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Full Length Research Paper

Effects of ultrasonic-assistant extraction parameters on total flavones yield of *Selaginella doederleinii* and its antioxidant activity

Shuang Li[1,2], Ronghua Zhu[1], Ming Zhong[1], Yuping Zhang[1], Kelong Huang[1]*, Xu Zhi[1] and Shuting Fu[1]

[1]College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan 410083, P. R. China.
[2]Guilin Institute for Food and Drug Control, Guilin, Guangxi 541002, P. R. China.

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This paper, applied the central composite design combined with response surface methodology to optimize the parameter of ultrasonic-assisted extraction the total flavonoids from *Selaginella doederleinii* Hieron (SD). The results indicate that the maximum extract of total flavonoids was 4.414 mg/g by using 70% ethanol as solvent, extract 50 min at 65°C. Antioxidant activity of extracts, determined by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay, ranged from 20.22 to 46.64 mg/ml of raw SD. The optimization condition for antioxidant activity was similar to extract of total flavonoids. Yield increase of the total flavonoids depends on the cooperative effect of three selected parameters in the process of ultrasonic-assisted extract. Antioxidant activity more reckon on the ethanol concentration. Moreover, it was also found that there was not significant correlation between the bioactivities and content of total flavonoids.

Key words: *Selaginella doederleinii* Hieron, response surface methodology, flavonoi, antioxidant activity.

INTRODUCTION

The genus *Selaginella* is composed of about 700 species and belongs to the family *Selaginellaceae*. In particularly, SD has been used as a traditional Chinese medicine (Lu et al., 2004), which is a well-known perennial Pteridophyte plant growing in South and Southwestern China at low altitude (Lin et al., 1994). It has been used to treat cancer and cardiovascular diseases (Lu et al., 2004; Lin et al., 1994; Lin, 1987). Several biflavonoids, lignans, and alkaloids have been reported from this plant (Mori et al., 1988; Mori et al., 1989; Lin et al., 1991; Pan et al., 2001; Lee et al., 2008) together with some biological activities such as cytotoxicity (Mori et al., 1988), anti-human immunodeficiency virus (HIV) activity, inhibition of human DNA polymerase, and anti-inflammatory effects (Mori et al., 1988; Mori et al., 1989; Lin et al., 1991; Pan et al., 2001).

It has been reported that maceration usually has been applied to extract flavonoids from SD (Mori et al., 1988; Mori et al., 1989; Lin et al., 1991; Pan et al., 2001; Lee et al., 2008). However, the method may reduce the yield of flavonoids due to extract incompletely as well as footless time (Scalia et al., 1998; Diouf et al., 2009). In recent years, various novel extraction techniques have been developed for the extract of nutraceutical and medicine from plants.

The technique of ultrasound-assisted extraction has attracted much attention due to simple, rapid, efficient and low cost. Ultrasound-assisted extraction from medicine herb is based on the interaction among chemical compounds present in the plant with extracting media. Thus, ultrasound exerts a mechanical effect, allowing greater penetration of solvent into the tissue of fiber, increasing the contact surface area between fiber and liquid phase. Moreover, ultrasonic waves pass over the material media producing expansions and compression cycles, which may produce negative pressure in the

*Corresponding author. E-mail: lishuang_5058@yahoo.com.cn, huangkelong@yahoo.com.cn. Tel: +86 731 8887 9850. Fax: +86 731 8887 9850.

Table 1. Selected factors and their response in the ultrasonic-assisted extraction of *S. doederleinii* Hieron.

Runs	Symbols and coded levels			Yields: (mg/g)	DPPH (mg/ml)
	X_1 (°C)	X_2 (%)	X_3 (min)	Total flavonoids	ED_{50}value
1	55	70	70	2.213	28.06
2	65	70	50	3.682	26.84
3	65	80	30	2.593	34.83
4	75	60	50	1.897	20.75
5	75	80	50	2.346	44.23
6	65	60	30	1.010	24.95
7	65	70	50	3.776	20.59
8	65	80	70	2.806	37.29
9	55	80	50	2.686	26.53
10	75	70	30	2.171	35.04
11	65	70	50	3.775	27.73
12	65	70	50	4.414	20.22
13	65	60	70	1.511	25.12
14	55	60	50	1.158	33.23
15	55	70	30	1.964	45.22
16	75	70	70	2.542	46.64
17	65	70	50	3.791	20.69

X_1:Temperature(°C), X_2: ethanol concentration (%), X_3: extraction times (min).

liquid, generating cavities format in liquid phase and bubbles. The compression cycles gases and molecular in the vapor phase can be expelling from bubbles. Successive expansions-compressions cycles results in the collapse of these structures and increase the temperatures and pressures. It would enhance the efficient extraction (Santos et al., 2006; Vilkhu et al., 2008).

Traditionally, many techniques could be used for optimization the parameter of extract flavonoids from different medicine herb (Bhujbal et al., 2008; Thoo et al., 2010; Huang et al., 2009). But these methods have been carried out study on each variable separately, some methods are time-consuming and its major disadvantage is that it does not include the interactive effects among the studied variables. In our research, the response surface methodology was selected to optimizes the parameters of extract flavonoids from SD, due to this method, the method allow more than one variable to be optimized simultaneously and provide the effect each other of these variables (Santos et al., 2006; Vilkhu et al., 2008; Huang et al., 2009; Ma et al., 2008; Khan et al., 2010; Yang and Zhang, 2008)

MATERIALS AND CHEMICALS

SD samples purchased from Guilin Pharmaceutical Co. (Guilin, China) was identified by Professor Guangzhao Li in Guangxi Institute of Botany, the Chinese Academy of Sciences. The voucher specimens were deposited in the College of Chemistry and Chemical Engineering, Central South University, Changsha, China. The air-dried samples were smashed into powder and stored in exsiccator. 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was purchased from Sigma Co. (MO, USA). Rutin (>90%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). All other reagents and solvents were analytical reagent grade and used without purification. All aqueous solution was prepared using newly distilled water.

METHODS

Ultrasonic-assisted extraction

For the ultrasound-assisted extraction experiments, an ultrasonic bath (KQ-50DE, Kunshan Ultrasound Co. Ltd., China) was used as an ultrasound source, to which 40 kHz transducers were annealed at the bottom. The bath power rating was 50 W on the scale of 40 - 99%. The extraction of total flavonoids was performed by adding 2.0 g of SD powder into 50 ml flask with 40 ml solvent (Table 1). The flask was then partially immersed into the ultrasonic bath, which contains adequate water. Water in the ultrasonic bath was circulated and regulated at constant desired temperature to avoid the water temperature rise, caused by ultrasonic exposure. The extractions solution under corresponding condition were filtered and concentrated by reduced-pressure. The residue was diluted to 10 ml with 70% ethanol as sample stock solution preparation.

Experimental design

The extract efficient of flavonoids may be affected by many factors such as solvent composition, extraction time, extraction temperature, solvent to solid ratio, and extraction pressure, and so on (Scalia et al., 1998; Bhujbal et al., 2008; Thoo et al., 2010; Huang et al., 2009; Lee and Lin, 2007). In our research, temperature,

the ethanol concentration, and extraction time were selected as important extraction parameters by a series of preliminary experiments (data not shown here). To investigate the effects of temperature (55 - 75°C), the concentration of ethanol (60 - 80%), and extraction of times (30 - 70 min) on total flavones yield and antioxidant activity of flavones-rich extraction of SD, the Experimental design consisted of two sections as follows: (a) the effects of three independent variables and levels of ultrasonic extraction on total flavonoids yield by a Box-Behnken design (Table1) (Box and Behnken, 1960). Experimental design, data analysis, and quadratic model building were conducted using the software Design Expert (Version 6.0.5 trial, Stat-Ease Inc., Minneapolis, MN, USA). Five repetitions at the centre design point were performed to estimation the pure error sum of squares. (b) The same procedure is employed to research the effect of these three independent variables on total antioxidant activity of flavonoid-rich extraction.

UV-absorption analysis

Determination of total flavonoids

The rutin can be considered as suitable compound for the quality the total flavonoid of most botanical (The State Pharmacopoeia Commission of P. R. China, 2005; Abouzid and Elsherbeiny (2008). It was used as the standard for a calibration curve. So, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml rutin standard stock solution (0.2 $mg.ml^{-1}$) was placed in a 10 ml volumetric flask, respectively. Distilled water was added to make 3 and 0.3 ml $NaNO_2$ (1:20) were added. 0.3 ml $AlCl_3$ (1:10) were added 6 min later. After 6 min, 4 ml NaOH ($1mol.L^{-1}$) were added and the total was made up to 10 ml with distilled water. The solution was mixed well again and incubated for 15 min at room temperature. 0.5 ml sample stock solution was transferred to 10 ml volumetric flask. The same procedure is applied to prepare sample solution. The solutions were scanned by UV-2450 spectrophotometer using quartz cuvettes (1.0 cm) (Shimadzu, Japan) at 506 nm against a blank.

The linear equation can be calculated by the absorption as y-axis and the content of rutin as abscissa.

$$A = 11.59C - 0.0192 \quad r = 0.9994 \qquad (1)$$

Where A is the absorption, C is the flavonoids content in $mg.ml^{-1}$and r is the relative coefficient.

The flavonoids content was calculated using the following linear Equation (1).

Antioxidant activity determination

DPPH assay is a robust, facile antioxidant assay *in vitro*. The molecule of DPPH is a stable radical that can accept an electron from the compound to analyze. We used a modification of the assay condition reported by the described method (Thuong et al., 2007; Kumar et al., 2008). 0, 2, 3.5, and 5 µl sample stock solution of each extraction was added to 1.325 mM DPPH (14 µl) solution in 96 well plates with in presence 230 µl ethanol, respectively. The resulting solution was mixed for 2 min and incubated in the dark for 30 min at 37°C. Than the absorbance of the reaction mixture was recorded at 517 nm on a microplate reader. Exaction solvents of corresponding volumes were used as controls for each solvent extract. The percent activity calculated by the following Equation (2), by substituting the mean of the absorbance values, which were recorded in triplicate:

$$\%\text{scavenging activity} = 100 \times [\text{control-sample}]/\text{control} \qquad (2)$$

ED_{50} values (concentration required to quench 50% of the radicals under the experimental conditions in this assay) were calculated for several SD extracts showing high levels of scavenging activity.

Statistical analysis

Differences between variables were tested for significance using the one-way of analysis of variance (ANOVA) procedure (SPSS for Windows 13.0, SPSS Inc. Chicago, IL; Design Expert 6.0.5 trial, Stat-Ease Inc., Minneapolis, MN, USA). Significant differences were accepted at $P \leq 0.05$.

RESULTS AND DISCUSSION

Effects of temperature, the contents of alcohol, and extraction of times on total flavones yield

Table 1 shows the experimental design and the total flavones yields obtained under different testing conditions. A Box–Behnken model and three-dimension (3D) response surface (Figures 1a – c) were also used for information. As can be seen, in general, the best results in terms of total flavonoids yield were obtained working at the 50°C, 70% ethanol and 50 min extraction of times (4.414 mg/g) on raw SD in run twelve and 3.776 mg/g of raw SD in run seven.

The statistical model, representing the total flavones yield from SD as a function of the independent variables within the region under investigation, can be expressed by the following quadratic Equation (3):

$$Y = 3.89+0.12X_1+0.61X_2+0.17X_3-0.27X_1X_2+0.030X_1X_3-0.072X_2X_3-0.81X_1^2-1.05X_2^2-0.85X_3^2 \quad (3)$$

Where Y is the total flavones yield from SD (mg/g), X_1, X_2, and X_3 are the coded variables for temperature, ethanol concentration and extraction time, respectively.

In general, the validity of the model could be judged by the lack of fit to check the adequacy of the model (Table 2). The P-value of the model was significant ($P = 0.0001 < 0.05$) while the lack of fit value of the model was 0.7952 ($P > 0.05$, not significant). Both the values indicated that the model developed for total flavones yield prediction exhibited a good fit. Also, a good agreement with the experimental results for the coefficient (r^2) of 0.9709 was found.

According to the model (Equation 3), temperature (X_1), the concentration of ethanol (X_2), the extraction of times (X_3), and the interaction between temperature and the extractions time (X_1X_3) have positive influences on the yield of total flavones. By contraries, the quadratic term of them (X_1^2, X_2^2, and X_3^2), the interaction between temperature and the concentration of ethanol (X_1X_2), the concentration of ethanol and the extraction times (X_2X_3) exhibited negative influences.

It is well-known that higher temperature promotes the solubility of the solute and increases the yield by the high mass transfer of solute in the matrix and/or from the matrix to the solvent due to the effect. At the same time,

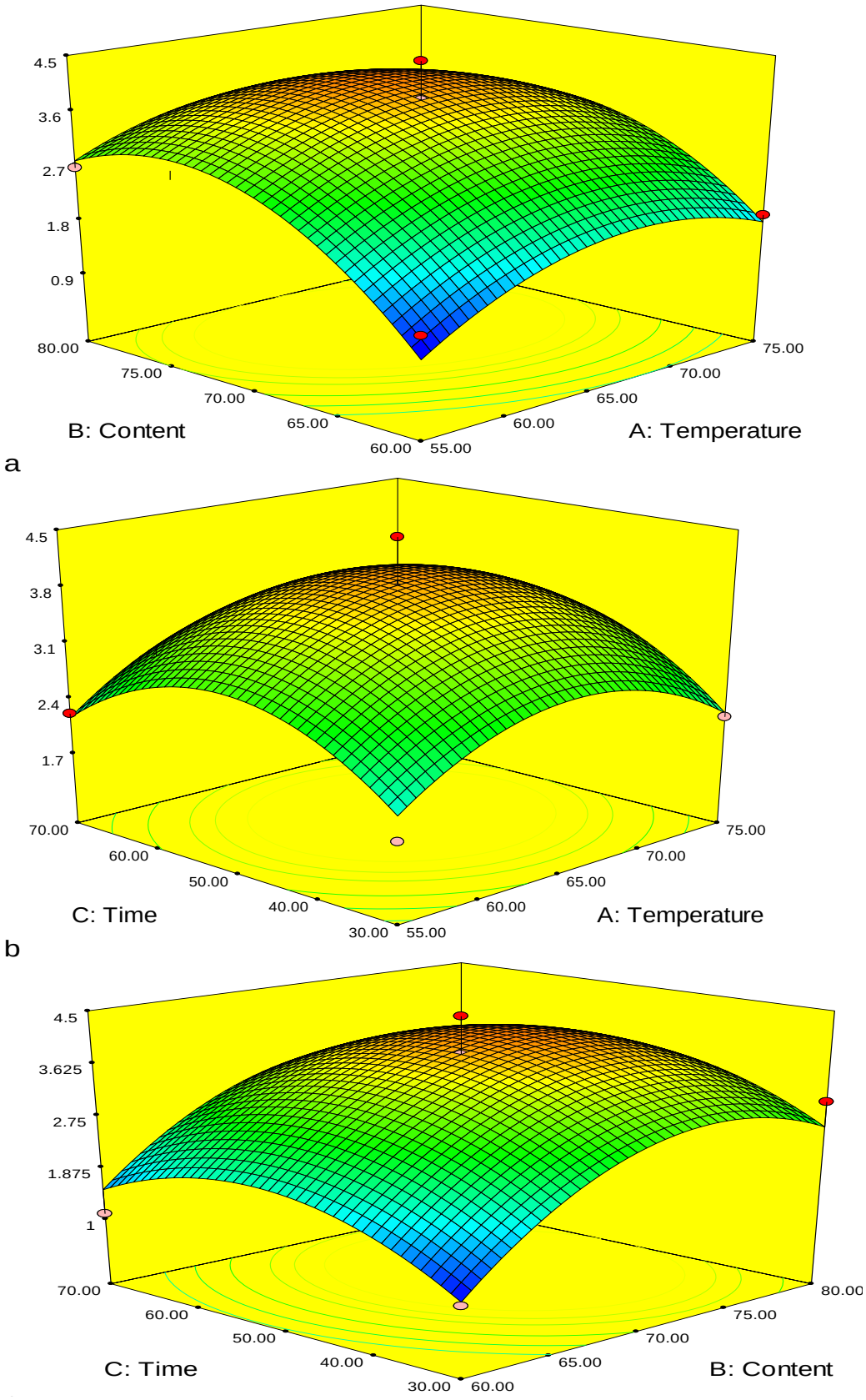

Figure 1. 3D response surface plot showing effects of ethanol concentration and temperature (a), extraction time and temperature (b), ethanol concentration and extraction time (c) on ultrasonic-assisted extraction total flavonoids from *S. doederleinii* Hieron.

Table 2. Analysis of variance (ANOVA) of the response surface quadratic model for the total flavones yields and the value ED_{50}value of total antioxidant of *S. doederleinii* Hieron.

Source	Degrees of freedom	Sum of squares	Mean square	*F* value	Prob > *F*
Total flavones yield	9	15.33	1.70	25.99	0.0001
Residual	7	0.46	0.066		
Lack of fit	3	0.10	0.035	0.40	0.7641
Pure error	4	0.35	0.088		
Total	16	15.79			
Total antioxidant activity	9	11.73	1.30	14.32	0.0010
Residual	7	0.64	0.091		
Lack of fit	3	0.13	0.044	0.35	0.7952
Pure error	4	0.51	0.13		
Total	16	12.37			

increasing temperature reduces the solvent density and consequently decreases the yield of total flavonoids at constant pressure (Marsili and Callahan, 1993; Wang et al., 2008). Therefore, the increase in temperature could have either a positive or a negative effect. Even as (Figures 1a, b) shown, our study exhibited a similar results. The effects of temperature on yield of total flavonoids demonstrated quadratic distribution. The effects of ethanol–water compositions on the extraction yield of total flavonoids obtained by ultrasound-assisted extraction 50 min as temperature from 55 to 75°C and after 30 - 70 min of ultrasound-assisted extraction at 65°C were shown in Figure1a and c. The extraction yields of total flavonoids increased with the increasing percentage of ethanol up to 70%. This was probably due to the relative polarity and the increase in effective swelling of the plant by water, which helped increase the surface area for solute–solvent contact (Li et al., 2004). However, it was also observed in experiment that as ethanol concentration increases up to 80%, the yield of total flavonoids reduced. It may be seen that 70% ethanol could product the highest extraction yield. The results suggest that 70% ethanol could offer the suitable vapour pressure, viscosity and surface tension of extraction solvent, which are very important physical characteristics of solvent influencing efficiency of sonication activity, for the flavonoid extract. With ethanol concentration more increases, the polarity of solvent change, which might extract more impurity (Huang et al., 2009; Mason et al., 1996; Hemwimon et al., 2007; Zhu and Tao, 2007).

The extraction yield is also time-dependent (Figure1, b, c), under the condition of ultrasonic-assisted extraction, the total flavonoids extraction yield increases with the extraction time for the first 50 min, and than decrease, alternatively the ethanol concentration keep in 70% or the temperature at 65°C. As the extraction time prolongs, the flavonoids compounds was not stable during long times at thermal process. The chemical decomposition of bioactive compound present in extract may occur, resulting in a decrease in extraction yield (Huang et al., 2009; Wang et al. 2008).The optimized results show that using 70% ethanol as extraction solvent and 70 min extraction time at 65°C temperature, the highest extraction ratio of total flavonoids could arrive 4.414 mg/g in term of raw material. All of them exhibited similar effect trendy on the yield total flavonoids from SD.

Effects of temperature, concentration of alcohol, and extraction time on antioxidant activity

It was similar to the total flavonoids, the total antioxidants of SD as a function of the independent variables within the region under investigation, can be expressed by the following quadratic Equation (4):

$$ED_{50}=2.34+0.17X_1+0.49X_2-0.037X_3+0.75X_1X_2+0.72X_1X_3+0.057X_2X_3+0.80X_1^2-0.021X_2^2+0.73X_3^2 \quad (4)$$

Where ED_{50} is the total antioxidant activity of extraction from the SD (mg/ml), X_1, X_2, and X_3 are the coded variables for temperature, the ethanol concentration, and extraction of time, respectively.

As shown in Table 2, the lack of fit was 0.7952 ($P > 0.05$) and the model of total antioxidant was 0.001 ($P<0.05$), which indicated the responses were sufficiently explained by the regression equation. The regression models allowed the prediction of the effects of the three parameters on total antioxidant of SD fractions. The extraction of times and the quadratic term of ethanol concentration shown positive influences on the total antioxidant, but the other parameter exhibited negative influences as shows in Equation (4).

The 3D response surface curves were plotted to explain the results of temperature, ethanol concentration, and extraction time effect on total antioxidant activities of the extraction under different circumstances (Figures 2 a–c). It was found that the selected parameters had obvious operation difference between total flavonoids yield and antioxidant by comparative Figures 1 and 2.

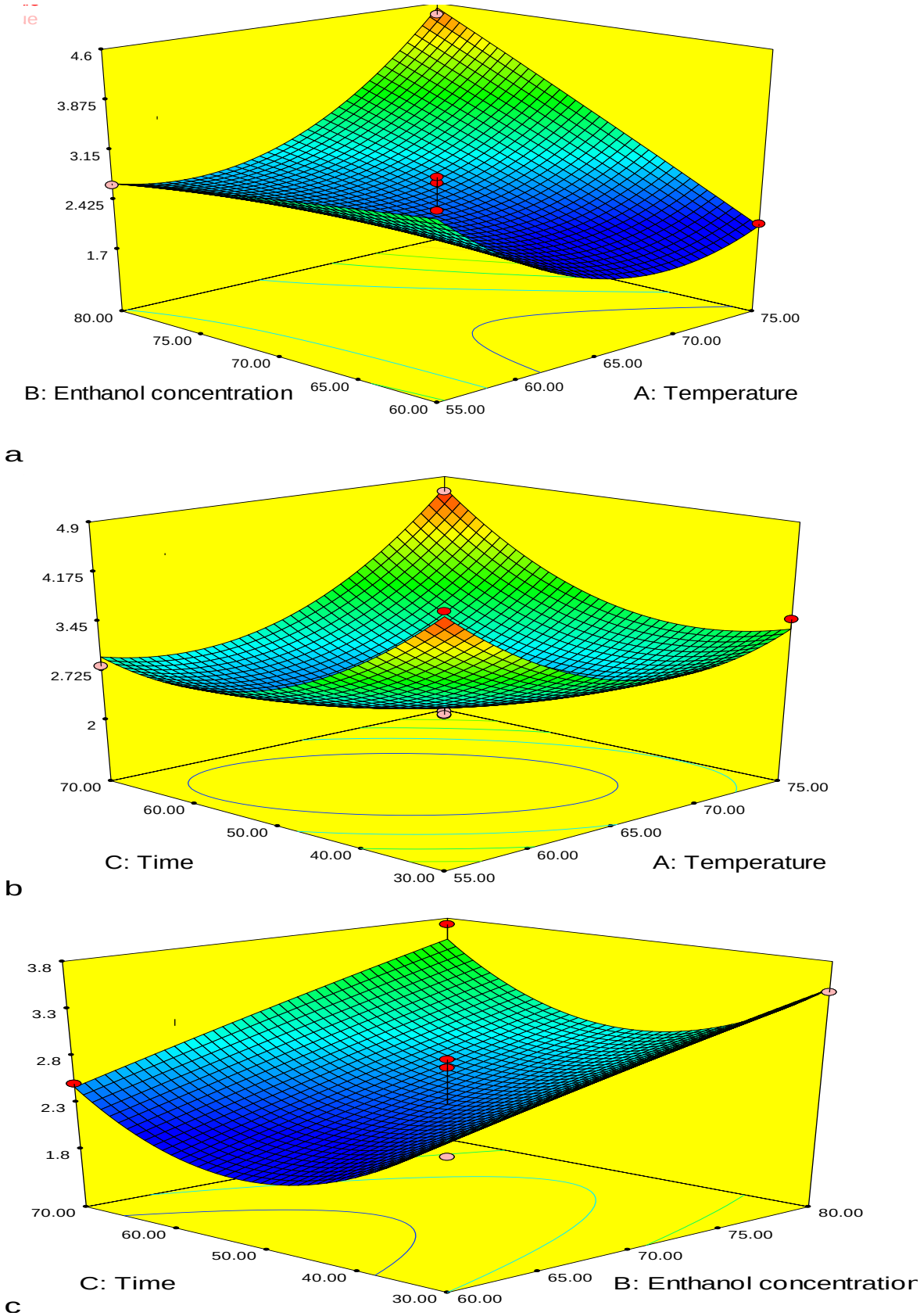

Figure 2. Response surface plots for the effects of ethanol concentration and temperature (a), extraction time and temperature (b), ethanol concentration and extraction time (c) on total antioxidant activity of *S. doederleinii* Hieron.

It can be seen from Figure 2a, the response surface was generated as a function of ethanol concentration and temperature as the extracts time kept in 50 min. The ethanol concentration and temperature demonstrated quadratic effects on the response; hence antioxidant increased up to about 65 - 70% ethanol concentrations at temperature less 65°C, than decline with its further increase. This change trendy was coincided with the antioxidant of soft wheat bran and grape cane extracts (Liyana-Pathirana and Shahidi, 2005; Karacabey and Mazza, 2010).

As shows Figure 2b, the temperature and the extract time influenced the antioxidant response in a quadratic manner while keeping the ethanol concentration constant at 70%. The response surface was generated as a function of solvent temperature (55 - 75°C) and time (30 – 70 min) under the circumstance. Hence total antioxidant decreased with increasing the temperature and extract time up to a certain level (approximately 65°C and 50 min, respectively).

Figure 2c showed the antioxidant parameter ED_{50} as a function of solvent composition and extracts time. Linear term of the ethanol concentration had significant effects on the response. It was shown a clear trend towards as the total antioxidant increased with the concentration decrease from 80 to 60%. Therefore, the lower the ethanol concentration applied to extract of SD, the lower ED_{50} values found. However, temperature demonstrated a non-linear effect on total antioxidant. When the extracts prolong to 50 min, the total antioxidant of extraction has highest power, and than decline with its further increase, which was in agreement in recent study that has shown those observed in the process of total antioxidant (Hayat et al., 2009; Azizah et al., 1999).The analysis of the surface response revealed that the stationary point for total antioxidant was a ridge analysis was performed to determine the critical levels of the design variables that may produce the maximum response. The critical values in terms of coded variables for total were 65, 70% and 50 min.

Correlations of the yield of total flavonoids with antioxidant activity

Flavonoids have been reported to be responsible for the antioxidant activities of botanical extracts. The DPPH assay has been used to measure antioxidant activity and the results of methods had a good correlate with those of the total flaconoids contents [14]. From Table1, it could found that highest yield of total flavonoids with lowest ED_{50} value, but lowest yields of total flavonoids with mediate ED_{50} value. There was no significant correlation between radical-scavenging activity and the content of total flavonoids, which demonstrated a bad relative coefficient($r = 0.3238$) by linear fit analysis. It can be explained that phenols compounds may be extracted under the selected condition, which were also responsible for the antioxidant activities.

Verification experiments

Verification experiments performed at the predicted conditions derived from ridge analysis of RSM demonstrated that experimental values were reasonably close to predicted values confirming the validity and adequacy of the predicted models. Moreover, the verification experiments also proved that the predicted values of total flavonoids and antioxidant for the model with SD fraction could be satisfactorily achieved within 95% confidence interval of experimental values (data not show here).

Conclusion

Central composite rotatable design combined with response surface methodology can be used to assess the optimization condition of ultrasound-assisted extract the total flavonoids from SD and provide bioactivity information about extracts of SD for development and application this resource.

REFERENCES

Lu YP, Chen YG, Wen J (2004). A new bioflavone *Selaginella doederleinii*. Yunnan Zhiwu Yanjiu., 26(2): 226-228.

Lin RC, Skaltsounis AL, Seguin E, Tillequin F, Koch M (1994). Phenolic constituents of *Selaginella doederleinii*. Planta. Med., 60(2): 168-170.

Lin RC, Elisabeth S, Francois T, Michel K (1987). New alkaloid glycosides from *Selaginella doederleinii.* J. Nat. Prod., 50(3): 422-426.

Mori H, Xu QA, Sakamoto O, Uesugi Y, Ono Y, Koda A, Nishioka I (1988). Immunological mechanisms of antitumor activity of some kinds of Chinese herbs: Meth A-induced delayed type hypersensitivity. Japanese. J. pharm., 48(1): 37-46.

Mori H, Xu Q, Sakamoto O, Uesugi Y, Koda A, Nishioka I (1989).Mechanisms of antitumor activity of aqueous extracts from Chinese herbs: their immunopharmacological properties. Japanese J. pharm., 49(3): 423-431.

Lin RC, Peyroux J, Elisabeth S, Koch M (1991). Hypertensive effect of glycosidic derivatives of hordenine isolated from *Selaginella doederleinii* hieron and structural analogs in rats. Phytother. Res., 5(4): 188-190.

Pan KY, Lin JL, Chen JS (2001). Severe reversible bone marrow suppression induced by *Selaginella doederleinii*. J. Toxicol. Clin. Toxicol., 39(6): 637-639.

Lee NY, Min HY, Lee J, Nam JW, Lee YJ, Han AR, Wiryawan A, Suprapto W, Lee SK, Seo EK (2008). Identification of a New Cytotoxic Biflavanone from *Selaginella doederleinii.* Chem. Pharm. Bull., 56(9): 1360-1361

Scalia S, Giuffreda L, Pallado P(1998). Analytical and preparative supercritical fluid extraction of Chamomile flowers and its comparison with conventional methods. J.Pharm. Biomed. Anal., 21(3): 549-558,

Diouf PN, Stevanovic T, Boutin Y (2009). The effect of extraction process on polyphenol content, triterpene composition and bioactivity of yellow birch (Betula alleghaniensis Britton) extracts. Industrial Crops Products, 30(2): 297-303.

Santos JD, Krug FJ, Pereira Korn M (2006). Currents on ultrasounds-assisted extraction for sample preparation and spectroscopic analytes determination. Appl. Spectrosc. Rev., 41:305-321.

Vilkhu K, Mawson R, Simons L, Bates D (2008). Applications and opportunities for ultrasound assisted extraction in the food Industry- A review, Innovative Food Sci. Emerging Technologies. 9: 161-169.

Bhujbal SS, Chitlange SS, Suralkar AA, Shinde DB, Patil MJ (2008). Anti- inflammatory activity of an isolated flavonoid fraction from *Celosia argentea* Linn. J. Med. Plant.Res., 2(3): 052-054.

Thoo YY, Ho SK, Liang JY, Ho ChW, Tan ChP (2010). Effects of binary solvent extraction system, extraction time and extraction temperature on phenolic antioxidants and antioxidant capacity from mengkudu (Morinda citrifolia). Food Chemistry. 120(1): 290-295.

Huang W, Xue A, Niu H, Jia Zh, Wang JW (2009). Optimised ultrasonic-assisted extraction of flavonoids from Folium eucommiae and evaluation of antioxidant activity in multi-test systems *in vitro*. Food Chem.,114(3): 1147-1154.

Ma YQ, Ye XQ, Hao YB, Xu GN, Xu GH, Liu DH (2008). Ultrasound-assisted extraction of hesperidin from Penggan (Citrus reticulata) peel. Ultrasonics Sonochemistry. 15(3): 227-232.

Khan MK, Abert-Vian M, Fabiano-Tixier AS, Dangles O, Chemat F (2010). Ultrasound-assisted extraction of polyphenols (flavanone glycosides) from orange (*Citrus sinensis* L.) peel, Food Chem., 119(2): 851-858

Yang Y, Zhang F(2008). Ultrasound-assisted extraction of rutin and quercetin from Euonymus alatus (Thunb.) Sieb. Ultrasonics Sonochemistry. 15(4): 308-313.

Lee MH, Lin Ch-Ch(2007). Comparison of techniques for extraction of isoflavones from the root of Radix Puerariae: Ultrasonic and pressurized solvent extractions. Food Chem., 105: 223-228.

Abouzid SF, Elsherbeiny GM (2008). Increase in flavonoids content in red onion peel by mechanical shredding. J. Med. Plant. Res., 2(9): 258-260.

Thuong PT, Kang HJ, Na MK, Jin WY, Youn UJ, Seong YH, Song KS, Min BS, Bae KH (2007). Anti-oxidant constituents from *Sedum takesimense*. Phytochemistry, 68: 2432-2438.

Kumar N, Ahmad KKM, Dang R Husain A (2008). Antioxidant and antimicrobial activity of propolis from Tamil Nadu zone. J. Med. Plant. Res., 2(12): 361-364.

Marsili R, Callahan D (1993). Comparison of a liquid solvent extraction technique and supercritical fluid extraction for the determination of α- and β-carotene in vegetable. J. Chrom. Sci., 31: 422-428.

Wang LZh, Yang B, Du XQ, Yi Ch (2008). Optimisation of supercritical fluid extraction of flavonoids from Pueraria lobata. Food Chem., 108(2): 737-741.

Li H, Pordesimo L, Weiss J (2004). High intensity ultrasound-assisted extraction of oil from soybeans. Food Res. Int., 37: 731-738.

Mason TJ, Paniwnyk L, Lorimer JP (1996). The uses of ultrasound in food technology. Ultrasonics Sonochemistry, 3: S253-S260.

Hemwimon S, Pavasant P, Shotipruk A (2007). Microwave-assisted extraction of antioxidative anthraquinones from roots of *Miranda citrifolia*. Separation Purification Technol., 54: 44-50.

Zhu Y-Ch, Tao WY(2007). Ultrasound-assisted extraction of flavonoids from the lotus leaves. Appl. Chem. Industry. 36(11): 1106-1109.

Wang L, Li D, Bao ChL, You JY, Wang ZM, Shi YH, Zhang HQ(2008). Ultrasonic extraction and separation of anthraquinones from Rheum palmatum L. Ultrasonics Sonochemistry, 15(5): 738-746.

Liyana-Pathirana C, Shahidi F (2005). Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem., 93: 47-56.

Karacabey E, Mazza G (2010). Optimisation of antioxidant activity of grape cane extracts using response surface methodology. Food Chem., 119(1): 343-348.

Hayat K, Hussain S, Abbas S, Farooq U, Ding BM, Xia ShQ, Jia ChSh, Zhang XM, Xia WSh (2009). Optimized microwave-assisted extraction of phenolic acids from citrus mandarin peels and evaluation of antioxidant activity in vitro. Separation Purification Technol., 70(1): 63-70.

Azizah AH, Nik Ruslawati NM, Tee TS (1999). Extraction and characterization of antioxidant from cocoa by-products. Food Chem., 64(2): 199-202.