethanol, and 1g/10 mL (p>0.05).

Antibacterial activity

Thus, in the present study, antibacterial properties of extracts were evaluated against *L. monocytogenes*, *E. coli*, *S. enterica* and *S. aureu.* The results are presented in Table 3. A wide range of antibacterial activity was observed from 1.7±0.58 mm in treatment 3 for *E. coli* and 5.7±0.57 mm in treatment 4 for *S. aureus.* Antibacterial properties were observed in all treatments, although inhibition zone was in the wide range between 1.7±0.58 mm inhibition zone in treatment 3 for *E. coli* and 5.7±0.57 mm inhibition zone in treatment 4 for *S. aureus.*

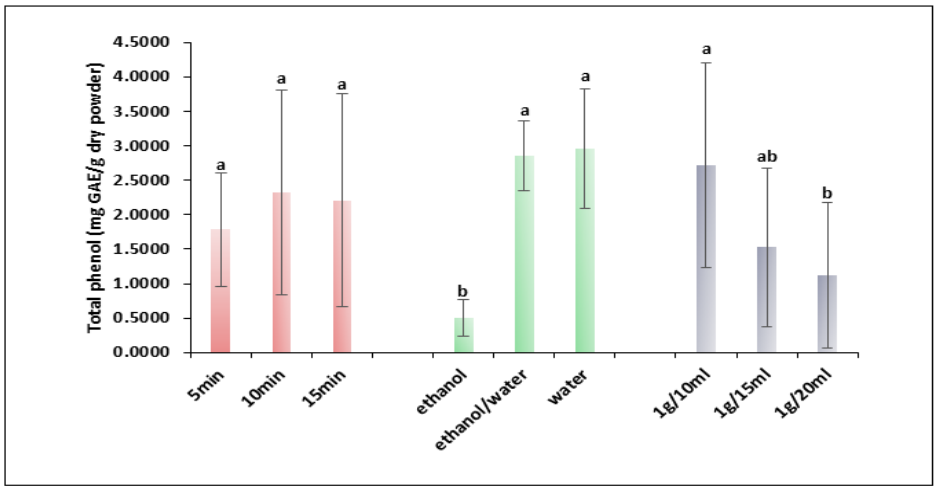

Figure 1: The effect of sonication time, solvent and dry powder to solvent ratio on total phenolic compounds.

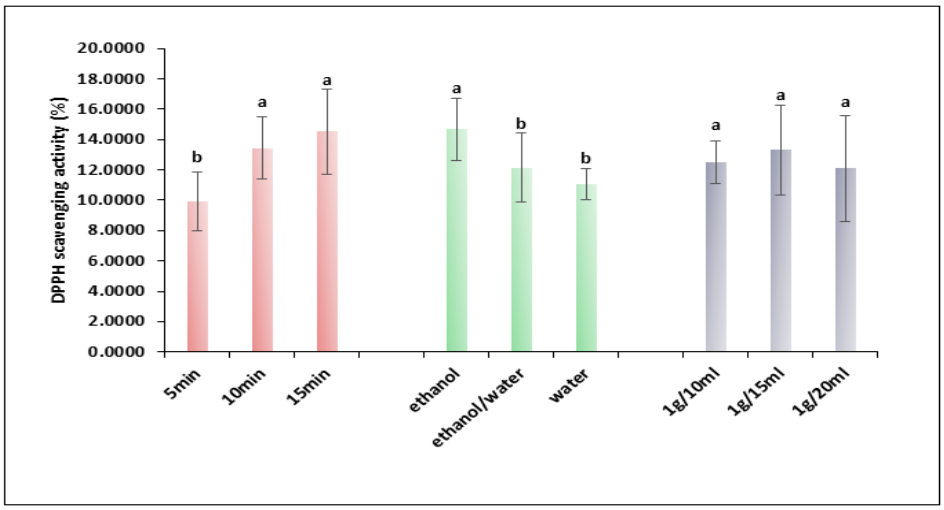

Figure 2: The effect of sonication time, solvent and dry powder to solvent ratio on DPPH scavenging activity (%).

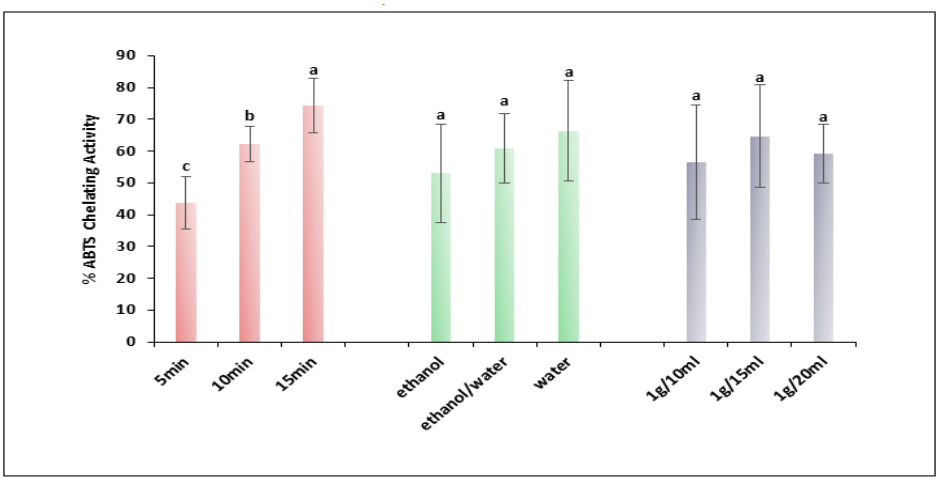

Figure 3: The effect of sonication time, solvent and dry powder to solvent ratio on ABTS radical scavenging activity.

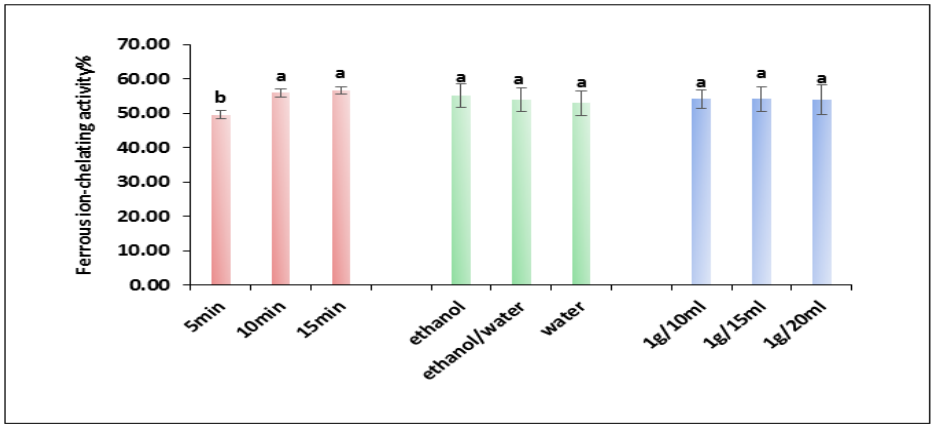

Figure 4: The effect of sonication time, solvent and dry powder to solvent ratio on Ferrous ion-chelating activity.

Table 3: Antibacterial activity of extracts by different treatments.

Runs	*L. monocytogenes*	*E. coli*	*S. enterica*	*S. aureus*
1	4.7± 0.57 ^{ab}	2.7± 0.58 ^{cd}	4.7± 0.58 ^{a}	4.7±0.57 ^{ab}
2	5.3± 0.57 ^{a}	3.7±0.58 ^{bc}	5.7±0.58 ^{a}	5.3±1.15 ^{a}
3	3.7± 0.57 ^{b}	1.7±0.58 ^{d}	2.7±0.58 ^{b}	3.0±1 ^{c}
4	3.7±0.57 ^{b}	4.7±0.58 ^{ab}	5.3±1.16 ^{a}	5.7±0.57 ^{a}
5	4.7± 0.57 ^{ab}	5.0±1.00 ^{ab}	4.7±0.58 ^{a}	5.0±1 ^{ab}
6	4.3± 0.57 ^{ab}	4.7±0.58 ^{ab}	4.3±0.58 ^{a}	4.7±0.57 ^{ab}
7	5.0± 1 ^{a}	5.3±1.16 ^{a}	4.7±0.58 ^{a}	5.3±0.57 ^{a}
8	4.3± 0.57 ^{ab}	5.3±0.58 ^{a}	4.3±0.58 ^{a}	4.7±0.57 ^{ab}
9	5.3± 0.57 ^{a}	4.3±0.58 ^{ab}	4.3±1.16 ^{a}	3.7±0.57 ^{bc}
Tetracycline	not determined	not determined	21.33±2.08	20.67±2.08
Gentamicin	34.33±2.52	21.33±1.53	not determined	not determined
DMSO	15.00±00	15.00±00	15.00±00	15.00±00

All treatments are performed in triplicate and the data represent mean ± SD.
Values represent inhibition zone in mm.

Discussion

Optimization of Extraction Conditions by Taguchi L9 Design

Taguchi optimization was used for the extraction of bioactive compounds from various sources. Taguchi method has proved to be beneficial since it requires fewer experiments than a complete experimental design (Olalere *et al.*, 2018; Moghbeli *et al.*, 2020).

The yield of extraction

Today, it is obvious that the method of extraction including conventional and non-conventional methods and the condition of extraction can affect the yield of extraction of bioactive compounds from various sources (Muñiz-Márquez *et al.*, 2013; Nipornram *et al.*, 2018).

Amounts of total phenolic compounds (TPC)

Phenolic compounds are considered as the most secondary important metabolites in plants found in mono and polysaccharides or can occur as ester or methyl ester derivatives (Drevelegka

and Goula 2020). Among all phenolic acids, flavonoids, and tannins are regarded as the main dietary phenolic compounds (Manzano Durán *et al.*, 2020). Studies have shown that there is a strong positive correlation between the phenolic contents and the antioxidant potential of natural compounds (Ganesan *et al.*, 2011). Nevertheless, the antioxidant activity of phenolic compounds is attributed to the scavenging capacity of free radicals, donating hydrogen and electrons, and chelating cationic metals (Arteaga-Crespo *et al.*, 2020). Molecular structures, particularly the number and positions of the hydroxyl groups, and the nature of substitutions on the aromatic rings, confers to phenolic compounds the capacity of inactivating free radicals, referred as structure-activity relationship (SAR) (Drevelegka and Goula, 2020; Oroian *et al*., 2020). The results obtained from some studies demonstrated that sonication time is a fundamental roll in the extraction of phenolic compounds, which are consistent with our results (Drevelegka and Goula, 2020). Other studies showed multiple behaviors of sonication time in the extraction of bioactive compounds related to ultrasonic waves affecting the mass transfer rate, mainly in the solvent penetration stage (Chemat *et al.*, 2017; Drevelegka and Goula, 2020). Solvent choice is one of the main factors of the extraction of antioxidant compounds from plants, such as flavonoids, anthocyanins, and phenolic compounds, because these compounds are easily dissolved by non-polar organic solvents such as methanol, ethanol, and acetone, with different concentrations (Alves Filho *et al.*, 2020; Oroian et al., 2020). In this study, due to low toxicity, easy availability and financial accessibility of ethanol, we used ethanol, water and ethanol/water (50/50) to extract (Alves Filho *et al.*, 2020). Previous studies have proved that the extraction solvents have a significant effect on TPC, and it is also clear that the effect of solvents on TPC is in following order of water> water/methanol> methanol> ethanol (López *et al.*, 2011). Our results in accordance with those of similar research on the same issue (López *et al.*, 2011). The dry powder to solvent ratio parameter is another key factor in the extraction of bioactive compound from the plant, while different results are shown in study of Medina-Torres *et al.* (2017). The amount of TPC was decreased by increasing solvent to dry powder ratio, which this unusual effect could be due to the fact that a large solvent volume might decrease the energy absorption of material (Drevelegka and Goula, 2020). A high solvent/solid ratio may result in insufficient energy in facilitating the cell wall breakage for effective leaching out of the extracted compounds (Han *et al.*, 2011).

DPPH scavenging activity (%)

One of the general tests for determining antioxidant compounds is a DPPH scavenging activity, which has some useful properties, such as short time to

do, and enough sensitivity at low concentration (Ganesan *et al*., 2011). Wong *et al*. (2014) used binary solvent extraction system from kenaf seeds (*Hibiscus cannabinus L*.) by a pulsed ultrasonic-assisted extraction, and the results showed that with increasing ultrasonication time (extraction time) the percent of DPPH scavenging activity increased in all treatments. Moreover, they found that using binary solvent, such as ethanol-water, could more effective than a monosolvent system (water or pure ethanol) (Wong *et al.*, 2014). However, their study proved that the concentration of ethanol had no irregular effects on DPPH scavenging activity, and also they found that the mixture of solvents could help to extract the rate of antioxidant compounds (Wong *et al.*, 2014). In another study, DPPH scavenging activity increased as sonication time increased from 10 to 25 min, which is believed that increasing sonication time could be effective in cell well and help to extract different compounds from cell plants (Altemimi *et al.*, 2016). When we used ultrasound to extract the bioactive compounds, the acoustic cavitation produced by the ultrasound system can pass the ultrasound waves, which are strong enough to generate voids in the solvent. These phenomenon is cavitation bubbles that are capable to improve and decrease size during pressure fluctuations reaching a critical point where they collapse and release large amounts of energy with an increase in the temperature and pressure of the medium (Wong *et al.*, 2014; Chemat *et al.*, 2017).

ABTS radical scavenging activity

This test is well-known test practiced to evaluate antioxidant properties. In addition, ABTS radical scavenging activity can be applied in both organic and aqueous solvent systems, and requires a short time to do. Nevertheless, because of these aspects of ABTS radical scavenging activity is more effective than DPPH radical scavenging assay in experiments (Cho *et al.*, 2013; Arteaga-Crespo *et al.*, 2020). In line with our results, Alonso-Carrillo *et al.* (2017) showed a significant difference between the used assays with respect to the antioxidant activity behavior which might be due to the nature of the radical used for measuring this property. The clear difference between DPPH and ABTS results could be attributed to the polarity of the solvents. Noteworthy, ABTS radical cation ($ABTS^{\cdot+}$) could be reactive towards most antioxidants, and it is also soluble in both aqueous and organic solvents, and it can be applied over a wide pH and/or ionic strength range. While another assay is based on electron-transfer that have characters including production of a violet solution in ethanol reduced in the presence of an antioxidant molecule, stable at room temperature, as well as giving rise to colorless ethanol solution (Kuda *et al.*, 2005; Kwon *et al.*, 2013; Alonso-Carrillo *et al.*, 2017).

Ferrous ion-chelating activity

The antioxidant properties of the natural plant extracts can determine from their ability to chelate transition metal ions, especially Fe^{2+} and Cu^{2+}. Because of it, this test was used in this study and the obtained results showed that the highest When oxidative stress has occurred, the generated oxyradicals can have a damaging effect on healthy cells. Consequently, the metal chelating activity of antioxidant compounds can play a vital role in inhabiting the generated oxyradical. (Kumar *et al.*, 2008). Kumar *et al.* (2008) reported that the methanolic extracts from *Kappaphycus alvarezii* showed a powerful inhibition of metal. A large number of studies found that the capacity of metal chelating activity in seaweeds can be attributed to a number of hydroxyl group, and also is dependent on location of hydroxyl group in phenolic structure (Ganesan *et al*., 2011;Foujdar *et al.*, 2020) (Fig. 4).

Antibacterial activity

Dramatic use of synthetic antibiotics has inevitably resulted in the development of antibiotic resistance strains (Cox *et al.*, 2010; Mortezaei *et al.*, 2020). Thus, identification of novel and safe alternatives has drawn researchers attention to discovering the medicinal plants and their extracts as promising candidates (Cox *et al.*, 2010). Such plants have a wide range of biological activities such as antibacterial, antifungal, and antioxidant activities. Therefore, the plant extracts despite their nutritional values may be a common replacement for antibiotics (Park *et al.*, 2005; Cox *et al.*, 2010). Many factors contribute to antimicrobial plant extracts, including concentration, extraction method, solvent type, and culture media (Ravi *et al.*, 2018; Oroian *et al*., 2020). A wide range of antibacterial activity against *E. coli and S. aureus* was assumed that the antibacterial properties of seaweeds might be attributed to the type of algal species, type of solvent used and tested bacterial species. In their study, Abd El-Aty *et al*. (2014) examined the antibacterial activity of *Azolla caroliniana* extracts by chloroform, methanol, and ethanol and the results showed the highest antibacterial activity was observed in acetone extracted *Salmonella senftenberg* (24 mm inhibition zone). Other studies have shown that the antibacterial activity of seaweed might be due to their antioxidant activity, which is known to possess efficient antibacterial activity (Zhang *et al.*, 2013). Marzouk *et al.* (2011) reported that polar solvents such as methanol provide better and more efficient extraction than the other solvents. In this regard, antibacterial effects of ethanolic extract of *Stinging nettle* were significant against *Staphylococcus aureus* (Mirtaghi *et al.*, 2016). In another study on *Solano unigram* plant, different solvents of ethyl acetate, ethanol, methanol, diethyl ether, chloroform and hexane were used and antibacterial activity of the extracts was tested against several Grams

positive and Gram negative bacteria of *Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Proteous vulgaris, Pseudomonas putrida, klebsiella pneumonia*, and *E. coli* (Sridhar and Naidu, 2011). The results showed a range of bactericidal against all strains and the highest effects were observed in ethyl acetate extraction from the seed. Ikigai *et al.* (1993) found that gram negative bacteria are more susceptible to polyphenols due to a repulsive force shaped between lipopolysaccharide layer and polyphenols.

Acknowledgments

The authors would like to thank University of Guilan and Tarbiat modares for their support.

References

Abd El-Aty, A.M., Choi, J.H., Rahman, M.M., Kim, S.W., Tosun, A. and Shim, J.H., 2014. Residues and contaminants in tea and tea infusions: a review. *Food Additives and Contaminants: Part A*, 31(**11**), 1794-1804. DOI:10.1080/19440049.2014.958575

Alboofetileh, M., Rezaei, M., Tabarsa, M., Rittà, M., Donalisio, M., Mariatti, F., You, S., Lembo, D. and Cravotto, G., 2019. Effect of different non-conventional extraction methods on the antibacterial and antiviral activity of fucoidans extracted from *Nizamuddinia zanardinii*. *International Journal of Biological Macromolecules,* 124(**4**) 131-137. DOI:10.1016/j.ijbiomac.2018.11.201

Alonso-Carrillo, N., de los Ángeles Aguilar-Santamaría, M., Vernon-Carter, E.J., Jiménez-Alvarado, R., Cruz-Sosa, F. and Román-Guerrero, A., 2017. Extraction of phenolic compounds from Satureja macrostema using microwave-ultrasound assisted and reflux methods and evaluation of their antioxidant activity and cytotoxicity. *Industrial Crops and Products*, 103(**9**), 213-221. DOI: 10.1016/j.indcrop.2017.04.002

Altemimi, A., Watson, D.G., Choudhary, R., Dasari, M.R. and Lightfoot, D.A., 2016. Ultrasound assisted extraction of phenolic compounds from peaches and pumpkins. *PloS one*, 11(**2**), e0148758. DOI: 10.1371/journal.pone.0148758

Alves, F., Elenilson, G., Sousa, V.M., Rodrigues, S., de Brito, E.S. and Fernandes, F.A.N., 2020. Green ultrasound-assisted extraction of chlorogenic acids from sweet potato peels and sonochemical hydrolysis of caffeoylquinic acids derivatives. *Ultrasonics sonochemistry*, 63, 104911. DOI: 10.1016/j.ultsonch.2019.104911

Arteaga-Crespo, Y., Radice, M., Bravo-Sanchez, L.R., García-Quintana, Y. and Scalvenzi, L., 2020. Optimisation of ultrasound-assisted extraction of phenolic antioxidants from *Ilex guayusa Loes*. leaves using response surface methodology. *Heliyon*, 6(**1**), e03043. DOI: 10.1016/j.heliyon.2019.e03043

Babakhani Lashkan, A., Rezaei, M., Rezaei, K. and Seifabadi, S.J.,

2012. Optimization of extraction of antioxidant compounds in microwave-assisted extracts of brown algae *Sargassum angustifolium*. *Journal of Fisheries*, 65(**3**), 243-255. DOI:10.22059/jfisheries.2012.29488

Balboa, Elena M., Conde, E., Moure, A., Falqué, E. and Domínguez, H., 2013. In vitro antioxidant properties of crude extracts and compounds from brown algae. *Food chemistry*, 138(**2-3**), 1764-1785. DOI: 10.1016/j.foodchem.2012.11.026

Chemat, F., Rombaut, N., Sicaire, Anne-Gaëlle, M.A., Fabiano-Tixier, A.S. and Abert-Vian, M., 2017. Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrasonics sonochemistry*, 34, 540-560. DOI: 10.1016/j.ultsonch.2016.06.035

Cho, M.L., Lee, D.J., Lee, H.S., Lee, Y.J. and You, S.G., 2013. LPS-induced NO inhibition and antioxidant activities of ethanol extracts and their solvent partitioned fractions from four brown seaweeds. *Ocean Science Journal*, 48(**4**), 349-359. DOI: 10.1007/s12601-013-0033-y

Ciric, A., Krajnc, B., Heath, D. and Ogrinc, N., 2020. Response surface methodology and artificial neural network approach for the optimization of ultrasound-assisted extraction of polyphenols from garlic. *Food and Chemical Toxicology*, 135, 110976. DOI: 10.1016/j.fct.2019.110976

Cox, S., Abu-Ghannam, N. and Gupta, Sh., 2010. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. *International Food Research Journal*, 17(**1**), 205-220. DOI: 10.21427/D7HC92

Drevelegka, I. and Goula, A.M., 2020. Recovery of grape pomace phenolic compounds through optimized extraction and adsorption processes. *Chemical Engineering and Processing-Process Intensification*, 149, 107845**.** DOI: 10.1016/j.cep.2020.107845

Espada-Bellido, E., Ferreiro-González, M., Carrera, C., Palma, M., Barroso, C.G. and Barbero, G.F., 2017. Optimization of the ultrasound-assisted extraction of anthocyanins and total phenolic compounds in mulberry (*Morus nigra*) pulp. *Food chemistry*, 219, 23-32. DOI: 10.1016/j.foodchem.2016.09.122

Foujdar, R., Bera, M.B. and Chopra, H.K., 2020. Optimization of process variables of probe ultrasonic-assisted extraction of phenolic compounds from the peel of *Punica granatum Var*. Bhagwa and it's chemical and bioactivity characterization. *Journal of Food Processing and Preservation*, 44(**1**), e14317. DOI: 10.1111/jfpp.14317

Ganesan, K., Kumar, K.S. and Rao, P.V. Subba, 2011. Comparative assessment of antioxidant activity in three edible species of green seaweed, Enteromorpha from Okha, Northwest coast of India. *Innovative food science and emerging technologies*, 12(**1**), 73-78. DOI: 10.1016/j.ifset.2010.11.005

Gofar, N., Diana, A. and Setianingsih, E., 2018. Potential of

Anabaena Azollae Extract as Antimicrobial Agent For Paddy Crop Disease. *Science and Technology Indonesia*, 3(**4**), 183-188. DOI: 10.26554/sti.2018.3.4.183-188

Han, D., Zhu, T. and Row, K.H., 2011. Ultrasonic extraction of phenolic compounds from Laminaria japonica Aresch using ionic liquid as extraction solvent. *Bulletin of the Korean Chemical Society*, 32(**7**), 2212-2216. DOI: 10.5012/bkcs.2011.32.7.2212

Ikigai, H., Nakae, T., Hara, Y. and Shimamura, T., 1993. Bactericidal catechins damage the lipid bilayer. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1147(**1**), 132-136. DOI: 10.1016/0005-2736(93)90323-R

Jovanović, A.A., Đorđević, V.B., Zdunić, G.M., Pljevljakušić, D.S., Šavikin, K.P., Gođevac, D.M. and Bugarski, B.M., 2017. Optimization of the extraction process of polyphenols from *Thymus serpyllum* L. herb using maceration, heat-and ultrasound-assisted techniques. *Separation and Purification Technology*, 179, 369-380. DOI: 10.1016/j.seppur.2017.01.055

Kuda, T., Tsunekawa, M., Goto, H. and Araki, Y., 2005. Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *Journal of food composition and analysis*, 18(**7**), 625-633. DOI: 10.1016/j.jfca.2004.06.015

Kumar, K. Suresh, Ganesan, K. and Rao, P.V. Subba, 2008. Antioxidant potential of solvent extracts of Kappaphycus alvarezii (Doty) Doty–An edible seaweed. *Food chemistry*, 107(**1**), 289-295. DOI: 10.1016/j.foodchem.2007.08.016

Kwon, T.H., Kim, T.W., Kim, Ch.G. and Park, N.H., 2013. Antioxidant activity of various solvent fractions from edible brown alga, Eisenia bicyclis and its active compounds. *Journal of food science*, 78(**5**), C679-C684. DOI: 10.1111/1750-3841.12109

López, A., Rico, M., Rivero, A. and de Tangil, M.S., 2011. The effects of solvents on the phenolic contents and antioxidant activity of *Stypocaulon scoparium* algae extracts. *Food Chemistry*, 125(**3**), 1104-1109. DOI: 10.1016/j.foodchem.2010.09.101

Mandal, V., Mohan, Y. and Hemalatha, S., 2008. Microwave assisted extraction of curcumin by sample–solvent dual heating mechanism using Taguchi L9 orthogonal design. *Journal of pharmaceutical and biomedical analysis*, 46(**2**), 322-327. DOI: 10.1016/j.jpba.2007.10.020

Marzouk, B., Marzouk, Z., Mastouri, M., Fenina, N. and Aouni, M., 2011. Comparative evaluation of the antimicrobial activity of *Citrullus colocynthis* immature fruit and seed organic extracts. *African Journal of Biotechnology*, 10(**11**), 2130-2134. DOI: 10.5897/AJB10.1412

Manzano Durán, R., Sánchez, J. E. F., Velardo-Micharet, B., & Gómez, M. J. R. (2020). Multivariate optimization of ultrasound-assisted extraction for the determination of phenolic compounds in plums (*Prunus salicina* Lindl.) by high-performance

liquid chromatography (HPLC). *Instrumentation Science and Technology*, *48*(**2**), 113–127. DOI: 10.1080/10739149.2019.1662438

Medina-Torres, N., Ayora-Talavera, T., Espinosa-Andrews, H., Sánchez-Contreras, A. and Pacheco, N., 2017. Ultrasound assisted extraction for the recovery of phenolic compounds from vegetable sources. *Agronomy*, 7(**3**), 47. DOI: 10.3390/agronomy7030047

Mirtaghi, S.M., Torbati Nejad, P., Mazandarani, M., Livani, F. and Bagheri, H., 2016. Evaluation of antibacterial activity of *Urtica dioica* L. leaf ethanolic extract using agar well diffusion and disc diffusion methods. *Medical Laboratory Journal*, 10(**5**), 15-21. DOI: 0.18869/acadpub.mlj.10.5.15

Moghbeli, S., Jafari, S.M., Maghsoudlou, Y. and Dehnad, D., 2020. A Taguchi approach optimization of date powder production by spray drying with the aid of whey protein-pectin complexes. *Powder Technology*, 359, 85-93. DOI: 10.1016/j.powtec.2019.10.013

Morales-Medina, R., Tamm, F., Guadix, A.M., Guadix, E.M. and Drusch, S., 2016. Functional and antioxidant properties of hydrolysates of sardine (*S. pilchardus)* and horse mackerel (*T. mediterraneus*) for the microencapsulation of fish oil by spray-drying. *Food chemistry*, 194, 1208-1216. DOI: 10.1016/j.foodchem.2015.08.122

Mortezaei, F., Royan, M., Allaf Noveirian, H., Babakhani, A., Alaie Kordghashlaghi, H. and Balcázar, J.L., 2020. In vitro assessment of potential probiotic characteristics of indigenous *Lactococcus lactis* and *Weissella oryzae* isolates from rainbow trout (*Oncorhynchus mykiss* Walbaum). Journal of applied microbiology, 129(**4**), 1004-1019. DOI: 10.1111/jam.14652

Muñiz-Márquez, D.B., Martínez-Ávila, G.C., Wong-Paz, J.E., Belmares-Cerda, R., Rodríguez-Herrera, R. and Aguilar, C.N., 2013. Ultrasound-assisted extraction of phenolic compounds from Laurus nobilis L. and their antioxidant activity. *Ultrasonics sonochemistry*, 20(**5**), 1149-1154. DOI: 10.1016/j.ultsonch.2013.02.008

Nipornram, S., Tochampa, W., Rattanatraiwong, P. and Singanusong, R., 2018. Optimization of low power ultrasound-assisted extraction of phenolic compounds from mandarin (*Citrus reticulata* Blanco cv. Sainampueng) peel. *Food chemistry*, 241, 338-345. DOI: 10.1016/j.foodchem.2017.08.114

Olalere, O.A., Abdurahman, H.N., Yunus, R. bin Mohd, Alara, O.R., Ahmad, M.M., Zaki, Y.H. and Abdlrhman, H.S. Mohamed, 2018. Parameter study, antioxidant activities, morphological and functional characteristics in microwave extraction of medicinal oleoresins from black and white pepper. *Journal of Taibah University for Science*, 12(**6**), 730-737. DOI: 10.1080/16583655.2018.1515323

Oroian, M., Dranca, F., & Ursachi, F. (2020). Comparative evaluation of maceration, microwave and

ultrasonic-assisted extraction of phenolic compounds from propolis. *Journal of Food Science and Technology*, *57*(**1**), 70–78. DOI: 10.1007/s13197-019-04031-x

Park, J. H., Gyung, J. C., Kyoung, S. J., He, K. L., Heung, T. K., Kwang, Y. C., & Kim, J. C. (2005). Antifungal activity against plant pathogenic fungi of chaetoviridins isolated from *Chaetomium globosum*. *FEMS Microbiology Letters*, *252*(**2**), 309–313. DOI: 10.1016/j.femsle.2005.09.013

Pouil, S., Samsudin, R., Slembrouck, J., Sihabuddin, A., Sundari, G., Khazaidan, K., Kristanto, A. H., Pantjara, B., & Caruso, D. (2020). Effects of shading, fertilization and snail grazing on the productivity of the water fern *Azolla filiculoides* for tropical freshwater aquaculture. *Aquatic Botany*, *160* (September 2019), 103150. DOI: 10.1016/j.aquabot.2019.103150

Ravi, R., Husna Zulkrnin, N. S., Rozhan, N. N., Nik Yusoff, N. R., Mat Rasat, M. S., Ahmad, M. I., Hamzah, Z., Ishak, I. H., & Mohd Amin, M. F. (2018). Evaluation of Two Different Solvents for *Azolla pinnata* Extracts on Chemical Compositions and Larvicidal Activity against *Aedes albopictus* (Diptera: Culicidae). *Journal of Chemistry*, *2018*(July 2017). DOI: 10.1155/2018/7453816

Shemami, M. R., Tabarsa, M., & You, S. G. (2018). Isolation and chemical characterization of a novel immunostimulating galactofucan from freshwater *Azolla filiculoides*. *International Journal of Biological Macromolecules*, *118*, 2082–2091. DOI:10.1016/j.ijbiomac.2018.07.06

Sridhar, T. M., & Naidu, C. V. (2011). In Vitro Antibacterial Activity and Phytochemical Analysis of *Solanum nigrum* (Linn.) - An Important Antiulcer Medicinal Plant. *Journal of Phytology*, *3*(2), 78–82.

Wong, Y. H., Lau, H. W., Tan, C. P., Long, K., & Nyam, K. L. (2014). Binary solvent extraction system and extraction time effects on phenolic antioxidants from kenaf seeds (*Hibiscus cannabinus* L.) extracted by a pulsed ultrasonic-assisted extraction. *The Scientific World Journal*, *2014*. DOI: 10.1155/2014/789346

Xu, D. P., Zheng, J., Zhou, Y., Li, Y., Li, S., & Li, H. Bin. (2017). Ultrasound-assisted extraction of natural antioxidants from the flower of *Limonium sinuatum*: Optimization and comparison with conventional methods. *Food Chemistry*, *217*, 552–559. DOI: 10.1016/j.foodchem.2016.09.013

Yuan, C., Zhu, T., Cao, T., Xi, Y., Zhang, X., & Ni, L. (2019). Antioxidant systems of aquatic macrophytes in three life forms: a case study in Lake Erhai, China. *Journal of Oceanology and Limnology*, *37*(**2**), 665–674. DOI: 10.1007/s00343-019-8025-0

Zhang, G., Hu, M., He, L., Fu, P., Wang, L., & Zhou, J. (2013). Optimization of microwave-assisted enzymatic extraction of polyphenols from waste peanut shells and evaluation of its antioxidant and antibacterial activities in vitro. *Food and Bioproducts Processing*, *91*(**2**), 158–168. DOI: 10.1016/j.fbp.2012.09.003