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Full Length Research Paper

Optimization of ultrasonic-assisted extraction of chlorogenic acid from *Folium eucommiae* and evaluation of its antioxidant activity

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***Folium eucommiae* is a traditional Chinese medicinal herb. Ultrasonic-assisted extraction (UAE) technique was developed for the fast extraction of chlorogenic acid from *F. eucommiae* leaves. Several influential parameters of the UAE procedure (solvents, extraction cycles, extraction temperature, solvent to material ratio, irradiation time and solvent concentration) were investigated for the optimization of the chlorogenic acid extract using single factor and Box-Behnken experimental design. Response surface methodology analysis showed good correspondence between experimental and predicted values. It was found that the most effective parameter was solvent to material ratio, which was in good agreement with the experimental value. The adjusted coefficient of determination (R_{adj}^2) for the model was 96.1%. Probability value ($P < 0.001$) demonstrated a very high significance for the regression model. The maximum yield of chlorogenic acid of 0.77 mg/g with UAE was obtained by dual extraction with ethanol to material ratio of 17/1 v/m, irradiation time of 25 min, ethanol concentration of 52%, extraction temperature of 50°C and ultrasonic frequency of 5 kHz. The optimal yield with UAE was slightly lower than that of Soxhlet extraction and higher than that of heat reflux extraction. The extracts exhibited high scavenging effects on DPPH radical with increasing concentration from 0.1 to 0.9 mg/ml. The extracts also exhibited a quite strong concentration-dependent inhibition of hydroxyl radical.**

Key words: *Folium eucommiae*, chlorogenic acid, ultrasonic-assisted extraction, response surface methodology.

INTRODUCTION

Folium eucommiae is one of the oldest tonic herbs in traditional Chinese Medicine. Cortex of *F. eucommiae* has been extensively used in China, Japan, and Korea for strengthening tendons and bones, reinforcing muscle, benefiting liver and kidney, preventing miscarriage and anti-ageing (Hsieh and Yen, 2000; Hung et al., 2006; Zhou et al., 2009). Recently, *F. eucommiae* was proved to be rich in chlorogenic acid. It has been known as a functional health food and commonly used in the treatments of hypertension as well as fatty liver (Zhang et al., 2008). The main active constituents of *F. eucommiae* leaves include flavonoids, chlorogenic acid, saponins, polysaccharides and trace elements (Cheung et al., 2003; Guiming et al., 2007). Recent studies reveal that chlorogenic acid of *F. eucommiae* show strong antioxidant activity and pharmacological properties (Geun et al., 2004).

Ultrasonic-assisted extraction (UAE) has been proven to be one of the important techniques for extracting the

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bioactive compounds from herbs, and it is quite adaptable on a small or large scale (Kamaljit et al., 2008). The application of UAE offers many advantages including the reduction of solvents, temperature and the time for extraction, which is very useful for the extraction of thermolabile and unstable compounds. As well-known, ultrasound extraction is the new technology that attracts much more attention in the department of separation and extraction in recent years. Ultrasonic cavitation creates shear forces that break cell walls mechanically and improve material transfer. This effect is being used in the extraction of liquid compounds from solid cells (Huang et al., 2009; Zhang et al., 2009). Compared with other extraction techniques such as microwave-assisted extraction, the ultrasonic device is cheaper and its operation is much easier.

The use of large amounts of organic hazardous solvents in sample preparation and the increasing cost of solvent waste disposal have created a growing demand for better and more environment friendly extraction methods. The use of polar solvent as an extraction solvent for phenolic compounds has been proposed. Many reports have been published on the application of UAE of secondary metabolites from plants. Nevertheless, few publications on chlorogenic acid extraction from *F. eucommiae* by UAE are available till now.

In the present study, ultrasound-assisted extraction was employed to extract chlorogenic acid from *F. eucommiae*. Response surface methodology was used to build a model between the chlorogenic acid yield and these independent factors, and to optimize the extraction conditions. The UAE process was also compared with other methods. Furthermore, the antioxidant activity of chlorogenic acid extract from *F. eucommiae* was also determined. The objective of the work is to establish the optimized condition of ultrasound-assisted extraction for the chlorogenic acids-enriched extract from *F. eucommiae* and provide bioactivity information about chlorogenic acid for development and application of the resource.

MATERIALS AND METHODS

Materials and apparatus

F. eucommiae was kindly supplied from Kaihua Du Kang Co., Ltd., in Zhejiang province. The cut pieces were ground with a blade-mill to obtain a relatively homogenous drug powder and then sieved through 10-mesh screen. The powder was dried at 50℃ until constant weight and was well blended before use. Chlorogenic acid was purchased from National Institute for the Control of Pharmaceutical and Biological Product (Beijing, China). All other chemicals were of analytical grade.

Ultrasonic cleaner (SB5200DTD, Ultrasonic Instrument Co. Kunshan, Jiangsu, China) modified in our laboratory was used for ultrasonic extraction of chlorogenic acid. DK-S24 thermostate water bath (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) for heating extraction of chlorogenic acid. Agilent 1100 HPLC system (Agilent, United States) for chlorogenic acid analysis of sample. Evaporator (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China) for concentration of sample and FD-3 freeze drier (Beijing Boyikang Experimental Instrument CO., Ltd., Shanghai, China) for dryness of the concentrated sample. Water was purified using a Milli-Q gradient.

UAE extraction method

UAE was performed in ultrasonic cleaning bath apparatus using closed vessel system. 20 g of drug powder of *F. eucommiae* leaves varied according to the water to material ratio at ultrasonic frequency 5 kHz were put into extraction vessel under different UAE conditions. After extraction, the vessels were allowed to cool at room temperature, and filtered off through 0.45 µm microporous membrane. The filtrate was collected and the solid was extracted some times with the same volume of fresh solvent. Extracts were obtained by vacuum drying.

Several influential parameters of the UAE procedure (solvent, extraction temperature, extraction cycles, solvent to material ratio, irradiation time and solvent concentration) were investigated for the optimization of the extraction using single factor and Box-Behnken experimental design (Jiang et al., 2006; Shao et al., 2009).

Quantitative analysis of chlorogenic acid

The authentic standard of chlorogenic acid was weighted accurately then dissolved in methanol and diluted to 1 mg/ml. Standard solution was stored in the dark under refrigeration at 4℃ and was found to be stable for a month. A series of working standard solutions were prepared by the appropriate dilution of the above-mentioned standard solution with water to create chlorogenic acid concentration. Quantitative analysis of chlorogenic acid concentration was carried out by HPLC on Agilent 1100 HPLC system composed of a quaternary pump with a degasser, a thermostatted column compartment, a variable wavelength detector, an autosampler and 1100 ChemStation software. Sample analyses were performed on an Alltech C18 column (250 × 4.6 mm I.D., 5 µm) at a column temperature of 25℃. The mobile phase was methanol-water-acetic acid (20:78.4:1.6, v/v/v) at a flow rate of 1 ml/min, and the effluent was monitored at 327 nm by UV detector and sample volume injected was 10 µl.

Experimental design

Firstly, the single factor experiment for UAE was performed with the analysis of the effects of three factors (solvent, extraction temperature and extraction cycles) on chlorogenic acid yield from *F. eucommiae* leaf. Secondly, the optimization of extraction process parameters of solvent to material ratio, irradiation time and solvent concentration was performed using Box-Behnken design and a model was developed. Finally, the model validation and comparement with Soxhlet and heat flux extraction on chlorogenic acid yield were carried out.

Box-Behnken design

A Box-Behnken design was employed to study the response Y, namely chlorogenic acid yield. The independent variables were X_1, X_2, and X_3 representing solvent to solid ratio, irradiation time and solvent concentration, respectively. Three repeated experiments at the center (0, 0, 0) of the design were performed to allow the estimation of the pure error. The experimental design was shown in Table 1. All experiments were carried out in a randomized order to minimize the effect of unexpected variability in the observed

Table 1. Independent variables and their levels for Box-Behnken design.

Independent variables	Codes	Variable levels		
		-1	0	+1
Solvent to solid ratio (ml/g)	X_1	10:1	15:1	20:1
Irradiation time (min)	X_2	10	25	40
Solvent concentration (%)	X_3	30	45	60

Table 2. Effect of solvent type on the extraction of chlorogenic acid from *F. eucommiae.*

Solvent	Extraction of chlorogenic acid (10^{-1}mg/g)			Mean (10^{-1}mg/g)
CH_3OH	5.52	5.47	5.39	5.47
C_2H_5OH	4.22	4.36	4.41	4.33
H_2O	1.84	1.95	1.86	1.88

[a.] Extraction was carried out under sonication for 30 min; solvent/sample ratio 20/1 (v/w).

response due to extraneous factors.

As for the optimization for chlorogenic acid yield, the responses were analyzed using Matlab software (Alejandro et al., 2004). A quadratic polynomial regression model was assumed for predicting response. The model proposed for each response of Y was

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_1X_3 + A_6X_2X_3 + A_7X_1X_1 + A_8X_2X_2 + A_9X_3X_3 \quad (1)$$

Where A_0 is constant; A_1, A_2, and A_3 are linear coefficients; A_4, A_5, and A_6 are cross-product coefficients; A_7, A_8, and A_9 are quadratic coefficients.

Free radical scavenging activity on DPPH

The DPPH (purchased from Sigma Corp.) radical scavenging test was carried out. 2 ml of 0.2 mmol/L ethanol solution of DPPH (freshly prepared daily) was mixed with 2 ml of various dilutions of the crude chlorogenic acid extract. The mixture was deposited in the dark at room temperature for 30 min, and the decrease of absorbance at 517 nm was measured. The scavenging rate was calculated as follows:

$$[1-(A_S-A_0)/A],$$

Where A_S is the OD value of chlorogenic acid extract; A_0 is the OD value of the blank sample (without DPPH); A is the OD value of the control (without chlorogenic acid extract) (Chattip et al., 2008).

Hydroxyl radical-scavenging activity

The hydroxyl radical-scavenging assay was carried out. Both 0.3 ml 1.10-phenanthroline (75 mmol/l) and $FeSO_4$ dissolved in 1 ml Tris-HCl buffer solution were thoroughly mixed with 0.3 ml extract sample and 0.2 ml H_2O_2 (75 mmol/l). The reaction mixture was incubated at 37℃ for 60 min, and the absorbance was measured at 510 nm. The scavenging activity of hydroxyl radical was expressed using the following equation:

$$\text{Hydroxyl radical-scavenging activity (\%)} = (As-A_1)/(A_0 -As) *100$$

Where As is absorbance of the sample, A_1 is absorbance of the control solution containing 1,10-phenanthroline, $FeSO_4$ and H_2O_2, A_0 is absorbance of the blank solution containing 1.10-phenanthroline and $FeSO_4$ (Elena et al., 2009).

Statistical analysis

All trials were carried out in triplicate and the averages of chlorogenic acid yield were taken as responsive value. Analyses of variance (ANOVA) were used to determine the significant difference in chlorogenic acid yield extracted from *F. eucommiae* under different conditions. A second-order polynomial regressed equation was established on the basis of analysis of Box-Behnken experimental data, and the optimal conditions for extraction were found using the software of Matlab 7.0 version.

RESULTS AND DISCUSSION

Effect of solvents extraction

It is known that the chlorogenic acid is a compound containing many hydroxyl groups conferring thus a high solubility in water or alcohols. The pieces were used in the extraction of chlorogenic acid from fresh leaves of *F. eucommiae* under sonication.

As solvents, water, methanol and ethanol were tested to extract chlorogenic acid from fresh leaves of *F. eucommiae* under sonication. The results are shown in Table 2, from which we can find that the mean extraction efficiency of chlorogenic acid decreases in the order:

$$CH_3OH > C_2H_5OH > H_2O$$

Allowing for use of large amounts of methanol solvents in sample preparation created a growing demand for better and more environment friendly extraction. Ethanol is an interesting alternative to the usual solvents, particularly in view of its low cost, polarity and non-toxic character (Devanand et al., 2007). The use of ethanol as an extraction solvent for bioactive compounds has been

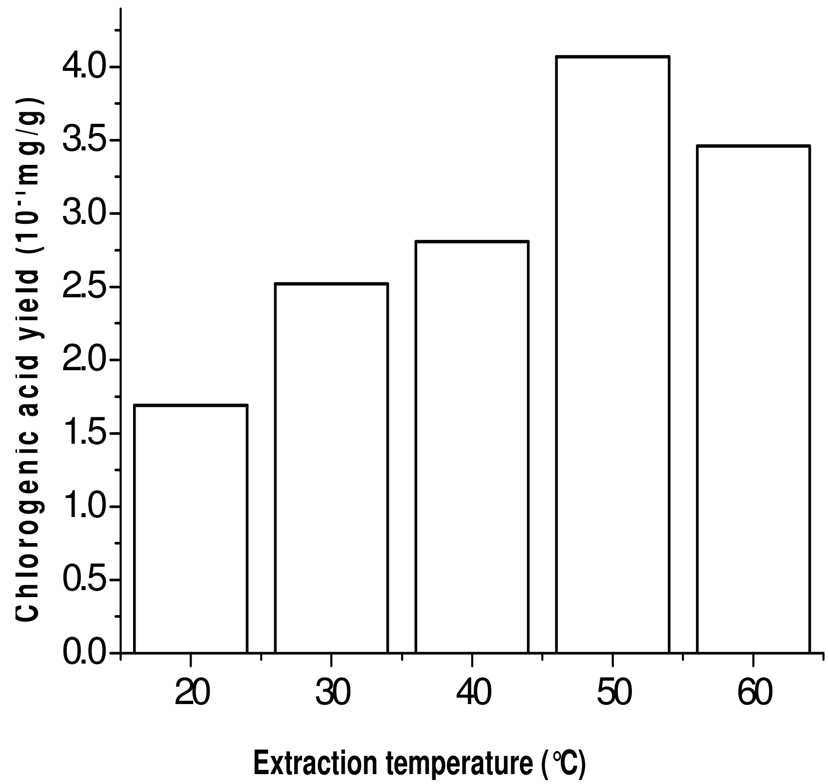


Figure 1. Effects of extraction temperature on chlorogenic acid yield (ethanol concentration 30%, solvent to material ratio 15:1, irradiation time 15 min).

proposed.

Effect of extraction temperature

Figure 1 showed that the chlorogenic acid yield rise as extraction temperature increased from 30 to 50℃, and approached the peak value at 50℃. This was probably due to the improvement of the mass transfer resulting from the increased solubility of chlorogenic acid and the decreased viscosity of the solvent. However, when extraction temperature was above 50℃, the extraction yield started to decrease. This may be ascribed to the decrease of number of acoustic cavitation bubbles created by ultrasound and the thermal degradation of chlorogenic acid. In addition, high extraction temperature might result in improvement of energy cost and enhancement of impurities.

Effect of extraction cycles

The effect of extraction cycle was investigated in this experiment (Figure 2). An amount of 20.0 g drug powder was extracted for 20 min under solvent to material ratio 20 ml/g. The residue was taken back and re-extracted three times under the above-mentioned conditions. The yield of chlorogenic acid in three cycles was 0.52 mg/g. The yields of two cycles accounted for 93.0% of the yield of three cycles and the third extraction accounted for only 7.0% of the yield of three cycles.

RSM model fitting

The statistical combination of the independent variables in coded and natural values along with the predicted and experimental response was presented of chlorogenic in Table 3. The analysis of variance (ANOVA) acid extraction was shown in Table 4. The experimental data for extraction ratio has correlation coefficient (R^2) of 0.99 with the calculated model. The calculated model was also able to explain 99% of the results. The greater similarity of the experimental and predicted values indicated the accuracy of prediction of model for chlorogenic acid yield. The results suggested that the model could work well for the prediction of chlorogenic acid enriched extract from *F. eucommiae* by ultrasonic-assisted method.

In ANOVA analysis, the P-values were used as a tool to check the significance of each coefficient, which also indicated the interaction strength of each parameter. The smaller the p-values were, the bigger the significance of

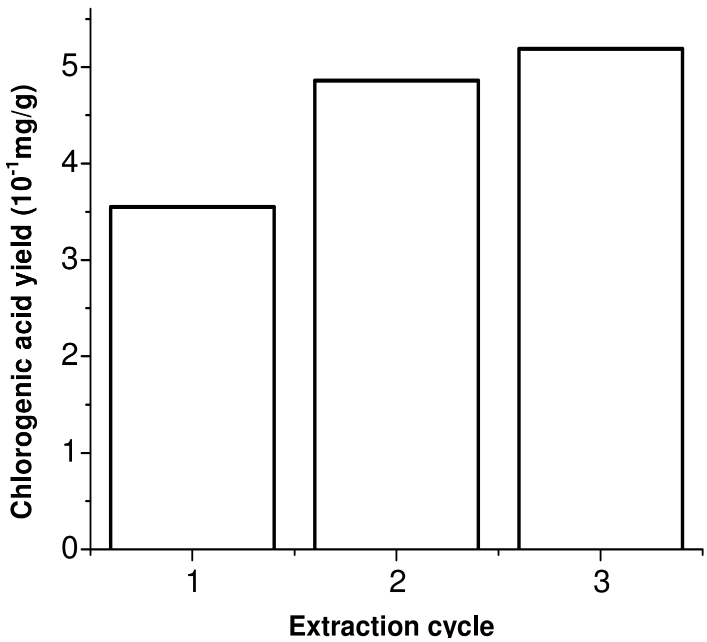


Figure 2. Effects of extraction cycle on chlorogenic acid yield.

Table 3. Box-benhen design and experiment data.

Run	X_1	X_2	X_3	True model	RSM model	Relative error (%)
1	-1	-1	0	2.15	2.04	5.11
2	-1	1	0	4.82	4.93	2.28
3	1	-1	0	5.64	5.53	1.95
4	1	1	0	5.31	5.62	5.52
5	0	-1	-1	3.29	3.41	3.52
6	0	-1	1	4.83	5.03	3.97
7	0	1	-1	5.49	5.29	3.64
8	0	1	1	6.44	6.32	1.86
9	-1	0	-1	3.60	3.79	5.01
10	1	0	-1	5.35	5.34	0.19
11	-1	0	1	4.48	4.49	0.24
12	1	0	1	7.49	7.30	2.54
13	0	0	0	7.31	7.42	1.50
14	0	0	0	7.54	7.42	1.59
15	0	0	0	7.40	7.42	0.27

Table 4. Analysis of variance (anova) for the fitted quadratic polynomial model.

Source	Sum of squares	d.f.	Mean square	F-value	Probability (P)
Model	37.1485	9	4.1276	39.8310	0.0004**
Linear	18.1133	3	6.0377	58.2636	0.0003**
Quadratic	16.3013	3	5.4338	52.4353	0.0003**
Cross Product	2.7339	3	0.9113	8.7940	0.02*
Lack of fit	0.4913	3	0.1636	12.1940	0.08
Pure error	0.0269	2	0.0135		
Total	37.6667	14			
R^2 = 98.6%, Adj R^2 = 96.1%					

[a]** Significant at 1% level.

Table 5. Results of regression analysis of a full second order polynomial model.

Term	Coefficients estimated	p-Value
Intercept	7.4167	
X_1	1.0925	0.0002**
X_2	0.7937	0.0009**
X_3	0.6637	0.002**
X_1X_2	-0.75	0.0055**
X_1X_3	0.315	0.1077
X_2X_3	-0.1475	0.4015
X_1^2	-1.3596	0.0004**
X_2^2	-1.5771	0.0002**
X_3^2	-0.8270	0.0043**

[a]* Significant at 5% level; [b]** Significant at 1% level.

the corresponding coefficient. Here, the p-value of the model was smaller than 0.001, which indicated that the model was suitable for use in this experiment. The P-value of "lack of fit" was 0.08 (P > 0.01), which indicated that "lack of fit" was insignificant relative to the pure error. The values indicated that, the accuracy and general availability of the polynomial model were adequate (Wu et al., 2007).

The regression coefficients and the corresponding p-values were presented in Table 5. From the p-values of each model term, it could be concluded that the regression coefficients of the linear term X_1, X_2, X_3, and all the quadratic terms had significant effect on chlorogenic acid yield. They were all significant at 1% level.

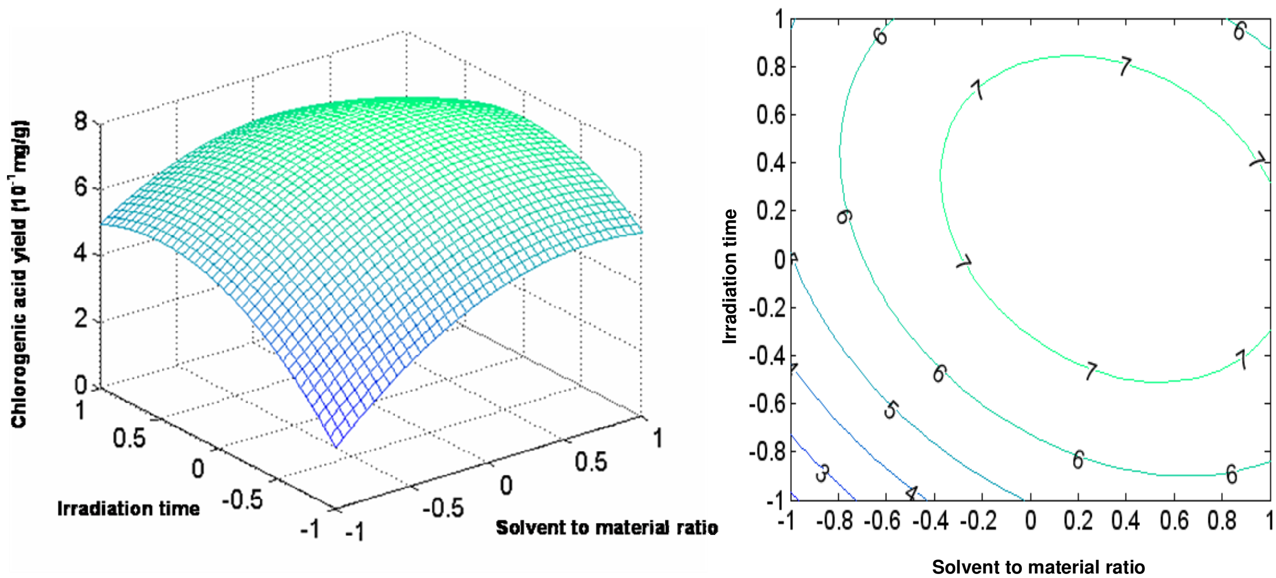
Using the designed experimental data (Table 3), the polynomial model for the chlorogenic acid yield was regressed and shown as below (in term of coded factors):

$$Y = 7.4167 + 1.0925X_1 + 0.7937X_2 + 0.6637X_3 - 1.3596X_1X_1 - 0.75X_1X_2 + 0.315X_1X_3 - 1.5771X_2X_2 - 0.1475X_2X_3 - 0.8270X_3X_3 \quad (2)$$

Many parameters can influence the performance of chlorogenic acid yield from *F. eucommiae*. Equation 2 showed that chlorogenic acid yield had a complex relationship with independent variables that encompass both first and second-order polynomials. The best way of expressing the effect of any parameter on the yield within the experimental space under investigated was to generate response surface plots of the equation.

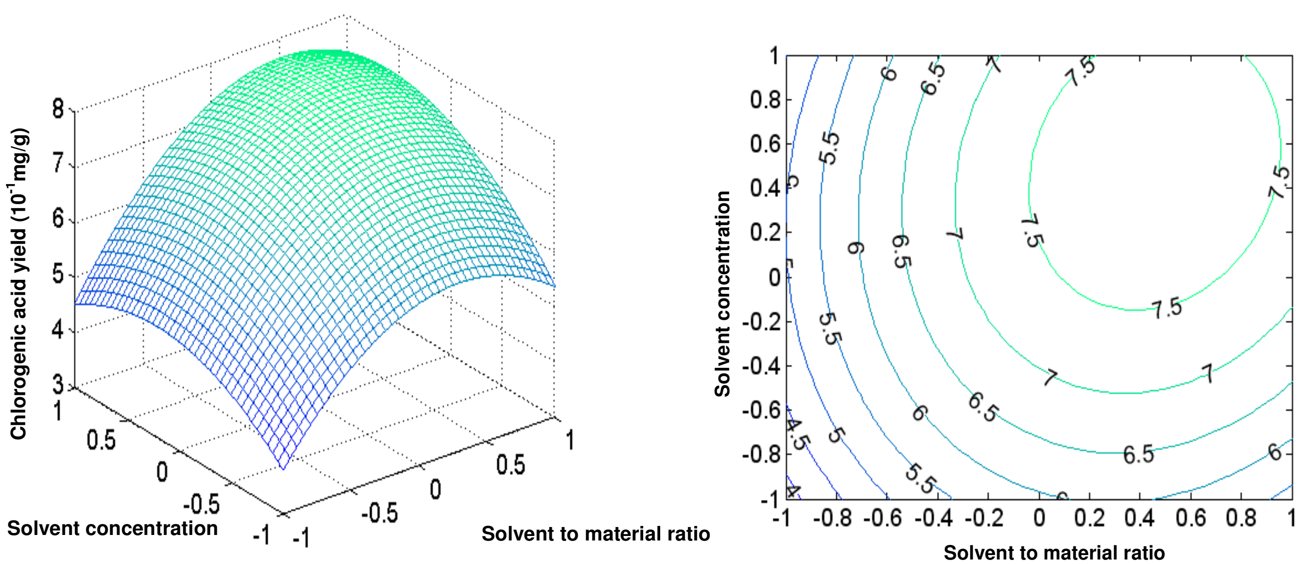
To investigate the interactive effects of operational parameters on chlorogenic acid-enriched extract, the three dimensional profiles of multiple non-linear regression model were depicted in Figures 3 and 4, respectively, when the other parameter was kept constant. From the shape of contour plots, the significance of the mutual interactions between the independent variables could be estimated. An elliptical profile of the contour plots indicates remarkable interaction between the independent variables. Contour plot and response surface curve showing predicted response surface of chlorogenic acid yield as a function of solvent to material ratio and irradiation time was shown in Figure 3. As evident from Figure 3, increase of solvent to material ratio from 10:1 to 17:1 with increase of extraction time from about 10 to 25 min enhance chlorogenic acid yield, while more than 25 min extraction appeared to be disadvantaged on the extract. It could be explained that, as the extraction time prolongs, the chemical decomposition of chlorogenic acid compound present in extract may occur, resulting in a decrease in extraction yield. It implies that although increase in liquid to solid ratio could accelerate mass transfer ratio between solid and liquid, which could be favorable to increase in chlorogenic acid extraction ratio, the advantage could be offset by decomposition of chlorogenic acid with prolong of the irradiation time and solid to liquid ratio arrived at the optimal values (Jing et al., 2008). It was indicated that yield was sensitive to irradiation time. An increase in chlorogenic acid yield was observed with the increasing of irradiation time at first. It is clear in Figure 3 that a maximum level of chlorogenic acid could be achieved at the optimum point (solvent to material ratio 17:1, irradiation time 25 min).

Figure 4 indicates that the highest extraction ratio could be achieved when using about 52% ethanol concentration. As showed in Figure 4, increase of ethanol concentration from 30 to 52% with increase of liquid to solid ratio from 10/1 to 17/1 accelerate chlorogenic acid extraction ratio, and the initial increase of the extraction ratio is followed by a decrease after ethanol concentration of more than 50% at liquid to solid ratio below 20/1. It was also observed in the experiment that as ethanol concentration increases, the polarity of solvent



(A) (B)

Figure 3. Response surface curve (a) and contour plot (b) showing predicted response surface of chlorogenic acid yield as a function of material ratio and irradiation time (solvent concentration = 45%).



(A) (B)

Figure 4. Response surface curve (a) and contour plot (b) showing predicted response surface of chlorogenic acid yield as a function of solvent to material ratio and solvent concentration (irradiation time = 25 min).

increases, which would extract more impurity (Veličković et al., 2006). It may be seen that about 52% ethanol could produce the highest extraction yield. The results suggest that 52% ethanol could offer the suitable vapor pressure, viscosity and surface tension of extraction solvent, which are very important physical characteristics of solvent influencing efficiency of sonication activity, for the flavonoids-enriched extract. On the other hand, presence of suitable water in solvent could facilitate increase at the extraction yields since water could helpful to enhance swelling of plant material, which is favorable to increase the contact surface area between the plant matrix and the solvent, resulting in increase of the extraction ratio.

Validation of the model

The model was processed by partial differential coefficient, and equal to zero. The point of curved surface was determined (X_1 = 0.50, X_2 = 0, X_3 = 0.50). According to RSM result, an experiment with solvent to material ratio of 17/1, irradiation time of 25 min, and solvent concentration value of 52% was conducted in order to investigate the effect of RSM. The experiment was carried out at the optimized conditions.

Chlorogenic acid yield of 0.77 mg/g was obtained and was in good agreement with the predicted one. The accuracy of the model was validated with triplicate experiments under the aforementioned optimal reaction conditions. As a result, the model was considered to be accurate and reliable for predicting the chlorogenic acid yield.

Free radical scavenging activity on DPPH and hydroxyl radical-scavenging activity test provide an easy and rapid way to evaluate the antiradical activities of chlorogenic acid extract. DPPH is a very stable free radical. It exhibits the absorption at 515 nm, but upon reduction by an antioxidant, the absorption disappears. The chlorogenic acid extract could scavenge it effectively as shown in Figure 5. With increasing concentration between 0.10 and 0.90 mg/ml, the scavenging effects increased significantly. When the scavenging rate achieved 63.2%, it lay in a steady state, even if concentration increased.

Hydroxyl radicals are considered to cause the ageing of human body and some diseases. Figure 6 indicates that the chlorogenic acid extract exhibited a quite strong concentration-dependent inhibition of hydroxyl radical (R^2 = 0.99) .The results from the test systems suggest that the chlorogenic acid extract had significant antioxidant activity and the effect increased with increase of extract concentration.

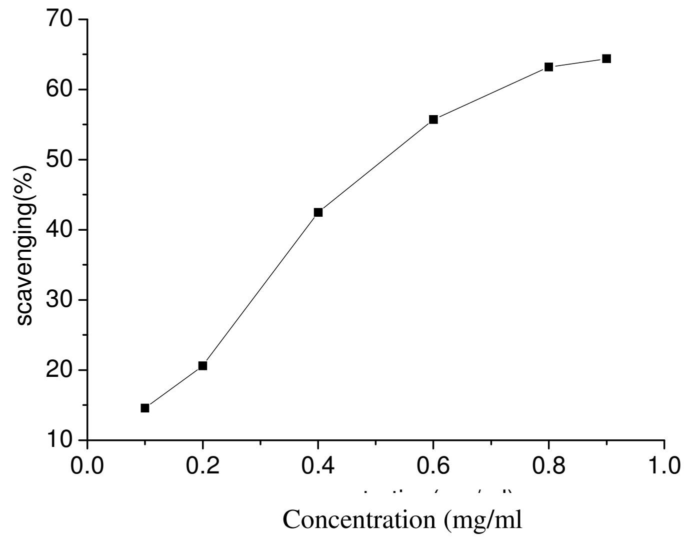


Figure 5. DPPH radical scavenging activity of chlorogenic acid extract.

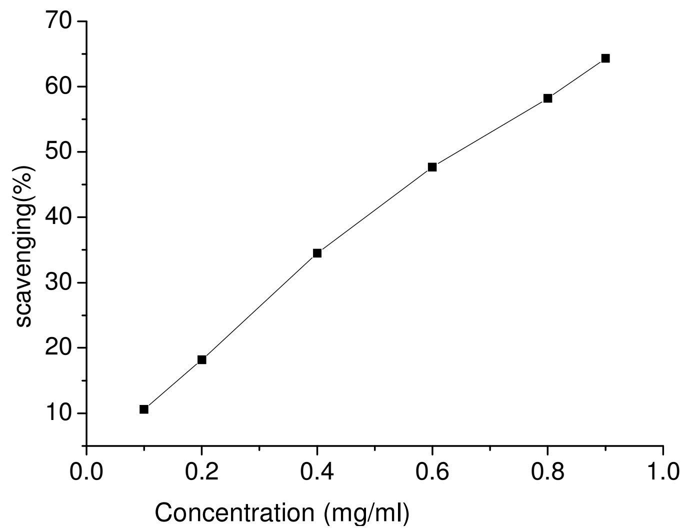


Figure 6. Hydroxyl radical-scavenging activity of chlorogenic acid extract.

Comparison of UAE with conventional extraction

Soxhlet extraction was performed in a Soxhlet apparatus. 20.0 g drug powder was placed in an extraction bag filter, and impregnated with ethanol.

Extraction was performed at 85℃ for about 4 h with 360 ml ethanol. Heat reflux extraction was conducted in a water bath at 80℃. An amount of 20.0 g rug powder were placed into a 1000 ml glass flask with 360 ml 85% (v/v) aqueous ethanol and extracted for two 2 h cycles.

It can be seen in Table 6 that the chlorogenic acid yield of Soxhlet method is maximum among the three methods and UAE is the second highest yield method. Though the chlorogenic acid yield was slightly lower than that of Soxhlet, UAE took only one by eighth time of Soxhlet. The chlorogenic acid yield of UAE is much higher than that of heat reflux extraction. Therefore, UAE can save a lot of time as compared to Soxhlet and heat reflux method and bring higher yield of chlorogenic acid. This shows that UAE extraction of chlorogenic acid from *F. eucommiae* has a relatively higher commercial value. The UAE method showed obvious advantages in terms of short duration time and high efficiency to extract chlorogenic acid from *F. eucommiae*.

Conclusion

Ultrasonic-assisted extraction technique was developed for the fast extraction of chlorogenic acid from *F. eucommiae* leaf. The antioxidant activity including

Table 6. Comparison of UAE with other extraction methods.

Extraction methods	Extraction time	Extraction solvent	Chlorogenic acid yield (10^{-1}mg/g)
Soxhlet	4 h	85% ethanol	8.19
Heat reflux extraction	2 h*2	85% ethanol	4.61
UAE	30 min	50% ethanol	7.57

hydroxyl radical-scavenging activity, free radical scavenging activity on DPPH of the chlorogenic acid extract was evaluated. The results showed good correspondence between experimental and predicted values.

It was found that the most effective parameter was solvent to material ratio, which was in good agreement with the experimental value. The adjusted coefficient of determination (R_{adj}^2) for the model was 96.3%. Probability value ($P < 0.001$) demonstrated a very high significance for the regression model. The maximum yield of chlorogenic acid 0.77 mg/g with UAE was obtained by dual extraction with solvent to material ratio of 17/1, irradiation time of 25 min, solvent concentration of 52%, extraction temperature of 50℃ and ultrasonic frequency 5 kHz. The optimal yield with UAE was slightly lower than that of Soxhlet extracted and higher than that of heat reflux extraction with water. The extracts exhibited high scavenging effects on DPPH radical with increasing concentration between 0.1 and 0.9 mg/ml. The extracts also exhibited a quite strong concentration-dependent inhibition of hydroxyl radical.

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REFERENCES

Alejandro CO, Héctor CG, Fernando AI (2004). An Integrated Matlab tool box for first order multivariate calibration. Chemom. Intell. Lab. Syst., 73: 189-197.

Chattip P, Wanchai De-Eknamkul (2008). Extraction of flavonoids and carotenoids from Thai silk waste and antioxidant activity of extracts. Sep. Purif. Technol., 62: 444-448.

Cheung HY, Lai WP, Cheung MS (2003). Rapid and simultaneous analysis of some bioactive components in *Eucommia ulmoides* by capillary electrophoresis. J. Chromatogr,, 989(2): 303-310.

Devanand LL, Ronita B, Savithiry N (2007). Comparison of extraction solvents and techniques used for the assay of isoflavones from soybean. Food Chem,, 105(1): 325-333.

Elena Pastor-Cavada, Rocio J, Julio E, Pastor (2009). Antioxidant activity of seed polyphenols in fifteen wild *Lathyrus* species from South Spain. LWT., 42: 705-709.

Geun, WL, Hyun CY, Sang YB (2004). Inhibitory effect of *Eucommia ulmoides* Oliver on adipogenic differentiation through proteome analysis. Enzym. Microb. Technol., 35: 632-638.

Guiming F, Yin W, Shuo Z, Zuowei Z (2007). Supercritical fluid extraction technology of flavonoids in *Evcommia ulmoides* Oliv. Leaves and components analysis by LC-MS. Chin. Food Chem., 28: 128-131 (in Chinese).

Hsieh CL, Yen GC (2000). Antioxidant actions of Du-zhong (*Eucommia ulmoides* Oliv.) toward oxidative damage in biomolecules. Life Sci., 66(15): 1387-1400.

Huang W, Xu eA, Niu H (2009). Optimised ultrasonic-assisted extraction of flavonoids from *Folium eucommiae* and evaluation of antioxidant activity in multi-test systems *in vitro*. Food Chem., 114: 1147-1154.

Hung MY, Timothy Yu-Chi Fu, Shih PH (2006). Du-Zhong (*Eucommia ulmoides* Oliv.) leaves inhibits CCl4-induced hepatic damage in rats. Food Chem. Toxicol., 44(8): 1424-1431.

Jiang ST, Shao P, Pan LJ, Zhao YY (2006). Molecular Distillation for Recovering Tocopherol and Fatty Acid Methyl Esters from Rapeseed Oil Deodoriser Distillate. Biosyst. Eng., 93(4): 383-391.

Jing W, Baoguo S, Yanping C, Yuan T, Xuehong L (2008). Optimisation of ultrasound assisted extraction of phenolic compounds from wheat bran. Food Chem., 106: 804-810.

Kamaljit V, Raymond M, Lloyd S, Darren B (2008). Application and opportunities for ultrasound assisted extraction in the food industry-A Rev. Innov. Food Sci. Emerg. Technol., 9(2):, 161-169.

Shao P, He JZ, Sun PL, Jiang ST (2009). Process optimisation for the production of biodiesel from rapeseed soapstock by a novel method of short path distillation. Biosyst. Eng., 102(3): 285-290.

Wu Y, Steve WC, Tang J, Gu XH (2007). Optimization of extraction process of crude polysaccharides from boat-fruited sterculia seeds by response surface methodology. Food Chem., 105(4): 1599-1605.

Zhang B, Yang RY, Zhao Y (2008). Separation of chlorogenic acid from honeysuckle crude extracts by macroporous resins. J. Chromatogr. B., 867: 253-258.

Zhang HF, Yang XH, Zhao LD, Wang Y (2009). Ultrasonic-assisted extraction of epimedin C from fresh leaves of Epimedium and extraction mechanism. Innov. Food Sci. Emerg.Technol., 10(1): 54-60.

Zhou YQ, Liang M, Li WZ (2009). Protective effects of *Eucommia ulmoides* Oliv. bark and leaf on amyloid β-induced cytotoxicity. Environ. Toxicol. Pharmacol., 28(3): 342-349.

Veličković DT, Milenović DM, Ristić MS, Veljković VB (2006). Kinetics of ultrasonic extraction of extractive substances from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage. Ultrason. Sonochem., 13(2): 150-156.