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Short communication

Microwave-assisted extraction of tanshinones from *Salvia miltiorrhiza bunge* with analysis by high-performance liquid chromatography

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Abstract

A novel microwave-assisted extraction (MAE) method has been developed for the extraction and determination of tanshinones (tanshinone IIA, cryptotanshinone and tanshinone I) from the root of *Salvia miltiorrhiza bunge* with analysis by HPLC. Various experimental conditions were investigated to optimize the percentage extraction. Under appropriate MAE conditions, such as ethanol concentrations of 95% (v/v), MAE for 2 min, liquid/solid ratio of 10:1 (ml/g), the percentage extraction can reach high in a short time. The percentage extraction (tanshinone IIA: 0.29%; cryptotanshinone: 0.23%; tanshinone I: 0.11%) by MAE was the same or even higher than conventional extraction methods. MAE only needs 2 min, but extraction at room temperature, heat reflux extraction, ultrasonic extraction and Soxhlet extraction need 24 h, 45 min, 75 min and 90 min, respectively. MAE was also available in pilot plant form for larger scale extraction. © 2001 Published by Elsevier Science B.V.

Keywords: Microwave-assisted extraction; Extraction methods; *Salvia miltiorrhiza bunge*; Pharmaceutical analysis; Plant material; Tanshinones; Diterpenes; Terpenes

1. Introduction

Dried root of *Salvia miltiorrhiza bunge* (Tan-shen in Chinese) is an ancient Chinese drug. It is commonly used in traditional Chinese medicine for promoting blood circulation to remove blood stasis, clearing away heat, relieving vexation, nourishing blood, tranquilizing the mind, cooling the blood to relieve carbuncles, treating hemorrhages, menstrual disorders and miscarriages [1,2].

Tan-shen contains abietane-type diterpenes (tanshinones), such as tanshinone IIA, cryptotanshinone and tanshinone I (Fig. 1) [2]. They have some activity as a broad-spectrum bactericide, can dilate coronary arteries and increase coronary flow, but have cytotoxic activity and can modulate mutagenic activity. They also protect the myocardium against ischaemia [3].

Microwave-assisted extraction (MAE) has been used for the extraction of biologically active compounds from different matrices, such as extraction of lupin alkaloid (sparteine) and drug metabolites from seeds and rat faeces [4], extraction of taxanes from *Taxus* biomass [5], extraction of azadirachtin-related

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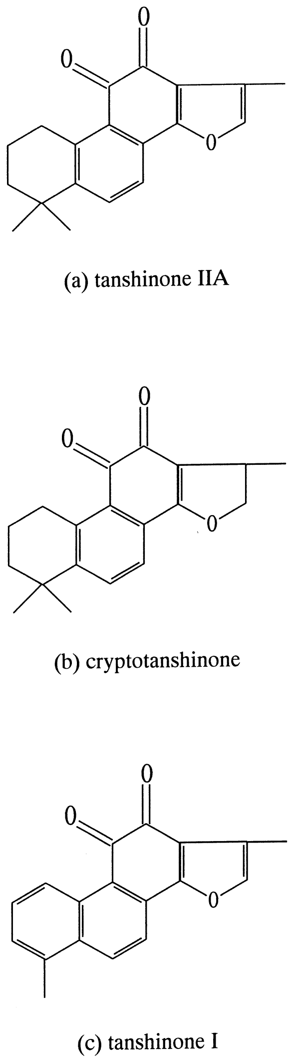

Fig. 1. Molecular structure of tanshinone IIA, cryptotanshinone and tanshinone I.

limonoids from neem seed kernel [6], extraction of felodipine tablets [7], and extraction of glycyrrhizic acid from licorice root [8].

Extraction of tanshinones from the root of *Salvia miltiorrhiza bunge* and subsequent analysis by high-performance liquid chromatography has been reported [9–11]. However, no report has been done on the use of MAE for the extraction of tanshinones from the root of *Salvia miltiorrhiza bunge*. The purpose of this work was to develop a novel MAE method and evaluate MAE and conventional extraction methods for the extraction and determination of tanshinones from the root of *Salvia miltiorrhiza bunge*.

2. Materials and methods

2.1. Plant materials

Dried root of *Salvia miltiorrhiza bunge* (Tan-shen in Chinese) was purchased from Tongrentang Medicine Cooperation (China) and cut into pieces about 3–8 mm diameter and thickness of about 3–5 mm.

2.2. Reagents

Ethanol, methanol, acetone, *n*-butanol, ethyl acetate, *n*-butyl acetate and glacial acetic acid used in the experimental work were all of analytical reagent grade chemicals. Methanol and tetrahydrofuran used in the HPLC analysis were all of reagent grade for HPLC. Tanshinone IIA, cryptotanshinone and tanshinone I standards were purchased from the China Institute for Drugs and Biological Products Identification.

2.3. MAE

A household microwave oven was modified in our laboratory [8]. Pieces of *Salvia miltiorrhiza bunge* root were mixed with an appropriate solvent (100 ml). The suspensions were irradiated with 25 s of power-on to give the desired temperature of about 80°C and then 2 s of power-on for heating and 10 s of power-off for cooling, and so on, to the pre-setting extraction time, without allowing the suspensions to super-boil.

2.4. HPLC analysis

The suspensions following MAE and other conventional extraction methods were centrifuged and filtered through a 0.5-μm membrane and then analyzed by HPLC (HP1090 liquid chromatograph, Hewlett-Packard) using a Zorbax-SB guard column and Zorbax-ODS (5 μm, 150×4.6 mm, DuPont) column. Elution was with methanol–tetrahydrofuran–glacial acetic acid–water (16:37.5:1:45.5, v/v) at 1 ml min^{-1}. The elution was monitored at 254 nm. The three tanshinones were completely separated from other compounds. Retention times of cryptotanshinone, tanshinone I, tanshinone IIA were, respectively, about 4.8, 5.7 and 9.3 min (Fig. 2). The detector response was linear from 0.16 to 1.4 μg of cryptotanshinone (y=2360.74x-142.56, R=0.9998, n=9), tanshinone I (y=3642.48x-195.2, R=0.9999, n=9), tanshinone IIA (y=2547.4x-180.3, R=0.9997, n=9) and was used to give quantitative data. This method is sensitive and accurate with good reproducibility. The analytical operation can be completed in 25 min.

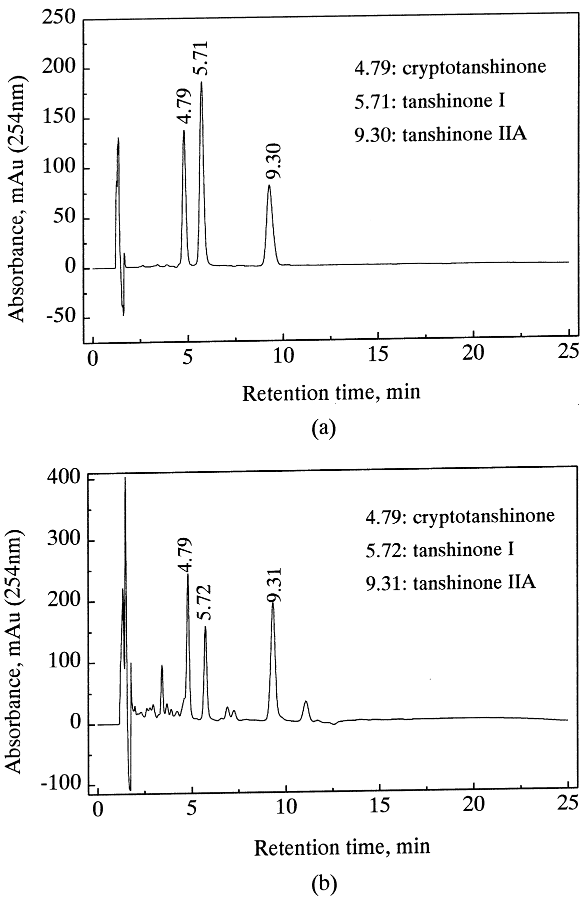

Fig. 2. High-performance liquid chromatograms. (a) Tanshinone standards; (b) root of salvia miltiorrhiza bunge. Separation conditions: HP1090 liquid chromatograph, Zorbax-SB guard column, Zorbax-ODS analytical column (5 μm, 150×4.6 mm, DuPont), UV detection at 254 nm, elution with methanol–tetrahydrofuran–glacial acetic acid–water (16:37.5:1:45.5, v/v) at 1 ml min^{-1}.

In the present work, the percentage extraction of tanshinones was defined as follows:

$$\text{Percentage extraction (w/w)} = \frac{\text{Mass of tanshinone (in extracted solution)}}{\text{Mass of material (root of salvia miltiorrhiza bunge)}} \cdot 100\%$$

3. Results and discussion

3.1. Effect of different solvents on percentage extraction of tanshinones

The experimental results show that except *n*-butyl acetate, ethanol, methanol, acetone, *n*-butanol, ethyl acetate and tetrahydrofuran can reach almost the same high percentage extraction of tanshinones. As the ethanol is non-toxic and can be mixed with water in different ratio, so it is chosen to extract tanshinones from *Salvia miltiorrhiza bunge*.

3.2. Effect of ethanol concentration on percentage extraction of tanshinones

Fig. 3 shows that the percentage extraction of tanshinones from the root of *Salvia miltiorrhiza bunge* was increased with the increase of ethanol concentration. The highest percentage extraction of tanshinones occurs at 95% (v/v) ethanol concen-

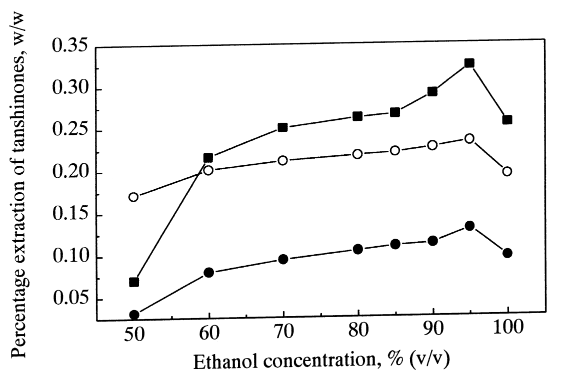

Fig. 3. Effect of ethanol concentration on percentage extraction of tanshinones. Solvent: 100 ml, pieces of *Salvia miltiorrhiza bunge* root: 10 g, MAE for 4 min, liquid/solid ratio: 10:1, ml/g. (■) Tanshinone IIA, (○) cryptotanshinone, (●) tanshinone I.

tration. Because water was easy to penetrate into the root of *Salvia miltiorrhiza bunge*, it can enhance the root of *Salvia miltiorrhiza bunge* to absorb microwave energy. The mass transfer of tanshinones from the root of *Salvia miltiorrhiza bunge* to the solvent was enhanced, therefore, 95% (v/v) ethanol can reach a higher percentage of extraction of tanshinones than pure ethanol and 90% (v/v) ethanol. The 95% (v/v) ethanol concentration in water was used in the following experiments.

3.3. Effect of MAE time on percentage extraction of tanshinones

Fig. 4 shows the effect of MAE time on percentage extraction of tanshinones. The results indicated that the percentage extraction of tanshinones was increased with the increase of MAE time. MAE reached the highest percentage extraction in 2 min. If MAE time was longer than 2 min, the percentage extraction of tanshinones was decreased with the increase of MAE time because tanshinones were easy to decompose at high temperature for long periods of time [12]. So MAE time for 2 min was used in the following experiments.

3.4. Effect of liquid/solid ratios on percentage extraction of tanshinones

Fig. 5 shows that the percentage extraction of tanshinones was increased with the increase of

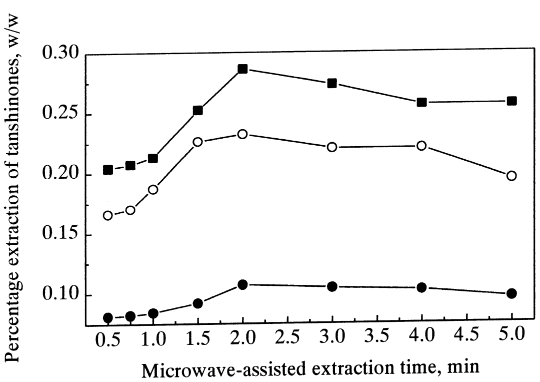

Fig. 4. Effect of MAE time on percentage extraction of tanshinones. Solvent: 95% (v/v) ethanol 100 ml, pieces of *Salvia miltiorrhiza bunge* root: 10 g, liquid/solid ratio: 10:1, ml/g. (■) Tanshinone IIA, (○) cryptotanshinone, (●) tanshinone I.

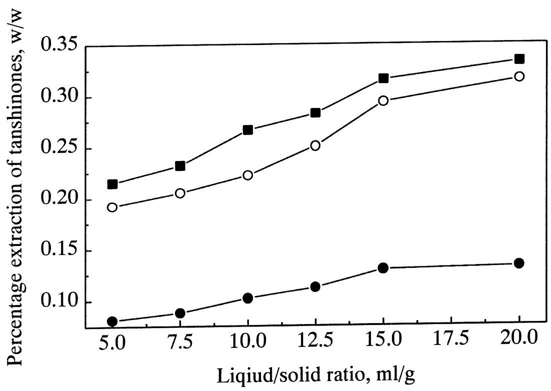

Fig. 5. Effect of liquid/solid ratio on percentage extraction of tanshinones. Solvent: 95% (v/v) ethanol 100 ml, MAE for 2 min. (■) Tanshinone IIA, (○) cryptotanshinone, (●) tanshinone I.

liquid/solid ratios. It is obvious that more energy is required to carry out the leaching under high ratio. The liquid/solid ratio of 10:1 (ml/g) was sufficient to reach the high percentage extraction, and it was used afterwards.

3.5. Effect of pre-leaching time before MAE on percentage extraction of tanshinones

Pre-leaching time at room temperature before MAE for 2 min influenced the percentage extraction of tanshinones (Fig. 6). If pre-leaching time was 45 min before MAE, the percentage extraction of tan-

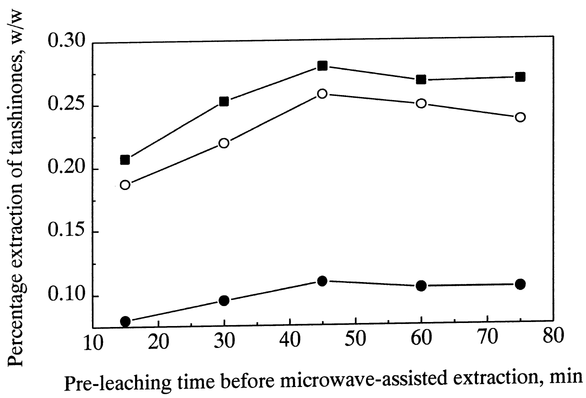

Fig. 6. Effect of pre-leaching time before MAE on percentage extraction of tanshinones. Solvent: 95% (v/v) ethanol 100 ml, pieces of *Salvia miltiorrhiza bunge* root: 10 g, MAE for 2 min, liquid/solid ratio: 10:1, ml/g. (■) Tanshinone IIA, (○) cryptotanshinone, (●) tanshinone I.

Table 1
Comparison of the results of extraction with MAE and conventional extraction methods (averages of the three replicates)

Extraction method	Extraction time	Percentage extraction, w/w		
		Tanshinone IIA	Cryptotanshinone	Tanshinone I
MAE	2 min	0.29	0.23	0.11
Extraction at room temperature	24 h	0.24	0.22	0.10
Heat reflux extraction	45 min	0.25	0.24	0.11
Ultrasonic extraction	75 min	0.28	0.25	0.10
Soxhlet extraction	90 min	0.33	0.25	0.12

Solvent: 95% (v/v) ethanol 100 ml; pieces of *Salvia miltiorrhiza bunge* root: 10 g; liquid/solid ratio: 10:1 ml/g

shinones can reach the highest. If the pre-leaching time was more than 45 min, tanshinones had been partly transferred from the root of *Salvia miltiorrhiza bunge* to solvent before MAE. They were easy to decompose at high temperature in the solvent [12], so percentage extraction of tanshinones was decreased when pre-leaching time was more than 45 min. Pre-leaching for a short time before MAE was of benefit for improving the percentage extraction.

3.6. Comparison of MAE and conventional extraction methods

Table 1 shows that MAE only for 2 min, extraction at room temperature for 24 h, ultrasonic extraction for 75 min, heating reflux extraction for 45 min, Soxhlet extraction for 90 min, MAE can reach almost the same or even higher percentage extraction of tanshinones. MAE obviously can reduce the extraction time in order to reach the same percentage extraction.

4. Conclusions

Conditions for MAE of tanshinone IIA, cryptotanshinone and tanshinone I from the root of *Salvia miltiorrhiza bunge* have been studied. Compared with the conventional methods, the MAE procedure employed provides high extraction efficiency within a short time, and is less labor intensive. Because tanshinones easily decompose at a high temperature for long period of time, MAE can reach high percentage extraction within a short time, it was a novel alternative extraction method for fast extraction and determination of tanshinones from the root of *Salvia miltiorrhiza bunge*. It was also available in pilot plant form for larger scale extraction, so the prospect of MAE of tanshinones from the root of *Salvia miltiorrhiza bunge* was very well.

Acknowledgements

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