African Journal of Biotechnology Vol. 10(65), pp. 14637-14643, 24 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1841
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Optimization for ultra high pressure extraction of *Scutellaria barbata* by central composite design-response surface methodology

Sheng-tan Zhang[1,2#], Zhao-yu Wang[3#], Guo-hua Cheng[4#], Tie-shan Wang[1], Zheng Ni[1] and Jing-ming Lin[1]*

[1]Department of Pharmacy, Zhujiang Hospital, Southern Medical University, Guangzhou, 510282, China.
[2]Department of Pharmacy, Cang zhou Hospital of Integrated Traditional Chinese and Western Medicine of Hebei Province, Cangzhou,061001,China.
[3]College of Life Science and Biopharmacology, Guangdong Pharmaceutical University, Guangzhou, 510006, China.
[4]Department of Pharmacy, Cancer Hospital, Guangzhou Medical University, Guangzhou, 510095, China.

Accepted 15 September, 2011

A high performance ultra high pressure (UHP) technique was developed for high yield extraction of bioactive components from *Scutellaria barbata* at lower temperatures, within a short time, and for less power consumption. The UHP process of scutellarin, luteolin and apigenin from *S. barbata* was optimized by using central composite design-response surface methodology (CCD-RSM). The optimum result of UHP was compared with those of the conventional extraction methods of circulation reflux, ultrasonic and microwave. The optimal conditions for extraction were: extracting pressure of 204.72 MPa, ethanol concentration of 61.44% and solid-liquid ratio of 1:78.79. The extraction yield of scutellarin, luteolin and apigenin from *S. barbata* were: 12.38, 1.76 and 0.23 mg g^{-1}, respectively and an integrated score of 7.18. This showed that the UHP was more effective than the conventional extraction methods.

Key words: Ultra high pressure, central composite design-response surface methodology, *Scutellaria barbata*, high performance liquid chromatography.

INTRODUCTION

Scutellaria barbata D. Don (Ban Zhi Lian, BZL), is a traditional Chinese medicine for clearing heats, relieving toxicity, reducing swelling, curing sores and abscesses (Dharmananda, 2004). Its anti-cancer property has recently been reported and the clinical trial of its extract for advanced breast cancer treatment was conducted in US (Fong et al., 2008). Previous investigations showed that this plant had over 30 flavonoids, more than 10 neo-clerodane-type diterpenoids, triterpenoids and sterol glucosides, some of which exhibited interesting biological activities against tumor and viruses, immune adjustment, Wang et al., 2010).There are various of methods for etc. (Yu et al., 2004; Hanna et al., 2010; Dai et al., 2010; extracting salidroside such as reflux extraction, ultrasonic extraction and Soxhlet extraction (Zhang et al., 2007). Besides long extracting time, most of these methods employ heating which could easily decompose some thermo-sensitive ingredients or transformation.

Ultra high pressures (UHP) are widely used in the ceramics, casting industry, pharmaceutics, metallurgy, plastics making and civil engineering. Literatures reported that UHP technique could shorten processing time and reach high extraction yields, without any adverse side effects on the activity and structure of the bioactive components.

The application of high pressure to plant materials was initially reported (Prasada et al., 2009). UHP has been use for the extraction of flavonoids, anthocyanins, ginse-

*Corresponding author. E-mail: linjm1231@21cn.com.

#These authors contributed equally to this paper.

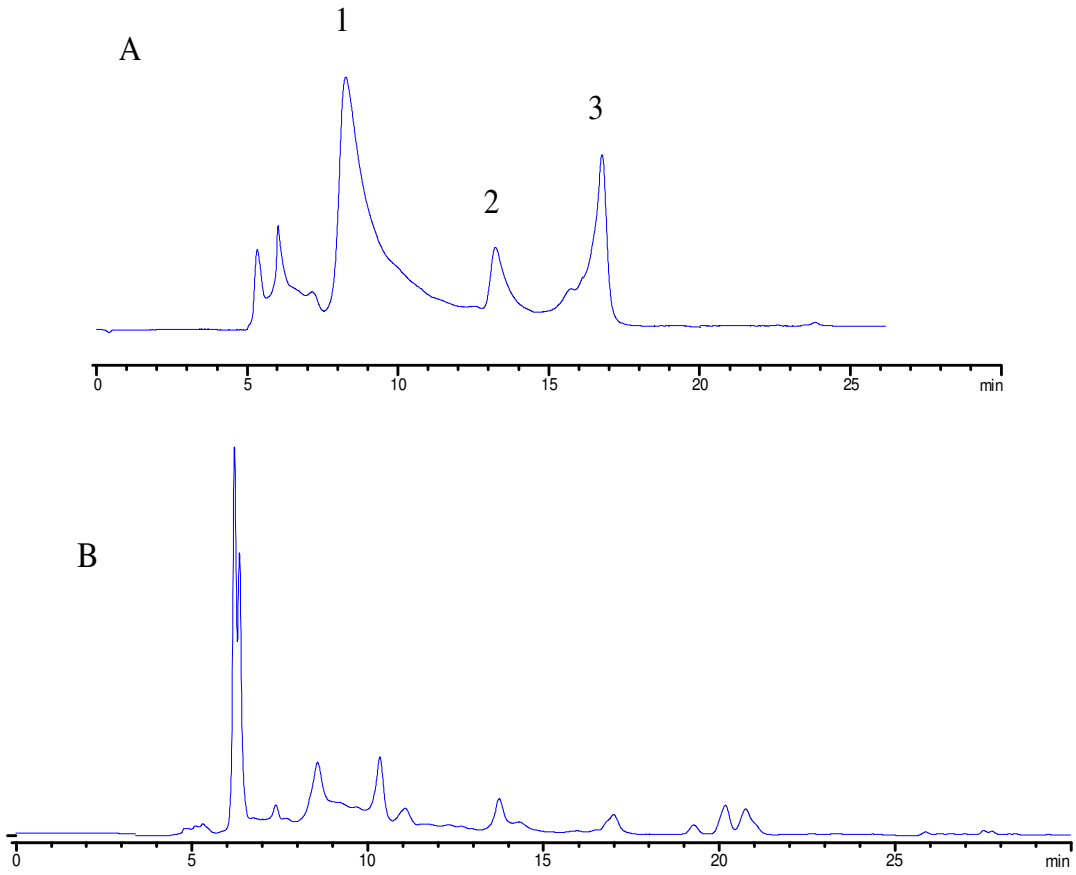

Figure 1. A: HPLC of mixed reference standard. 1, Scutellarin; 2, apigenin; 3, luteolin. B: HPLC of *S. barbata* sample.

noside, flavones and salidroside (Prasada et al., 2009, 2010; Corrales et al., 2008; Xi et al., 2009; Angela et al., 2011).

This research used the UHP extraction technique and CCD-RSM to extract scutellarin, luteolin and apiolin from S. barbata, and also compared UHP with the extracting method of circulation reflux, ultrasonic and microwave.

MATERIALS AND METHODS

DL700-0.55 mm × 1.5 mm Ultrahigh pressure extraction device (Shanghai Dalong machine device company); DFT-100 high-speed smash device (Wenling Lin Tai Machinery Co., Ltd.); MA-110 electronic scale (precision 0.1mg, Shanghai Scale Instrument company); Agilent1100 high performance liquid chromatograph (American Agilent Technologies Co. Ltd.); KQ-600DE Nc ultrasonic apparatus (Kunshan ultrasonic machine Co. Ltd) and microwave oven (Guangdong Galanz Co. Ltd) were used. *S. barbata* (Henan) was authenticated by Zhang Shouyao, the Dean of Pharmacy Department of Zhujiang Hospital, Guangdong province. Scutellarin (China pharmaceutical and biological products inspection, No: 110842-200605), luteolin (Shanghai crystal pure reagent Co. Ltd, NO: 22806-1109851), apiolin (Shanghai crystal pure reagent Co. Ltd, NO: 22803-1109847), methyl alcohol were chromatographic pure, ethanol and the other reagents were analytically pure (Tianjin Damao chemical reagent Co. Ltd).

Extraction of active ingredient from *S. barbata* by UHP

The *S. barbat*a was crushed using a 24 mesh screen. About 2.0 g plant material was set in a sealed bag and solvent was added, the bubbles were sealed, the pressure was increased, keeping pressure for 90 s, then the pressure was removed and extracts were obtained, filtered using a 0.22 μm filter membrane, and then stored at 4°C.

Determination of scutellarin, luteolin and apigenin

High performance liquid chromatography analysis

Hypersil ODS C18 column (4.6 × 250 mm, 5 μm Dalian Yi Lite); a mobile phase of methanol (A) - acetic acid water (B pH 2.4), elution gradients of 0 to 60 min, A 22 to 64%; 0 to 60 min, B 78 to 36%; detection wavelength of 335 nm, flow rate of 1 ml·min^{-1}, and column temperature of 30°C were used. In this chromatography conditions, the chromatogram of reference substance and *S. barbata* samples are shown in Figure 1.

Preparation of standard curve

0.01378 g scutellarin, 0.00463 g luteolin and 0.00596 g of apigenin were added into a 50 ml flask, accurately, and appropriate amount of methanol was added to the mark to dissolve. 0.05, 0.1, 0.5, 1.0, 2.0 and 5.0 ml of mixture control solution were transferred into a 10

Table 1. The level of form factors (r = 1.732).

Factor	-r	-1	0	1	r
extraction pressure (X_1)	100	142.6	200	257.7	300
solvent concentration (X_2)	25%	40%	60%	80%	95%
solid-liquid ratio (X_3)	5	35.6	77.5	119.3	150

Table 2. Experimental design.

S/N	X_1	X_2 (%)	X_3
1	142.6	40	35.6
2	200.0	40	35.6
3	142.6	80	35.6
4	257.7	80	35.6
5	142.6	40	119.3
6	257.7	40	119.3
7	142.6	80	119.3
8	257.7	80	119.3
9	100.0	60	77.5
10	300.0	60	77.5
11	200.0	25	77.5
12	200.0	95	77.5
13	200.0	60	5
14	200.0	60	150
15	200.0	60	77.5
16	200.0	60	77.5
17	200.0	60	77.5
18	200.0	60	77.5
19	200.0	60	77.5
20	200.0	60	77.5

ml volumetric flask, respectively. Then, an amount of methanol was added to the mark for dilution. 30 µl of the mixture control solutions of different concentrations was taken for analysis. With the concentration of sample as abscissa and the peak area value as vertical axis, the regression equation was calculated. Regression curve of scutellarin was $Y_1 = 2876.3X_1 + 3.9815$ (r = 0.9991). Scutellarin showed good linear relationship in the range of 0.04134 to 4.13425 µg. Regression curve of luteolin was $Y_2 = 3572X_2 + 8.9952$ (r = 0.9996). Luteolin showed good linear relationship in the range of 0.01389 to 1.38923 µg. Regression curve of apigenin was $Y_3 = 4027.3X_3 + 7.8629$ (r = 0.9994). Apigenin showed good linear relationship in the range of 0.01788 to 1.78816 µg.

Determination of extraction sample

Precise amount of sample filtration (30 µl) was analyzed with the earlier mentioned chromatographic conditions and measured three times for each sample. The average peak area value of scutellarin, luteolin and apigenin was gotten, respectively. The data were taken into the standard curve and the content of the sample was calculated.

Optimization of the UHP process of *S. barbata* using the CCD-RSM

Literature (Zhang et al., 2007; Xi et al., 2011; Corrales et al., 2008) showed that the main factors affecting the extraction efficiency are pressure, concentration solvent and solid-liquid ratio. Extraction pressure (X_1), ethanol concentration (X_2) and solid-liquid ratio (X_3) were therefore selected as the main factors according to the CCD-RSM principles in this study to optimize the combination. The levels of the independent variables were -r, -1, 0, 1 and r, the experimental factors and levels of coding are shown in Table 1. The experiment design is shown in Table 2. The Design-Expert 8.0.4 software was used to for RSA analysis.

RESULTS

The results which were analyzed by Design-Expert 8.0.4 software are shown in Table 3. A multiple regression of three major factors (extraction pressure (X_1), ethanol concentration (X_2) and solid-liquid ratio (X_3)) in 20 trials,

Table 3. Results of design/response surface methodology.

Test number	Extraction ratio of scutellarin (mg·g^{-1})	Extraction ratio of luteolin (mg·g^{-1})	Extraction ratio of apigenin /mg·g^{-1}	Composite score
1	9.48	1.39	1.67	5.49
2	9.67	1.43	1.75	5.61
3	8.53	1.21	1.5	4.93
4	8.08	1.36	1.81	4.81
5	9.92	1.52	1.92	5.80
6	10.08	1.48	1.88	5.86
7	8.39	1.22	1.47	4.86
8	8.18	1.01	1.12	4.62
9	11.75	1.59	1.85	6.72
10	12.02	1.66	2.01	6.91
11	7.01	0.88	0.93	3.96
12	7.98	1.08	1.21	4.56
13	12.18	1.65	2.04	6.99
14	12.23	1.64	1.89	6.99
15	12.38	1.68	2.18	7.13
16	12.33	1.66	2.20	7.10
17	12.40	1.70	2.14	7.13
18	12.30	1.74	2.19	7.11
19	12.38	1.69	2.19	7.13
20	12.35	1.71	2.15	7.12

and the zero experiment repeated six times to obtain *S. barbata* extract provided the following quadratic regression model:

$$R = 7.14 + 0.055X_1 + 0.17X_2 + 0.021X_3 - 0.067X_1X_2 - 0.022X_1X_3 - 0.10X_2X_3 - 0.26X_1^2 - 1.11X_2^2 - 0.20X_3^2 - 0.62X_1^2X_2 - 0.077X_1X_2^2$$

Responses of experimental and composite scores in the regression equation predicted that the determination coefficient was 0.9648 (R^2 = 0.9648), showing that the model fitted well. Analysis of variance showed that the *F* value of the overall model test revealed a significant response (P <0.05) and that the effect of ethanol concentration on extraction rate of the three major components of *S. barbata* was also significant (P <0.05). The determination coefficient of the model (R^2 = 0.9246) showed that the model could explain 92.46% of the response value of the change, and that the model fitted better, confirming that the analysis could permit a forecast of the main components of *S. barbata*. The CCD-RSM was adopted with the extraction rate of scutellarin (a), luteolin (b) and apigenin (c) of integrate scores as evaluation index, optimizing the ultra high pressure extraction process. The results are shown in Table 3 and the ANOVA results are in Table 4. The integrated score = $0.5X_1 + 0.3X_2 + 0.2X_3$.

The results of regression equation coefficient are shown in Table 4, which showed that X_2 was very significant and X_1, X_3 were not significant in this model, from the F values, it could be seen that the single factor of the order: X_2> X_1> X_3, the ethanol concentration > extraction pressure > solid-liquid ratio; X_2^2 was significantly quadratic, the other factors were not significant. X_1, X_2, X_3 and their interaction effects on the response are shown in Figures 2 to 4, which directly reflected the interaction of various factors on the response values. Graphics showed that the solid-liquid ratio and ethanol concentration affected most significantly, the extraction yield of *S. barbata*, showing a steep curve; while extraction pressure had the lowest significant impact. With the increasing or decreasing of the extraction pressure, the response value of corresponding curves did not change significantly.

Verification test

The optimal extraction conditions were obtained by Design-Expert 8.0.4 software which was: extraction pressure conditions of 204.72 MPa, ethanol concentration of 61.44% and solid-liquid ratio of 1:78.79, then the integrate score of 7.15. 2.0 g of the three samples of *S. barbata* were set in a sealed bag, and 157.6 ml of 61.44% ethanol was added accurately, sealed, pressurized to 204.72 MPa, for 90 s, treated according to the earlier mentioned methods, then with 0.22 μm filter membrane, chromatographic conditions were determined as earlier mentioned, the extraction efficiency of scutellarin, luteolin and apigenin were 12.38, 1.76 and 2.31 mg g^{-1}, respectively, and with integrated score of

Table 4. Response surface regression analysis results.

Variance source	df	Sum of square	Mean square	F	P
X_1	1	0.018	0.018	0.070	0.798
X_2	1	1.810	1.810	6.580	0.0080
X_3	1	6.429E	6.429E	0.025	0.8785
X_1 X_2	1	0.036	0.036	0.14	0.7167
X_1 X_3	1	4.050E	4.050E	0.016	0.9034
X_2X_3	1	0.084	0.084	0.330	0.5837
X_1^2	1	1.040	1.040	4.010	0.0801
X_2^2	1	19.34	19.34	74.98	< 0.0001
X_3^2	1	0.620	0.620	2.391	0.1603
Model	11	21.490	1.950	7.580	0.0040
Error	5	2.883E	5.767E		
Total	19	23.56			

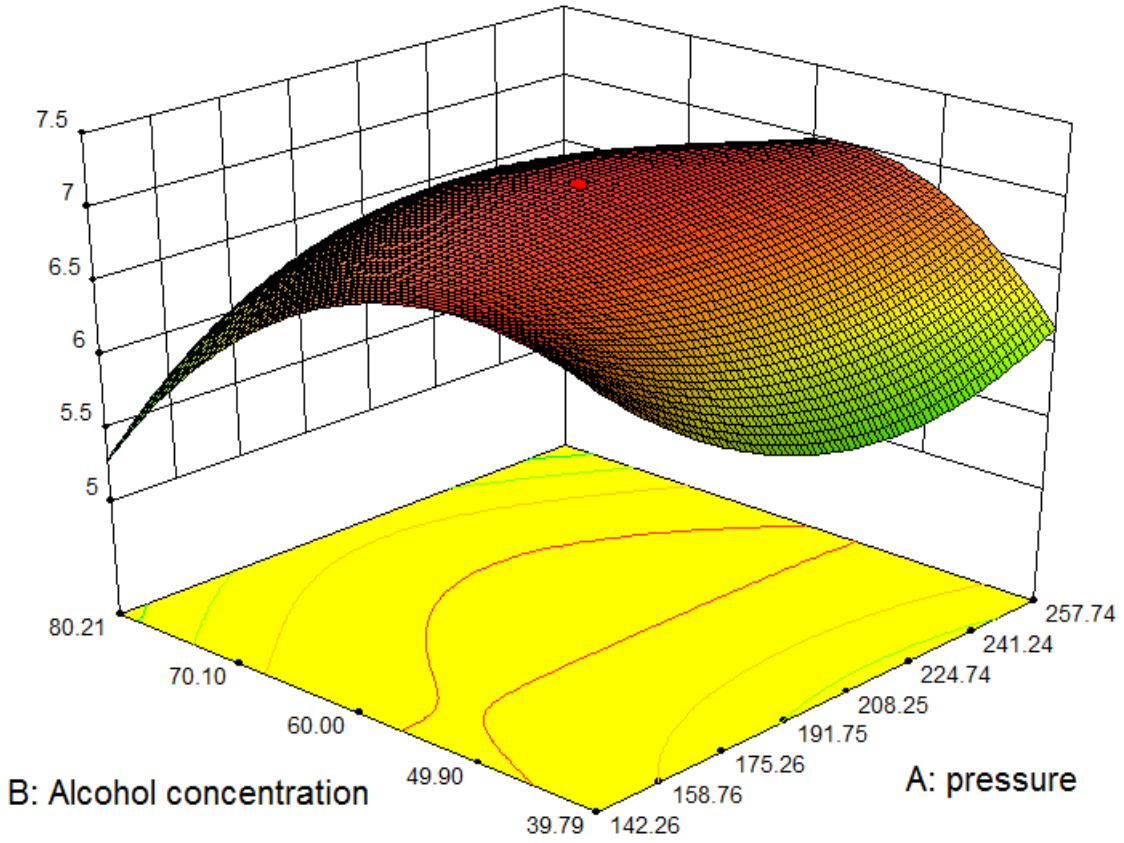

Figure 2. Effects of alcohol concentration and extraction pressure on extraction yield.

7.18. When compared with composite design verification test results, the prediction was closer and better.

Comparison of different extraction methods

About 2.0 g of four samples of *S. barbata* were set in a sealed bag, and solvent (157.6 ml) of 61.44% ethanol was added, respectively. The *S. barbata* samples were extracted by reflux extraction, ultrasonic extraction, microwave extraction and UHP method. The extraction conditions and the results are shown in Table 5, which showed that the contents of three main components in extracts were the highest by the UHP method.

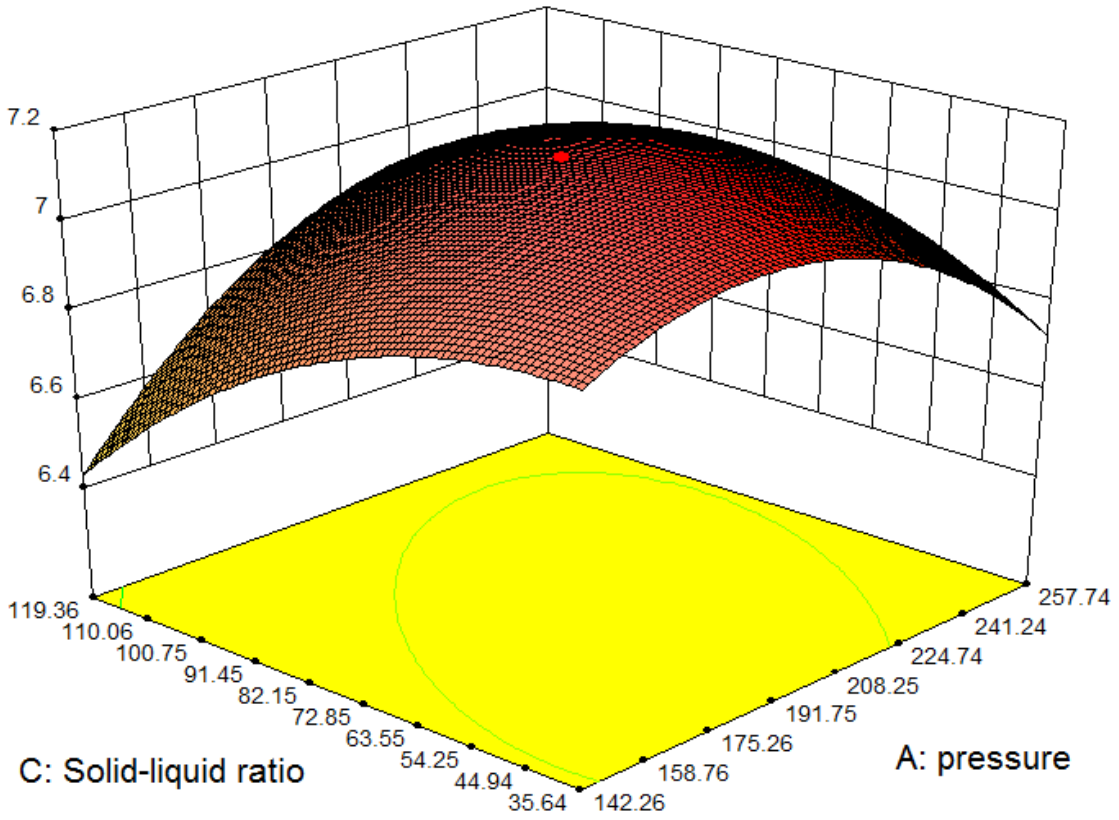

Figure 3. Effects of solid-liquid ratio and extraction pressure on extraction yield.

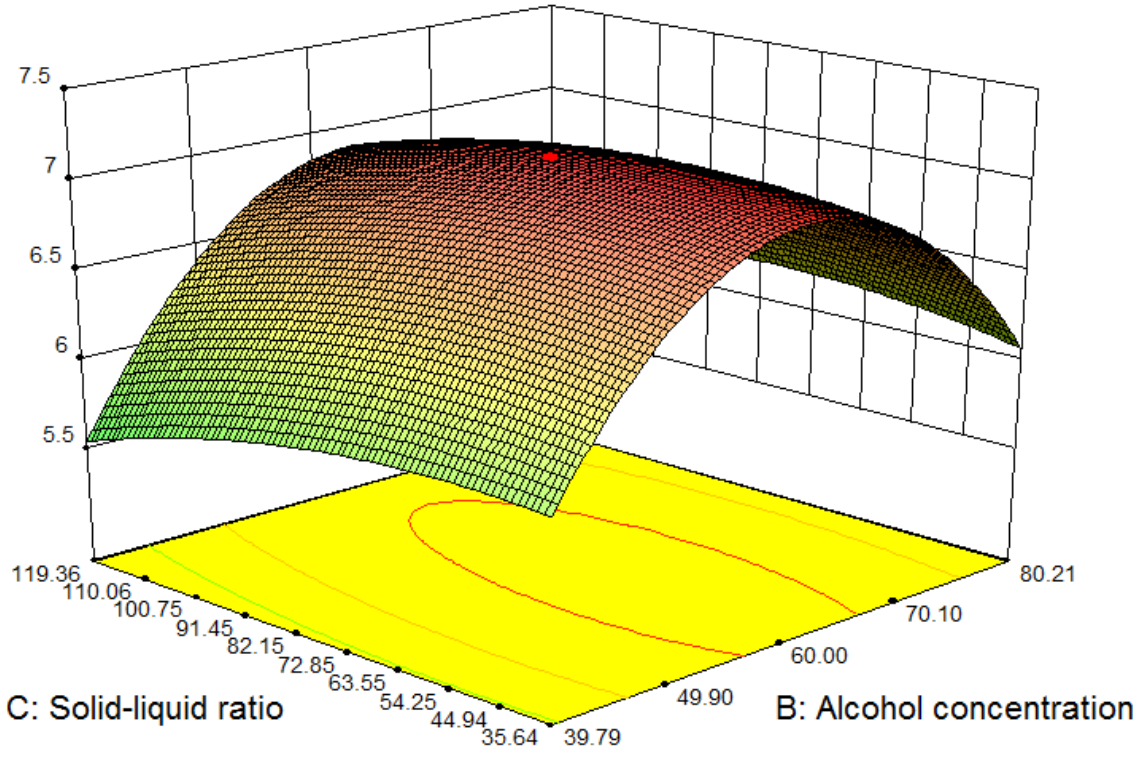

Figure 4. Effects of solid-liquid ratio and ethanol concentration on extraction yield.

Table 5. Comparison results for different extraction methods.

Extraction method	Extraction condition	Extraction ratio of Scutellarin (mg·g^{-1})	Extraction ratio of luteolin (mg·g^{-1})	Extraction ratio of apigenin (mg·g^{-1})	Composite score
Refluxing extraction	60℃ , 4 h	7.56	1.14	1.49	4.42
Ultrasonic extraction	40 kHz, 40 min	9.13	1.28	1.96	5.34
Microwave extraction	2450 MHz, 3 min	9.26	1.36	1.85	5.41
UHP extraction	200 MPa, 90 s	12.35	1.77	2.28	7.16

DISCUSSION

In this study, the possibility of using ultra-high-pressure assisted extraction to recover antioxidant and antity-rosinase compounds was investigated. These results indicate that the sample of ultra-high-pressure assisted extraction possessed higher phenolic contents and exhibited stronger antioxidant activities than other samples (Prasada et al., 2010). As a new plant extraction technology in recent years, UHP provides a new approach for traditional Chinese medicine extraction (Liao et al., 2010). The advantages of this method are as follows: 1, lower operating temperature; 2, wide range of application, water or alcohol soluble, fat-soluble and polar, weakly polar or non-polar active ingredients can be extracted using this technique; 3, short extraction time, usually only 1 to 20 min; 4, high extraction efficiency, low energy consumption; 5, fewer impurities, can significantly reduce the microbial content of raw materials, thus extending the storage time; 6, extraction process is simple, no environmental pollution, and the high degree of mechanization, is suitable for modern production. Literature (Zhang et al., 2004) suggested that in the extraction process of some herbs whose cell walls are easy to rupture, 100 MPa pressure was enough to break the cell wall for most of the herbs. For maximum extraction, solvent is the most important factor, followed by the liquid - solid ratio, and lastly, the extraction time and pressure. When compared with the conventional methods, ultra-high-pressure extraction time is short, only 2 min, much lesser than conventional methods, and has the advantages of higher extraction rate, simple operation, low energy consumption etc. The active ingredient of *S. barbata* extract can prevent heat loss and pharmacological effects or activity loss. In the next step, the pharmacodynamic comparison validation by different extraction methods will be studied. This effective extraction technologies and low-cost raw materials represent an environmental and economical alternative to conventional extraction methods where large amounts of organic solvents and long extraction times are required. The use of extraction technologies will reduce raw materials, processing wastes and facilitate the production of natural valuable products. Meanwhile, the crude extract should be subjected to further separation and purification. The composition and the pharmacological properties of flavonoids need to be further studied.

Furthermore, the UHP technique could be used in combination with other techniques such as ultrasonic extraction and enzymatic extraction to improve the extracting rate and efficiency.

REFERENCES

Angela SJ , Corinna ER , Ramón G (2011). Influence of ultra-high pressure homogenisation on antioxidant capacity, polyphenol and vitamin content of clear apple juice. Food Chem. 127: 447–454.

Corrales M, Toepfl S, Butz P (2008). Extraction of anthocyanins from grape by-products assisted by ultrasonics, high hydrostatic pressure or pulsed electric fields: A comparison. Innovat. Food Sci. Emerg. Technol. 9: 85–91.

Dai SJ, Qu GW, Yu Q (2010). New neo-clerodane diterpenoids from *Scutellaria barbata* with cytotoxic activities. Fitoterapi, 81: 737–741.

Dharmananda S (2004). Oldenlandia and Scutellaria, Antitoxin and Anticancer Herbs. Institute for Traditional Medicine, Portland, OR.

Fong S, Shoemaker M, Cadaoas J (2008). Molecular mechanisms underlying selective cytotoxic activity ofBZL101, an extract of *Scutellaria barbata*, towards breast cancer cells. Can. Biol. Ther. 7: 577–586.

Hanna L, Yu JK, Inho C (2010). Two novel neo-clerodane diterpenoids from *Scutellaria barbata*. Bioorganic Med. Chem. Lett., 20: 288-290.

Liao GP, He S, Zhang ZY (2010). Optimization of ultrahigh pressure extraction for polydatin and resveratrol from Polygonum cuspidatum by using uniform design. China J. Chin. Mater. Med. 35: 3282-3286.

Prasada KN, Bao Y, John S (2010). Enhanced antioxidant and antityrosinase activities of longan fruit pericarp by ultra-high-pressure-assisted extraction. J. Pharm. Biomed. Anal. 51: 471–477.

Prasada KN, Bao Y, Mouming Z (2009). High pressure extraction of corilagin from longan(Dimocarpus longan Lour.) fruit pericarp. Separat. Purif. Technol. 70: 41–45.

Wang P, Geng XT, Pi GP (2010). Preparative separation of four individual flavonoids in *Scutellaria barbata* D. Don based on high selectivity polymeric adsorbents with different polarities. J. Chromatogr. B. 878: 3375–3381.

Xi J, Shen DJ, Li Y, Zhang R (2011). Comparison of *in vitro* antioxidant activities and bioactive components of green tea extracts by different extraction methods. Int. J. Pharm. 408: 97-101.

Xi J, Shen DJ, Zhao S (2009). Characterization of polyphenols from green tea leaves using a high hydrostatic pressure extraction. Internati. J. Pharm. 382: 139–143.

Yu J, Lei J, Yu H (2004). Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. Phytochemistry, 65: 881-884.

Zhang SQ, Bi HM, Liu CJ (2007). Extraction of bio-active components from *Rhodiola sachalinensis* under ultrahigh hydrostatic pressure. Separat. Purif. Technol. 57, 277–282.

Zhang SQ, Chen RZ, Wang CZ (2007). Experiment study on ultrahigh pressure extraction of Ginsenosides. J. Food Eng. 79: 1-5.

Zhang SQ, Zhu JJ, Wang CZ (2004). Novel high pressure extraction technology. Int. J. Pharm. 278: 471-47.