Journal of Medicinal Plants Research, Vol. 4(4) pp. 304-308, 18 February, 2010
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2010 Academic Journals

Full Length Research Paper

Microwave-assisted extraction of flavonoids from Chinese herb *Radix puerariae (Ge Gen)*

Yan-ling Wang[1]*, Guang-Sheng Xi[1], Yong-Chun Zheng[1] and Fu-Sheng Miao[2]

[1]Department of Chinese Medicine, Jilin Agricultural Science and Technology College ,Jilin, Jilin Province, 132101, People's Republic of China.
[2]Physical Education College, Liaoning Normal University, Dalian, Liaoning Province, 116029, People's Republic of China.

Accepted 27 January, 2010

Microwave-assisted extraction (MAE) technique was developed for the extraction of flavonoids from *Radix puerariae* (FRP). Several influential parameters of the MAE procedure (ethanol concentration, solvent volume, microwave power and extraction time) were studied through single factor experiments and orthogonal experiment for the optimization of the extraction protocol. The optimal conditions of MAE were: ethanol concentration 70%, solvent volume 35 ml microwave power 255W and extraction time 6.5 min, while extraction yield of FRP was 8.37 mg/g. The developed MAE method provided a good alternative for the extraction of flavonoids from *R. puerariae*.

Key words: Microwave-assisted extraction **(**MAE), flavonoids, *Radix puerariae.*

INTRODUCTION

Radix puerariae is the dried root of *Pueraria lobata* (Willd.) Ohwi and *Pueraria thomsonii* Berth. In China, *R. puerariae* is known as '*Ge Gen*', and has been used as a traditional Chinese herb, which is also widely accepted by consumers over the world for its healthy effects. It has been used for the management of various diseases, including cardiovascular disorders (Yeung et al., 2006; Wang et al., 2008), or as an antimicrobial, pain-releasing and appetite-inducing agent (Keung and Vallee, 1998). It has also proven useful in the treatment of alcohol abuse and hypertension (Fan et al., 1985; Keung et al., 1997; Lukas et al., 2005). Flavonoids, derivatives of benzo-ç-pyrone, are widespread in plants (Burda and Oleszek, 2001) and they are considered to be an active ingredient in *R. puerariae* (Li et al., 2003). Xu and He (2007) have indicated that puerarin (daidzein 8-C-glucoside), daidzein and rutin are the major flavonoids in *R. puerariae*. In recent years, flavonoids have attracted the interest of researchers because they show promise of being powerful antioxidants which can protect the human body from free radicals (Bors et al., 1996; Halliwell and Gutteridge, 1999).

Conventional techniques as heating, boiling, or refluxing can be used to extract flavonoids, however, the disadvantages are the loss of flavonoids due to ionization, hydrolysis and oxidation during extraction as well as the long extraction time (Li et al., 2005; Huang et al., 2009). Other techniques which include supercritical carbondioxide extraction, subcritical water extraction, ultrasonic assisted extraction (UAE) and microwave-assisted extraction (MAE) have also become of interest as alternatives for the conventional methods. Among these, MAE is the simplest and the most economical technique for extraction of many plant derived compounds (Zhang et al., 2005; Hemwimon et al., 2007). The enhancement of product recovery by microwave is generally attributed to its heating effect, which occurs due to the dipole rotation of the solvent in the microwave field. This causes the solvent temperature to rise, which then increases the solubility of the compound of interest. Specifically, solvent heating by microwave occurs when molecules of the polar solvent could not align themselves quickly enough to the high frequency electric field of microwave. This discrepancy causes the solvent molecules to dissipate the absorbed energy in the form of heat (Hemwimon et al., 2007). Nevertheless, no reports on MAE of flavonoids from *R. Puerariae* (FRP) have been published.

In the present study, MAE for the flavonoids from *R.*

*Corresponding author. E-mail: ylwxgs@yahoo.cn. Tel: +86-0432-3509060. Fax: +86- 0432-3509711.

puerariae (FRP) were investigated and the operational parameters were optimised using single factor experiments and orthogonal experiment design. The objective of the work is to establish the optimised condition of MAE for FRP for development and application of the medicinal plant resource.

MATERIALS AND METHODS

Plant material

R. puerariae were purchased from a commercial market in Jilin, China. A voucher specimen (No. 2036) was deposited at the Museum, Traditional Chinese Medicine Sciences, Jilin Agricultural Science and Technology College. Samples were pulverised in a knife mill and were passed through a 20-mesh sieve.

Microwaves-assisted extraction (MAE)

MAE experiments were performed with a MSP-100E multimode microwave extraction system. The MSP-100E instrument has an internal temperature control system with a fibre temperature probe and a pressure control system, which respectively monitors the temperature and the pressure inside the vessel. In all of the experiments, the pressure was set under 300 kPa to prevent the dissolution of the target compound (Liu et al., 2007; Zhang et al., 2009). A 15 ml ethanol solution was added to 0.5 g of dried sample powder placed in an inner vessel. The extraction was carried out with different extraction conditions. The extract was filtered and the filtrate was collected and freeze-dried for determination of flavonoids.

Determination of flavonoids

The UCARY-100 spectrophotometer (Varian, USA) was used to determine the content of flavonoids in the above isolated product at 510 nm (Zhuang et al., 1992; Chen and Li, 2007). The flavonoid content was calculated using the following linear equation based on the calibration curve prepared by rutin, range from 8.0 to 40 μg ml^{-1}.

$A = 11.237C – 0.0141$, $R2 = 0.9997$

Where; A is the absorbance, C is the flavonoid content in μg ml^{-1}.

RESULTS AND DISCUSSION

Effect of ethanol concentration on extraction yield of FRP

The factors concerning MAE include ethanol concentration, solvent volume, microwave power and extraction time. The influence of each factor was studied by single factor experiments (Chen et al., 2006; Zhang et al., 2009).

The selection of the most suitable solvent for extracting the analytes of interest from the sample matrix is a fundamental step in developing any extraction method. Methanol has a relatively higher dissipation factor, which means that it could absorb much of the microwave energy and transform it into heat better than other solvents.

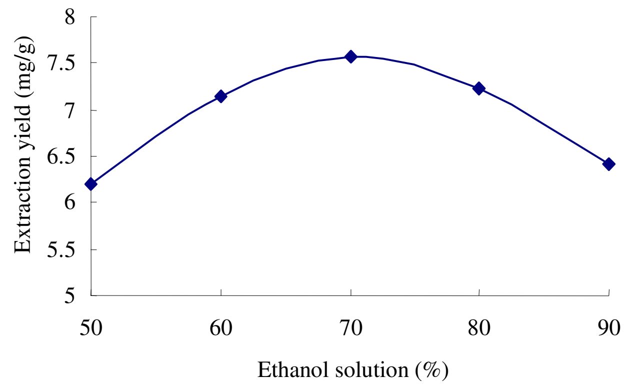

Figure 1. Effect of ethanol concentration on the extraction yield of FRP.

However, methanol was not tested in this study, because it is highly toxic and is not practical for use in food and pharmaceutical processing (Hemwimon et al., 2007). For this reason, mixtures of ethanol–water were tested under the same conditions. The results were summarized in Figure 1. It can be observed that the extraction yield of FRP were greatly influenced by the ethanol concentration. The extraction yield of FRP increased sharply with the increase of ethanol concentration up to 70%. When ethanol concentration increased from 70 to 90 %, however, extraction yield slightly decreased. It was in accordance with existed studies (Duan et al., 2001; Ma et al., 2005). From these results, it is clear that the addition of some amount of water enhances the extraction efficiency. One possible reason for the increased efficiency with a presence of some water might be due to the increase in swelling of plant material by water, which increased the contact surface area between the plant matrix and the solvent (Chen et al., 2008). Therefore, further studies were conducted with 70% ethanol.

Effect of solvent volume on the extraction yield of FRP

Generally in conventional extraction techniques, a higher volume of solvent will increase the recovery, but in MAE, a higher solvent volume may give lower recoveries (Eskilsson and Bjorklund, 2000; Guo et al., 2001; Xiao et al., 2008).

To investigate the influence of solvent volume on extraction yield of FRP, experiments were performed by increasing the extractant solvent volume from 20 to 60 ml under the experimental conditions described above. It is seen in Figure 2 that the extraction yield of FRP increased with the increase of solvent volume and reached its maximum at 30 ml/g. It decreased as solvent volume was above 30 ml/g. This was probably due to the larger volume of 70% ethanol causing excessive swelling of the material by water and absorbing the effective con-

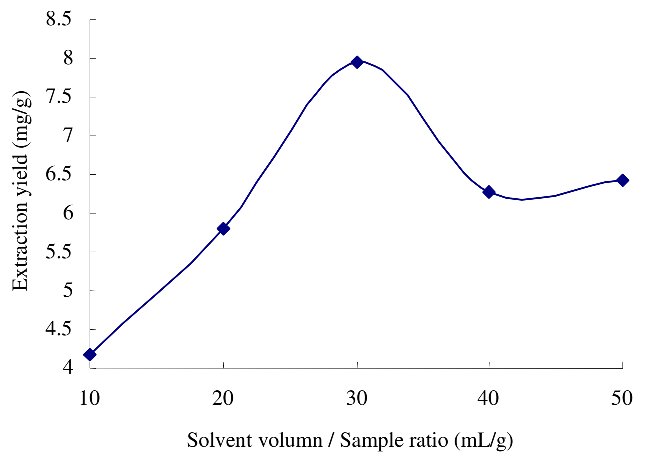

Figure 2. Effect of solvent volume on the extraction yield of FRP.

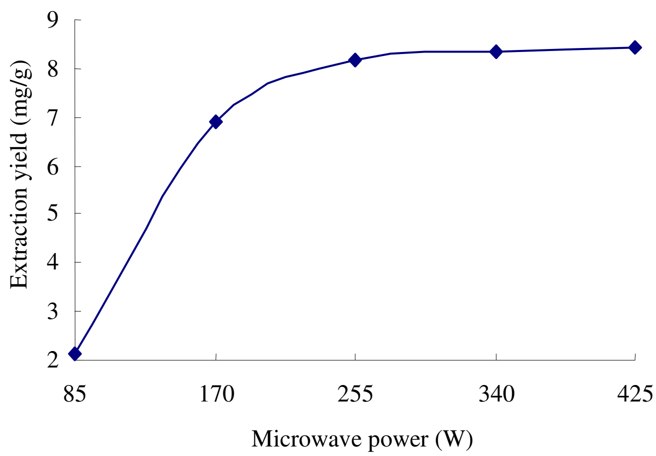

Figure 3. Effect of microwave power on the extraction yield of FRP.

stituent (Xiao et al., 2008). Therefore, a volume of 30 ml was enough for extraction.

Effect of microwave power on the extraction yield of FRP

Microwave irradiation energy disrupts hydrogen bonds, because of microwave-induced dipole rotation of molecules and migration of dissolved ions. Microwave irradiation energy can enhance the penetration of the solvent into the matrix and deliver efficiently to materials through molecular interaction with the electromagnetic field and offer a rapid transfer of energy to the solvent and matrix, allowing the dissolution of components to be extracted (Duan et al., 2001; Hu, 2003; Guo et al., 2003).

In order to evaluate the effect of microwave power MAE, the different microwave powers were controlled for 6 min, e.g., 85, 170, 255, 340 and 425 W. The results are shown in Figure 3. The experimental results demonstrate that the extraction yield of FRP increase with the enhancement of the microwave forward power when the processing forward power is in the range of 85 – 255 W, and then the extraction yield of FRP are not significantly changed from 255 to 425 W. This is due to the fact that the accelerated extraction of flavonoids by increasing microwave power is related to the direct effects of microwave energy on biomolecules by ionic conduction and dipole rotation which result in power dissipated inside the solvent and plant material and then generate molecular movement and heating (Chen et al., 2008). More electromagnetic energy was transferred to the extraction system quickly and improved the extraction efficiency when the microwave power increased from 85 to 255 W. Therefore, it was decided to use 255 W microwave power in the following experiments.

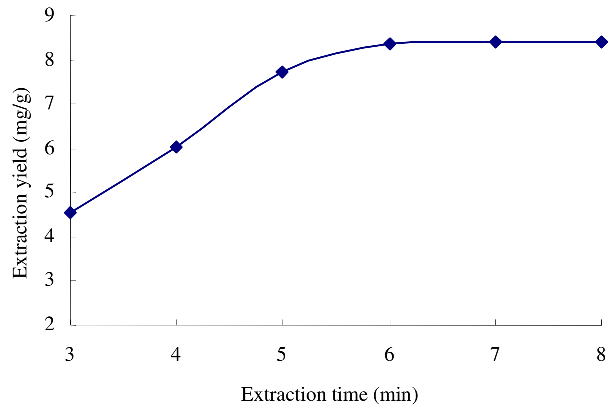

Figure 4. Effect of extraction time on the extraction yield of FRP.

Effect of extraction time on the extraction yield of FRP

Extraction time is a factor that must be studied to increase the effectiveness of extraction of analytes employing MAE (Zhou et al., 2007). Studies were carried out at different times, e.g., 3, 4, 5, 6,7and 8 min. The results are shown in Figure 4. The experimental results demonstrates that the extraction yield of FRP with the increase of the extraction time from 3 to 8 min, do not change from 6 to 8 min. The significance of the extraction time can be presumed to be related to the time required for the desorption process to take place (Chen et al., 2008). Therefore, 6 min was considered as the appropriate extraction time.

Optimization of MAE conditions of FRP

Since various parameters potentially affect the MAE process, the optimization of the experimental conditions represents a critical step in the development of a MAE method (Pan et al., 2008). In this work, four parameters were evaluated: (A) ethanol concentration, (B) solvent

Table 1. The factors and levels for the orthogonal design.

Levels	Ethanol concentration	Solvent volume	Microwave power	Extraction time
	A	B	C	D
1	65	25	170	5.5
2	70	30	255	6.5
3	75	35	340	6.5

Table 2. The results of orthogonal experiment L_9 (3^4).

Test numbers	A	B	C	D	Extraction yield of FRP
1	1	1	1	1	7.18
2	1	2	2	2	7.92
3	1	3	3	3	8.03
4	2	1	2	3	8.15
5	2	2	3	1	8.11
6	2	3	1	2	7.96
7	3	1	3	2	7.93
8	3	2	1	3	7.81
9	3	3	2	1	8.06
K_1	7.710	7.753	7.650	7.783	
K_2	8.073	7.947	8.043	7.937	
K_3	7.933	8.017	8.023	7.997	
R	0.363	0.264	0.393	0.214	

volume, (C) microwave power, (D) extraction time. On the basis of the effects of single factor in above chapters, for each variable, three levels were set to detect the most suitable extraction conditions as described in Table 1. In general, a full evaluation of the effect of four parameters at four levels on the yield would require 81(3^4) experiments. In order to reduce the number of experiments, an L_9 (3^4) orthogonal design graph was used (Table 2). In this way, only 9 experiments were necessary. As seen from Table 2, we can find that the influence to the mean extraction yields of FRP decreases in the order: C > A > B> D according to the R values. Microwave power was found to be the most important determinant of extraction yield of FRP. The best combination shown was $A_2B_3C_2D_3$ which is in specific, ethanol concentration was 70%, solvent volume was 35 ml, microwave power was 255 W and extraction time was 6.5 min. That was also the optimal extraction condition, while extraction yield of FRP was 8.37 mg/g.

Conclusion

Microwave-assisted extraction was determined to be an effective method for extracting flavonoids from *R. Puerariae*. Extraction yield of FRP were affected by ethanol concentration, solvent volume, microwave power and extraction time. The optimal condition through single factor experiments and orthogonal experiment was determined as followings: ethanol concentration 70%, solvent volume 35 ml, microwave power 255 W and extraction time 6.5 min. This showed great potential for industrial application in the near future.

ACKNOWLEDGEMENT

Partial support was received with gratitude from the education department of China's Jilin province (Project No. 2007239).

REFERENCES

Burda S, Oleszek W (2001). Antioxidant and Antiradical Activities of Flavonoids. J. Agric. Food Chem. 49: 2774-2779.

Bors W, Heller W, Michel C, Stettmaier K (1996). Flavonoids and polyphenols: chemistry and biology. In: Cadenas, E., Paker, L. (Eds.), Handbook of Antioxidants. Marcel Dekker Inc, New York, pp. 409–466.

Chen JJ, Li XG (2007). Hypolipidemic effect of flavonoids from mulberry leaves in triton WR-1339 induced hyperlipidemic mice. Asia Pacific J. Clin. Nutr. 16: 290-294.

Chen LG, Ding L, Zhang HR, Li J, Wang YT, Wang XP, Qu CL, Zhang HQ (2006). Dynamic microwave-assisted extraction coupled with on-line spectrophotometric determination of safflower yellow in Flos carthami. Anal. Chim. Acta 580: 75–82.

Chen LG, Jin HY, Zhang HR, Li J, Qu CL, Zhang HQ (2008). Dynamic microwave-assisted extraction of flavonoids from Herba Epimedii. Separation Purif. Technol. 59: 50–57.

Duan R, Wang B, Shi HX (2001). A Study on the Flavonoid-extracting Technology by Microwave from Ginkgo Biloba Leaves. J. Huaihai Institute Technol. 10: 46–48.

Eskilsson CS, Bjorklund E (2000). Analytical-scale microwave-assisted extraction. J. Chromatogr. A, 902: 227–250.

Fan LL, O'Keefe DD, Powell WWJ (1985). Pharmacologic studies on radix puerariae: effect of puerarin on regional myocardial blood flow and cardiac hemodynamics in dogs with acute myocardial ischemia. Clin. Med. J. 98: 821–832.

Guo Z, Fan G, Duan Y, Qin C, Wen M (2001). Microwave-assisted extraction of effective constituents from a Chinese herbal medicine Radix puerariae. Anal. Chim. Acta 436: 41–47.

Guo ZS, Li M, Feng YS (2003). Extraction of *pueraria* flavonid from pueraria lobata by microwave. J. Qiqihar Univ. (Natural Science Edition). 19: 14–15.

Halliwell B, Gutteridge JMC (1999). Free Radicals in Biology and Medicine. Oxford University Press Inc., New York. 225p.

Hemwimon S, Pavasant P, Shotipru A (2007). Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. Separat. Purif. Technol. 54: 44–50.

Hu QG (2003). A Study on the Flavonoid-extracting Technology by Microwave from Mulberry Leaves. J. Huaibei Ind. Teach. Coll. (Natural Sciences Edition). 24: 32–34.

Huang W, Xue A, Niu H, Jia Z, 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: 1147–1154.

Keung WM, Klyosov AA, Vallee BL (1997). Daidzin inhibits mitochondrial aldehyde dehydrogenase and suppresses ethanol intake of Syrian golden hamsters. Proceedings of the National Academy of Sciences of the United States of America, 94: 1675–1679.

Keung WM, Vallee BL (1998). Kudzu root: An ancient Chinese source of modern antidipsotropic agents. Phytochemistry 47: 499–506.

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

Li MQ, Sheng X, Shao XG (2003). Separation and Determination of Active Components of Pueraria Lobata Isoflavonoid Extract by Reversed Phase High Performance Liquid Chromatography. Chin. J. Anal. Chem. 31: 178–180.

Liu XYU, Zhang J, Wang RX (2007). Study on Extracting Process of Pueraria Flavone and Evaluation of Anti-oxidant Activity. Food Sci. 28: 232-237.

Lukas SE, Penetar D, Berko J, Vicens L, Palmer C, Mallya G (2005). An extract of the Chinese herbal root kudzu reduces alcohol drinking by heavy drinkers in a naturalistic setting. Alcoholism Clin. Exp. Res. 29: 756–762.

Ma HL, Wang C, Liu WM (2005). Microwave-assisted extracting of flavonoids from Radix Pueraria. J. Jiangsu Univ. (National Science Edition). 26: 98–100.

Wang LZ, Yang B, Dua XQ, Yi C (2008). Optimisation of supercritical fluid extraction of flavonoids from *Pueraria lobata*. Food Chem. 108: 737–741.

Xiao WH, Han LJ, Shi B (2008). Microwave-assisted extraction of flavonoids from Radix Astragali. Separat. Purif. Technol. 62: 614–618.Xu HN, He CH (2007). Separation and purification of puerarin with solvent extraction. Separat. Purif Technol. 56: 397–400.

Yeung DKY, Leung SWS, Xu YC, Vanhoutte PM, Man RYK (2006). Puerarin, an isoflavonoid derived from Radix puerariae, potentiates endothelium-independent relaxation via the cyclic AMP pathway in porcine coronary artery. Eur. J. Pharmacol. 552: 105–111.

Zhang F, Chen B, Xiao S, Yao S (2005). Optimization and comparison of different extraction techniques for sanguinarine and chelerythrine in fruits of *Macleaya cordata* (Willd) R.Br. Separat Purif. Technol. 42: 283–290.

Zhang F, Yang Y, Su P, Guo ZK (2009). Microwave-assisted Extraction of Rutin and Quercetin from the Stalks of *Euonymus alatus* (Thunb.) Sieb Microwave-assisted Extraction of Rutin and Quercetin. Phytochem. Anal. 20: 33–37

Zhou YM, Hou XL, Tian CR (2007). Optimal Micro-extraction Conditions Determination of Tartary Buckwheat Bran Flavonoids by RSA. Food Sci. 28: 253–257.

Zhuang XP, Lu YY, Yang GS (1992). Extraction and determination of flavonoid in ginkgo. Chin. Herb. Med. 23: 122-124.

Pan YM, Wang K, Huang SQ, Wang HS, Mu XM, He C, Ji XW, Zhang J, Huang FJ (2008). Antioxidant activity of microwave-assisted extract of Longan (*Dimocarpus Longan* Lour.) peel. Food Chem. 106: 1264–1270.