Carbohydrate Polymers 106 (2014) 238–246

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Optimization of polysaccharides from Zagros oak leaf using RSM: Antioxidant and antimicrobial activities

Saeed Tahmouzi*

Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Ramin Agriculture and Natural Resources University, Mollasani, Ahvaz, Iran

ARTICLE INFO

Article history:
Received 4 January 2014
Received in revised form 6 February 2014
Accepted 8 February 2014
Available online 17 February 2014

Keywords:
Quercus brantii
Polysaccharide
Optimization
Antioxidant
Antimicrobial

ABSTRACT

Ultrasonic assisted-extraction technique was applied to extract the polysaccharide from Zagros oak (*Quercus brantii* Lindl). The effects of four independent factors (ultrasonic power (X_1: 150–300 W), extraction temperature (X_2: 50–90 °C), extraction time (X_3: 30–90 min), and the ratio of water to raw material (X_4: 15–45)) on the extraction yield of polysaccharide from the leaves of *Q. brantii* Lindl (QBLP) were optimized using response surface methodology. The experimental data obtained were fitted to a second-order polynomial equation. The optimal extraction conditions for QBLP were determined as follows: X_1: 205.8 W, X_2: 81.9 °C, X_3: 55.6 min and X_4: 23.4. Under these optimal conditions, the experimental yield was 19.42 ± 0.53%, which was well matched with the value predicted by the model 19.61%. The results indicated that polysaccharide has strong scavenging activities in vitro on DPPH and hydroxyl radicals. In addition, the QBLP showed good antimicrobial activity at 1.5–2.5 mg/mL.

1. Introduction

A variety of extraction techniques have been employed for the separation of valuable compounds from the plant materials. Conventionally, polysaccharides are extracted by heating reflux extraction. Nowadays, various novel extraction techniques have been developed for the extraction of polysaccharides from natural plants. Compared with other advanced extraction methods such as supercritical fluid extraction, ion-pair extraction, enzymatic extraction and microwave-assisted extraction, the ultrasonic extraction is rapid, cheaper, and its operation is much easier (Chen et al., 2008; Li, Chen, & Yao, 2005; Wang & Weller, 2006). The principles of ultrasonic treatment include thermal and mechanical effects and cavitation, which greatly facilitate mass transfer between immiscible phases through a super agitation (Vinatoru et al., 1997).

Quercus brantii, commonly known as oaks, belongs to the family *Fagaceae*. It is regarded as being a species well adapted to dry and low-temperature climates and is found in the mixed pine-oak forests in Zagros, Iran (Luna-José, Montalvo-Espinosa, & Rendón-Aguilar, 2003). Oaks have spirally arranged leaves, with lobate margins in many species. *Q. brantii* extract has been reported to have great hepatoprotective, anti-tumor and anti-aging activity, may be related to the antioxidant capacity of their constituents and effect on expression of CYP2E1 (Rivas-Arreola et al., 2011; Rocha-Guzmán et al., 2009). It has been also reported to have anti-inflammatory activity after oral or topical administration (Almeida, Fernandes, Lima, Costa, & Bahia, 2008).

Polysaccharides are polymeric carbohydrate molecules that can be extracted under appropriate conditions (Lin & Lai, 2009). Isolated polysaccharides from plentiful of plants are utilized to great extent in food processing, as: thickening or gel-setting agents; stabilizers for emulsions and dispersions; film-forming, coating substances to protect sensitive food from undesired change; and inert fillers to increase the proportion of indigestible ballast substances in a diet (Lai & Lin, 2004; Salazar-Montoya, Ramos-Ramirez, & Delgado-Reyes, 2002).

In recent years, ultrasonic-assisted extraction technique has been developed for the extraction of polysaccharides from natural plants (Zhong & Wang, 2010).

Some diseases such as cancer, atherosclerosis and rheumatoid, can be directly induced by oxygen-derived free radicals (•OH), while the radical scavenging activity is one of the important functional properties for bioactive compounds (Yang, Zhao, Shi, & Yang, 2008). The radical scavenging activity and inhibition zone formation are often used to evaluate the capacity of antioxidant

* Correspondence to: Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Ramin Agriculture and Natural Resources University, Mollasani, Ahvaz, Iran. Tel.: +98 9124374860.
E-mail addresses: saeed_tahmouzi@yahoo.com, stahmouzi@gmail.com

http://dx.doi.org/10.1016/j.carbpol.2014.02.028
0144-8617/

and antimicrobial compounds, respectively (Prior & Cao, 1999). Recent studies demonstrated many plant polysaccharides have great antioxidant activities and should be paid more attention to exploring them as novel potential antioxidants (Ramarahnam, Osawa, Ochi, & Kawaishi, 1995; Wang, Chen, Jia, Tang, & Ma, 2012; Xu et al., 2009).

Response surface methodology (RSM) is a collection of empirical and mathematical techniques used to evaluate the interactions between multiple parameters and optimize processes (Zhong, Lin, Wang, & Zhou, 2012). This procedure reduces the number of experimental trials and overall cost (Myers & Montgomery, 2002).

To the best of our knowledge, there were no reports available in the literature regarding the optimization of ultrasonic-assisted extraction of polysaccharide from the leaves of *Q. brantii* by response surface methodology. In this work, the ultrasonic-assisted extraction variables (ultrasonic power, extraction temperature, extraction time and ratio of water to raw material) of polysaccharide (QBLP) (%) from the leaves of *Q. brantii* was firstly investigated and optimized using a BBD (4 factors and 3 levels). Antioxidant and antimicrobial activities of QBLP were investigated by various in vitro assays.

2. Materials and methods

2.1. Materials

The leaves of *Q. brantii* were collected from Kermanshah province, Iran, washed, freeze-dried, and powdered for this study. All other chemicals and solvents used were of analytical grade and obtained from Merck Co., Germany.

2.2. Ultrasonic extraction of crude polysaccharides from Q. brantii leaves

The extraction of polysaccharides from *Q. brantii* leaf was conducted by the method of Pan et al. (2010) with some modifications. The powder of *Q. brantii* leaf was firstly extracted with 80% ethanol at 60 °C in a water bath to deactivate the endogenous enzymes and remove interference components, including free sugars, amino acids and polyphenols in the sample. The protein contaminations were removed as described previously (Matthaei, Jones, Martin, & Nirenberg, 1962). Protein content in crude polysaccharide was determined according to the method of Bradford. It was lower than 0.8 g/100 g crude (SD = 0.03). Therefore, the interference of protein on the experiment was inconsiderable. The ethanolic mixture extract was centrifuged (7000 × *g*, 15 min at 20 °C) (PM180R, ALC International, Milan, Italy). The ethanol extraction was washed by precipitation using 85% ethanol and acetone, respectively. The combined extract was collected and freeze-dried (MCFD5505, SIM, Newark, DE, USA), and it was suspended in the water and sonicated with ultrasonic bath at the temperature of 50–90 °C, the ratio of water to raw material 15–45 and ultrasound power of 150–300 W for 30–90 min. The QBLP were weighted with a balance (AUY 220, Shimadzu, Japan). The percentage polysaccharide yield (%) is calculated as follows:

$$\text{QBLP extraction yield} \quad \% \quad (\text{w/w}) = \frac{S_0}{S} \tag{1}$$

So (g) is the dried QBLP weight; S (g) is the dried powder of *Q. brantii* leaf weight.

2.3. Experimental design

To determine the effect of ultrasonic power, extraction temperature, extraction time, and the ratio of water to raw material on the extraction yield of polysaccharide from the leaves of *Q. brantii* Lindl, response surface methodology was applied with a BBD design. This design led to studying the effects of four factors in a single block of 29 sets of test conditions (Tables 1 and 2). Statistical analysis was performed with the software package 'Design-Expert version 7.0.' Quadratic polynomial model was defined to fit the response (polysaccharide yield %):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4 \tag{2}$$

where β_0 is a constant coefficient of the models. The regression coefficients (β_1, β_2, β_3 and β_4), (β_{11}, β_{22}, β_{33} and β_{44}) and (β_{12}, β_{13}, β_{14}, β_{23}, β_{24} and β_{34}) respectively represent linear, quadratic and interaction effects of the model estimated by multiple regression analysis. X_1 (ultrasonic power), X_2 (extraction temperature), X_3 (extraction time) and X_4 (the ratio of water to raw material) are coded variables ranging from −1 to +1.

Table 1
Independent variables and their levels used in the response surface design (BBD).

Levels	Variables			
	Ultrasonic power (W)	Temperature (°C)	Extraction time (min)	Ratio of water to raw material
−1	150	50	30	15
0	225	70	60	30
+1	300	90	90	45

2.4. Antioxidant activities

2.4.1. Assay of hydroxyl radicals scavenging activity

The hydroxyl radical-scavenging activity of QBLP was measured by the method of Xiao et al. (2012). Polysaccharides were dissolved in deionized water to form final concentrations of 0.5, 1,

Table 2
Experimental results of the response surface methodology.

Trial	X_1 (W)	X_2 (°C)	X_3 (min)	X_4	Extraction yield (%)	
					Experimental	Predicted
1	225	70	60	30	19.08	19.38
2	150	70	60	15	16.43	16.57
3	225	70	30	15	13.67	13.8
4	150	70	60	45	9.56	9.87
5	150	70	30	30	12.96	12.82
6	225	70	90	45	8.65	8.53
7	150	70	90	30	9.14	9.23
8	300	90	60	30	19.08	19.22
9	225	70	60	30	19.40	19.43
10	225	50	90	30	6.44	6.55
11	225	50	30	30	6.70	6.93
12	225	50	60	15	9.56	9.73
13	300	70	90	30	17.66	17.54
14	225	90	90	30	12.12	12.3
15	300	70	60	15	18.45	18.54
16	300	50	60	30	13.12	13.14
17	225	70	90	15	9.64	9.56
18	300	70	30	30	16.38	16.32
19	225	90	60	15	18.18	18.1
20	150	50	60	30	8.34	8.2
21	300	70	60	45	16.08	16.14
22	225	70	30	45	9.73	9.88
23	225	70	60	30	18.65	18.89
24	150	90	60	30	15.32	15.18
25	225	70	60	30	19.35	19.43
26	225	50	60	45	8.43	8.7
27	225	90	30	30	14.37	14.01
28	225	90	60	45	13.18	13.15
29	225	70	60	30	18.69	18.88

1.5, 2, 2.5 and 3 mg/mL, respectively.1 ml of sample solution was mixed with 2 ml 0.05 M reaction buffer (pH 7.4), 1.5 mL 5 mM 1,10-phenanthroline, 1 mL 7.5 mM $FeSO_4$ and 1 ml H (0.1%) were added into a tube. After incubation at room temperature for 20 min, the absorbance of the mixture was measured at 536 nm, vitamin C as a positive control. The scavenging activity of hydroxyl radicals was expressed as following:

$$\text{Scavenging ability}\ (\%) = \frac{A_0 - A_1}{A_0} \times 100\,(\%) \quad (3)$$

A_0 and A_1 are the absorbance of control (without sample) and QBLP, respectively.

2.4.2. Assay of DPPH radical scavenging activity

The DPPH radical scavenging activity of QBLP was investigated according to the method based on Lai, Wen, Li, Wu, and Li (2010). Briefly, different volumes of the QBLP solution with different concentrations (0.5, 1, 1.5, 2, 2.5 and 3 mg/mL) were added to 2 ml DPPH solution and the final reaction volume was made up to 4 mL with 70% ethanol. After shaking vigorously, the mixture was incubated at 25 °C in the dark for 30 min. The absorbance was measured at 517 nm by ultraviolet–visible spectrometer. The same procedure was repeated with the synthetic antioxidant, butylated hydroxytoluene (BHT), as a positive control. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The ability to scavenge the DPPH radical was calculated according to the following equation:

$$\text{Scavenging effect}\ (\%) = \frac{B_0 - B_1}{B_0} \times 100\ (\%) \quad (4)$$

B_0 and B_1 are the absorbance of control (without sample) and QBLP, respectively.

2.5. Antimicrobial activity

Antimicrobial activity of QBLP was measured by the filter disk diffusion plate method with a few modification (Xie et al., 2012). Bacteria (*Salmonella typhi* and *Staphylococcus aureus*), yeast (*Candida albicans*) and fungi (*Penicillium citri*) were purchased as test organisms. After the inoculum solution (100 μL) of each test organism containing 10^6 cells/mL was added to the medium, filters (5 mm in diameter and 1.5 mm thick) containing samples (0.5, 1, 1.5, 2, 2.5 and 3 mg/ml) were placed in the center of the plate after the top agar had solidified. Nutrient agar served as a control. Bacterial plates were incubated at 37 °C for 24 h, while yeast and fungi plates were incubated at 28 °C for 48 h. The antimicrobial activity was assayed with calipers and expressed by the diameter (mm) of the inhibition zones.

2.6. Statistical analysis

All the data were shown as the means ± standard deviations of three parallel measurements and the data of RSM were analyzed using SAS (SAS Institute Inc., Cary, NC, USA). Results were evaluated by ANOVA, *p*-values of less than 0.05 were considered to be statistically significant.

3. Results and discussion

3.1. Extraction yield of QBLP

3.1.1. Effect of ultrasonic power on extraction yield of QBLP

To investigate the influence of different ultrasonic powers on extraction yield of QBLP, extraction process was carried out using the different powers of 100, 125, 150, 175, 200, 225, 250, 275 and 300 W (Fig. 1a). The extraction temperature, extraction time and the ratio of water to the raw material were fixed at 70 °C, 60 min and 30, respectively. As shown in Fig. 1a, the maximum extraction yield of QBLP was observed when the ultrasonic power was 300 W. This was agreed closely with reports of other authors in extracting polysaccharides (Yan et al., 2011). Although the extraction yield of polysaccharides was also high at 300 W, increasing ultrasound power will bring about the increase in cost for the extraction process from the industrialization point of view. Therefore, 100–300 W

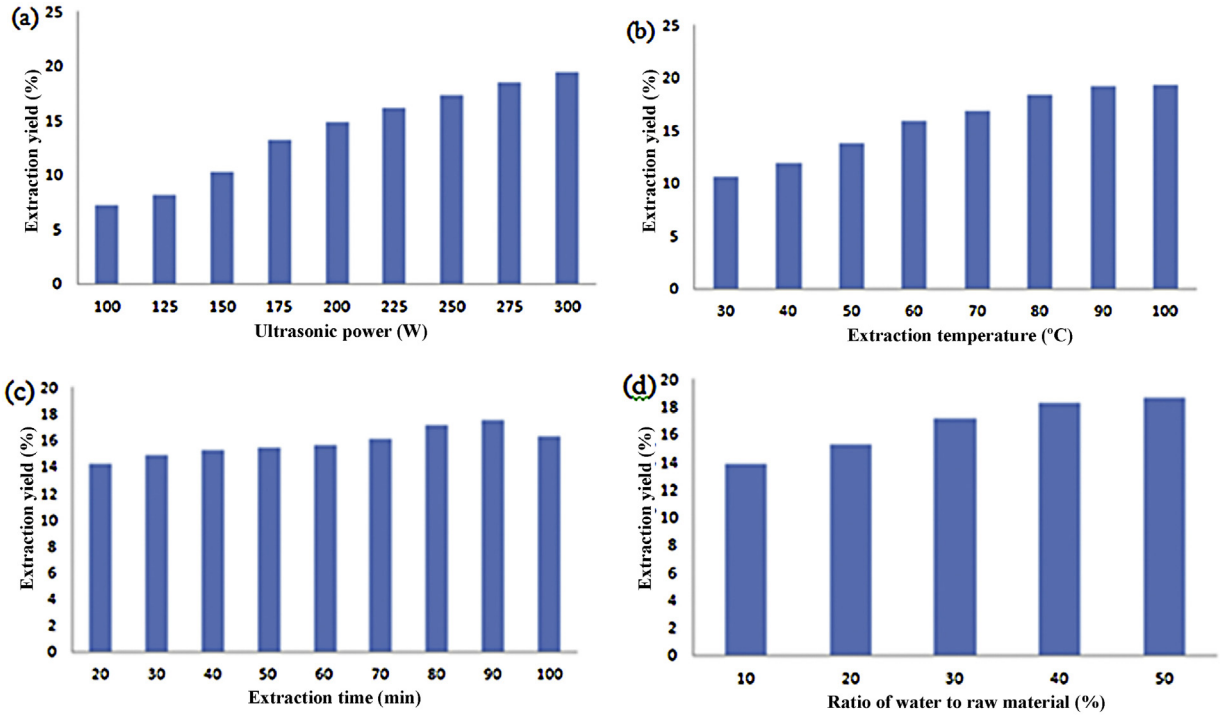

Fig. 1. Effects of ultrasonic power (a), extraction temperature (b), extraction time (c), and the ratio of water to raw material (d) on the extraction yield of *Q. brantii* leaf (%).

was considered in the present study. The increase of the polysaccharides diffusion coefficient and the enhanced solubility of the polysaccharides in the extracting solvent at higher powers caused the increase of the polysaccharides mass going out from the oak leaves into the solution. The extraction coefficient increased with increasing the ultrasonic power due to the increase of the polysaccharides solubility.

3.1.2. Effect of extraction temperature on extraction yield of QBLP

Extraction temperature is one of the important variables affecting the extraction yield of QBLP and it is necessary to select an optimum extraction temperature to assure the maximum extraction of QBLP by ultrasonic-assisted extraction; other factors were set as follows: ultrasonic power 225 W, extraction time 70 min and the ratio of water to the raw material 30. From the results, it is observed that, the extraction yield was increased and reached the maximum until 90 °C and then declined (Fig. 1b). Similar results were observed in the extraction of polyphenols from orange peel (Luengo, Álvarez, & Raso, 2013). Therefore, extraction temperature of 50–90 °C was investigated in the present work.

3.1.3. Effect of extraction time on extraction yield of QBLP

Different extraction time was set at 20, 30, 40, 50, 60, 70, 80, 90 and 100 min to evaluate the effect of extraction time on the extraction yield of QBLP when the other extraction conditions were set as follows: ultrasonic power 225 W, extraction temperature 70 °C and the ratio of water to the raw material 30 (Fig. 1c). Fig. 1c indicates that the extraction yield of QBLP was correlated with the increase of extraction time, and it reached 17.48 ± 0.5% when the extraction time was 90 min (Eskilsson, Davidsson, & Mathiasson, 2002). When extraction time was higher than 90 min, the yield declined with the further increase of time. These data suggest that applying a higher extraction time may lead to degradation of the QBLP. Therefore, extraction time of 90 min was adopted in the present study.

3.1.4. Effect of the ratio of water to the raw material on extraction yield of QBLP

The effect of the ratio of water to the raw material (10, 20, 30, 40 and 50) on the extraction yield of QBLP were investigated when the other three factors (ultrasound power, extraction time and extraction temperature) were fixed at 225 W, 60 min and 70 °C. Fig. 1d shows the ratio of water to the raw material had a significant effect on the extraction yield of QBLP. When the ratio was increased from 10 to 50, the extraction yield of QBLP increased significantly. The result implied the extraction yield of QBLP was significantly increased to the value (18.32%) at the ratio of 40. In this study, the extraction yield of QBLP decreased after the ratio of water to the raw material 45. Thus, 45 was chosen as the optimum ratio of water to the raw material.

According to the single-parameter study, it was adjusted ultrasound power 150–300 W, extraction temperature 50–90 °C, extraction time 30–90 min and the ratio of water to the raw material 15–45 for response surface methodology experiments.

3.2. Model fitting and adequacy checking

The influences of four ultrasonic-assisted extraction conditions, including ultrasonic power, extraction temperature, extraction time, and the ratio of water to raw material on the yield of QBLP were evaluated using Box–Behnken design (Table 2). A second order polynomial equation was applied to build a mathematical model to find the optimum conditions that maximize the extraction yield of QBLP and study the combined relationships between the independent variables and the response. The developed second-order model in term of coded variable is given below:

$$Y = 19.20 + 2.42X_1 + 3.23X_2 - 0.78X_3 - 1.61X_4 + 1.20X_1X_3 + 1.08X_1X_4 - 0.98X_2X_4 - 4.09X_2^2 - 5.14X_3^2 - 3.26X_4^2 \quad (5)$$

where X_1 is ultrasonic power (W), X_2 extraction temperature (°C), X_3 extraction time (min), X_4 the ratio of water to raw material and Y yield of QBLP (%), respectively.

Table 3
ANOVA for response surface quadratic model analysis of variance.

Source	CE[a]	SS[b]	DF[c]	SE[d]	MS[e]	*F* value	*p*-value
Model	19.20	516.53	14	0.40	36.90	46.19	<0.0001
X_1	2.42	70.23	1	0.26	70.23	87.93	<0.0001
X_2	3.23	124.87	1	0.26	124.87	156.34	<0.0001
X_3	−0.78	7.25	1	0.26	7.25	9.08	0.0093
X_4	−1.61	31.07	1	0.26	31.07	38.90	<0.0001
X_{12}	−0.22	0.20	1	0.45	0.20	0.25	0.6224 ns
X_{13}	1.20	5.78	1	0.45	5.78	7.24	0.0176
X_{14}	1.08	4.62	1	0.45	4.62	5.79	0.0305
X_{23}	−0.33	0.44	1	0.45	0.44	0.55	0.4691 ns
X_{24}	−0.98	3.84	1	0.45	3.84	4.81	0.0457
X_{34}	0.54	1.18	1	0.45	1.18	1.47	0.2448 ns
X_1^2	−0.64	2.67	1	0.35	2.67	3.35	0.0888 ns
X_2^2	−4.09	108.34	1	0.35	108.34	135.64	<0.0001
X_3^2	−5.14	171.66	1	0.35	171.66	214.92	<0.0001
X_4^2	−3.26	68.80	1	0.35	68.80	86.14	<0.0001
Residual		11.18	14		0.80		
Lack of fit		9.76	10		1.08	12.88	0.056
Pure error		0.34	4		0.084		
Cor total		527.71	28				
SD		0.89		R^2		0.978	
Mean		13.77		Adj-R^2		0.957	
C.V. (%)		6.49		Pred-R^2		0.880	
Press		62.99		Adequate precision		21.14	

ns: not significant.
[a] Coefficient estimate.
[b] Sum of squares.
[c] Degree of freedom.
[d] Standard error.
[e] Mean square.

Table 3 summarized the results of the ANOVA for the experimental results of the BBD. The analyses of variance were used to determine the coefficient of determination, lack of fit and the significance of the linear, quadratic and interaction effects of the independent variables on the response.

The significance of coefficient was tested using the *p*-value in Table 3. The corresponding variables become more effective as the *p*-value becomes smaller. Also, the *p*-value can be employed to check the interaction strength between independent factors (Muralidhar, Chirumamilla, Ramachandran, Marchant, & Nigam, 2001). The R^2_{adj} and R^2 for Eq. (5) were 0.957 and 0.978 respectively, indicating a better degree of correlation between experimental and theoretical values of the QBLP yield (Zhong & Wang, 2010). Low values of coefficient of variance (6.49) clearly indicated that the model was reproducible and reliable (Mason, Gunst, & Hess, 1989; Prakash Maran, Mekala, & Manikandan, 2013). From the analysis, the *F*-value of 46.19 and *p*-value <0.0001 indicates the response surface quadratic model was significant. Furthermore, results of the ANOVA indicated that the lack of fit was insignificant.

3.3. Optimization of extraction conditions of QBLP

Independent variables were optimized by RSM using BBD to obtain the maximum extraction yield of QBLP (Prakash Maran, Manikandan, Vigna Nivetha, & Dinesh, 2013). The aim of optimization was to find out the ultrasonic-assisted extraction conditions which give the maximum predicted yield of QBLP (Table 4).

In Fig. 2a, when the plots were developed for the extraction yield of QBLP with different ultrasonic power and extraction temperature at fixed extraction time 60 min and the ratio of water to the raw material 30. The yield of QBLP was increasing evidently as the increasing of ultrasonic power and extraction temperature. Maximum yield of QBLP can be achieved when ultrasonic power and extraction temperature were around 222 W and 68 °C, respectively.

In Fig. 2b, when the plots were developed for the extraction of QBLP with varying ultrasonic power and extraction time at fixed extraction time 70 °C and the ratio of water to the raw material 30, it can be seen that maximum recovery of QBLP can be achieved when ultrasonic power and extraction time were 295 W and 60 min, respectively. Fig. 2c shows the effect of the ultrasonic power and the ratio of water to the raw material on the extraction yield of QBLP at a fixed extraction temperature of 70 °C and extraction time of 60 min.

The plots in Fig. 3d, which give the extraction yield of QBLP as a function of extraction temperature and extraction time at fixed ultrasonic power 225 W and the ratio of water to raw material 30, indicated that the extraction yield of QBLP increased rapidly with the increasing of the extraction temperature from 70 to 90 °C, and the extraction yield of QBLP was found to increase with the increase of extraction time from 75 to 90 min.

Fig. 3e shows the plots at a different extraction temperature and the ratio of water to raw material at fixed extraction time 60 min and ultrasonic power 225 W. The extraction yield of QBLP increased rapidly within the extraction temperature from 80 to 90 °C, and the yield extraction of QBLP increased with the increase of the ratio of water to raw material from 37 to 45.

Fig. 3f showed the plots at varying extraction time and the ratio of water to raw material at a fixed extraction temperature 70 °C and ultrasonic power 225 W. It can be observed that maximum extraction yield of QBLP could be achieved when the extraction time and the ratio of water to raw material were 70 min and 30, respectively.

The maximum yield of QBLP and the optimum extraction conditions were obtained from desirability function approach was ultrasonic power of 205.8 W, extraction temperature of 81.9 °C, extraction time of 55.6 min, ratio of water to raw material was 23.4 and the model predicted a maximum response of 19.61% with a desirability value of 0.982.

3.4. Validation of the model

To validate the adequacy of the model equation, six confirmation experiments were conducted under the optimized conditions (ultrasonic power of 205.8 W, extraction temperature of 81.9 °C, extraction time of 55.6 min, ratio of water to raw material was 23.4), and then responses were measured. Extraction yield of QBLP was obtained as 19.42 ± 0.53% (which is the same as that predicted using RSM (19.61)). This value is very close to the corresponding predicted value (Table 4). The results indicated that the RSM approach was effective for optimizing the conditions for the extraction of QBLP from Zagros oak, and also suggested that the regression model was accurate and adequate for the extraction of QBLP.

3.5. Antioxidant activity

3.5.1. Scavenging activity of hydroxyl radicals

Hydroxyl radicals are the highly toxic species, can react with almost all the biological molecules by setting of free radical chain reactions (Rollet-Labelle et al., 1998). These radicals are very dangerous to organisms. Therefore, the removal of hydroxyl radical is important for antioxidant defense in cell or food systems. Polysaccharides from leaves represent one of the most promising classes of antioxidants for inhibiting oxidative reaction in foods and living systems due to their wide availability and low toxicity. Some studies have explained that polysaccharides isolated from the oak have considerable bioactivities, such as anticoagulant and hydroxyl radical scavenging abilities. Their activities are closely related to the presence of polyanionic charges (Rivas-Arreola et al., 2011; Xiao et al., 2012). The antioxidant activity not only depended on the plant origin but also on the molecular weight, chemical structure and arrangement of the active polysaccharide. Complicated carbohydrates, such as polysaccharide–peptide combinations, have also shown a potent antioxidant capacity (Li et al., 2012). QBLP was found to have the ability to scavenge hydroxyl radicals at concentrations between 0.5 mg/mL and 3 mg/mL. As shown in Fig. 4a, the scavenging activity of QBLP on hydroxyl radicals was in a concentration-dependent manner. The scavenging capacity of QBLP on hydroxyl groups was higher than positive control vitamin C. The scavenging ability of QBLP is strong at higher concentration, with 93% at the concentration of 2.8 mg/mL, but decline quickly with lower concentration. For hydroxyl radical scavenging of QBLP, there are two models of antioxidation mechanisms: one suppresses the generation of hydroxyl radical, and the other scavenges the hydroxyl radicals produced (Qi et al., 2006). Finally, antioxidant value was expressed as IC_{50}, the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial radical concentration IC_{50} was derived from the % disappearance versus

Table 4
Predicted and experimental values of the responses at optimum conditions.

Optimum condition				Extraction yield (%)		
Ultrasonic power (W)	Temperature (°C)	Extraction time (min)	Ratio of water to raw material	Experimental	Predicted	Desirability
205.8	81.9	55.6	23.4	19.42 ± 0.53	19.61	0.982

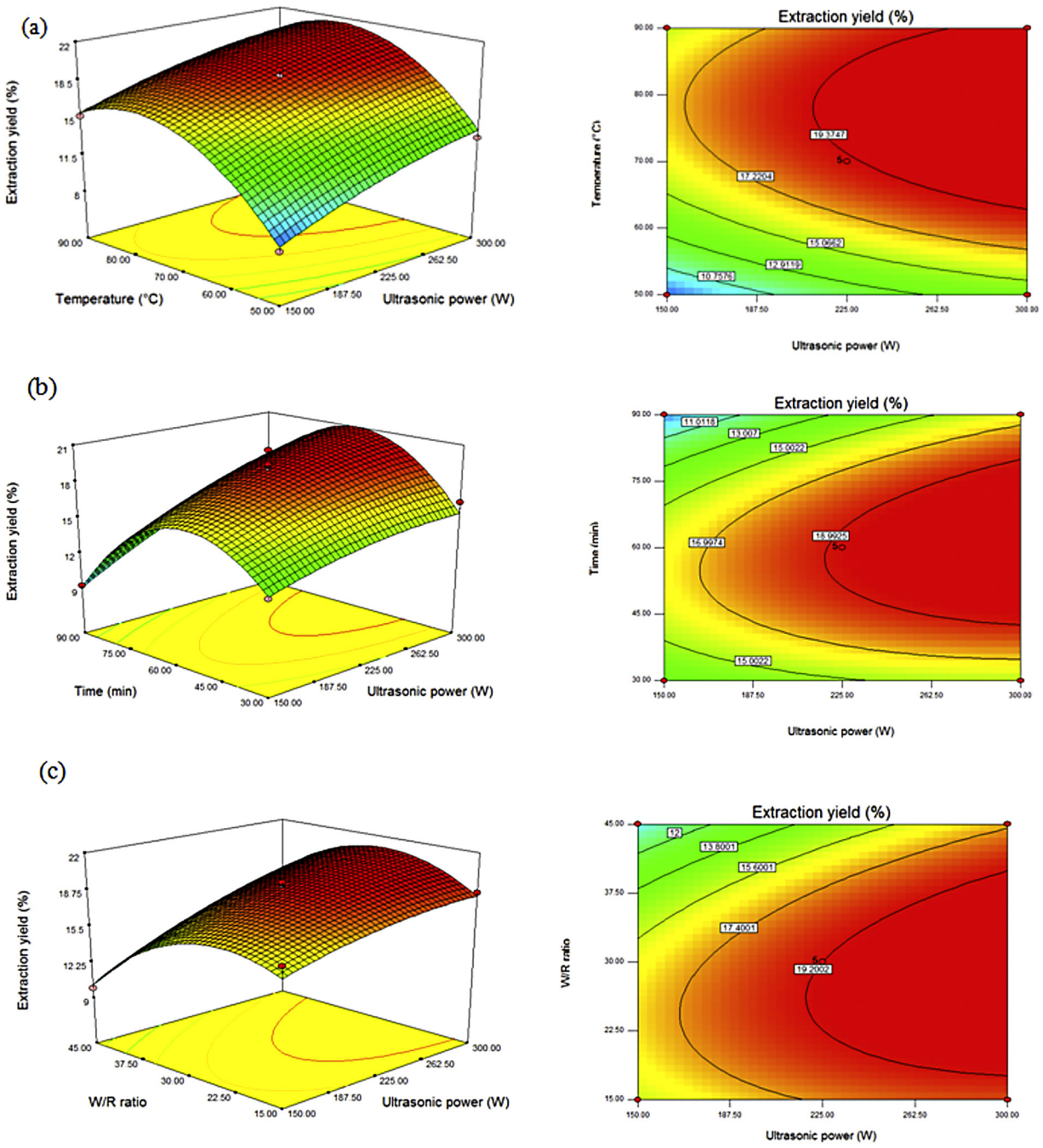

Fig. 2. Effect of (a) ultrasonic power and extraction temperature, (b) ultrasonic power and extraction time and (c) ultrasonic power and the ratio of water to raw material on extraction yield of *Q. brantii* leaf.

concentration plot. Half-effective inhibition concentrations of QBLP for scavenging of hydroxyl radical was lower (0.52 ± 0.01 mg/mL) than vitamin C (0.89 ± 0.09 mg/mL) as positive control. Results suggest that QBLP has an excellent scavenging capacity on hydroxyl radicals.

3.5.2. DPPH scavenging activity

The DPPH free radical is a stable free radical, which has been widely applied as a tool for measuring the free-radical scavenging capacity of the antioxidants (Ionita, 2005). Oak extract has been reported to have good DPPH scavenging ability, may be related to the antioxidant activity of their constituents and influence on expression of CYP2E1 (Rivas-Arreola et al., 2011; Rocha-Guzmán et al., 2009). The present work revealed the DPPH scavenging capacity of QBLP. Hence, the mechanism may be due to the supply of hydrogen by polysaccharides from oak leaf, which combines with radicals and it forms a stable radical to terminate the radical chain reaction (Lai et al., 2010). The change of concentration of QBLP was monitored to investigate the antioxidant effect of polysaccharide through the DPPH scavenging ability test. Fig. 4b presents the results. BHT was used as a positive control. As shown in Fig. 4b, QBLP exhibited notable radical scavenging activity, and the DPPH scavenging effects were increased by increasing concentration of QBLP. Compared with BHT, QBLP showed a higher degree of nitrogen

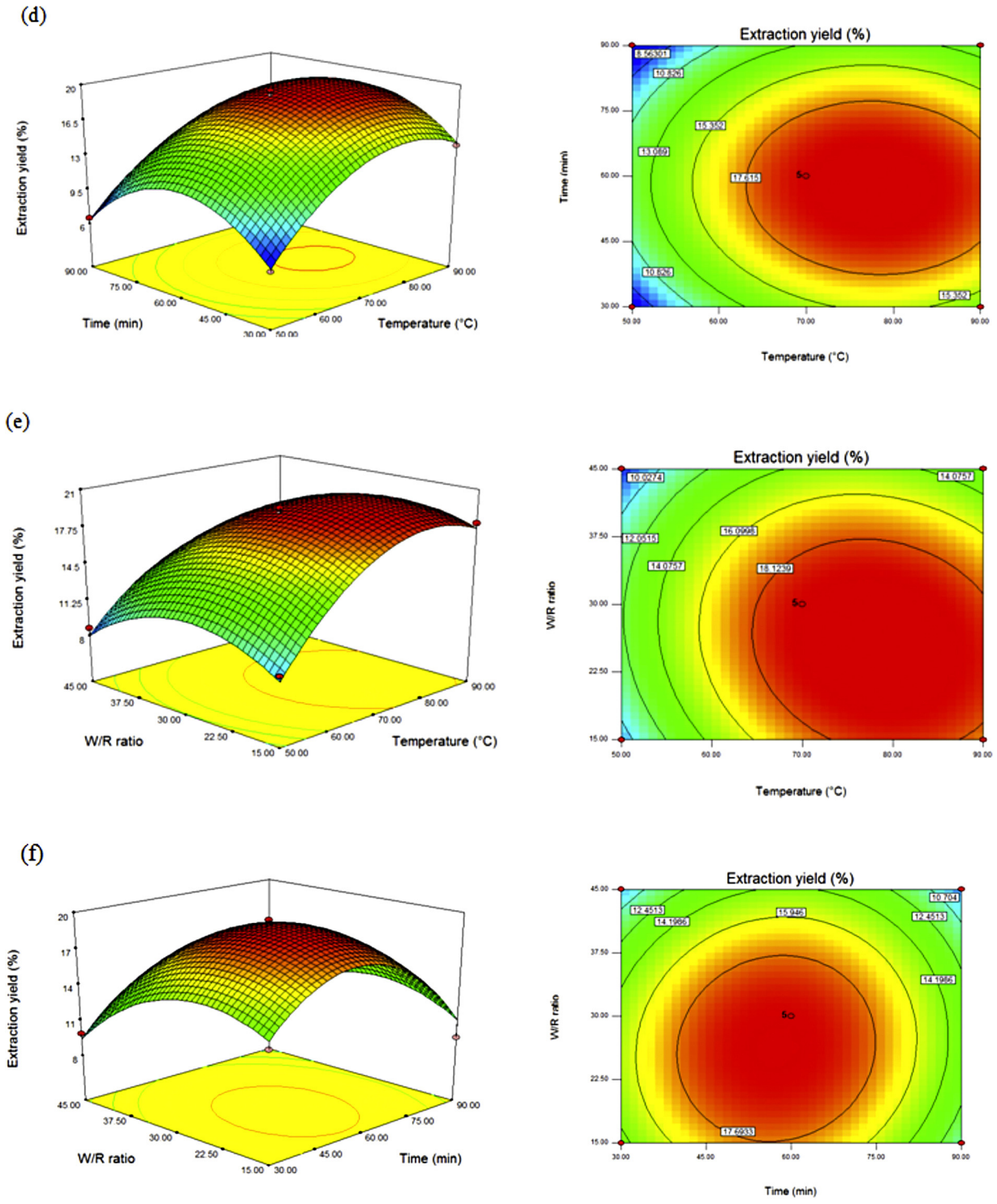

Fig. 3. Effect of (d) extraction temperature and extraction time, (e) extraction temperature and the ratio of water to raw material and (f) extraction time and the ratio of water to raw material on extraction yield of *Q. brantii* leaf.

radical-scavenging activity under the same conditions. The possible mechanism of QBLP might be explained to the supply of hydrogen (Lai et al., 2010). At 2.8 mg/mL concentration, QBLP was observed to possess obviously ($p < 0.05$) higher (94%) free radical-scavenging activity when compared to BHT (64%), suggesting and presents that has stronger DPPH radical-scavenging activity. In general, QBLP showed higher antioxidant activity ($IC_{50} = 0.41 \pm 0.02$ mg/mL) when compared to BHT ($IC_{50} = 1.15 \pm 0.10$ mg/mL).

3.6. Antimicrobial activity

Antimicrobial activity of QBLP against four microorganisms is showed in Fig. 4c. Of all selected organism, the QBLP were found to be the most effective against *C. albicans*, following by bacteria and *P. citri*. QBLP indicated the antimicrobial activities against *S. typhi*, *S. aureus*, *C. albicans* and *P. citri* with diameters of the inhibition zones of 7.92 mm, 8.64 mm, 10.76 mm and 6.51 mm, respectively.

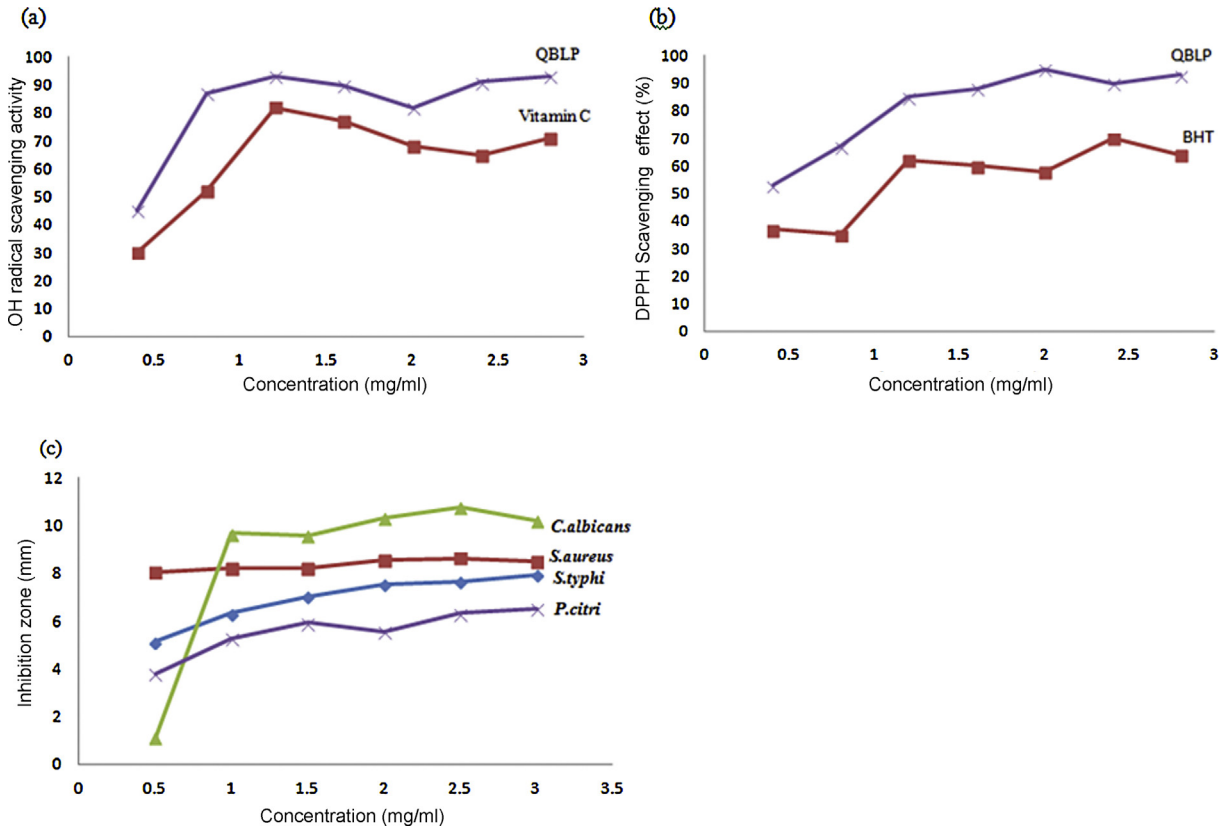

Fig. 4. scavenging effect of QBLP on (a) hydroxyl radicals and (b) DPPH radicals compared with that of vitamin C and butylated hydroxytoluene (BHT). (c) Antimicrobial activity of QBLP on *S. typhi*, *S. aureus*, *C. albicans* and *P. citri*.

However, QBLP had no inhibitory activity on *C. albicans* at a concentration of 0.5 mg/mL. The minimum inhibitory concentrations of QBLP were 1.5 (*S. typhi*), 1 (*S. aureus*), 2 (*C. albicans*) and 1.5 (*P. citri*) mg/mL. As the concentration increasing, the antimicrobial activities of QBLP increased significantly (He, Yang, Yang, & Yu, 2010). The mechanisms involved in antimicrobial activity of polysaccharide are worthy further investigation.

4. Conclusion

In this study, we had investigated an ultrasonic-assisted technique to extract polysaccharides from the oak (*Q. brantii* Lindl.) leaf via response surface methodology. Extraction temperature and ultrasonic power were the most important variables on the experimental yield of polysaccharides. A second-order using polynomial model was used to optimize polysaccharides extraction from *Q. brantii leaf* by ultrasonic-assisted method. The optimal extraction conditions were performed as following: ultrasonic power 205.8 W, extraction temperature 81.9 °C, extraction time of 55.6 min and the ratio of water to raw material of 23.4. Under the optimized conditions, the experimental yield of QBLP was 19.42%, which was closely agreed with the predicted yield (19.61%). The scavenging ability of QBLP was strong at higher concentration, with 93% at the concentration of 2.8 mg/mL. The DPPH scavenging effects of oak extract were increased by increasing concentration of QBLP. The extract of oak leaf indicated the antimicrobial activities against *S. typhi*, *S. aureus*, *C. albicans* and *P. citri* with different diameters of the inhibition zones. In conclusion, polysaccharide from *Q. brantii* leaf could be employed as potential natural antioxidant and antimicrobial in foods and drugs manufacturing.

Acknowledgement

The author wishes to thank Ramin Agricultural and Natural Resources University for scientific and financial supports of this research (Project No: 922/60).

References

Almeida, I. F., Fernandes, E., Lima, J. L. F. C., Costa, P. C., & Bahia, M. F. (2008). Protective effect of *Castanea sativa* and *Quercus robur* leaf extracts against oxygen and nitrogen reactive species. *Journal of Photochemistry and Photobiology B: Biology*, *91*(1), 87–95.

Chen, J. H., Wang, F. M., Liu, J., Lee, F. S. C., Wang, X. R., & Yang, H. H. (2008). Analysis of alkaloids in *Coptis chinensis* Franch by accelerated solvent extraction combined with ultra performance liquid chromatographic analysis with photodiode array and tandem mass spectrometry detections. *Analytica Chimica Acta*, *613*(2), 184–195.

Eskilsson, C. S., Davidsson, R., & Mathiasson, L. (2002). Harmful azo colorants in leather: Determination based on their cleavage and extraction of corresponding carcinogenic aromatic amines using modern extraction techniques. *Journal of Chromatography A*, *955*, 215–227.

He, F., Yang, Y., Yang, G., & Yu, L. J. (2010). Studies on antibacterial activity and antibacterial mechanism of a novel polysaccharide from *Streptomyces virginia* H03. *Food Control*, *21*(9), 1257–1262.

Ionita, P. (2005). Is DPPH stable free radical a good scavenger for oxygen active species? *Chemical Papers*, *59*(1), 11–16.

Lai, F., Wen, Q., Li, L., Wu, H., & Li, X. (2010). Antioxidant activities of water-soluble polysaccharide extracted from mung bean (*Vigna radiata* L.) hull with ultrasonic assisted treatment. *Carbohydrate Polymers*, *81*(2), 323–329.

Lai, L. S., & Lin, P. H. (2004). Application of decolourised hsian-tsao leaf gum to low-fat salad dressing model emulsions: A rheological study. *Journal of the Science of Food and Agriculture*, *84*(11), 1307–1314.

Li, H., Chen, B., & Yao, S. (2005). Application of ultrasonic technique extracting chlorogenic acid from *Eucommia ulmodies* Oliv. (*E. ulmodies*). *Ultrasonics Sonochemistry*, *12*, 295–300.

Li, N., Li, L., Fang, J. C., Wong, J. H., Ng, T. B., Jiang, Y., et al. (2012). Isolation and identification of a novel polysaccharide-peptide complex with antioxidant, anti-proliferative and hypoglycaemic activities from the abalone mushroom. *Bioscience Reports*, *32*, 221–228.

Lin, H. Y., & Lai, L. S. (2009). Isolation and viscometric characterization of hydrocolloids from mulberry (*Morus alba* Linn.) leaves. *Food Hydrocolloids*, *23*, 840–848.
Luengo, E., Álvarez, I., & Raso, J. (2013). Improving the pressing extraction of polyphenols of orange peel by pulsed electric fields. *Innovative Food Science & Emerging Technologies*, *17*, 79–84.
Luna-José, A. D. L., Montalvo-Espinosa, L., & Rendón-Aguilar, B. (2003). Los usos no Le nosos de los encinos en México. *Boletin Sociedad Botanica de Mexico*, *72*, 107–117.
Mason, R. L., Gunst, R. F., & Hess, J. L. (1989). *Statistical design and analysis of experiments: With applications to engineering and science, Wiley series in probability and mathematical statistics*. New York: John Wiley and Sons.
Matthaei, J. H., Jones, O. W., Martin, R. G., & Nirenberg, M. W. (1962). Characteristics and composition of RNA coding units. *Proceedings of the National Academy of Sciences of the United States of America*, *48*(4), 666–667.
Muralidhar, R. V., Chirumamilla, R. R., Ramachandran, V. N., Marchant, P., & Nigam, P. (2001). Racemic resolution of RS-baclofen using lipase from *Candida cylindracea* [*Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen*]. *Mededelingen*, *66*, 227–232.
Myers, R. H., & Montgomery, R. C. (2002). *Response surface methodology, process and product optimization using design experiment*. New York: Wiley.
Pan, Y., Dong, S., Hao, Y., Zhou, Y., Ren, X., & Wanga, J. (2010). Ultrasonic-assisted extraction process of crude polysaccharides from Yunzhi mushroom and its effect on hydroxyproline and glycosaminoglycan levels. *Carbohydrate Polymers*, *81*, 93–96.
Prakash Maran, J., Mekala, V., & Manikandan, S. (2013). Modeling and optimization of ultrasound-assisted extraction of polysaccharide from *Cucurbita moschata*. *Carbohydrate Polymers*, *92*, 2018–2026.
Prakash Maran, J., Manikandan, S., Vigna Nivetha, C., & Dinesh, R. (2013). Ultrasound assisted extraction of bioactive compounds from *Nephelium lappaceum* L. fruit peel using central composite face centered response surface design. *Arabian Journal of Chemistry*, http://dx.doi.org/10/1016/j.arabjc.2013.002.007
Prior, R. L., & Cao, G. (1999). In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radical Biology and Medicine*, *27*, 1173–1181.
Qi, H., Zhang, Q., Zhao, T., Hu, R., Zhang, K., & Li, Z. (2006). In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from *Ulva pertusa* (Chlorophyta). *Bioorganic & Medicinal Chemistry Letters*, *16*, 2441–2445.
Ramarahnam, N., Osawa, T., Ochi, H., & Kawaishi, S. (1995). *Trends in Food Science & Technology*, *6*, 75–82.
Rivas-Arreola, M. J., Rocha-Guzmán, N. E., Gallegos-Infante, J. A., Gonzalez-Laredo, R. F., Rosales-Castro, M., & Bacon, J. R. (2011). Antioxidant activity of oak (Quercus) leaves infusions against free radicals and their cardioprotective potential. *Pakistan Journal of Biological Sciences*, *13*(3), 537–545.
Rocha-Guzmán, N. E., Gallegos-Infante, J. A., González-Laredo, R. F., Reynoso-Camacho, R., Ramos-Gómez, M., & Garcia-Gasca, T. (2009). Antioxidant activity and genotoxic effect on HeLa cells of phenolic compounds from infusions of *Quercus resinosa* leaves. *Food Chemistry*, *115*(8), 1320–1325.
Rollet-Labelle, E., Grange, M. J., Elbim, C., Marquetty, C., Gougerot-Pocidalo, M. A., & Pasquier, C. (1998). Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free Radical Biology and Medicine*, *24*(4), 563–572.
Salazar-Montoya, J. A., Ramos-Ramirez, E. G., & Delgado-Reyes, V. A. (2002). *Carbohydrate Polymers*, *49*, 387–791.
Vinatoru, M., Toma, M., Radu, O., Filip, P. I., Lazurca, D., & Mason, T. J. (1997). The use of ultrasound for the extraction of bioactive principles from plant materials. *Ultrasonics Sonochemistry*, *4*, 135–139.
Wang, L., & Weller, C. L. (2006). Recent advances in extraction of nutraceuticals from plants. *Trends in Food Science & Technology*, *17*, 300–312.
Wang, R. F., Chen, P., Jia, F., Tang, J., & Ma, F. (2012). *International Journal of Biological Macromolecules*, *50*, 331–336.
Xiao, J., Sun, J., Yao, L., Zhao, Q., Wang, L., & Wang, X. (2012). Physicochemical characteristics of ultrasonic extracted polysaccharides from cordyceps cephalosporium mycelia. *International Journal of Biological Macromolecules*, *51*, 64–69.
Xie, J. H., Shen, M. Y., Xie, M. Y., Nie, S. P., Chen, Y., & Li, C. (2012). Ultrasonic-assisted extraction, antimicrobial and antioxidant activities of *Cyclocarya paliurus* (Batal.) Iljinskaja polysaccharides. *Carbohydrate Polymers*, *89*(1), 177–184.
Xu, W. T., Zhang, F. F., Luo, Y. B., Ma, L. Y., Kou, X. H., & Huang, K. L. (2009). *Carbohydrate Research*, *344*, 217–222.
Yan, Y. L., Yu, C. H., Chen, J., Li, X. X., Wang, W., & Li, S. Q. (2011). Ultrasonic-assisted extraction optimized by response surface methodology, chemical composition and antioxidant activity of polysaccharides from *Tremella mesenterica*. *Carbohydrate Polymers*, *83*(1), 217–224.
Yang, B., Zhao, M., Shi, J., Yang, N., & Jiang, Y. (2008). Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. *Food Chemistry*, *106*, 685–690.
Zhong, K., & Wang, Q. (2010). Optimization of ultrasonic extraction of polysaccharides from dried longan pulp using response surface methodology. *Carbohydrate Polymers*, *80*(1), 19–25.
Zhong, K., Lin, W., Wang, Q., & Zhou, S. (2012). Extraction and radicals scavenging activity of polysaccharides with microwave extraction from mung bean hulls. *International Journal of Biological Macromolecules*, *51*, 612–617.