

Optimisation of ultrasound-assisted extraction conditions for phenolic content and antioxidant activities of the alga *Hormosira banksii* using response surface methodology

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Abstract This study aimed to optimise ultrasound-assisted extraction (UAE) conditions of the brown alga *Hormosira banksii* for total phenolic content (TPC) and antioxidant activities including total antioxidant capacity (ABTS), DPPH free radical scavenging capacity (DPPH) and ferric reducing antioxidant power (FRAP) using response surface methodology (RSM). Box–Behnken design was employed to assess the effect of ultrasonic temperature, time and power on the TPC and antioxidant activities of the extracts. The results showed that RSM was an accurate and reliable method in predicting TPC and antioxidant activities (ABTS, DPPH and FRAP) of the extracts with R^2 values of 0.97, 0.96, 0.92 and 0.94, respectively. The ultrasonic temperature and time had the significant impact on TPC and antioxidant capacities. The optimal UAE conditions for the maximal values of TPC and antioxidant activities were of 30 °C, 60 min and power 60%, or 150 W. The values of TPC and antioxidant activities (ABTS, DPPH, FRAP) achieved under these parameters were 23.12 (mg GAE g⁻¹), 85.64 (mg TE g⁻¹), 47.24 (mg TE g⁻¹) and 12.56 (mg TE g⁻¹), respectively. UAE was found to be more efficient in comparison to conventional extraction, with shorter time for extraction and higher of TPC level and antioxidant activities. Therefore, ultrasonic-assisted extraction

using RSM is effective for extraction and further isolation and purification of phenolic compounds from *H. banksii*. In addition, this alga could be a potential rich source of natural antioxidants applied in food and pharmaceutical fields.

Keywords *Hormosira banksii* · Phaeophyceae · Ultrasonic-assisted extraction (UAE) · Optimisation · RSM

Introduction

Hormosira banksii (Turner) Decaisne, a brown alga (Fucales: Phaeophyta), is widely distributed along the coastal areas of New South Wales, Australia (Millar and Kraft 1994). In recent years, marine resources have attracted attention in the search for natural products in order to develop new drugs and healthy foods (El Gamal 2010; Mohamed et al. 2012) and marine algae have been reported as a potential source for the extraction of bioactive compounds (Gupta and Abu-Ghannam 2011). Many metabolites have been isolated from brown algae, including pigments (fucoxanthin, carotenoids, etc.), phenolic compounds (phlorotannins), sulfated polysaccharides (fucoidan), bromophenols and meroditerpenoids. These bioactive compounds have been linked to various health benefits such as being anti-allergic (Miyake et al. 2006), preventing cardiovascular diseases (Shi et al. 2010), neuroprotective effects (Alghazwi et al. 2016), anti-diabetes (Okada et al. 2004), and against several types of cancers (Cabrita et al. 2010; De Souza et al. 2009; Ferreres et al. 2012; Kumar et al. 2013; Kwak 2014).

Recently, conventional extraction was considered to have some drawbacks due to long time, high cost and degradation of product quality, while using organic solvents has to be minimised for extraction because of potential health and environmental concerns (Azmir et al. 2013; Polshettiwar and

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Varma 2008). Until now, various novel techniques have been developed for extracting components from algae (Kadam et al. 2013) such as pressurised liquid extraction (PLE) (Onofrejová et al. 2010), supercritical fluid extraction (SFE) (Tanniou et al. 2013), enzyme-assisted extraction (EAE) (Wijesinghe and Jeon 2012) and ultrasound-assisted extraction (UAE) (Roselló-Soto et al. 2015). UAE is considered an effective method in comparison with the others due to its low energy requirements and low solvent consumption (Chemat and Khan 2011). Ultrasound enhances extraction efficacy by using an ultrasonic wave that helps increase penetration of solvent into the materials and the contact surface area between solid and liquid phases. Moreover, the improvement in the extraction process using ultrasound is related to the destruction of the cell walls, reduction of the particle size and enhancement of the mass transfer through the cell wall due to the collapse of bubbles produced by cavitation (Wang et al. 2008; Macías-Sánchez et al. 2009; Teh and Birch 2014). The advantages and drawbacks of UAE from plants have been highlighted in previous studies (Romanik et al. 2007; Chemat and Khan 2011).

Response surface methodology (RSM) is a useful technique for optimising processes or products by establishing a statistical and mathematical model. This model allows the evaluation of multiple parameters and their interactions using quantitative data, leading to a reduction in the number of experimental trials required (Pompeu et al. 2009; Wu et al. 2012). Thus, it is also faster and more economical than other approaches required for an optimisation process.

This study aimed to optimise the ultrasonic conditions for extraction of total phenolic content (TPC) and antioxidant activities of the *H. banksii* using RSM. Conventional extraction was also applied to compare its effectiveness with the optimum ultrasonic extraction. The findings of this study have potential to be applied for further isolation and purification of phenolic compounds from *H. banksii*.

Materials and methods

Materials

The brown alga *H. banksii* was collected in March 2016 from Bateau Bay rocky shore, NSW, Australia. After collection, the sample was washed with seawater to remove natural residues (sand and epifauna), kept in a box to protect from light and immediately transported to the laboratory. The alga was then washed thoroughly with fresh water, immersed in liquid nitrogen, and freeze-dried for 48 h using a freeze dryer (Thomas Australia Pty. Ltd., Australia) with a drying chamber pressure of 2×10^{-1} mbar and a cryo-temperature of -50 °C. The dried sample was ground to give ≤ 0.6 -mm particle size using a 0.6-mm EFL 2000 stainless

steel mesh sieve (Endecotts Ltd., England) and stored at -20 °C for further analysis.

Ultrasound-assisted extraction (UAE)

The freeze-dried alga was extracted using ethanol 70% (v/v) with a solvent to material ratio of 50 (mL g⁻¹). UAE was conducted using an ultrasonic bath (Soniclean, 220 V, 50 Hz and 250 W, Soniclean Pty Ltd., Australia). The experimental parameters applied for extracting were designed by response surface methodology (RSM) (JMP software, version 13). After ultrasonic extraction, the extracts were immediately cooled on ice to room temperature (RT), filtered using a 0.45- μ m cellulose syringe filter (Phenomenex Australia Pty. Ltd., Australia) and diluted to the required volume for quantitative analysis.

Conventional extraction

Conventional extraction was conducted according to the method of Hossain et al. (2012) with some modifications. The dried and ground sample (0.5 g) was extracted with ethanol (70%) and the ratio of solvent to material (50/1 mL g⁻¹) at 30 °C. The samples were shaken for 12 h using a shaking water bath. The extracts were then immediately cooled on ice to RT, filtered and diluted to the required volume for analysis.

Response surface methodology (RSM)

RSM with a Box-Behnken design was employed for designing experimental conditions to determine the influence of the three independent parameters including ultrasonic temperature, time and power on the TPC and antioxidant activities of the extracts. The optimal ranges of temperature (30–50 °C), time (20–60 min) and power of ultrasound (60–100% or 150–250 W) were determined based on preliminary experiments (data not shown). The independent variables and their code variable levels are shown in Table 1. To express the TPC value or antioxidant activities as a function of the independent variables, a second-order polynomial equation (Eq. 1) was used as follows and previously described by Vuong et al. (2014):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j + \sum_{i=1}^k \beta_{ij} X_{ij}^2 \quad (1)$$

where various X_i values are independent variables affecting the responses Y ; β_0 , β_i , β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively; and k is the number of variables. The three independent ultrasonic parameters

Table 1 The independent variables and their levels used for Box-Behnken design

X_i	Factor levels		
	-1	0	+1
Temperature (X_1) (°C)	30	40	50
Time (X_2) (min)	20	40	60
Power of ultrasound (X_3) (%)	60	80	100

were assigned as X_1 (temperature, °C), X_2 (time, min) and X_3 (% ultrasonic power). Thus, the function containing these three independent variables was expressed as follows (Eq. 2):

$$\begin{aligned}
 Y = & \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{12} X_1 X_2 \\
 & + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 \\
 & + \beta_{33} X_3^2
 \end{aligned}
 \tag{2}$$

Determination of total phenolic content

Total phenolic content (TPC) The extract was diluted to fit within the optimal absorbance range for colorimetric assessment. TPC was determined according to the method of Vuong et al. (2013). Briefly, 0.5 mL of diluted sample, 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent was added, followed by the addition of 2 mL of NaCO₃ 7.5% (w/v), then mixed well on a vortex vibrator for 2 min and incubated in the dark at room temperature (RT) for 1 h before the absorbance was measured at 765 nm. Gallic acid was used as the standard for the construction of a calibration curve, with the results expressed as mg of gallic acid equivalents per gram of dried material (mg GAE g⁻¹).

Determination of antioxidant activities

ABTS total antioxidant capacity (ABTS) The extract was diluted to fit within the optimal absorbance range for colorimetric assessment. Total antioxidant capacity was measured using 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay as described by Thaipong et al. (2006). Methanol was used as control and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as a standard. The standard curve was linear between 25 and 1000 μM trolox. Results were expressed as mg of trolox equivalents per gram of dried material (mg TE g⁻¹).

DPPH free radical scavenging capacity (DPPH) The extract was diluted and analyzed using 1,1-diphenyl-2-picrylhydrazyl

(DPPH) assay as described by Brand-Williams et al. (1995). Methanol and trolox were used as a control and standard for the assay. The standard curve was linear between 25 and 1000 μM trolox. Results were expressed in mg TE g⁻¹ dried material.

Ferric reducing antioxidant power (FRAP) The extract was diluted and its iron chelating capacity was analysed using the FRAP assay as described by Benzie and Strain (1999). Trolox was used as the calibration standard. The standard curve was linear between 25 and 1000 μM trolox, and the results were expressed in mg TE g⁻¹ dried material.

Statistical analysis

RSM experimental design and analysis were conducted using JMP software (version 13). The software was also used to establish the model equation to graph the 3D- and 2D contour plots of variable response and to predict optimum values for the three response variables. All measurements were taken in triplicate ($n = 3$). Results were expressed as mean values with standard deviations, and significant differences between treatments were tested using analysis of variance and the LSD post hoc test with a 95% significance level ($P < 0.05$). Correlations among data were calculated using Pearson’s correlation coefficient and expressed as R^2 . The SPSS version 16.0 statistical package was used for all analyses.

Results

Fitting of the models

Fitting the models for the TPC values and antioxidant activities is important to assess how precisely the RSM mathematical model can predict the ideal variances and determine the correlations of the selected parameters to ultrasonic extractions. Therefore, analysis of variance was undertaken to evaluate the reliability of the RSM mathematical model. The TPC values and antioxidant activities of the extract obtained from all the experiments are presented in Table 2. Analysis of variance (ANOVA) indicated that the coefficient of multiple determination (R^2) for the response of TPC was 0.97, suggesting that there was a close correlation between the actual and predicted values of the TPC model data. The value of lack of fit was used to determine the adequacy of the model and was not significant ($P > 0.05$), indicating that the model could adequately fit the experimental data (Table 3) (Wu et al. 2012). Furthermore, the P value and t ratio were used to determine the significance of the coefficients of the quadratic polynomial models. The smaller P value and

Table 2 Experimental (Exp.) and predicted (Pred.) values of total phenolic compounds (TPC) and antioxidant activities: total antioxidant capacity (ABTS), DPPH free radical scavenging capacity (DPPH) and ferric reducing antioxidant power (FRAP) of *H. banksii* obtained from Box-Behnken design

Exp. run	Ultrasonic conditions			Antioxidant activity							
	X_1 (°C)	X_2 (min)	X_3 (%)	TPC (mg g ⁻¹)		ABTS (mg g ⁻¹)		DPPH (mg g ⁻¹)		FRAP (mg g ⁻¹)	
	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.	
1	30	40	60	21.41	20.95	83.34	81.75	46.88	48.04	12.77	12.15
2	30	20	80	18.38	18.52	72.25	74.05	40.46	40.60	10.07	10.13
3	30	60	80	22.25	22.59	84.55	86.90	47.73	45.51	12.13	12.00
4	30	40	100	21.85	21.83	75.69	73.13	40.53	41.45	9.15	9.85
5	40	40	80	18.28	18.08	70.34	71.66	42.43	41.54	7.97	7.67
6	40	20	100	18.36	18.24	65.27	66.03	32.77	31.71	6.61	5.86
7	40	60	60	20.68	20.80	75.18	74.42	37.76	38.82	7.49	8.24
8	40	20	60	14.46	14.78	68.63	68.41	36.71	35.41	5.46	6.02
9	40	40	80	18.48	18.08	71.05	71.66	41.83	41.54	7.88	7.67
10	40	60	100	18.31	17.99	71.91	72.13	34.36	35.66	6.94	6.38
11	40	40	80	17.48	18.08	73.58	71.66	40.37	41.54	7.15	7.67
12	50	60	80	18.76	18.61	62.66	60.86	37.11	36.97	6.67	6.61
13	50	40	60	18.68	18.70	53.65	56.22	38.47	37.55	6.68	5.98
14	50	20	80	17.24	16.90	63.95	61.60	32.29	34.51	5.61	5.74
15	50	40	100	18.01	18.47	58.58	60.17	38.46	37.30	5.63	6.25
Valid.	30	60	60	23.12 ± 1.01a	24.07 ± 1.66a	85.64 ± 2.07a	88.97 ± 8.89a	47.24 ± 0.65a	46.44 ± 6.30a	12.56 ± 0.43a	13.02 ± 2.71a

In validation row (valid), all the values are means ± standard deviations ($n = 4$) and the similar letters between Exp. and Pred. values are not significantly different ($P > 0.05$). The Pred. values were predicted by response surface methodology using JMP software (version 13)

Table 3 Regression coefficients of the fitted quadratic equation for total phenolic compound (TPC) and antioxidant activities (ABTS, DPPH and FRAP)

Regression coefficients	TPC		ABTS		DPPH		FRAP	
	Regression coefficient	<i>t</i> ratio	Regression coefficient	<i>t</i> ratio	Regression coefficient	<i>t</i> ratio	Regression coefficient	<i>t</i> ratio
β_0	18.08	57.29	71.67	42.42	41.54	34.18	7.67	14.92
Linear								
β_1	-1.40***	-7.24	-9.62***	-9.30	-3.66**	-4.92	-2.44***	-7.76
β_2	1.45***	7.48	3.02*	2.92	1.84	2.47	0.68	2.18
β_3	0.16	0.84	-1.17	-1.13	-1.71	-2.30	-0.51	-1.62
Interaction								
β_{12}	-0.59	-2.15	-3.39	-2.32	-0.61	-0.58	-0.25	-0.56
β_{13}	-0.28	-1.02	3.15	2.15	1.59	1.51	0.64	1.44
β_{23}	-1.57**	-5.74	0.02	0.02	0.14	0.13	-0.43	-0.96
Quadratic								
β_{11}	1.56**	5.47	-1.62	-1.06	1.77	1.62	1.44*	3.12
β_{22}	-0.48	-1.68	0.81	0.53	-3.92*	-3.57	-0.49	-1.06
β_{33}	0.35	1.23	-2.22	-1.46	-2.22	-2.03	-0.55	-1.19
R^2	0.97		0.96		0.92		0.94	
<i>P</i> value of lack of fit	0.51		0.09		0.27		0.22	
<i>P</i> values of models	0.002		0.006		0.027		0.012	

β_0 is a constant; β_p , β_{ij} and β_{ii} are the linear, interactive and quadratic coefficients of the second-order polynomial equation, respectively

*, **, ***Significantly differences with $P < 0.05, 0.01$ and 0.001 , respectively

larger *t* ratio would indicate a more significant effect on the corresponding variables (Kha et al. 2013). The *P* value of the model was found to be 0.002 (showing the significance of the model considering a confidence interval of 99%) and the *t* ratio of the model was 57.29, revealing that the mathematical model was reliable for predicting TPC values, following the second-order polynomial formula (Eq. 3).

$$\begin{aligned}
 Y_{TPC} = & 18.08 - 1.40X_1 + 1.45X_2 \\
 & + 0.16X_3 - 0.59X_1X_2 - 0.28X_1X_3 - 1.57X_2X_3 \\
 & + 1.56X_1^2 - 0.48X_2^2 + 0.35X_3^2 \quad (3)
 \end{aligned}$$

Fitting of the models for the three different antioxidant assays was also investigated. The results indicated that R^2 values for the models of ABTS, DPPH and FRAP were 0.96, 0.92 and 0.94, respectively, revealing the strong correlations between actual and predicted data (Table 3). The values for lack of fit were 0.19, 0.15 and 0.15, indicating that there was no significant difference between the predicted and experimental values for

ABTS, DPPH and FRAP, respectively ($P > 0.05$). Moreover, the *P* values of the models were 0.006, 0.027 and 0.012 and *t* ratio (42.42, 34.18 and 14.92) for different antioxidant activities (ABTS, DPPH and FRAP, respectively) further confirming that there was a strong reliability of these mathematical models in predicting the antioxidant activity for the following second-order polynomial formulas (Eqs. (4), (5) and (6):

$$\begin{aligned}
 Y_{ABTS} = & 71.67 - 9.62X_1 + 3.02X_2 - 1.17X_3 - 3.39X_1X_2 \\
 & + 3.15X_1X_3 + 0.02X_2X_3 - 1.62X_1^2 \\
 & + 0.81X_2^2 - 2.22X_3^2 \quad (4)
 \end{aligned}$$

$$\begin{aligned}
 Y_{DPPH} = & 41.54 - 3.66X_1 + 1.84X_2 - 1.71X_3 - 0.61X_1X_2 \\
 & + 1.59X_1X_3 + 0.14X_2X_3 \\
 & + 1.77X_1^2 - 3.92X_2^2 - 2.22X_3^2 \quad (5)
 \end{aligned}$$

$$\begin{aligned}
 Y_{FRAP} = & 7.67 - 2.44X_1 + 0.68X_2 - 0.51X_3 - 0.25X_1X_2 \\
 & + 0.64X_1X_3 - 0.43X_2X_3 \\
 & + 1.44X_1^2 - 0.49X_2^2 - 0.55X_3^2 \quad (6)
 \end{aligned}$$

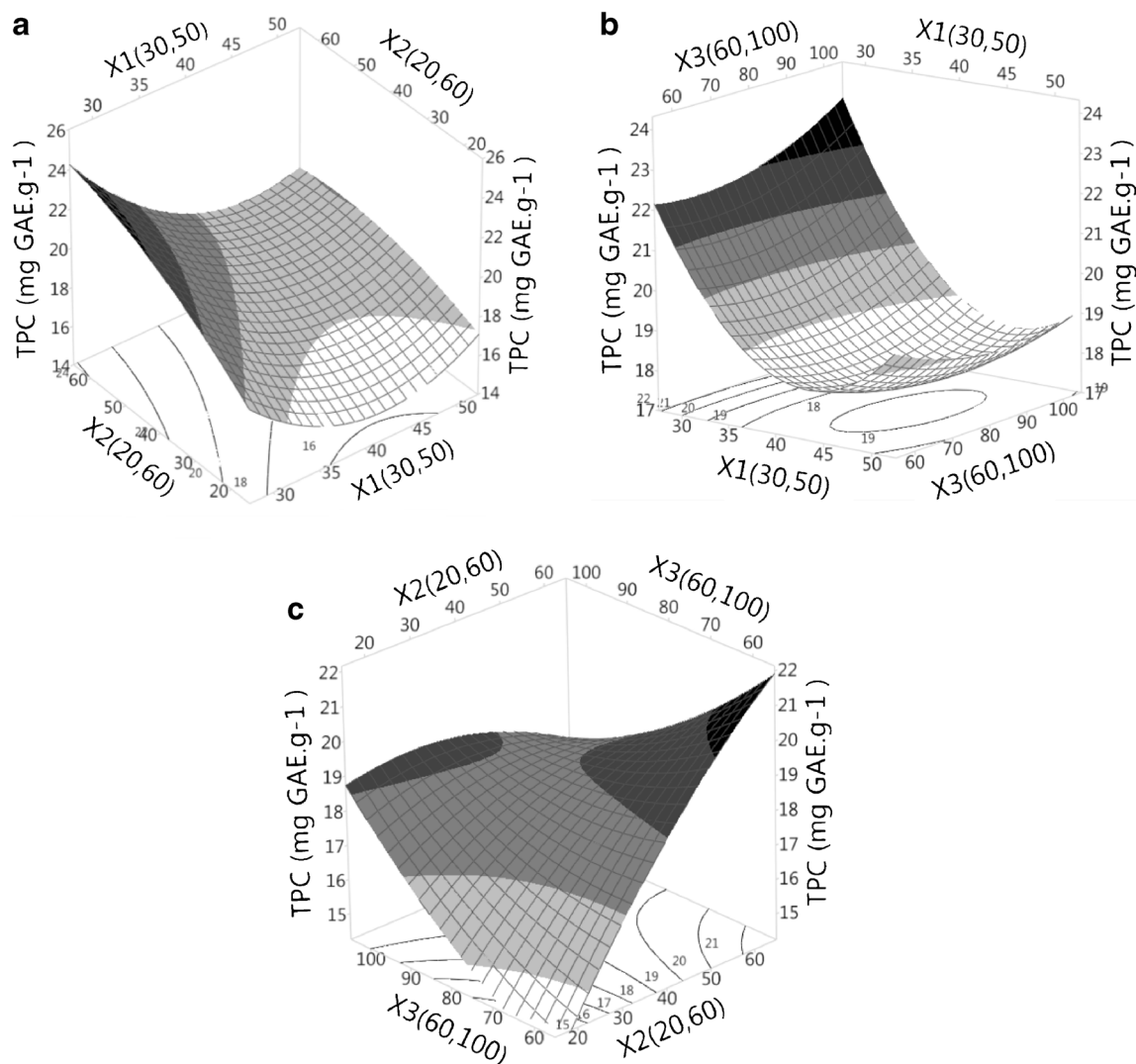


Fig. 1 The 3D response surface and 2D contour plots (a–c) of TPC (mg GAE g⁻¹) of *H. banksii* extract mutually affected by temperature (X_1 , °C), time (X_2 , min) and ultrasonic power (X_3 , %)

Effects of ultrasonic extraction conditions on the TPC

The optimal levels of the independent variables for the TPC were visualised by the 3D surface and 2D contour. The relationship between the independent variables and the responses was shown by response surface plots, while the contour plot indicated the shape of a response surface. The effect of the ultrasonic temperature, time and power on TPC is presented in Table 3 by the coefficients of quadratic models. As the results, the two independent variables (ultrasonic temperature and time) had significant impact on the TPC of *H. banksii* extract ($P < 0.05$). In addition, TPC was the most affected by ultrasonic time, followed by temperature and power of ultrasound. There was also the significant effect of the interaction (between independent variables: ultrasonic time \times power) and quadratic term of temperature on the TPC of the algal extract ($P < 0.01$). From Fig. 1a, it can be seen that there was a steady increase in

TPC of extract when temperature decreased from 50 to 30 °C and ultrasonic time increased between 20 and 60 min at the constant power of 80%. Figure 1b shows that TPC increased slightly when a decrease in temperature and an increase in level of ultrasonic power were applied at the constant time of 40 min. However, at the constant temperature of 40 °C, the long time and low power or short time and high power both led to the high level of TPC of the extract. The application of the long time and low power was found to be better for TPC (Fig. 1c).

Effects of ultrasonic extraction conditions on antioxidant activities

Total antioxidant capacity (ABTS)

For ABTS, all three parameters of ultrasonic temperature, time and power affected ABTS values of the *H. banksii* extracts

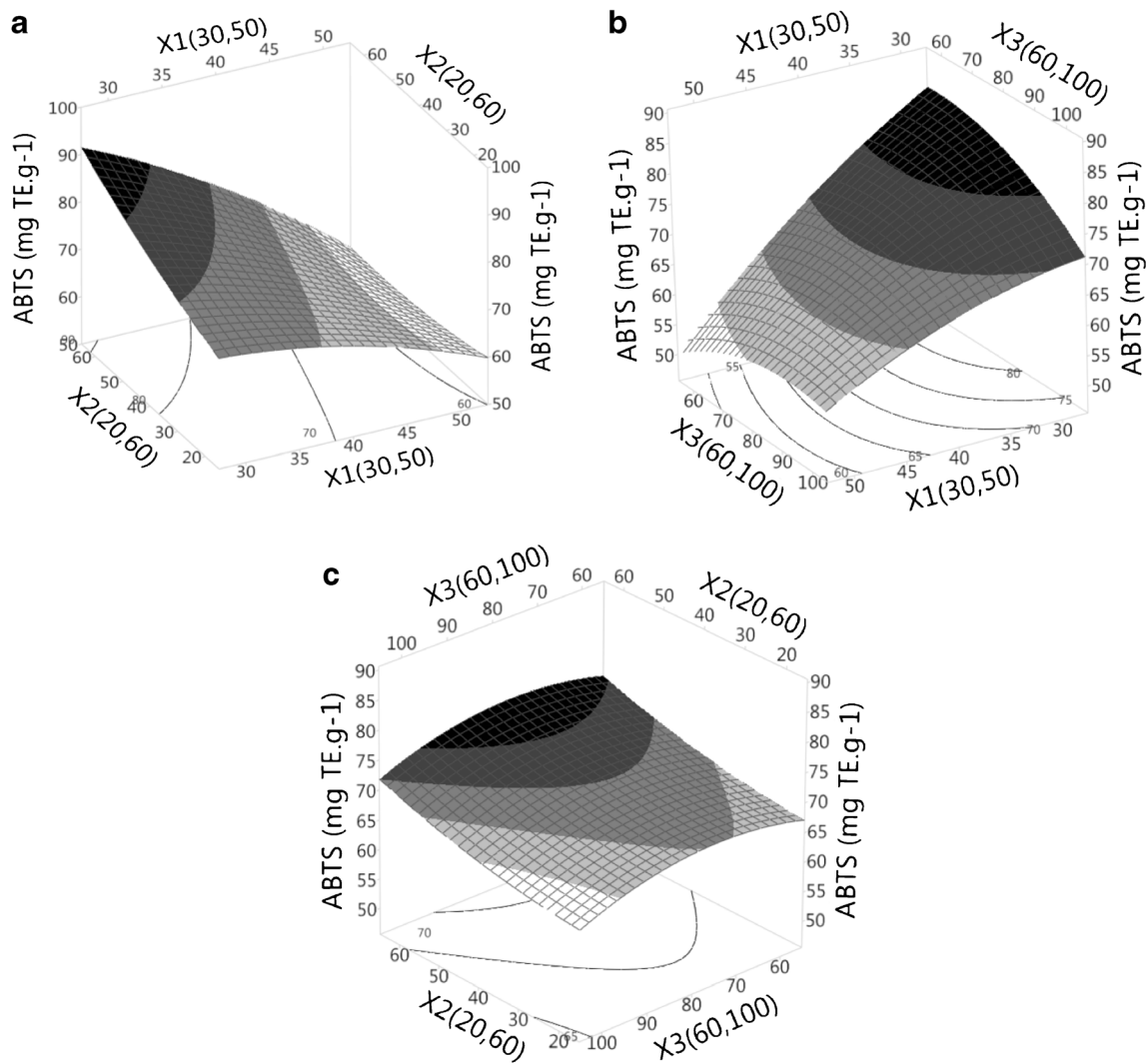


Fig. 2 The 3D response surface and 2D contour plots (a–c) of ABTS (mg TE g⁻¹) of *H. banksii* extract mutually affected by temperature (X_1 , °C), time (X_2 , min) and ultrasonic power (X_3 , %)

(Fig. 2a–c). The degree of the effect of the independent variables can be shown in the following order: temperature > time > power (based on the P values). The statistical results indicated that the ultrasonic temperature and time significantly affected the ABTS ($P < 0.05$, Table 3). In addition, Fig. 2a shows that ABTS values of *H. banksii* rapidly decreased when the temperature increased between 30 and 50 °C, while increasing moderately with ultrasonic time from 20 to 60 min at the constant power of 80%. A decrease in temperature and power resulted in the increase of ABTS in the algal extract at the constant time of 40 min (Fig. 2b). At the constant temperature of 40 °C, high ABTS was also observed when long time and low power of ultrasound was applied (Fig. 2c). Therefore, it was found that the highest value of ABTS (88.97 mg TE g⁻¹) from *H. banksii* extract could be obtained at the optimal ultrasonic parameters (temperature of 30 °C, time of 60 min and power of 60%).

The DPPH free radical scavenging capacity (DPPH)

Statistical results showed that only linearity of temperature and quadratic term of time significantly affected DPPH free radical scavenging capacity of the extract ($P < 0.05$; Table 3). The order of the influence of the parameters on the values of DPPH was temperature > time > power. Figure 3a illustrates that the DPPH value of *H. banksii* extract rose steadily when ultrasonic temperature went down from 50 to 30 °C and the ultrasonic time increased from 20 and 46.15 min at the power 80%. High DPPH was also observed at low temperature (30 °C) and power (60% or 150 W) at the constant time of 40 min (Fig. 3b). With the temperature at 40 °C, the moderate time (about 46 min) and low power resulted in the high value of DPPH of the extract (Fig. 3c). From the results, the ultrasonic parameters affected DPPH had the similar trend to ABTS of the extract except for a decrease of DPPH when time

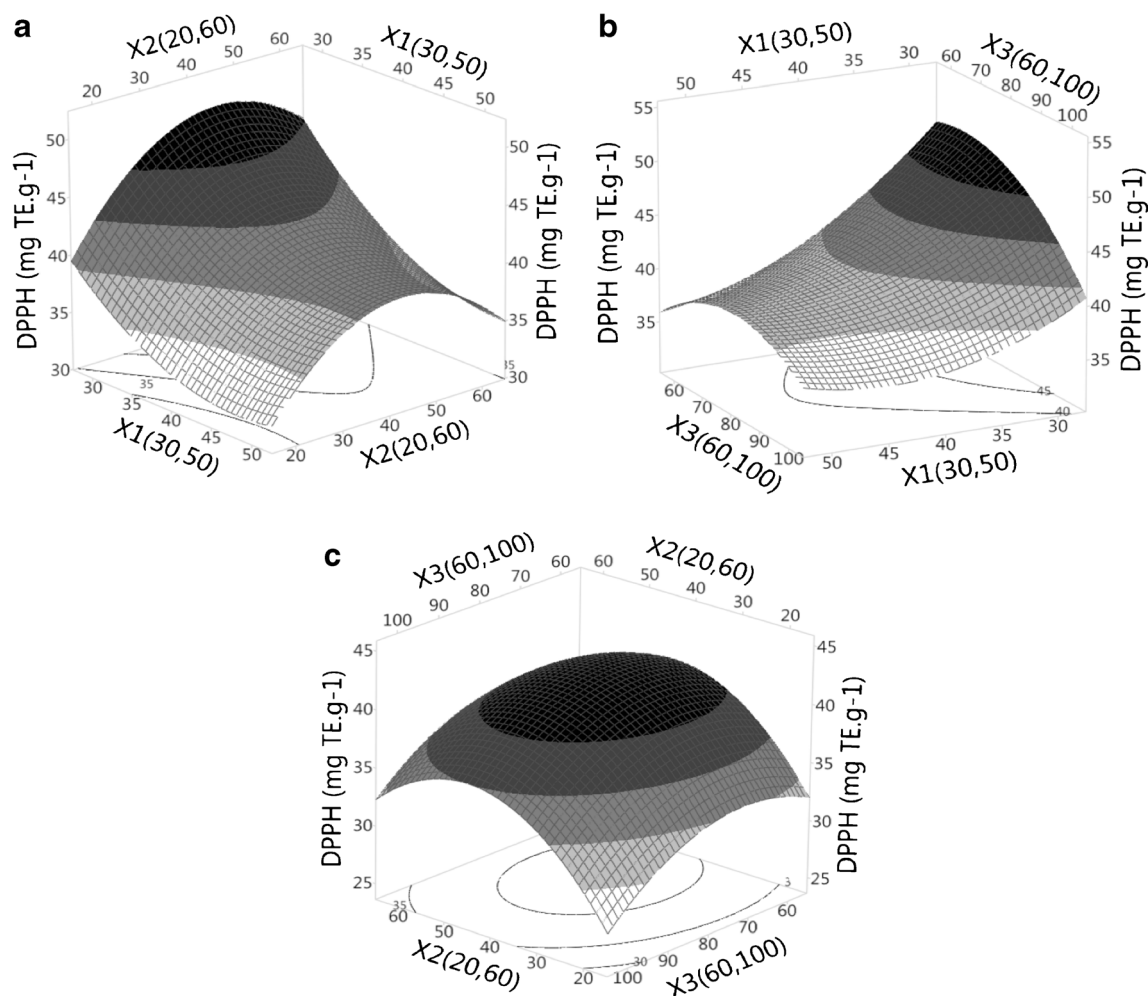


Fig. 3 The 3D response surface and 2D contour plots (a–c) of DPPH (mg TE g⁻¹) of *H. banksii* extract mutually affected by temperature (X_1 , °C), time (X_2 , min) and ultrasonic power (X_3 , %)

was from 46.15 to 60 min and the maximal value for DPPH (48.38 mg TE g⁻¹) could be obtained at the predicted conditions (temperature of 30 °C, time of 46.15 min and power of 60%).

Ferric reducing antioxidant power (FRAP)

Figure 4a–c and Table 3 outline the changes in FRAP of the extract under the different ultrasonic conditions. The FRAP was significantly affected by the linearity of temperature and the quadratic term of temperature ($P < 0.05$). FRAP was most affected by temperature, followed by time and power of ultrasound. An increase in the values of FRAP was observed as temperature decreased (between 50 and 30 °C) and time increased (between 20 and 60 min) at the moderate power of 80% (Fig. 4a). The antioxidant activity of the extract increased when both ultrasonic temperature and power decreased at the time of 40 min (Fig. 4b). As shown in Fig. 4c, the long time and low power resulted in the high value of FRAP for the extract at the temperature of 40 °C. From the model, it was

predicted that the maximum value of FRAP (13.02 mg TE g⁻¹) could be obtained with the conditions: ultrasonic temperature (30 °C), time (60 min) and the power (60% or 150 W).

Optimisation and validation of UAE conditions for TPC and antioxidant activities

The study aimed to determine the optimal conditions for TPC and antioxidant activities of the alga *H. banksii* using JMP 13 software. Through the process, the ultrasonic temperature, time and power were estimated to obtain the maximum values for TPC and antioxidant activities (ABTS, DPPH and FRAP). The results above and outlined in Fig. 5 indicated that the increase in temperature and power resulted in a decrease in TPC and antioxidant activities (except for the slight increase in TPC when power increased). A long time was also required for the high values of TPC and antioxidant activities (except for the slight decrease in DPPH when time increased between 46.15 and 60 min). The theoretical maximum values of TPC,

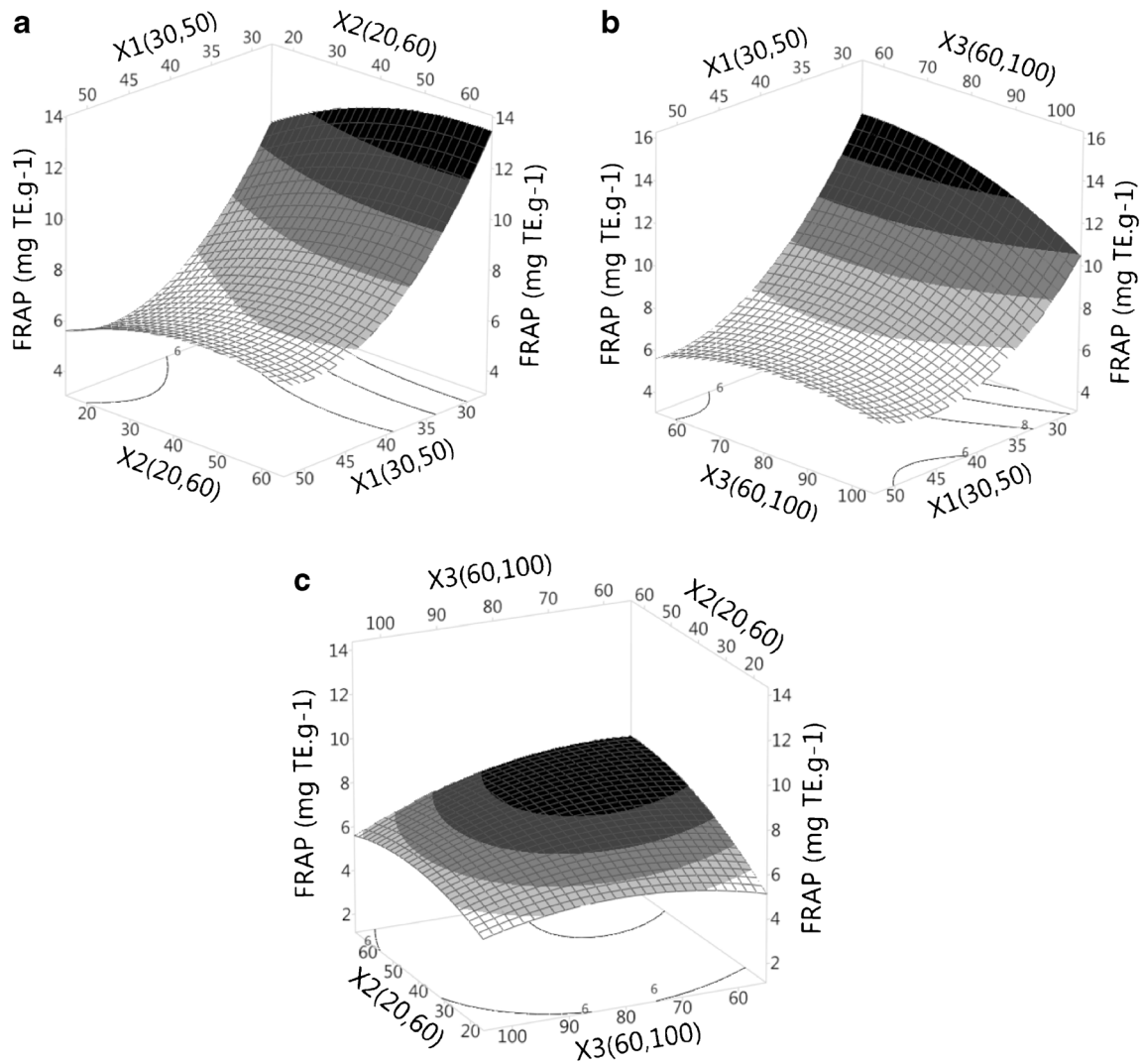


Fig. 4 The 3D response surface and 2D contour plots (a–c) of FRAP (mg TE g⁻¹) of *H. banksii* extract mutually affected by temperature (X_1 , °C), time (X_2 , min) and ultrasonic power (X_3 , %)

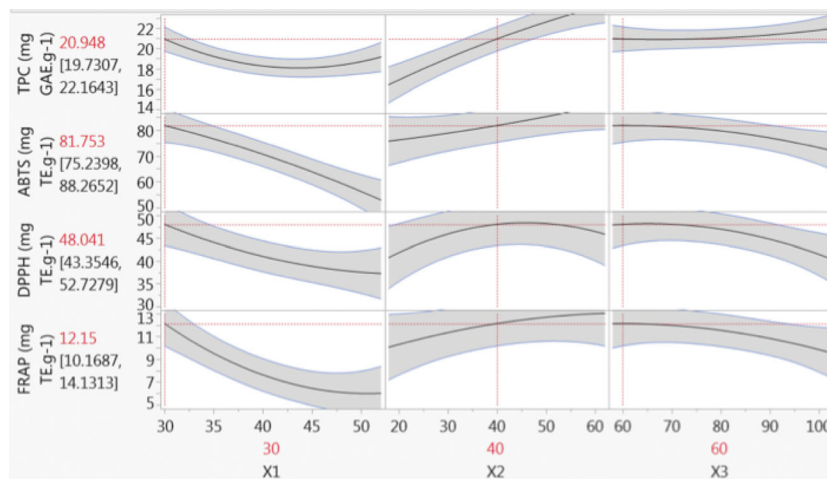


Fig. 5 The predicted profilers of TPC and antioxidant activities at the optimal conditions of the temperature (X_1 , °C), time (X_2 , min) and ultrasonic power (X_3 , %). Solid lines indicate predicted mean values of TPC and antioxidant activities. Red dashed lines show the values at each

condition, while blue lines indicate the 95% confidence intervals. The predicted profilers were predicted by response surface methodology using JMP software (version 13)

Table 4 The comparison between the UAE and conventional method

	Extraction methods	
	UAE	Conventional extraction
TPC (mg GAE g ⁻¹)	23.12 ± 1.01a	16.21 ± 0.56b
ABTS (mg TE g ⁻¹)	85.64 ± 2.07a	51.32 ± 2.33b
DPPH (mg TE g ⁻¹)	47.24 ± 0.65a	30.56 ± 2.26b
FRAP (mg TE g ⁻¹)	12.56 ± 0.43a	8.34 ± 0.12b

All the values are means ± standard deviations ($n = 4$) and the different letters in the same row are significantly different ($P < 0.05$)

ABTS and FRAP could be obtained by combining the ultrasonic temperature, time and power (Fig. 5). Based on the prediction of the model, the highest TPC, ABTS and FRAP were 24.07 mg GAE g⁻¹, 88.97 mg TE g⁻¹ and 13.02 mg TE g⁻¹, respectively, with the optimum conditions (temperature of 30 °C, time of 60 min and power of 60%). The DPPH value was 46.44 mg TE g⁻¹ with these parameters (gained 96% of the maximum value of DPPH). Therefore, the conditions above were chosen as the optimal conditions for extracting the alga *H. banksii*. In addition, the experiments were performed under these optimal conditions to validate the adequacy of the model prediction. The statistical results showed that there was no significant difference between predicted and measured responses of TPC and antioxidant activities (ABTS, DPPH and FRAP) ($P > 0.05$; Table 2) and that the measured values of the responses were found to be well fitted to the ones predicted by the regression model. Therefore, these conditions were suggested to extract the high yield of TPC and antioxidant activities of *H. banksii* for further isolation and utilisation. In addition, these findings further confirmed the appropriateness of the models used for optimising the extraction conditions using UAE technique.

Comparison of the extraction efficacy between the conventional and UAE methods

Conventional extraction techniques are usually applied to extract bioactive compounds from the materials. Therefore, this study conducted conventional extraction to compare the efficacy versus ultrasonic extraction under the optimum conditions. The results showed that the ultrasonic technique was effective to obtain significantly higher levels of TPC and antioxidant capacity when compared to the conventional one ($P < 0.05$; Table 4). The level of TPC using the ultrasound was 142.6% higher than the one by the conventional extraction. In terms of antioxidant capacity, the values for ABTS, DPPH and FRAP using the ultrasonic method were also higher (166.8, 154.6 and 150.6%, respectively) in comparison with those of the conventional one. It should be noted that the time applied for ultrasonic extraction was only 1 h, whereas

for the conventional conditions, 12 h was required. This further confirmed the advantage of the ultrasonic method in term of the efficacy, quality of the extracts for extracting biologically active compounds.

Discussion

Figure 1a–c and Table 3 show the effect of extraction conditions (temperature, time and power of ultrasound) on the recovery yield of phenolic compounds from *H. banksii*. The higher temperature created the higher solvent diffusion rate and mass transfer, while lower in the solvent viscosity and surface tension made more polyphenols dissolve into the extraction medium (Hossain et al. 2012), so the extraction yield increased. However, there was the limitation in using high temperature for extracting active components of plants, especially algae, due to the effect of high temperature on thermosensitive compounds (Shahidi et al. 1992; Le Lann et al. 2008). On the other hand, it could be that a reduction of the cavitation by high temperature resulted in the decrease in extraction yield as well as TPC (Dey and Rathod 2013). It can be seen that low temperature was suitable for extracting phenolic compounds from *H. banksii*.

In terms of time for extracting, it was shown that there were two stages in the extraction process using UAE. The soluble components on surfaces of the seaweed matrix were dissolved in solvents (washing stage) and mass transfer of the solute from the seaweed matrix into the solvent through diffusion and osmotic processes (slow extraction stage) (Kadam et al. 2015). Therefore, time was also required enough for solutes dissolving in solvents. Topuz et al. (2016) stated that the increase in TPC value when the long time was applied for the extraction of the red seaweed *Laurencia obtusa* with time 58 min. However, time needed to be optimised due to the efficacy of extract and the degradation of phenolic compounds in the extract. Han et al. (2011) showed that the maximum ultrasound time for extracting phenolic compounds from *Saccharina (Laminaria) japonica* was 60 min, while exceeding this ultrasonic time could lead to a decrease in TPC level due to degradation of bioactive compounds by ultrasonic wave.

Regarding to ultrasonic power, previous studies also found that the application of higher ultrasonic power increased the recovery yield of TPC in different algal species. The increase in TPC when higher ultrasonic power was applied could be explained by more damage to the cell wall under higher ultrasonic power (Kadam et al. 2015). Furthermore, it is believed that ultrasonic power acts as a driving force for the dispersion of solvent into solid samples, resulting in the increase in extraction yield (Han et al. 2011). However, this study found that there was only a slight increase in TPC of the extract when higher ultrasonic power was applied. This could be because,

as ultrasonic power was boosted, there was an increase in the bubble numbers in the solvent during cavitation, leading to a reduction in the efficacy of ultrasound energy transmission into the medium (Filgueiras et al. 2000; Zhao et al. 2007) and/or a decrease in TPC caused by ultrasonic wave with high power (Han et al. 2011; Teh and Birch 2014). The results from the model illustrated that critical values of temperature, time and power of ultrasound for TPC of algal extraction were of 30 °C, 60 min and 60%, respectively. At these conditions, the TPC value obtained was about 24.07 mg GAE g⁻¹.

A rich source of antioxidants (phlorotannins, tocopherols, carotenoids, ascorbic acid, fatty acids, etc.) can be found in a wide range of algal species (Gupta and Abu-Ghannam 2011). In this study, antioxidant activity of the extract *H. banksii* was evaluated by three simple, fast and reliable biochemical assays (ABTS, DPPH and FRAP) (Matanjan et al. 2008; Frankel and Meyer 2000; Tanniou et al. 2013). The results showed that the highest ABTS, DPPH and FRAP of extract obtained with the low ultrasonic temperature and power (30 °C and 60%) and the long time (60 min), except for DPPH with the highest value at 46.15 min.

There was a positive relationship between TPC and antioxidant activities (ABTS, DPPH and FRAP) when the extracting conditions changed (Fig. 5). This means that phenolic compounds are the main contributors on the antioxidant activities of this alga. It was confirmed by the strong correlations between TPC and antioxidant activities (ABTS, DPPH and FRAP) of the extract *H. banksii* ($r^2 = 0.992$, 0.993 and 0.920, respectively) in our previous study (Dang et al. 2016). This finding is supported by Matanjan et al. (2008) who indicated that the phenolic compounds mainly contributed to FRAP of the methanolic extracts ($r^2 = 0.96$). Previous studies also illustrated the role of phenolic compounds in antioxidant activity based on the positive correlation between phenolic hydroxyl groups and DPPH free radical scavenge activity in several brown algae: *Fucus vesiculocus*, *Laminaria ochroleuca*, *Sargassum muticum*, *Bifurcaria bifurcata*, *Laminaria* sp. and *Undaria pinnatifida* (Jiménez-Escrig et al. 2001; Le Lann et al. 2008; Amorim et al. 2012). On the other hand, Charoensiddhi et al. (2015) reported that the low correlation between TPC and ORAC in *Ecklonia radiata* may imply that not only phlorotannins but also other organic compounds accounted for antioxidant activity of this alga. Martins et al. (2013) reported that both *Amansia* sp. and *Cryptonemia seminervis* presented the most relevant antioxidant potential through DPPH assay, but they did not find any significant correlation between the antioxidant activity of the extracts and total phenolic content. In addition, it was found that antioxidant activity from *Spatoglossum schroederi* was mainly related to the present of flavonoids (Júnior et al. 2015). It can be seen that except for phenolic compounds, some other active compounds such as pigments (carotenoids, fucoxanthin, etc.), fatty acid, vitamins A, E or some organic

compounds in algal extracts may also contribute to the antioxidant properties (Matanjan et al. 2008). Other experiments are in progress for isolation and purification of bioactive compounds and it was found that both pigments and phenolic compounds (phlorotannins) had significantly affected the antioxidant activity of *H. banksii* extract. However, phenolic compounds showed the main role in antioxidant activity of this extract due to higher amount of phenolic compounds and antioxidant capacity in comparison to other compounds.

In conclusion, this study demonstrated that response surface methodology was appropriate for optimising the algal extracts with the greatest of TPC and antioxidant activities of the alga *H. banksii* using ultrasonic-assisted extraction. Ultrasonic temperature had the strongest influence on TPC and antioxidant capacity, followed by ultrasonic time, and then power. The optimal UAE conditions for TPC yield and antioxidant capacity of *H. banksii* were temperature of 30 °C, time of 60 min and power of 60% (150 W). Under these conditions, TPC and antioxidant activities (ABTS, DPPH, FRAP) achieved were 23.12 mg GAE g⁻¹, 85.64 mg TE g⁻¹, 47.24 mg TE g⁻¹, 12.56 mg TE g⁻¹, respectively. The extraction efficacy using UAE was significantly higher compared to the conventional approach. From the findings, the conditions of extraction by UAE using RSM has potential to be applied for further isolation and purification of phenolic compounds from alga *H. banksii* and application of phenolic compounds of this alga in food and pharmaceutical fields.

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