Journal of Medicinal Plants Research Vol. 6(10), pp. 1991-1996, 16 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1692
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Ultrasound-assisted extraction of oxymatrine from *Sophora flavescens*

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Accepted 17 January, 2012

Oxymatrine is one of main active compounds found in *Sophora flavescens*, which have been extensively used in China for the treatment of several diseases on viral hepatitis, cancer, cardiac and skin. The optimization of ultrasound-assisted extraction of oxymatrine from *S. flavescens* was investigated, and the extracts were analyzed by high-performance liquid chromatography (HPLC). Effects of several experimental parameters, such as concentration of extracting solvent, the ratio of liquid to material, extraction temperature, and extraction time on extraction efficiencies of oxymatrine from *S. flavescens* using ultrasound-assisted extraction were evaluated. The optimal extraction conditions were 1 g ground plant sample with 65% (v/v) ethanol solution in water, the ratio of liquid to material at 30:1 (ml/g) and extraction for 10 min at 50℃ under ultrasonic irradiation. Th e extraction rate of oxymatrine using ultrasound-assisted extraction was higher than those obtained using other methods, such as conventional solvent extraction, decocting method, Soxhlet extraction and microwave-assisted extraction. The results indicated that ultrasound-assisted extraction is a very useful method, and helpful for the full utilization of *S. flavescens*.

Key words: Ultrasound, extraction, oxymatrine and *Sophora flavescens.*

INTRODUCTION

The dry root of *Sophora flavescens* is an herbal medicine widely used for the treatment of several diseases, such as viral hepatitis, tumor, viral myocarditis, heart failure, and skin diseases (Liu et al., 2010; Jiang et al., 2009; Lin et al., 2011). Some studies showed the vasorelaxation effect of ethanol extract of *S. flavescens*, which relaxes vascular smooth muscle via endothelium-dependent NO-sGC-cGMP signaling pathway (Jin et al., 2011). It was also reported that the extract of *S. flavescens* could promote the hair growth (Roh et al., 2002). In addition, the extracts of *S. flavescens* have been formulated as pesticides either alone or in mixtures with conventional synthetic pesticides, which might be induced by its antimitotic effects (Akdeniz and Ozmen, 2011). Oxymatrine is the main alkaloid in *S. flavescens* (Gan et al., 2011). It has been proved owning various bioactivities, such as anti-inflammatory, antioxidant, anti-arthritic, antimicrobial, cure myocardial fibrosis, neuroprotection, antipruritic, antiandrogen, and protecting chronic heart failure (Yamaguchi-Miyamoto et al., 2003; Cui et al., 2011; Gou et al., 2011; Hu et al., 2011; Jin et al., 2010; Kuroyanagi et al., 1999; Shen et al., 2011). Compared to the cells without oxymatrine pre-treatment, oxymatrine can inhibit ducthe G (2) and M phase of H4IIE (Ho et al., 2009; Lin et al., 2009). Therefore, the improvement of extraction rate of oxymatrine from *S. flavescens* is very important, and helpful for the full utilization of this plant.

Several methods for extraction of oxymatrine from *S. flavescens* have been reported in the literature, such as maceration extraction, leakage method, decocting method, refluxing method and supercritical fluid extraction (Tan and Qin, 2006; Ma et al., 2008; Li and Wang, 2004; Zhang et al., 2007; Ling et al., 2007). However, the conventional extraction methods have

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some disadvantages, such as low extraction yield, time-consuming, large volume of solvent used. Recently, ultrasound-assisted extraction (UAE) has attracted increased attention because it can accelerate the extracting process and improve extraction yield of bioactive compounds (Dong et al., 2010; Boonkird et al., 2008; Bhandari et al., 2008; Li et al., 2005a, b; Chen et al., 2007; Wang et al., 2011; Wu et al., 2001; Li and Chen, 2004;Li et al., 2010; Xia et al., 2010; Salisova et al., 1997). The UAE allows the extraction solvent to penetrate cell walls, and the bubbles produced by acoustic cavitation aid in the disruption of the cell wall and the release of the targeted compounds (Wu et al., 2001). Therefore, UAE is a highly efficient and reduced solvent- and quick method. However, it is unknown whether the extraction rate of oxymatrine could be improved using UAE.

In this work, active ingredients in *S. flavescens* were extracted by the ultrasound-assisted extraction, and oxymatrine was used as a marker component of the plant, which was quantified by high-performance liquid chromatography (HPLC) with photodiode array detection. Effects of several experimental factors on extraction efficiencies of oxymatrine from this plant were studied. The crude extract obtained could be used as either components of some complex Chinese medicines or for further isolation and purification of bioactive compounds. The results obtained would be helpful for the full utilization of *S. flavescens*.

MATERIALS AND METHODS

Chemicals

Oxymatrine (98% purity) was bought from Siyi Biotechnology Company (Chengdu, China). Acetonitrile, ethanol and methanol were HPLC grade and purchased from Merck (Darmstadt, German). Phosphoric acid was bought from Tianjin Chemical Reagent Company (Tianjin, China). Deionized water was used throughout the experiment. A stock solution of oxymatrine (10 mg/ml) was prepared in methanol and was stored at 4℃. The calibration standards (10 to 500 µg/ml) were prepared from the stock solution by the serial dilution of methanol.

Instruments

The UAE was carried out in a KQ-600E ultrasonic device (Changzhou Nuoji Instrument Company, Changzhou, China) with an ultrasound power of 600 W, heating power of 800 W, and frequencies of 40 kHz, equipped with a digital timer and a temperature controller.

Plant materials and sample treatment

The dried root (with humidity of 1.8%) of *S. flavescens* Ait were purchased from a drugstore in Guangzhou, Guangdong Province, China, and ground into powder in a knife mill, and then stored at 4℃ in a refrigerator. The powder (1 g) of the root of *S. flavescens* Ait was accurately weighed, and placed in a capped glass tube, and then mixed with an appropriate amount of extraction solvent. After soaking for 30 min that permitted solvent to wet plant material, the tube with sample was immersed into water bath of ultrasonic device, and irradiated for the pre-set extraction temperature and time. After extraction, the sample was centrifuged at 9600 g for 10 min, and then the supernatant was collected for HPLC analysis.

For comparison, microwave-assisted extraction was carried out in an X-100A microwave extraction device (Xia et al., 2011a), and extracted at 50℃ for 50 min (10 min × 5 times) under microwave irradiation of 500 W (Xia et al., 2011b). In addition, Soxhlet extraction was done with a Soxhlet extraction apparatus at 90℃ for 4 h, and conventional solvent extraction was conducted with a mechanic shaker at room temperature (25℃ for 24 h (Xia et al., 2011c). The extracting solvent and the ratio of liquid to material were the same as those of ultrasound-assisted extraction under the optimal conditions.

HPLC analysis

A waters (Milford, MA, USA) 1525 binary HPLC pump separation module with a Waters 2996 photodiode array detector was used. An Agilent Zorbax Extend-C_{18} column (250 × 4.6 mm, 5 µm) and an auto-injector (10 µl) were used. The mobile phase was acetonitrile and 0.05 mol/L of phosphoric acid using isocratic elution (10:90, v/v) with a flow-rate of 1 ml/min (Jiang et al., 2009). The UV spectra were recorded between 190 and 400 nm for peak characterization, and the detection wavelength was set at 210 nm. The column temperature was kept at 27℃. The peak area was used to calculate the amount of oxymatrine from the standard curve. The chromatograms of oxymatrine in standard solution and in the sample are shown in Figure 1.

All the experiments were conducted in triplicate, and the average values ± SD (standard deviation) were reported.

RESULTS AND DISCUSSION

Effect of ethanol concentration on the extraction of oxymatrine

The extracting solvent has a strong impact on the extraction yield, and its choice was the first important step towards parameter optimization. Although methanol-water solution and chloroform-ammonia could be used as extracting solvents for extraction of oxymatrine from *S. flavescens* (Tan and Qin, 2006; Wu et al., 2009), water and ethanol-water solution were more widely used for extraction of oxymatrine because of toxicity of methanol and chloroform. In the present study, ethanol-water solution at different ratio was tested as extracting solvent for extraction of oxymatrine from *S. flavescens* under ultrasonic irradiation. Other experimental parameters were set as follows: the ratio of liquid to material, 10:1 (ml/g); extracting temperature, 25℃; and extractin g time, 20 min. The results revealed that the extraction yield was the highest when 65% ethanol was used as extracting solvent (Figure 2). Seen from Figure 2, when the concentration of ethanol was less than 65%, the yields increased with the increased concentration of ethanol. Then, the yields declined when ethanol concentration further increased. The results indicated that 65% ethanol was suitable for the extraction of oxymatrine from the

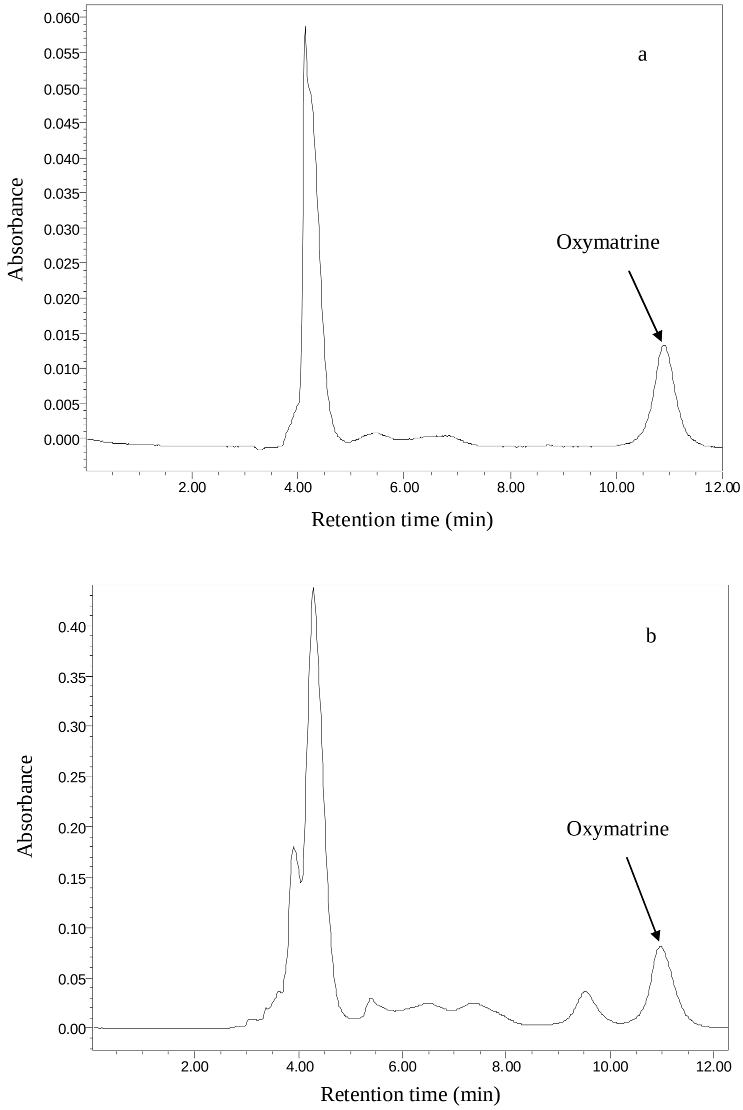

Figure 1. Chromatograms of oxymatrine in standard solution (a) and in the sample (b).

plant, which was chosen as the optimal concentration of ethanol.

Effect of the ratio of liquid to material on the extraction of oxymatrine

In order to obtain the maximum yield of extraction, effects of the ratio of liquid to material on the extracting yield of oxymatrine were studied. The amount of solvent was changed to get different solvent/mass ratio. Other experimental parameters were 65% ethanol, extracting temperature at 25℃, and 20 min of extracting time. The results are displayed in Figure 3. The yield increased with the increased ratio of liquid to material from 5:1 to 30:1, and kept almost unchanged from 30:1 to 40:1. The maximum yield was obtained at the ratio of 30:1 (Figure 3), which was chosen as the optimal ratio of liquid to material.

Effect of extracting temperature on the extraction of oxymatrine

Effects of extracting temperature on the yield of oxymatrine were investigated using 65% ethanol as extracting solvent with a ratio of liquid to material of 30:1, and 20 min of extracting time. The results are shown in Figure 4. The yield gradually increased when the temperature increased from 25 to 50℃, and then the

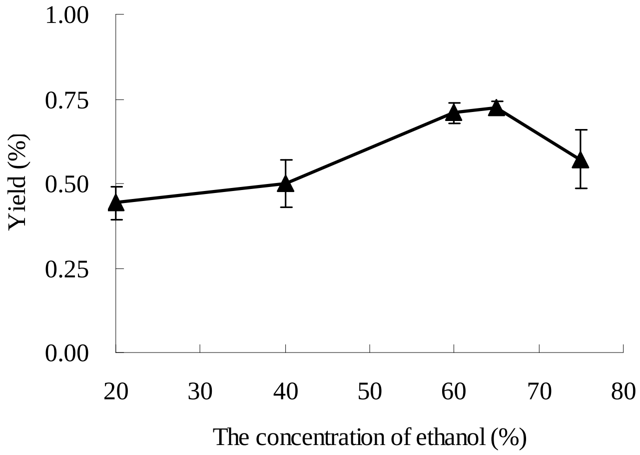

Figure 2. Effect of ethanol concentration on the extracting yield.

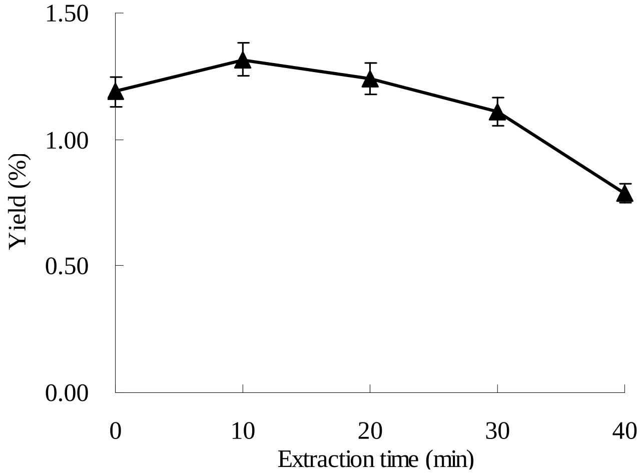

Figure 5. Effect of extracting time on the extracting yield.

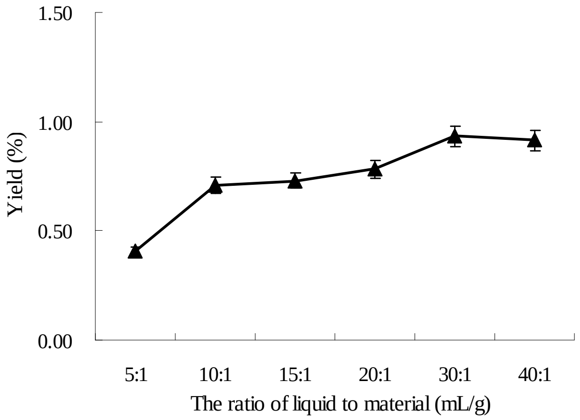

Figure 3. Effect of the ratio of liquid to material on the extracting yield.

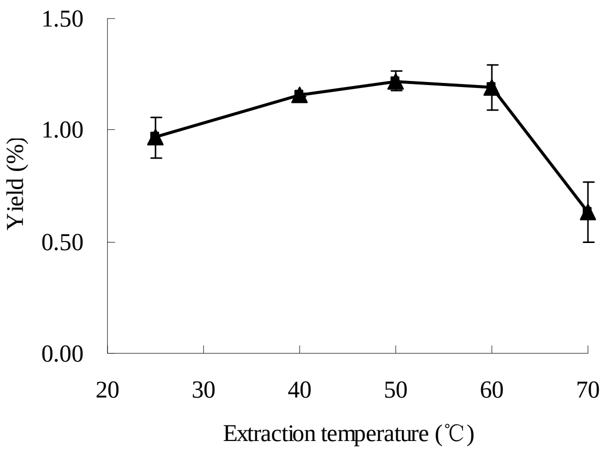

Figure 4. Effect of extracting temperature on the extracting yield.

yield decreased from 50 to 70℃. This result was ag reed with the findings reported in the literature (Kim et al., 2007), who extracted the bioactive compounds from ginseng. This enhanced yield of oxymatrine with the increase of extracting temperature might result from the increased diffusivity of the solvent into cells and enhanced desorption and solubility of oxymatrine from the cells (Vongsangnak et al., 2004). When the extracting temperature increased from 60 to 70℃, there was a markedly decrease in the yield, which might be due to the degradation of oxymatrine at high temperature under ultrasonic irradiation. Thus, the extracting temperature had a profound influence on the ultrasound-assisted extraction of bioactive compounds from plant materials, and the highest yield was obtained at 50℃.

Effect of ultrasound on the extraction of oxymatrine

Effect of ultrasonic time on the extraction yield of oxymatrine was firstly tested, and other experimental parameters were 65% ethanol with a ratio of liquid to material of 30:1, and extracting temperature at 50°C under ultrasonic irradiation. The results are displayed in Figure 5. There was slightly increase in the yield of oxymatrine from 0 to 10 min, and then the yield decreased with the increasing of irradiation time after 10 min. The maximum yield was obtained at 10 min. The results indicated that ultrasound might accelerate the extraction of oxymatrine from plant material in a short time (10 min), also could induce the yield to decrease for a longer time of ultrasonic irradiation because oxymatrine could be degraded.

In order to distinguish influence of the soaking from ultrasound, effects of the number of extraction on the recovery were studied, and the results are shown in Table 1. Compared with the soaking, effect of UAE on the

Table 1. Effects of the number of extraction on extraction rate of oxymatrine.

Number of extraction	Ultrasound-assisted extraction (10 min)		Soaking 30 min without UAE	
	Extraction rate (%)	RSD (%) (n = 3)	Extraction rate (%)	RSD (%) (n = 3)
1	67.18	1.03	50.49	4.9
2	25.91	2.92	7.53	1.9
3	4.86	1.70	3.62	7.0
4	1.45	5.40	0.83	9.8
5	0.60	8.85	0.33	8.6
Total	100		62.80	

recovery was very obvious if the number of extraction were 2 or 3, although the difference of UAE from the soaking was small if the number of extraction was only 1. This was because the soaking mainly dissolved molecules on surface of plant material. However, the UAE allowed the extraction solvent to penetrate plant material, and the bubbles produced by acoustic cavitation aided in disruption of cell wall, which could accelerate the release of the targeted compounds within plant material (Wu et al., 2001).

Comparison of ultrasound-assisted extraction with other extraction methods

For comparison, Soxhlet extraction was carried out at 90℃ for 4 h, and conventional solvent extraction was conducted at 25℃ for 24 h, and microwave-assisted extraction was done at 50℃ for 50 min (10 min × 5 times) (Xia et al., 2011b). If the extraction rate of oxymatrine obtained by UAE at 50℃ for 50 min (10 min × 5 times) was defined as 100% (Table 1), the rates obtained by conventional solvent extraction, Soxhlet extraction and microwave-assisted extraction were 66.8%, 78.1 and 86.1%, respectively. That is, the extraction rate of oxymatrine by UAE was higher than those by conventional solvent extraction, Soxhlet extraction and microwave-assisted extraction. Furthermore, the extraction rate of oxymatrine obtained by UAE was also higher than those (52.3 to 53.4%) obtained using both decocting and refluxing method in the literature (Tan and Qin, 2006), although UAE was carried out under the conditions of lower temperature and a shorter time.

Conclusion

The UAE of oxymatrine from *S. flavescens* has been studied by evaluating effects of several experimental parameters on the extracting yields of oxymatrine. The optimal extraction conditions were 65% ethanol, the ratio of liquid to material at 30:1, and extraction for 10 min at 50℃ under ultrasonic irradiation. Ultrasound could efficiently improve the extracting performance of oxymatrine. The extraction rate of oxymatrine using UAE was higher than those obtained using other methods, such as conventional solvent extraction, decocting method, Soxhlet extraction and microwave-assisted extraction. The results obtained are helpful for the full utilization of *S. flavescens*, which also indicated that the UAE is a very useful method for the extraction of important active compounds from plant materials.

ACKNOWLEDGEMENTS

This research was supported by the Hundred-Talents Scheme of Sun Yat-Sen University. The technical assistance from Ms. Ling Zheng is highly appreciated.

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