March 2010 ISSN : 0974-7419 Volume 9 Issue 1

Trade Science Inc.

Analytical CHEMISTRY

An Indian Journal

Full Paper

ACAIJ, 9(1) 2010 [190-195]

Application of cationic surfactant in the microwave-assisted extraction of alkaloids from *Rhizoma Coptidis*

Xubiao Luo*, Xinman Tu, Weipin Xiao, Shenglian Luo

College of Environmental and Chemical Engineering, Nanchang Hangkong University, Nanchang 330063, (P.R. CHINA)

E-mail: luoxubiao@126.com

Received: 11th February, 2010 ; Accepted: 21st February, 2010

ABSTRACT

The feasibility of employing cationic surfactant cetyltrimethyl ammonium bromide (CTAB) as an alternative and effective solvent for the microwave-assisted extraction of alkaloids from *Rhizoma Coptidis* was demonstrated. When compared with anionic and nonionic surfactant solvents, CTAB enhanced the extraction efficiency. The optimal extraction parameters based on the microwave-assisted micellar extraction technique were investigated. Under optimal conditions, i.e. 0.468 g/L CTAB, microwave-assisted extraction for 11 min at 70 °C, the extraction recovery of seven alkaloids reached over 91.6% in a one-step extraction. Furthermore, the combination of microwave-assisted extraction and cationic surfactant micellar extraction was shown to be a green, rapid and effective approach for alkaloids of *Rhizoma Coptidis* samples. © 2010 Trade Science Inc. - INDIA

KEYWORDS

Alkaloids;
Rhizoma;
Coptidis;
CTAB;
Microwave-assisted extraction.

INTRODUCTION

Rhizoma Coptidis (RC), known as 'Huanglian', is a commonly used Chinese herb drug with the effects of clearing heat, drying up dampness, purging toxicosis and detoxicification in China[1]. It is known to contain berberine, palmatine, coptisine, epiberberine, jatrorrhizine, columbamine and berberastine (structure are shown as Figure 1). The alkaloids exhibit a great variety of biological and pharmacological activities including anti-microbial, anti-diarrhoea, anti-diabetic, and anti-cancer[2-5]. Thus, the separation and purification of alkaloids from RC is necessary for us to evaluate the biochemical activity or take full advantage of its medical value.

Several extraction techniques have been reported for the separation and extraction of alkaloids in RC, including heating reflux extraction[6], maceration at room temperature, Soxhlet extraction[7] and microwave-assistant extraction (MAE)[8]. Traditionally, abundant volatile organic solvents, including methanol, ethanol, acetone, and some mixed solvents, were utilized to extract alkaloids from the Chinese herb. Microwave-assisted extraction (MAE) has been adapted for many scientific applications, including the extraction of pesticides, metals, pollutants and natural products[9-11]. Despite the advantages of reduced solvent usage and shorter extraction time, most of extractions are still make use of organic solvents. Thus, the development of highly efficient and environmental friendly pretreatment methods is of great interest.

Recently, microwave-assisted micellar extraction is receiving much attention because it is a simple, fast, low-cost, easy-to-handle and non-toxic procedure a

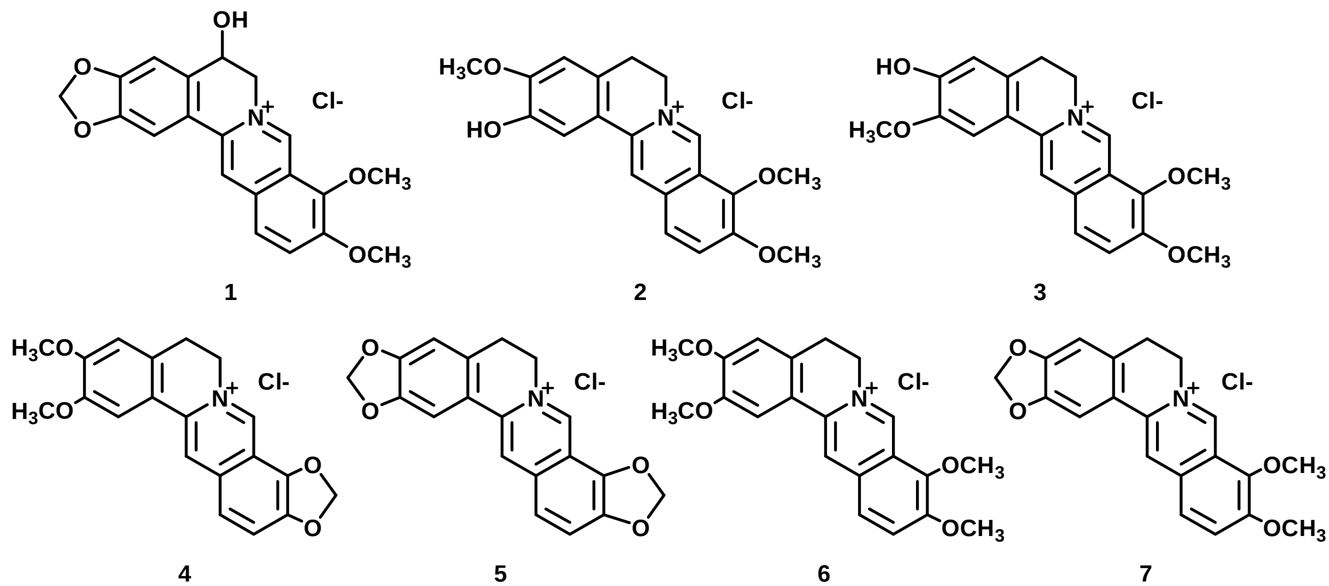


Figure 1 : Structures of seven alkaloids: 1.berberastine; 2. columbamine; 3. jatrorrhizine; 4. epiberberine; 5. coptisine; 6. palmatine; 7. berberine.

possible alternative for the extraction of different products from plant matrices[12-14]. Aqueous non-ionic surfactant as solvents in the MAE of organic compounds has drawn greater attention. However, aqueous cationic surfactant as solvents in the MAE of natural products has not been reported in the literature. Aqueous cationic surfactants maybe excel non-ionic surfactants in extraction efficiency of alkaloids because of the principle of similitude between solute and solvent.

This paper offers a novel method that is fast, simple and free of organic solvents, and seven alkaloids can be extracted with better recovery rate as compared to aqueous non-ionic surfactant as solvents. At the same time various experimental parameters such as temperature, time and concentration of the surfactant were evaluated.

EXPERIMENT

Materials and reagents

Rhizoma Coptidis was purchased from pharmaceutical company (huichun tang) in Nanchang, China. The dried sample was grinded and the powder was sieved with a width of 50 mesh. Berberine chloride, jatrorrhizine chloride and palmatine chloride were obtained from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Coptisine chloride, epiberberine chloride, columbamine chloride and berberastine chloride were isolated by the modified method described in the paper[15]. These alkaloids were identified by MS, IR and NMR. The purity of these four alkaloids are >98%. The non-ionic surfactants Triton X-100, cationic surfactants cetyltrimethyl ammonium bromide (CTAB), and anionic sodium dodecyl sulfate(SDS) were obtained from Sigma (St. Louis, MO, USA) . Its solution was prepared in de-ionized water. 1-Ethyl-3-methylimidazolium tetrafluoroborate ([EMIM]BF_4) was also obtained from Sigma (St. Louis, MO, USA) . HPLC-grade acetonitrile and methanol were obtained from Tedia Company, Inc. (Fairfield, Ohio, U.S.A). All other reagents were of analytical grade.

Microwave-assisted micellar extraction of alkaloids

Focused microwave-assisted extractions were performed at atmospheric pressure using a Microwave Synthesis System apparatus (XH-100A, Beijing Xianghu Science and Technology Development Co.,LTD, Beijing, China) equipped with an infrared temperature control system, stirring and cooling options. The cooling is carried out by means of a flow of water.

One-gram sample of RC were poured a glass vessel, 30 mL of different solvents (ethanol, pure water, 1.68 g/L SDS, 1% TX-100) containing various concentrations (v/v) of aqueous cationic surfactant solutions. The vessel was placed in the microwave oven and was heated at some preset extraction program. The

Full Paper

extracts were then centrifuged at 4000rpm for 5 min. The supernatant was transferred in a 100 ml volumetric flask, diluted to volume with extraction solution. The solution was filtered through a 0.45μm membrane prior to injection into the HPLC system.

The extraction recovery of alkaloids was defined as follows:

$$\text{recovery of alkaloid (\%)} = \frac{\text{the mass of compound in extraction solution}}{\text{the mass of compound in sample}} \times 100$$

The mass of each alkaloid in extraction solution (one step extraction) was analyzed by RP-HPLC. The mass of each alkaloid in sample was determined by analysis of the total extraction solutions by Soxhlet extraction with 70% ethanol solution for 6 h.

HPLC analysis

HPLC analysis was performed on HP1100 system (Agilent Technologies, California, USA) equipped with a manual injector and an ultraviolet detector. For data analysis and calculation Chemstation software was used (Agilent Technologies). The alkaloids were separated on a spherigel C_{18} analytical column (4.6mm × 250 mm, 5 μm, Johnsson Dalian, China) using eluents of 50 mM [EMIM]BF_4 and acetonitrile (V:V=6:4) in isocratic system. The injection volume was 10 μL. The detection wavelength was set 266 nm.

RESULTS AND DISCUSSION

Comparison of CTAB with conventional extraction solvents

Efficiency for extraction was compared between 0.468 g/L CTAB, 1.68 g/L SDS, 1% TX-100, ethanol, and pure water. From Figure 2, it can be seen that aqueous cationic surfactant solutions provides larger recoveries for seven alkaloids, in addition acidified solution (pH = l) can also increase the recovery of alkaloids due to solubility enhancement with chloride ionic form. Extraction efficiencies of aqueous surfactant solutions except for SDS solution are higher than pure water and ethanol. Presumably cationic and nonionic micelles swell the organic matters and more thoroughly diffuse into the solid matrix, increasing the mass-transfer coefficient during desorption of soluble ingredients from the plant matrix to water. However, anionic micelles swell also the organic matters and diffuse into the solid matrix, but produce precipitation with alkaloids and impede alkaloids solublization. During the experiment, it was found that cationic surfactant solutions provide higher recoveries than conventional nonionic surfactant solutions because of the principle of similitude between solute and solvent. Furthermore, aqueous cationic surfactant solutions for sample preparation are safer, less expensive and more environmentally friendly than organic solvents. Thus cationic surfactant solutions are selected for further studies.

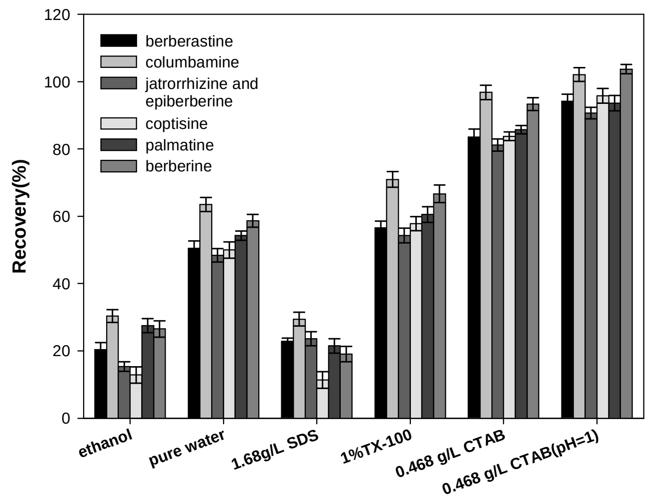


Figure 2 : Comparison of the extraction recovery using different solvents. (Solid / liquid = 1:30; extraction time:11 min; pH = 1 adjusting HCl).

Optimization of extraction conditions

Effect of extraction time on the extraction recovery of alkaloids

Extraction time is one of the key factors influencing the extraction recovery of alkaloids. The effect of extraction time on efficiency was observed. The result can be seen from Figure 3. The experimental results show that the extraction recovery of alkaloids from the *RC* increased rapidly with an increase of extraction time ranging from 3 min to 11 min. At s extraction time exceeding 11 min, the extraction recoveries of compounds decrease. These observations were similar for seven alkaloids under the same conditions. This is because the recovery of alkaloids increased as a function of time. On the other hand, if irradiation time is longer than 11 min, the extraction recovery is decreased because of decomposability of alkaloids at high temperature for long periods of time. So microwave extraction time for 11 min is appropriate for higher extraction recovery.

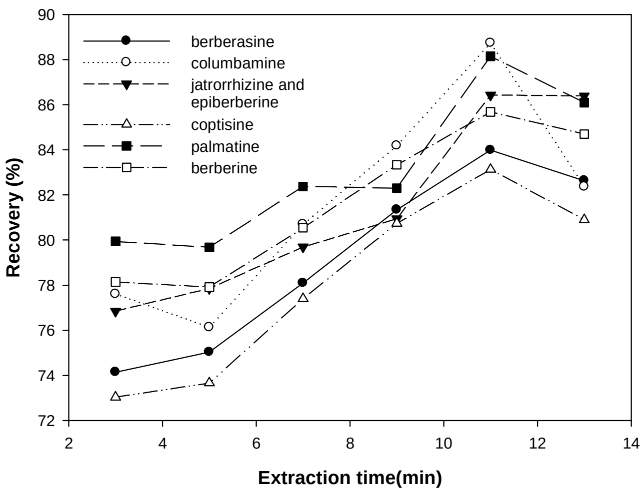


Figure 3 : Effect of extraction time on the extraction recovery of alkaloids

Effect of solid/liquid ratios on the extraction recovery of alkaloids

The solid/liquid ratio (solvent/matrix) play roles in the microwave-assisted micellar extraction process as other parameters. The effect of solid/liquid ratio on extraction efficiency was also investigated. It can be seen from Figure 4. The experimental results show that the solid/liquid ratio of 1:30 (g/mL) is sufficient for each compound to obtain the highest recovery, Hence the solid/liquid ratio of 1:30 is employed in the following experiments.

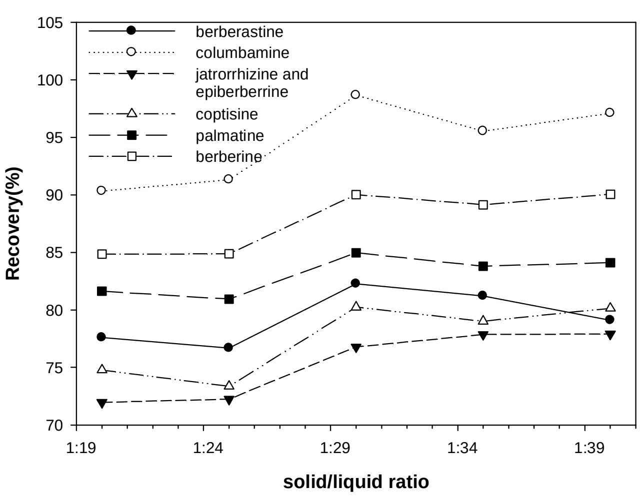


Figure 4 : Effect of liquid/solid ratio on the extraction recovery of alkaloids

Effect of the surfactant concentration on the percentage extraction of alkaloids

The properties of micellar phase influence alkaloids solubilisation. Among these, the nature of the surfactant and its concentration are major contributors affecting alkaloids solubilisation. The effect of the surfactant concentration of CTAB on extraction was examined. Figure 5 shows that the extraction recovery of alkaloids from the *Rhizoma Coptidis* increased rapidly with an increase of surfactant concentration ranging from 0.059 g/L to 0.468 g/L. At surfactant concentrations exceeding 0.468 g/L, the extraction recoveries of compounds decrease slowly. Seven alkaloids solubilisation are favoured as CTAB concentration increased because hydrophobic interaction was usually generated at higher surfactant concentrations. However, micellar clustering takes place above a certain concentration, which can reduce the interfacial area that is available to alkaloids. Thus 0.468 g/L of CTAB is selected for further studies.

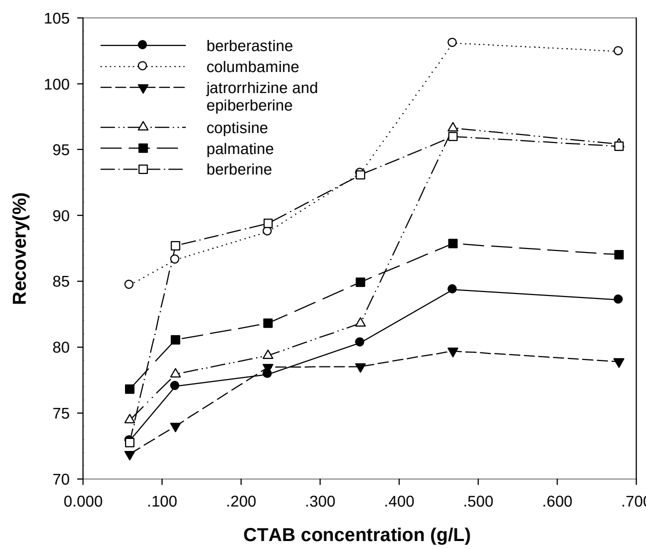


Figure 5 : Effect of concentration of CTAB (g/L) on the extraction recovery of alkaloids

PH effect on the percentage extraction of alkaloids

PH effect in the extraction efficiency of alkaloids was studied by adjusting the pH value of the micellar solution. Generally, the sample is acidified in order to make alkaloids ionization. In order to study this influence, we compared the results obtained using a different pH micellar solution with hydrochloric acid. The result was seen from Figure 6. For seven alkaloids, this study indicates that the extractions were better under acidic conditions than under neutral or alkaline conditions. The results led us to the conclusion that the extractions should be performed under acid conditions to ensure the cationic form of the target analytes. Therefore, pH =1 was chosen for higher extraction recovery.

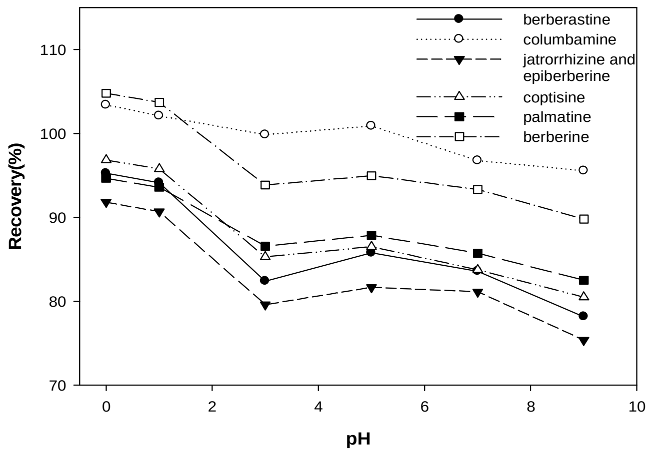
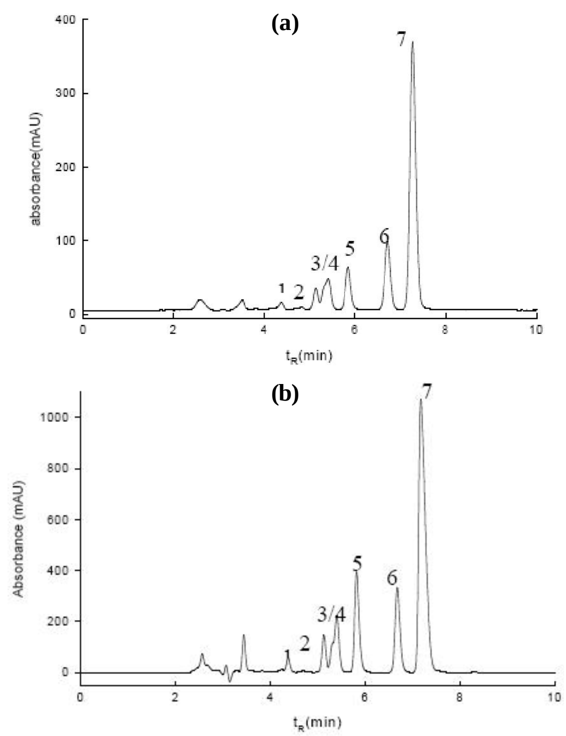


Figure 6 : pH effect on the extraction recovery of alkaloids

In summary, the optimum conditions in the microwave assisted extraction were: a radiation time of 11 min; solid/liquid ratio of 1:30, 0.468 g/L of CTAB micellar solution at pH = 1. A typical chromatogram containing seven alkaloids under optimal separation conditions, after microwave-assisted cationic micellar extraction was applied, is shown in Figure 7.



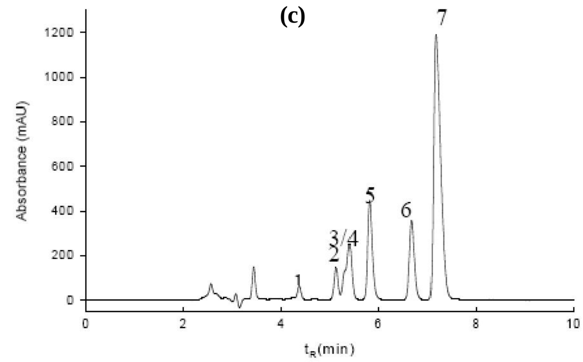


Figure 7 : HPLC chromatograms of the alkaloids extracted from the *Rhizoma Coptidis*. (a) chromatogram of water extraction: The peak symbols are as in Figure 1; (b) chromatogram of 1%TX-100; (c) chromatogram of CTAB extraction.

Comparison of MAE with other extraction methods

Efficiency for extraction was compared with shake flask extraction method, heat reflux extraction and microwave-assisted micellar extraction. The results were demonstrated that for only 11 minutes of microwave irradiation for microwave extraction in CTAB micellar solvent, the contents of alkaloids were higher than those of shake flask extraction for 5 hours((at room temperature, extraction recovery of alkaloids 70.3%) and those of heat reflux extraction for 1 hours (extraction recovery of alkaloids 85.7%). This result is not surprising because the inner molecular heating effect of microwave facilitated the interaction between the surfactant solution and the alkaloids in herbal material, so MAE can reach higher extraction efficiency in shorter extraction time. Therefore, the microwave extraction of alkaloid is more efficient, rapid and convenient than the reflux extraction and shake flask extraction.

Analytical performance characteristics

The performance method was evaluated under optimal detection conditions by determination of the linearity, detection limits and reproducibility. Calibration curves were obtained by using different concentration levels ranging from 2.5 to 100.0 μg/mL for berberine, palmatine, coptisine, epiberberine/jatrorrhizine, columbamine and berberastine. The results were listed in TABLE 1. Curves were linear over these concentration ranges. In all cases, the regression coefficients were over 0.999.

The limits of detection (LODs) of individual alkaloids were determined by calculating signal/noise ratio (S/N = 3) for each alkaloids (TABLE 1). The results are in line with those obtained by other authors.

The reproducibility of the method was evaluated through analysis of six replicates of RC sample. The relative standard deviations (RSDs) were under 3.5% for all the target compounds (TABLE 1). Therefore, the accuracy obtained using the proposed method is satisfactory.

TABLE 1 : Analytical parameters of the proposed method

Compound	Calibration curves	Regression coefficients	Limit of detection (μg/mL)	Relative standard deviation (%,n=3)
Berberastine	Y = 6.57+8.10X	0.9998	0.04	3.1
Columbamine	Y = 2.26+1.10X	0.9991	0.19	2.4
Epiberberine /Jatrorrhizine	Y = 8.25+4.76X	0.9994	0.53	2.7
Coptisine	Y = 21.12+5.05X	0.9993	0.18	3.4
Palmatine	Y = -3.54+5.05X	0.9998	0.11	2.8
Berberine	Y = -5.18+28.14X	0.9997	0.06	3.5

Recoveries of the method proposed, were determined by processing spiked RC samples with 10 μg/g of mixture of seven alkaloids through out the entire procedure. The average extraction efficiency for berberine, palmatine, coptisine, epiberberine /jatrorrhizine, columbamine and berberastine was 102.3%, 94.5%, 95.6%, 91.2%, 101.3%, and 94.2%, respectively. Therefore, the recoveries obtained using the proposed method is satisfactory.

CONCLUSION

Microwave-assisted cationic micellar extraction using aqueous CTAB solutions can be a possible alternative for extraction of alkaloids in *Rhizoma Coptidis* samples, without removing surfactant in the filtrate for its subsequent analysis by HPLC. The extraction recovery of alkaloids was above 91. 7%. The proposed technique is a green, simple, rapid, effective extraction method for separation of alkaloids from the Chinese herb. It is therefore considered that this method is promising and can be a good alternative to the traditional techniques.

ACKNOWLEDGEMENTS

This work was financially supported by a grant from National Science Fund for Distinguished Young Scholars (50725825), National Natural Science Foundation of China (50978132), Scientific Research Fund of Jiangxi Provincial Education Department (GJJ08200), and Opening Fund of Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education of China), Hunan Normal University (KLCBTCMR2008-6).

REFERENCES

[1] H.Y.Hsu, Y.P.Chen, S.J.Sheu, C.H.Hsu, C.C.Chen, H.C.Chang; 'Chinese Material Medica-A Concise Guide', Modern Drug Press, Taipei, **(1985)**.
[2] R.Marek, P.Seckarova, D.Hulova, J.Marek, J.Dostal, V.Sklenar; J.Nat.Prod., **66**, 481 **(2003)**.
[3] T.C.Birdsall, G.S.Kelly; Altern.Med.Rev., **2**, 94 **(1997)**.
[4] C.L.Kuo, C.W.Chi, T.Y.Liu; Cancer Lett., **203**, 127 **(2004)**.
[5] B.S.Ko, S.B.Choi, S.K.Park, J.S.Jang, Y.E.Kim, S.Park; Biol.Pharm.Bull., **28**, 1431 **(2005)**.
[6] C.O.Chan, C.C.Chu, D.K.Mok, F.T.Chau; Anal. Chim.Acta, **592**, 121 **(2007)**.
[7] G.Romanik, E.Gilgenast, A.Przyjazny, M.Kaminski; J.Biochem.Biophys.Methods, **70**, 253 **(2007)**.
[8] A.H.Chen, Y.T.Lee, C.Y.Chen; J.Chin.Chem.Soc., **52**, 781 **(2005)**.
[9] B.M.Pereira, M.J.G.Castro, S.M.Lorenzo, P.L.Mahia, D.P.Rodriguez, E.Femandez; J.Chromatoar.A, **1061**, 133 **(2004)**.
[10] L.Sun, H.K.Lee; J.Sep.Sci., **25**, 67 **(2002)**.
[11] X.B.Luo, B.Chen, S.Z.Yao; Phytochem.Anal., **17**, 431 **(2006)**.
[12] E.K.Paleologos, D.L.Giokas, M.I.Karayannis; Trends Anal.Chem., **24**, 426 **(2005)**.
[13] C.Sun, Y.C.Xie, H.Z.Liu; Chin.J.Chem.Eng., **15**, 474 **(2007)**.
[14] C.Sun, H.Z.Liu; Anal.Chim.Acta, **612**, 160 **(2008)**.
[15] D.B.Zhao, Y.L.Tang, H.Q.Wang; Zhongguo Zhong Yao Za Zhi, **30**, 1756 **(2005)**.