AIMS Agriculture and Food, 6(1): 32–48.
DOI: 10.3934/agrfood.2021003
Received: 03 October 2020
Accepted: 12 November 2020
Published: 30 November 2020

http://www.aimspress.com/journal/agriculture

Research article

Maximising phenolic compounds and antioxidant capacity from *Laurencia intermedia* using ultrasound-assisted extraction

Patrick A. Blamo Jr[1,2], Hong Ngoc Thuy Pham[1,*] and The Han Nguyen[1,*]

[1] Faculty of Food Technology, Nha Trang University, No. 02 Nguyen Dinh Chieu Street, Nha Trang City, Khanh Hoa Province, Viet Nam
[2] Department of Biological Sciences, University of Liberia Capitol Hill, P.O. Box 9020, Monrovia Liberia

*** Correspondence:** Email: ngocthuy@ntu.edu.vn or hongngocthuy.pham@uon.edu.au, hannt@ntu.edu.vn; Tel: +84905029643, +84975964605.

Abstract: *Laurencia intermedia*, which belongs to red algae (Rhodophyta), has been found in tropical shore areas. Recently, it has been reported to be a rich source of bioactive compounds; however, there have been limited studies on extraction techniques for recovering bioactive compounds from *L. intermedia*. Hence, this study was conducted to optimise the ultrasound extraction conditions for maximising recovery yield of total phenolic content (TPC) and antioxidants from *L. intermedia* using response surface methodology. The results showed that the ratio of sample to solvent had the strongest effect on TPC, while extraction temperature, extraction time, ethanol concentration and ratio of sample to solvent had significant influence on antioxidant power. The yield of TPC, DPPH scavenging ability and ferric reducing antioxidant power were 161.79 ± 3.52 mg GAE/100 g, 32.30 ± 1.20 mg TE/100 g and 87.77 ± 3.17 mg TE/100 g, respectively at the optimum extraction conditions (50 ℃, 60 min, 30% ethanol and sample to solvent ratio of 2 g/100 mL). These conditions were employed to prepare *L. intermedia* extract for subsequent fractionation step, which generated *n*-hexane, ethyl acetate and aqueous fractions. Among these fractions, ethyl acetate fraction was found to possess the highest yield of TPC and the greatest antioxidant capacity that could be used for further isolation and purification of individual compounds.

Keywords: *Laurencia intermedia*; phenolic compounds; ultrasound-assisted extraction; antioxidants; red seaweed

Abbreviations: ANOVA: Analyzes of Variance; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; TPTZ: 2,4,6-Tris(2-pyridyl)-*s*-triazine; DRSC: DPPH Radical Scavenging Capacity; FRAP: Ferric Reducing Antioxidant Power; RSM: Response Surface Methodology; TPC: Total Phenolic Content; UAE: Ultrasound-assisted extraction; UV: Ultraviolet Radiation; HCl: Hydrochloric Acid

1. Introduction

Seaweeds (Marine macroalgae) are a group of marine multicellular algae, rich in minerals, vitamins and polysaccharides. They are known as potential sources of bioactive substances with strong biological activities such as antibacterial, anticancer, antioxidant, anti-fungal and antiviral properties [1]. They are divided into three main groups based on their colors, including Rhodophyceae (red seaweed), Chlorophyceae (green seaweed) and Phaeophyceae (brown seaweed) [2]. They are abundant on the coastline of Vietnam and are used for food, medicinal and cosmeceutical purposes [3]. Additionally, recent studies on seaweeds have proven their usefulness as biofuels, fertilisers, fish feed, food ingredients, cosmeceuticals and their application in bioremediation and anti-biofilm activity [4–11]. Their pharmacological and biomedical application including antibiotics, antiviral, antifungal, anticancer, anticoagulant, anti-inflammatory and antioxidant properties have also been reported [12–17].

Red seaweeds considered as the largest group of marine macroalgae have been found to contain a variety of bioactive compounds including polysaccharides (alginate, agar, and carrageenan), lipids, phenolics, steroids, glycosides, saponins, alkaloids and triterpenoids [18]. Recent studies have reported that isolated compounds from red seaweeds possessed a range of biological activities such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory and anticancer properties [12,19–22]. These findings offer red seaweeds as a promising source of bioactive compounds which can be used in the cosmetic, pharmaceutical and food industries.

Ultrasound-assisted extraction (UAE) is one of recent novel extraction techniques. The method applies the cavitation phenomenon which occurs when an extraction solvent in contact with a sample, that is subjected to ultrasounds at high frequency pulses to generate local hotspots at macroscopic scale with high shear stress and temperature by producing cavitation bubbles [23,24]. The collision of cavitation bubbles created in the bath, results in pressure and temperature changes thereby facilitating the rate of mass transfer of analytes to the solvent. Although the method can successfully be applied at low temperature, short time and less solvent, high extract yield can be obtained.

Response surface methodology (RSM) is an effective tool for optimisation of the extraction process. It is a compilation of mathematical and statistical methods, helpful for developing the models as well as analysing the effects of parameters and their interactions [25,26]. RSM has been successfully used to optimise the extraction conditions of antioxidants and phenolic compounds from different seaweed species [27–29].

This study aimed to investigate total phenolic contents and antioxidant activities of different red seaweeds (*Galaxaura arborea*, *Mastophora rocea* and *Laurencia intermedia* Yamada). *L. intermedia* showed the most potential one and was selected to investigate the effects of ultrasound-assisted extraction conditions including temperature, time, solvent concentration and ratio of sample to solvent on the phenolics and antioxidant power. In addition, the optimum UAE conditions would be established for maximising the yields of TPC and antioxidants produced from *L. intermedia*. The crude extract and derived fractions (*n*-hexane, ethyl acetate and aqueous fractions) from *L. intermedia* were then prepared for examination of phenolic content and antioxidant activities.

2. Materials and methods

2.1. Sample collection and preparation

The red seaweed samples (*Galaxaura arborea*, *Mastophora rocea* and *Laurencia intermedia* Yamada) were harvested during March to July 2017 at the coast of Nha Trang Bay, Khanh Hoa Province, Vietnam. The samples were authenticated by Mr. Do Anh Duy (Research Institute for Marine Fisheries, Hai Phong City, Vietnam). The voucher specimens (NT-02) of *L. intermedia* Yamada was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy in Ho Chi Minh City, Vietnam. Samples were individually rinsed to remove particulates and epiphytes, and then air-dried in the shade. Dried seaweed samples were milled into a fine powder to fifty (50) micron. Dried powder was vacuum packaged and stored at −20 ℃ for phytochemical and biological analysis.

2.2. Chemicals

Folin-Ciocalteu's phenol reagent, 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) were obtained from Sigma- Aldrich Company (USA); 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Alfa Aesar (UK). The rest of the chemicals and reagents were of analytical grade.

2.3. Screening test to select the starting sample

A conventional extraction method was used in screening TPC and antioxidant activity of three seaweed species (*G. arborea*, *M. rocea* and *L. intermedia* Yamada). Briefly, 1 g of the dried seaweed sample was mixed with 50 mL of methanol 75%. The mixture were placed in the Orbital Shaking Water Bath (VS—1205 SW2) at 60 ℃ for 2 hours. Post-conventional extraction, the mixture was cool in a cold-water bath and centrifuged at 8500 rpm for 20 min at 4 ℃ (MEGA 17R Small High Speed refrigerated centrifuge) to obtain the extract. The total phenolic content and the antioxidant capacity of the extract were determined and the species with the highest phenolic content and antioxidant activity was selected for subsequent experiments.

2.4. Experimental design

Based on single factor experiments (data not shown), ethanol was found to be the suitable solvent for extraction of TPC and antioxidants from *L. intermedia*, and four key variables with optimal ranges were selected for RSM optimisation, including ethanol concentration (0–75%), temperature (40–60 ℃), time (20–60 min) and sample to solvent ratio (1–6 g/100 mL). The Box-Behnken design was employed to investigate the interactions of these variables on the extraction efficiency of TPC and antioxidant capacity. The design consisted of 27 experimental runs with three center points as shown in Table 3. The data were fitted into a second-order polynomial model as follows (Equation 1):

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2 \quad (1)$$

where k is the number of variables; X_i represents independent variables affecting the responses Y (TPC, DPPH and FRAP); and β_o, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively.

2.5. Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction was performed in an ultrasound bath (Branson 2510, 42 Hz, 100 W, Branson Ultrasonic Corp., Danbury, USA). Seaweed samples were mixed with ethanol with appropriate concentrations, placed into an extraction flask and sonicated for different times and temperatures. After the extraction, the flasks were immediately removed from the ultrasound bath and cooled to room temperature by cooling water. The seaweed extracts were centrifuged using a MEGA 17R small high-speed refrigerated centrifuge at 8500 rpm, 4 ℃ for 20 min. Extracts were collected and used for the determination of the TPC and antioxidant capacity.

2.6. Fractionation

The crude extract was obtained using the optimum extraction conditions for *L. intermedia* determined as above. The extract was condensed using a vacuum rotary evaporator (Laborota 4001, Heidolph, Germany). The crude extract was then fractionated using *n*-hexane and ethyl acetate to generate *n*-hexane, ethyl acetate and aqueous fractions, and their phenolic content and antioxidant activity were further evaluated.

2.7. Total phenolic content (TPC) determination

The TPC of the extracts was determined as described by Pham et al. [30]. To 0.5 mL of extract, 2.5 mL of 10% (v/v) Folin-Ciocalteu reagent was added and left at room temperature for 8 min, then thoroughly mixed with 2 mL of 7.5% (w/v) Na_2CO_3. The resulting mixture was incubated in the dark at room temperature for 1 hour. The absorbance was then measured at 765 nm using a UV–VIS spectrophotometer (Biochrom Libra S50, Biochemical Ltd., Cambridge, UK). Gallic acid was used as a standard with the results expressed as mg of gallic acid equivalents per 100 g of dried material (mg GAE/100 g).

2.8. DPPH radical scavenging capacity (DRSC) assay

DRSC was assessed as described by Pham et al. [30]. DPPH stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. The stock solution was kept in the dark at −20 ℃ for further use. To 10 mL of the stock solution, 45 mL methanol was added to prepare the working solution at absorbance value 1.1 ± 0.02 at 515 nm. To 2.85 mL of the working solution, 0.15 mL of the sample was added and left in the dark at room temperature for 3 hours. The absorbance was measured at 515 nm using a UV–VIS spectrophotometer (Biochrom Libra S50, Biochemical Ltd., Cambridge, UK). Using trolox as a standard, and the results were expressed as mg trolox equivalents per 100 g of dried weight (mg TE/100 g).

2.9. Ferric reducing antioxidant power (FRAP) assay

FRAP was measured according to the method of Pham et al. [31] with some modifications. FRAP working solution was prepared by mixing 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in the ratio of 10: 1: 1. To 0.15 mL of sample, 2.85 mL of the working FRAP solution was added and the absorbance was read at 593 nm after incubation in the dark for 30 min at room temperature. Results were expressed as mg trolox equivalents per 100 g of dried material (mg TE/100 g).

2.10. Statistical analysis

All experiments were conducted in triplicate. JMP 15 was used to design optimisation experiments, to generate the model equations, to graph the 3D and 2D contour plots of the responses and to predict the optimum values for the independent variables. Minitab 16 and excel statistical tools were used for data analysis.

3. Results

3.1. Screening antioxidant capacity and phenolic content of different red seaweeds

The data in Table 1 compare the antioxidant capacity and total phenolic content of three screened red seaweed's species (*G. arborea*, *M. rocea* and *L. intermedia* Yamada*).* According to the results obtained, *L. intermedia* Yamada demonstrated the highest total phenolic content and antioxidant activity. It also shows no significant difference of the DRSC and TPC of *G. arborea* and *M. rocea*. *L. intermedia* Yamada was therefore selected for subsequent investigation.

Table 1. TPC and antioxidant activity of three screened red seaweed's species.

Samples	TPC (mg GAE/100 g)	DRSC (mg TE/100 g)	FRAP (mg TE/100 g)
L. intermedia Yamada	52.76 ± 3.09^{a}	31.94 ± 1.55^{a}	49.73 ± 2.21^{a}
G. arborea	25.30 ± 3.36^{b}	6.073 ± 1.42^{b}	38.65 ± 3.78^{ab}
M. rocea	27.17 ± 2.21^{b}	6.22 ± 4.76^{b}	31.52 ± 0.35^{b}

The results are displayed as means ± standard deviations (n = 3). Means in the same column with different superscript letters are significantly different ($p < 0.05$). TPC—Total phenolic content. DRSC—DPPH radical scavenging capacity. FRAP—Ferric reducing antioxidant power. TE—Trolox equivalents. GAE—Gallic acid equivalents.

3.2. Fitting the models

Table 2 shows the Box-Behnken design (BBD) with the experimental and estimated data. The actual values strongly correlated with the estimated values, that was revealed through high R^2 values (0.95, 0.97 and 0.99 for TPC, DRSC and FRAP, respectively) (Table 3). Additionally, the p-values for lack of fit of TPC, DRSC and FRAP were 0.0909, 0.0768 and 0.1951, respectively, greater than 0.05, revealing the lack of fit was not significant. In Table 3, the estimated regression coefficients for the quadratic polynomial model and analyses of variance for the experimental results of TPC, DRSC and FRAP, and p-values of models are also displayed. The models developed to predict the TPC and

antioxidant activities were found to be highly reliable for predicting the responses. These models can be fitted into the following second-order polynomial equations (2–4):

$$Y_{TPC} = 126.40 + 2.79X_1 + 4.0X_2 - 3.15X_3 - 36.22X_4 + 1.76X_1X_2 + 0.68X_1X_3 - 1.66X_1X_4 - 0.76X_2X_3 - 0.55X_2X_4 + 8.98X_3X_4 + 0.45X_1^2 + 1.72X_2^2 - 6.98\ X_3^2 + 18.29X_4^2 \quad (2)$$

$$Y_{DRSC} = 31.53 + 3.57X_1 + 0.23X_2 + 7.83X_3 + 5.18X_4 - 0.60X_1X_2 + 0.93X_1X_3 + 0.23X_1X_4 - 0.025X_2X_3 + 0.1X_2X_4 - 4.45X_3X_4 - 3.54X_1^2 + 6.933X_2^2 - 3.05X_3^2 + 1.15X_4^2 \quad (3)$$

$$Y_{FRAP} = 83.68 + 1.59X_1 + 4.13X_2 - 13.50X_3 + 0.24X_4 + 0.23X_1X_2 - 2.50X_1X_3 + 0.13X_1X_4 - 1.41X_2X_3 + 0.00X_2X_4 + 0.84X_3X_4 - 6.36X_1^2 - 0.20X_2^2 - 8.65X_3^2 - 0.30X_4^2 \quad (4)$$

3.3. Influence of extraction parameters on TPC and antioxidant capacity

The influence of extraction temperature, extraction time, ethanol concentration and sample to solvent ratio on TPC and antioxidant capacity was shown in Table 3 and Figures 1–3. The results showed that in the tested narrow ranges of temperature (40–60 ℃), time (20–60 min), ethanol concentration (0–75%), TPC was not significantly affected when these parameters were changed ($p > 0.05$) with the exception of sample to solvent ratio. It was seen that temperature, ethanol concentration and sample to solvent ratio had significant effect on DPPH scavenging ability, while temperature, time and solvent concentration significantly affected FRAP ($p < 0.05$, Table 3). The data in Table 3 show minor interaction effect of the variables. The only interaction recorded are temperature and ethanol concentration for FRAP ($p < 0.05$, Table 3), and ethanol concentration and sample to solvent ratio for DRSC ($p < 0.05$, Table 3).

3.4. Optimisation and verification of the models

The optimal UAE conditions for maximum recovery of both phenolic compounds and antioxidant properties from *L. intermedia* were determined at fixed ultrasound power (100 W) and frequency (42 kHz) to be temperature of 50 ℃, time of 60 min, ethanol concentration of 30%, and sample to solvent ratio of 2 g/100 mL. Verification experiments were conducted under these optimal UAE conditions to verify and confirm the adequacy of the models, and the results were presented in Table 4. Under the optimum conditions, the experimental values of TPC, DRSC and FRAP were 161.79 mg GAE/100 g, 32.30 mg TE/100 g and 87.77 mg TE/100 g, respectively, while the predicted values of respective assays were 162.34 mg GAE/100 g, 33.78 mg TE/100 g and 90.09 mg TE/100 g (Table 4). The results of mean comparison show no significant difference between experimental and estimated values of all assays ($p > 0.05$), revealing the reliability and adequacy of the developed models.

Table 4. Model verification at optimum conditions.

	TPC (mg GAE/100g)	DRSC (mg TE/100g)	FRAP (mg TE/100g)
Experimental values	161.79 ± 3.52^a	32.30 ± 1.20^a	87.77 ± 3.17^a
Estimated values	162.34 ± 11.67^a	33.78 ± 2.72^a	90.09 ± 2.37^a

Means in the same column with same superscript letter show no significant difference ($p > 0.05$). TPC: Total phenolic content. DRSC: DPPH radical scavenging capacity. FRAP: Ferric reducing antioxidant power. GAE: Gallic acid equivalents. TE: Trolox equivalents.

Table 2. Box-Behnken design (BBD) with experimental versus estimated data for responses (n = 3).

Run	Extraction conditions				TPC (mg GAE/100 g)		DRSC (mg TE/100 g)		FRAP (mg TE/100 g)	
	X_1	X_2	X_3	X_4	Exp.	Est.	Exp.	Est.	Exp.	Est.
1	40	20	37.5	3.5	118.36	123.53	29.21	30.53	69.92	71.66
2	50	40	37.5	3.5	127.98	126.40	32.23	31.53	84.75	83.68
3	60	60	37.5	3.5	134.10	137.11	38.70	38.13	84.11	83.05
4	50	60	37.5	1.0	187.13	187.17	35.84	34.57	88.61	87.06
5	50	20	75	3.5	118.01	114.75	43.62	43.25	58.49	58.62
6	50	20	37.5	1.0	178.29	178.09	36.31	34.30	79.21	78.82
7	40	60	37.5	3.5	121.39	128.02	31.62	32.19	78.60	79.45
8	50	40	37.5	3.5	128.32	126.40	30.91	31.53	83.09	83.68
9	50	40	0	6.0	103.95	95.67	33.83	31.43	88.07	87.63
10	50	60	75	3.5	126.19	121.23	43.60	43.22	61.88	64.05
11	50	60	37.5	6.0	113.58	113.64	43.54	45.12	88.59	87.55
12	50	40	75	1.0	145.35	161.81	33.62	36.73	59.03	60.15
13	50	40	75	6.0	100.81	107.32	40.51	38.18	61.74	62.31
14	60	40	37.5	1.0	196.24	185.81	29.10	27.30	78.52	78.22
15	50	40	37.5	3.5	122.89	126.40	31.53	31.53	83.21	83.68
16	40	40	0	3.5	116.93	120.90	15.74	14.47	79.16	78.10
17	50	20	37.5	6.0	106.92	106.74	43.61	44.45	79.18	79.29
18	50	60	0	3.5	133.82	129.04	28.11	28.07	93.23	93.86
19	60	40	37.5	6.0	110.56	110.06	37.34	38.10	79.21	78.96
20	60	40	0	3.5	114.64	125.13	19.22	19.75	84.07	86.24
21	40	40	37.5	1.0	184.45	176.92	21.83	20.62	74.33	75.34
22	40	40	37.5	6.0	105.39	107.79	29.12	30.52	74.50	75.56
23	50	40	0	1.0	184.39	186.06	9.10	12.18	88.72	88.83
24	50	20	0	3.5	122.61	119.53	27.13	27.10	84.20	82.79

Continued on next page

Run	Extraction conditions				TPC (mg GAE/100 g)		DRSC (mg TE/100 g)		FRAP (mg TE/100 g)	
	X_1	X_2	X_3	X_4	Exp.	Est.	Exp.	Est.	Exp.	Est.
25	60	20	37.5	3.5	124.05	125.60	38.73	38.86	74.52	74.34
26	60	40	75	3.5	124.30	120.19	36.44	37.25	54.62	54.24
27	40	40	75	3.5	123.89	113.26	29.21	28.27	59.70	56.10

X_1–Temperature (℃), X_2–Time (min), X_3–Ethanol concentration (%), X_4–sample to solvent ratio (g/100 mL). TPC, DRSC and FRAP represent total phenolic content, DPPH radical scavenging capacity and ferric reducing antioxidant power, respectively. GAE and TE mean gallic equivalents and trolox equivalents, respectively. Exp. and Est. stand for experimental and estimated data, respectively.

Table 3. Estimated regression coefficients for the quadratic polynomial model and analyses of variance for the experimental results for TPC, DRSC and FRAP.

		TPC			DRSC			FRAP		
Effects	DF	Estimate	*F*-Value	*P*-Value	Estimate	*F*-value	*P*-Value	Estimate	*F*-Value	*P*-Value
Model	14	126.40		<0.0001[a]	31.53		<0.0001[a]	83.68		<0.0001[a]
Linear effect										
X_1	1	2.79	1.14	0.31	3.57	34.32	<0.0001[a]	1.57	8.80	0.0118[a]
X_2	1	4.00	2.35	0.15	0.23	0.15	0.7083	4.13	60.72	<0.0001[a]
X_3	1	−3.15	1.46	0.25	7.83	165.17	<0.0001[a]	−13.50	650.28	<0.0001[a]
X_4	1	−36.22	192.67	<.0001[a]	5.18	72.24	<0.0001[a]	0.24	0.20	0.6595
Interaction effect										
X_1X_2	1	1.76	0.15	0.70	−0.60	0.32	0.5799	0.23	0.06	0.8082
X_1X_3	1	0.68	0.02	0.88	0.93	0.77	0.3976	-2.50	7.42	0.0185[a]
X_1X_4	1	−1.66	0.13	0.72	0.23	0.05	0.8346	0.13	0.02	0.8896
X_2X_3	1	−0.76	0.03	0.87	−0.25	0.06	0.8166	−1.41	2.36	0.15
X_2X_4	1	−0.55	0.01	0.91	0.10	0.01	0.926	0.00	0.00	0.9979
X_3X_4	1	8.98	3.94	0.07	−4.45	17.81	0.0012[a]	0.84	0.84	0.3776
Quadratic effect										

Continued on next page

		TPC			DRSC			FRAP		
Effects	DF	Estimate	*F*-Value	*P*-Value	Estimate	*F*-value	*P*-Value	Estimate	*F*-Value	*P*-Value
$(X_1)^2$	1	0.45	0.38	0.91	−3.54	30.98	0.0022[a]	−6.36	40.65	<0.0001[a]
$(X_2)^2$	1	1.72	0.10	0.67	6.93	79.00	<0.0001[a]	−0.20	7.92	0.0156[a]
$(X_3)^2$	1	−6.98	12.55	0.10	−3.05	15.92	0.0058[a]	−8.65	130.50	<0.0001[a]
$(X_4)^2$	1	18.29	21.85	<.0001[a]	1.15	1.57	0.2335	−0.30	0.15	0.7091
Lack of fit	10		10.40	0.0909		12.41	0.0768		4.51	0.1951
R^2			0.95			0.97			0.99	
RMSE		9.0393			2.1092			1.833		

[a] Stands for statistical significance ($p < 0.05$). X_1–Temperature (°C), X_2–Time (min), X_3–Ethanol concentration (%), X_4–sample to solvent ratio (g/100 mL).

3.5. TPC and antioxidant activities of the fractions

The antioxidant activities and TPC of fractions derived from *L. intermedia* were assessed (Table 5). Ethyl acetate fraction demonstrated to exhibit the highest yield of phenolic content, over eighteen times higher than that of the aqueous fraction, and ten times higher than that of *n*-hexane fraction. Similar trend was found in the DRSC and FRAP assessments. Particularly, DRSC of the ethyl acetate fraction was over 53-fold and 81-fold greater than those of the aqueous and *n*-hexane fractions, respectively. The ethyl acetate was also found to possess the highest FRAP value compared to the aqueous and *n*-hexane fractions.

Table 5. Antioxidant activities and TPC of crude extract and fractions derived from *L. intermedia.*

Extract/Fraction	DRSC (mg TE/100 g)	FRAP (mg TE/100 g)	TPC (mg GAE/100 g)
n-Hexane	16.6 ± 2.7[b]	159.7 ± 5.7[b]	461.4 ± 12.9[b]
Ethyl acetate	1347.7 ± 17.5[a]	3694.5 ± 87.1[a]	4696.8 ± 70.8[a]
Aqueous	25.2 ± 3.8[b]	99.7 ± 1.7[b]	251.3 ± 0.3[c]

Means in the same column with different letters are significantly different ($p < 0.05$). DRSC–DPPH radical scavenging capacity. FRAP–Ferric reducing antioxidant power. TPC–Total phenolic content. TE–Trolox equivalents. GAE–Gallic acid equivalents.

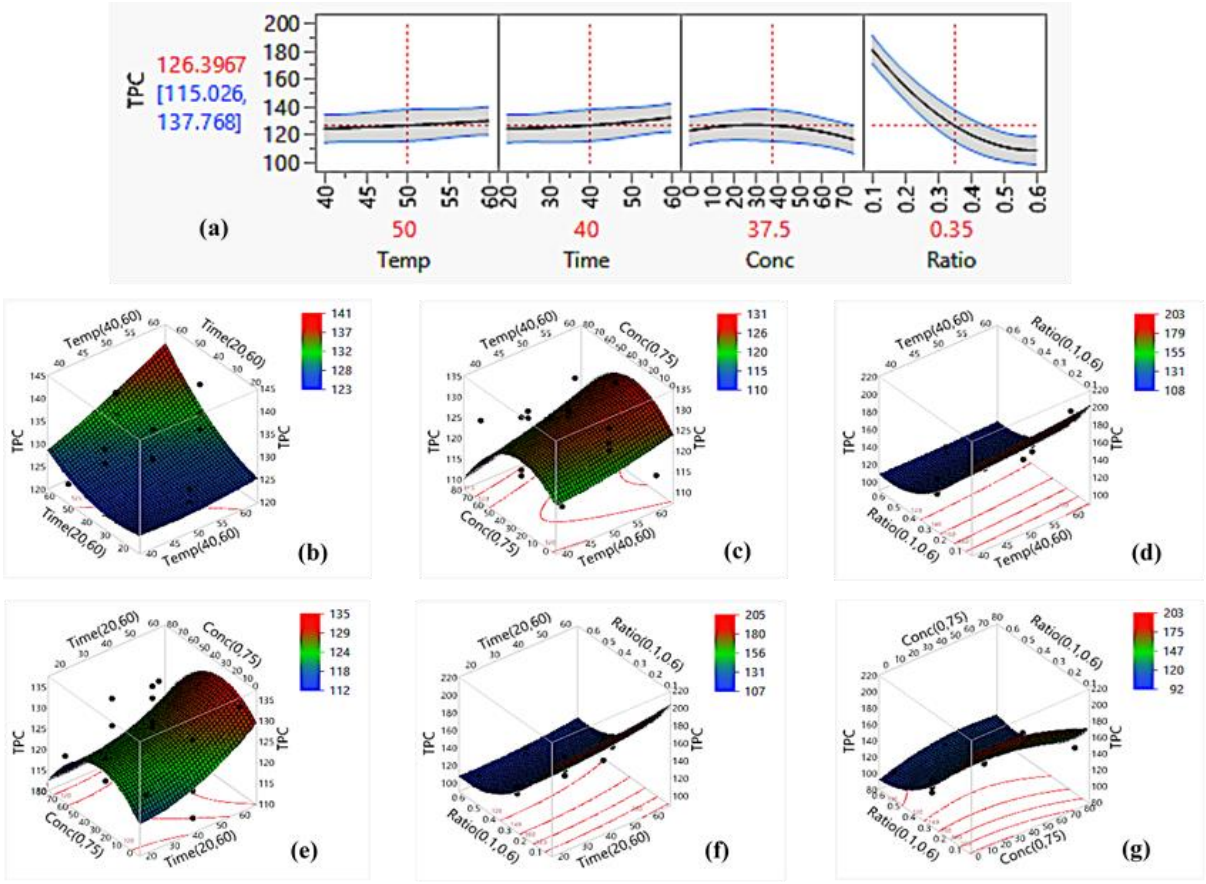

Figure 1. Prediction profiler (a) and response surface plots for TPC of *L. intermedia*. The interactions between (b) temperature and time, (c) temperature and ethanol concentration, (d) temperature and sample to solvent ratio, (e) time and ethanol concentration, (f) time and sample to solvent ratio and (g) ethanol concentration and sample to solvent ratio.

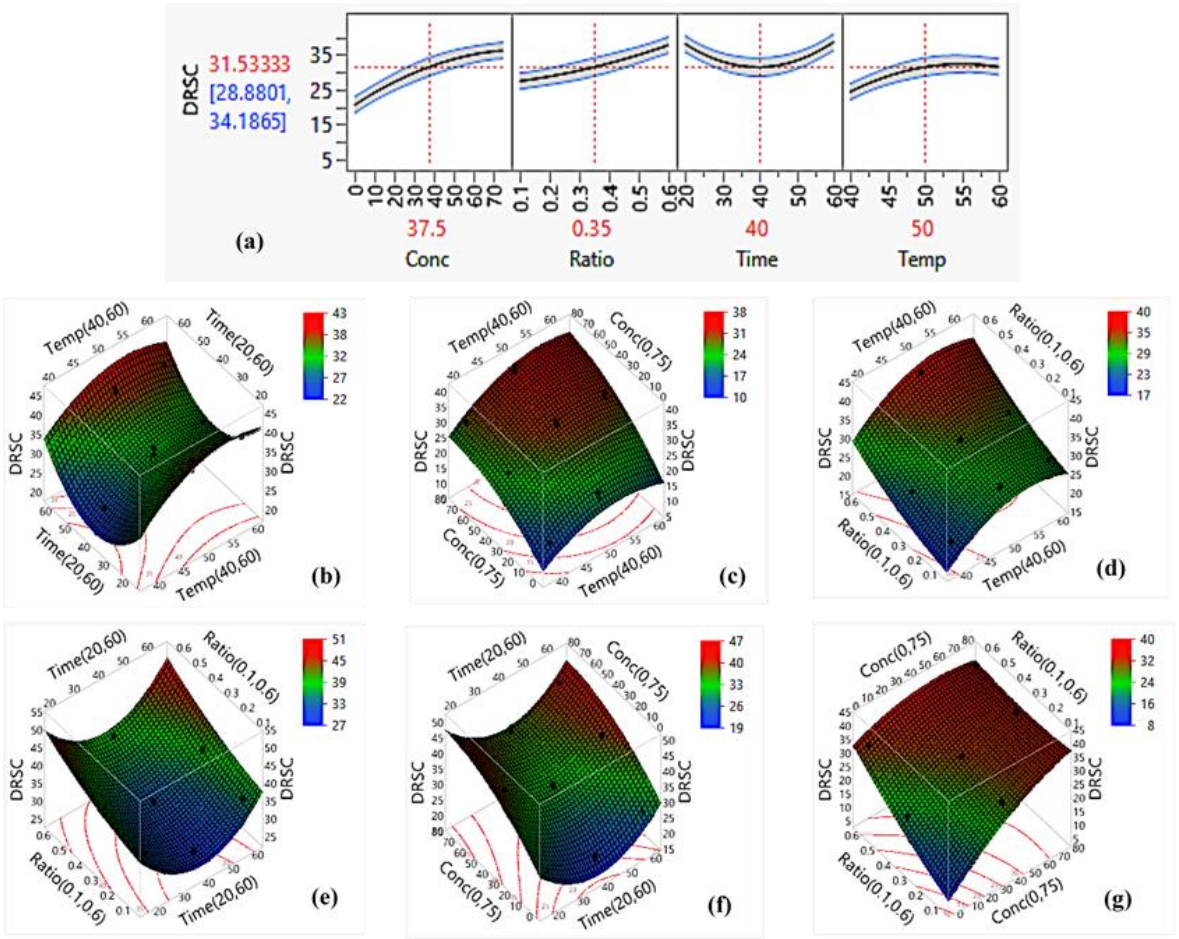

Figure 2. Prediction profiler (a) and response surface plots for DRSC of *L. intermedia*. The interactions between (b) temperature and time, (c) temperature and ethanol concentration, (d) temperature and sample to solvent ratio, (e) time and sample to solvent ratio, (f) time and ethanol concentration and (g) ethanol concentration and sample to solvent ratio.

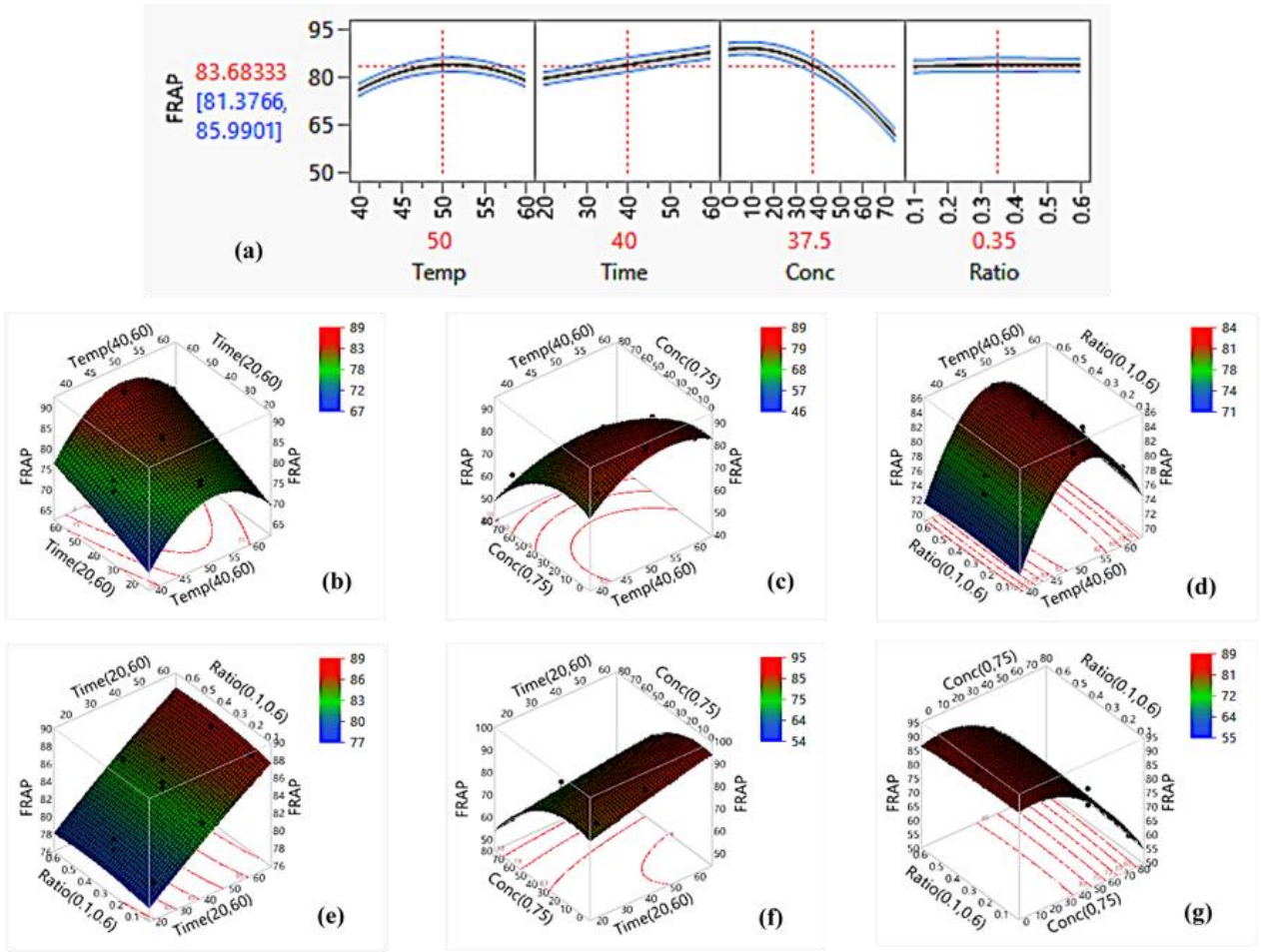

Figure 3. Prediction profiler (a) and response surface plots for FRAP of *L. intermedia*. The interactions between (b) temperature and time, (c) temperature and ethanol concentration, (d) temperature and sample to solvent ratio, (e) time and sample to solvent ratio, (f) time and ethanol concentration and (g) ethanol concentration and sample to solvent ratio.

4. Discussion

Determining the fit of the models strongly demonstrates the reliability of the developed mathematical models and the predicted conditions. It further confirms the strong correlation between the predicted and experimental values of the total phenolic content and antioxidant activity of *L. intermedia*.

The R^2 (determination coefficient) is the ratio of the explained variation to the total variation and it measures the degree of fit [32]. The variance analysis for the TPC showed R^2 (0.95), lack of fit test (0.091) and p-value < 0.0001, strongly indicating the model adequacy. The R^2 (0.97) for DRSC also demonstrates a good fit between the actual and predicted values. Moreover, there was no significance in the lack of fit (0.077) indicating the model accuracy. The p-value was found to be lower than 0.0001, further providing firm evidence of the reliability of DRSC model. Regarding FRAP, an insignificant lack of fit (0.1951) and the low p-value of the model (<0.0001) showed a

good fit and strong reliability of the model. The high R^2 value (0.99) show a very strong correlation between the actual and predicted values of FRAP (99% matching).

The influence of four extraction parameters, including temperature, time, ethanol concentration and sample to solvent ratio on the phenolic content of *L. intermedia* was analysed. Temperature, time and solvent concentration had no significant impact on the total phenolic content ($p > 0.05$). However, the linear term of sample to solvent ratio, and the quadratic term of sample to solvent ratio were noticed to have statistical effect on TPC ($p < 0.05$). These terms were considered as the most influential variable for the extraction of phenolic compounds from *L. intermedia*. In particular, increasing the ratio of sample to solvent led to a significant decrease in TPC. The rest of the terms show no significant effect ($p > 0.05$). The result was agreed by previous studies done by Ahmed et al. [33] and Bamba et al. [34], who reported that an increase in the total phenolic content of koreeb seeds flour and blueberry pomace could be obtained when the solid-solvent ratio was decreased. These findings are in line with the mass transfer principle, the higher the volume of solvent is used, the greater the concentration gradient will be, creating a driving force for the transfer of the solutes from the sample matrix to the external solvent [35].

The linear (X_1, X_3, X_4), quadratic (X_1^2, X_2^2, X_3^2) and interaction (X_3X_4) effects had significant impact on the DRSC ($p < 0.05$). They demonstrated to be the most influential variables for the DPPH radical scavenging activity from *L. intermedia*. The effects of the rest of the factors were insignificant ($p > 0.05$). The DRSC increased with a decrease in the interaction of ethanol concentration and sample to solvent ratio (X_3X_4) and the quadratic effects of temperature and ethanol concentration, favoring the scavenging capacity of *L. intermedia.* This is indicated by negative coefficients. This finding was supported by the results of Ahmed et al. [33].

According to the results for FRAP, the linear (X_1, X_2, X_3), quadratic (X_1^2, X_2^2, X_3^2) and interaction (X_1X_3) effects had significant impact on the ferric reducing antioxidant ability. The rest of the terms were insignificant with *p*-values higher than 0.05. Negative coefficients for the linear effect of ethanol concentration (X_3,), the interaction between temperature and ethanol concentration (X_1X_3), and between time and ethanol concentration (X_2X_3), as well as the quadratic effect of temperature, time, and ethanol concentration denotes that decreases in these variables resulted in an increase in FRAP value. Mokrani and Madani [36] reported the response of FRAP under these interactions similar to our findings.

The 3D response surface plots were constructed using Equations (2–4) in order to provide a better understanding of the interaction between factors. The graphs were generated in JMP 15 statistical software by plotting the responses using the z-axis against two independent variables, while keeping the other independent variables constant. The interaction between sample to solvent ratio and ethanol concentration for DRSC, and temperature and ethanol concentration for FRAP, were the only significant effects recorded.

Solvent fractionation is an important step to separate the compounds based on the polarity. Here, the optimised conditions for the extraction of phenolic compounds and antioxidants were used to obtain the crude extract for the fractionation. The highest yield of TPC and the strongest antioxidant activity were found in the ethyl acetate fraction, while the values of TPC, DRSC and FRAP in the *n*-hexane fraction were substantially lower. Ethyl acetate is a polar solvent though less polar than water, but the high phenolic content and antioxidant activity contain in the ethyl acetate fraction would suggest that most of the bioactive compounds contained in this species are polar. This better explains the reason why low solvent concentration favor high phenolic content and ferric reducing antioxidant

power in this current study. Similar results were reported by Hacke et al. [37] who found that ethyl acetate fractions derived from *Cymbopogon citratus* possessed significant quantities of phenolic acids (caffeic, chlorogenic, gallic and rosmarinic acids) and flavonoids (catechin, epicatechin, rutin, luteolin and apigenin). In the study done on *Anthemis praecox* Link aerial parts, Belhaoues et al. [38] similarly found ethyl acetate fraction possessed the highest amount of phenolic compounds. Mariem et al. [39] also reported that the highest antioxidant activity was found in the ethyl acetate fraction derived from medicinal halophyte *Retama raetam*. Hence, ethyl acetate fraction from *L. intermedia* could be further employed to isolate individual phenolic compounds and antioxidants.

Phenolic compounds exhibit free-radical scavenging properties, protecting algae thalli from negative effects of UV radiation [40]. High level of phenolic content is always coupled with antioxidant and antibacterial activities. In this study, there was a strong correlation found between the total phenolic content and the DPPH radical scavenging capacity with high R^2 value (0.865). TPC and FRAP also had a close correlation with R^2 value = 0.956. Similarly, phenolic compounds of *Helicteres hirsuta* leaf and stem were found to be well correlated with antioxidant capacity [30,31]. However, the study of Rahiman et al. (2013) [41] found that no correlation between the phenolic content and antioxidant activity of *Ocimum sanctum* (Lamiaceae), *Cucumis sativus*. (Cucurbitaceae), *Capsicum frutescens* (Solanaceae) and *Coriandrum sativum*. Terpinc and co-workers [42] reported similar findings when they investigated the correlation between total phenolic content and antioxidant capacity of oil cake extract. Variations in the correlation of phenolic contents and antioxidant properties can be explained due to the abundance and complexity of compounds contain in different materials. Also, it is difficult to predict this correlation as the result of the interactions that these compounds undergo under various extraction conditions [43].

5. Conclusions

In conclusion, the models developed to predict the TPC and antioxidant activities were found to be highly reliable for predicting the responses. The optimisation process of the total phenolic content (TPC), DPPH radical scavenging capacity (DRSC) and ferric reducing antioxidant power (FRAP) from *L. intermedia* using ultrasound-assisted extraction (UAE) methods was fully investigated in this study. The optimum conditions using UAE were determined to be temperature of 50 ℃, time of 60 min, ethanol concentration of 30%, and sample to solvent ratio of 2 g/100 mL at a fixed UAE power and frequency at 100 W and 42 kHz, respectively. It was found that only sample to solvent ratio had significant impact on the TPC, whereas temperature, ethanol concentration and sample to solvent ratio significantly affected DRSC. FRAP value was also influenced by extraction time and ethanol concentration. This is the first study reporting the optimisation of *L. intermedia* using UAE, which is very useful for further investigation on the species.

Acknowledgements

The authors gratefully thank the Project of Khanh Hoa Province (No. ĐT-2017-20902-ĐL to Dr. The Han Nguyen) for the financial support. The authors would like to thank all members of project KC.09.05/16-20 for their help in seaweed collection and preparation.

Conflict of interest

The authors declare no conflict of interest.

References

1. Gomez-Zavaglia A, Prieto Lage MA, Jimenez-Lopez C, et al. (2019) The potential of seaweeds as a source of functional ingredients of prebiotic and antioxidant value. *Antioxidants* 8: 406.
2. Kadam SU, Álvarez C, Tiwari BK, et al. (2015) Extraction of biomolecules from seaweeds. In: Brijesh KT, Declan JT. (Eds), *Seaweed sustainability*, *Food and Non-Food Applications*. London: Academic Press, 243–269.
3. Ha TTH, Huong LM, Cuong LH, et al. (2019) Evaluation of biological activities of some seaweed and seagrass species in the coastal area of Vietnam. *Vietnam J Mar Sci Technol* 19: 405–414.
4. Kim JK, Kottuparambil S, Moh SH, et al. (2015) Potential applications of nuisance microalgae blooms. *J Appl Phycol* 27: 1223–1234.
5. Milledge JJ, Nielsen BV, Bailey D (2016) High-value products from macroalgae: the potential uses of the invasive brown seaweed. *Sargassum muticum*. *Rev Environ Sci Biotechnol* 15: 67–88.
6. Uysal O, Uysal FO, Ekinci K (2015) Evaluation of microalgae as microbial fertilizer. *Eur J Sustainable Dev* 4: 77–82.
7. Thiyagarasaiyar K, Goh BH, Jeon YJ, et al. (2020) Algae metabolites in cosmeceutical: an overview of current applications and challenges. *Mar Drugs*18: 323.
8. Bwapwa JK, Jaiyeola AT, Chetty R (2017) Bioremediation of acid mine drainage using algae strains: A review. *S Afr J Chem Eng* 24: 62–70.
9. Jun JY, Jung MJ, Jeong IH, et al. (2018) Antimicrobial and antibiofilm activities of sulfated polysaccharides from marine algae against dental plaque bacteria. *Mar Drugs* 16: 301.
10. Gandhi AD, Vizhi DK, Lavanya K, et al. (2017) *In vitro* anti-biofilm and anti-bacterial activity of *Sesbania grandiflora* extract against *Staphylococcus aureus*. *Biochem Biophys Rep* 12: 193–197.
11. Zeraatkar AK, Ahmadzadeh H, Talebi AF, et al. (2016) Potential use of algae for heavy metal bioremediation, a critical review. *J Environ Manage* 181: 817–831.
12. Omar H, Al-Judaibiand A, El-Gendy A (2018) Antimicrobial, antioxidant, anticancer activity and phytochemical analysis of the red alga, *Laurencia papillosa*. *Int J Pharmacol* 14: 572–583.
13. Haq SH, Al-Ruwaished G, Al-Mutlaq MA, et al. (2019) Antioxidant, anticancer activity and phytochemical analysis of green algae, *Chaetomorpha* collected from the Arabian Gulf. *Sci Rep* 9: 18906.
14. Chingizova EA, Skriptsova AV, Anisimov MM, et al. (2017) Antimicrobial activity of marine algal extracts. *Int J Phytomedicine* 9: 113–122.
15. Martínez Andrade KA, Lauritano C, Romano G, et al. (2018) Marine microalgae with anti-cancer properties. *Mar Drugs* 16: 165.
16. Liu X, Wang S, Cao S, et al. (2018) Structural characteristics and anticoagulant property *in vitro* and *in vivo* of a seaweed sulfated Rhamnan. *Mar Drugs* 16: 243.

17. Delgado NG, Vázquez AIF, Sánchez CH, et al. (2013) Anti-inflammatory and antinociceptive activities of methanolic extract from red seaweed *Dichotomaria obtusata*. *Braz J Pharm Sci* 49: 65–74.
18. Cotas J, Leandro A, Pacheco D, et al. (2020) A comprehensive review of the nutraceutical and therapeutic applications of red seaweeds (Rhodophyta). *Life* 10: 19.
19. Nguyen TH, Nguyen TLP, Tran TVA, et al. (2019) Antidiabetic and antioxidant activities of red seaweed *Laurencia dendroidea*. *Asian Pac J Trop Biomed* 9: 501–509.
20. Trung DV, Truc NTT, Duy CNH, et al. (2019) Halogenated sesquiterpenes from the red alga *Laurencia intermedia* Yamada. *Vietnam J Chem* 57: 723–727.
21. Irie T, Suzuki M, Kurosawa E, et al. (1970) Laurinterol, debromolaurinterol and isolaurinterol, constituents of *Laurencia intermedia* Yamada. *Tetrahedron* 26: 3271–3277.
22. Makkar F, Chakraborty K (2017) Antidiabetic and anti-inflammatory potential of sulphated polygalactans from red seaweeds *Kappaphycus alvarezii* and *Gracilaria opuntia*. *Int J Food Prop* 20: 1326–1337.
23. Chemat F, Rombaut N, Sicaire AG, et al. (2017) Ultrasound assisted extraction of food and natural products. Mechanisms, techniques, combinations, protocols and applications. A review. *Ultrason Sonochem* 34: 540–560.
24. Pacheco-Fernández I, González-Hernández P, Rocío-Bautista P (2015) Main uses of microwaves and ultrasounds in analytical extraction schemes: An overview. In: Anderson JL, Pino V, Stalcup AM. (Eds), *Analytical Separation Science*. Hoboken, New Jersey, USA: John Wiley & Sons, 5: 1469–1502.
25. Box GE, Wilson KB (1951) On the experimental attainment of optimum conditions. *J R Stat Soc Series B Stat Methodol* 13: 1–38.
26. Montgomery DC, Runger GC (2010) *Applied statistics and probability for engineers*. 5th ed. New York: John Wiley & Sons, 784.
27. Kadam SU, Tiwari BK, Smyth TJ, et al. (2015) Optimization of ultrasound assisted extraction of bioactive components from brown seaweed Ascophyllum nodosum using response surface methodology. *Ultrason Sonochem* 23: 308–316.
28. Topuz OK, Gokoglu N, Yerlikaya P, et al. (2016) Optimization of antioxidant activity and phenolic compound extraction conditions from red seaweed (*Laurencia obtuse*). *J Aquat Food Prod Technol* 25: 414–422.
29. Dang TT, Vuong VQ, Schreider MJ, et al. (2017) Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant activities of the alga *Hormosira banksii* using response surface methodology. *J Appl Phycol* 29: 3161–3173.
30. Pham HNT, Nguyen VT, Vuong QV, et al. (2015) Effect of extraction solvents and drying methods on the physicochemical and antioxidant properties of *Helicteres hirsuta* Lour. Leaves. *Technologies* 3: 285–301.
31. Pham HNT, Tang NV, Vuong QV, et al. (2017) Bioactive compound yield and antioxidant capacity of *Helicteres hirsuta* Lour. Stem as affected by various solvents and drying methods. *J Food Process Preserv* 41: e12879.
32. Wang L, Wang Z, Li X (2013) Optimization of ultrasonic-assisted extraction of phenolic antioxidants from *Malus baccata* (Linn.) Borkh using response surface methodology: Sample preparation. *J Sep Sci* 36: 1652–1658.

33. Ahmed MI, Xu X, Sulieman AA, et al. (2020) Effect of extraction conditions on phenolic compounds and antioxidant properties of koreeb (*Dactyloctenium aegyptium*) seeds flour. *J Food Meas Charact* 14: 799–808.
34. Bamba BSB, Shi J, Tranchant CC, et al. (2018) Influence of extraction conditions on ultrasound-assisted recovery of bioactive phenolics from blueberry pomace and their antioxidant activity. *Molecules* 23: 1685.
35. Topuz OK, Gokoglu N, Yerlikaya P, et al. (2016) Optimization of antioxidant activity and phenolic compound extraction conditions from red seaweed (*Laurencia obtuse*). *J Aquat Food Prod Technol* 25: 414–422.
36. Mokrani A, Madani K (2016) Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. *Sep Purif Technol* 162: 68–76.
37. Hacke ACM, Marques JA, Vellosa JCR, et al. (2018) Ethyl acetate fraction of *Cymbopogon citratus* as a potential source of antioxidant compounds. *New J Chem* 42: 3642–3652.
38. Belhaoues S, Amri S, Bensouilah M (2020) Major phenolic compounds, antioxidant and antibacterial activities of *Anthemis praecox* Link aerial parts. *S Afr J Bot* 131: 200–205.
39. Mariem S, Hanen F, Inès J (2014) Phenolic profile, biological activities and fraction analysis of the medicinal halophyte *Retama raetam*. *S Afr J Bot* 94: 114–121.
40. Zubia M, Fabre MS, Kerjean V, et al. (2009) Antioxidant and antitumoural activities of some Phaeophyta from Brittany coasts. *Food Chem* 116: 693–701.
41. Rahiman S, Tantry BA, Kumar A (2012) Variation of antioxidant activity and phenolic content of some common home remedies with storage time. *Afr J Tradit Complement Altern Med* 10: 124–127.
42. Terpinc P, Čeh B, Ulrih NP, et al. (2012) Studies of the correlation between antioxidant properties and the total phenolic content of different oil cake extracts. *Ind Crops Prod* 39: 210–217.
43. Ulewicz-Magulska B, Wesolowski M (2019) Total phenolic contents and antioxidant potential of herbs used for medical and culinary purposes. *Plant Foods Hum Nutr* 74: 61–67.

AIMS Press