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ORIGINAL RESEARCH PAPER

Microwave-assisted aqueous two-phase extraction of piceid, resveratrol and emodin from *Polygonum cuspidatum* by ethanol/ammonium sulphate systems

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Abstract Microwave-assisted, aqueous two-phase extraction was investigated to obtain effective constituents, including piceid, resveratrol and emodin in *Polygonum cuspidatum*. An aqueous two-phase system consisting of 25% (w/w) ethanol 21% (w/w) $(NH_4)_2SO_4$ gave equal yields of piceid, and 1.1- and 1.9-times higher yields of resveratrol and emodin, respectively, than that achieved by microwave-assisted extraction and heat reflux extraction. Three-separate operations, extraction, clarification and concentration, are hereby integrated into a single step to get higher yields at lower cost. This is therefore a potentially useful method for the extraction and purification of target products.

Keywords Emodin · Ethanol/ammonium sulphate · Microwave-assisted aqueous two-phase extraction · Piceid · *Polygonum cuspidatum* · Resveratrol

Introduction

Polygonum cuspidatum, a Chinese traditional herbal medicine, is used for eliminating stasis to activate blood circulation, relieving pain, expelling phlegm and arresting coughing. The main components in it are resveratrol, piceid and emodin. Resveratrol and piceid are widely used in medicine, health products and cosmetic industries on account of their various pharmaceutical properties such as anti-inflammatory, anticancer (Bertelli et al. 1999) and cardioprotective activities (Li et al. 2000). Emodin has antibacterial (Chukwujekwu et al. 2006) and anti-tumor activities (Srinivas et al. 2003).

Traditional extraction of the three compounds is heat-reflux extraction by ethanol. However, large amounts of ethanol, as well as a long time, are needed (Liu et al. 2007). Moreover, filtration and concentration are also needed before purification by column chromatography (Xiang et al. 2005). This results in a low yield and high cost of the products. Microwave-assisted extraction (MAE) is an alternative method for the extraction of components from herbs due to its advantages such as a short time, using less solvent and its high efficiency (Pan et al. 2000).

Aqueous two-phase extraction (ATPE) using short chain alcohol/salt system has been applied to the purification of natural compounds for its low cost, easy recovery of alcohol by evaporation, etc. Ethanol/phosphate system was used to purify glycyrrhizin from extract of *Glycyrrhiza uralensis Fisch* and the highest partition coefficient got to 13 (Tan et al. 2002); Ethanol/$(NH_4)_2SO_4$ system was selected to separate resveratrol from extract of *Polygonum cuspidatum*, and the purity of resveratrol in the top phase reached 34.29% (Li et al. 2006). However, ATPE was only

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applied to the purification of components from crude extract, and there is no report emphasizing the extraction behavior of active components in raw herbs when aqueous two-phase system is used as solvent.

In this paper, microwave-assisted aqueous two-phase extraction (MAATPE) was performed for the extraction of bio-active components from the raw herb of *Polygonum cuspidatum*. This procedure integrates extraction, clarification and concentration into a single step to increase yield and at lower cost. This study is the first report presenting a simple and effective method for the extraction of compounds directly from herbs by an aqueous two-phase system.

Materials and methods

Chemicals and materials

Dried root of *Polygonum cuspidatum* was obtained from Xiancaotang Medicine Cooperation (Dalian, China), ground into powder and passed through a 60 mesh sieve. *trans*-Piceid and emodin were purchased from National Institute for Control of Biological and Pharmaceutical Drugs of China (Beijing, China), and *trans*-resveratrol from Sigma Chemical Co. Other reagents are of analytical grade.

Analytical methods

Diluted extracts were analyzed by HPLC, using a Jasco separation module with a Kromasil C_{18} column (5 μm, 4.6 × 250 mm) as the stationary phase. The mobile phase consisted of water (A) and acetonitrile (B). *trans*-Piceid, *trans*-resveratrol and emodin were eluted with a gradient time programme: 0–15 min: 31–50% B; 15–20 min: 50–98% B; 20–25 min: 98% B at room temperature at of 0.7 ml/min. The eluate was monitored with a gradient time programme: 0–18 min at 306 nm, where both *trans*-piceid and *trans*-resveratrol have absorbance maxima, 18–30 min at 254 nm, where emodin has its maximal absorbance.

Partition behavior of piceid, resveratrol and emodin in ethanol/$(NH_4)_2SO_4$ aqueous two-phase system

Dry powder of *Polygonum cuspidatum*, 8 g, were extracted by Microwave-assisted extraction (MAE) using 100 ml absolute ethanol for 2 min. The extract was centrifuged and the supernatant and solid $(NH_4)_2SO_4$ were mixed to form an aqueous two-phase system consisting of 15–25% (w/w) ethanol and 15–28% (w/w) $(NH_4)_2SO_4$. The mixture was held for at least 1 h at room temperature. The concentrations of compounds in the top and bottom phases were analyzed by HPLC. The partition coefficient is defined as the ratio of the concentration of compound in the top phase to that in the bottom phase. The recovery is the mass ratio of compound partitioned in the top phase to the total amount of compounds.

Microwave-assisted aqueous two-phase extraction (MAATPE) from raw herb

MAATPE were performed in a refitted household microwave oven with a condensor outside. *Polygonum cuspidatum* powder were (4 g) mixed with water, solid $(NH_4)_2SO_4$ and absolute ethanol. The suspension was placed in the microwave oven and irradiated at 650 W. The mixture was cooled at room temperature and the top phase was taken for analysis. To compare other procedures with MAATPE, $(NH_4)_2SO_4$ solution, ethanol solution and water were each mixed with the herb and irradiated by microwave until they boiled. Then absolute ethanol, solid $(NH_4)_2SO_4$, as well as absolute ethanol and solid $(NH_4)_2SO_4$ were added, respectively. The suspensions were shaken for 1 min and held for 5 min to form aqueous two-phase systems. MAE and heat-reflux extraction were also conducted.

Phase ratio was expressed as ratio of volume of top phase to that of bottom phase. Yield of compound was defined as ratio of quantity of the compound in the extractive to the total quantity of it in raw herb. Total quantity of compound was determined by MAE of raw herb with ethanol repeatedly until the extract was colorless.

Results and discussion

Partition behavior of piceid, resveratrol and emodin in ethanol/$(NH_4)_2SO_4$ aqueous two-phase system

As shown in Figs. 1–3, the partition coefficient and recovery of piceid, resveratrol and emodin increased dramatically with the increasing concentration of

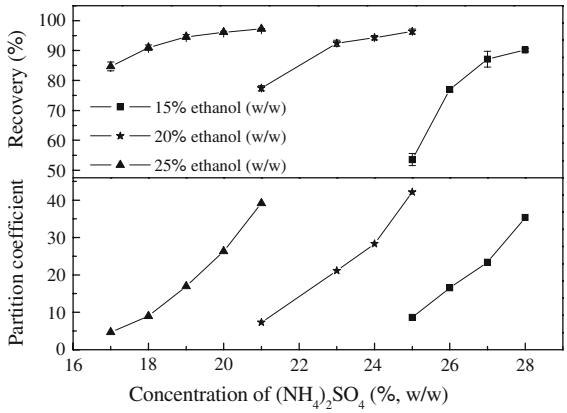

Fig. 1 Effect of concentration of $(NH_4)_2SO_4$ on partition coefficient and recovery of piceid

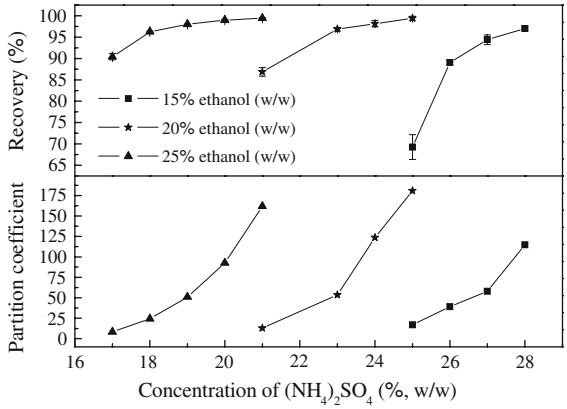

Fig. 2 Effect of concentration of $(NH_4)_2SO_4$ on partition coefficient and recovery of resveratrol

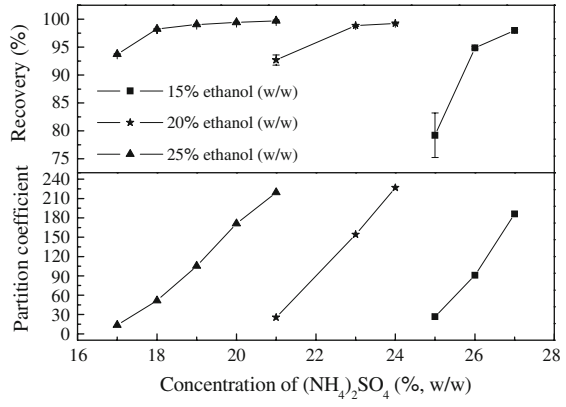

Fig. 3 Effect of concentration of $(NH_4)_2SO_4$ on partition coefficient and recovery of emodin

$(NH_4)_2SO_4$ and ethanol. The highest partition coefficients of piceid, resveratrol and emodin were 42, 180 and 226, respectively, indicating that almost all the three compounds were enriched in the top phase, but also, demonstrating that hydrophobic compounds tend to concentrate in the top phase. Ethanol is enriched in the top phase and $(NH_4)_2SO_4$ in the bottom phase, and piceid, resveratrol and emodin are all ethanol-soluble compounds that tend to concentrate in the top phase. Therefore, the ethanol/$(NH_4)_2SO_4$ system is an effective system for the concentration of the three components.

Microwave-assisted aqueous two-phase extraction

The high partition coefficient and recovery of the three compounds in ethanol/$(NH_4)_2SO_4$ system indicated that they are enriched in the top phase. Based on these results, MAE and ATPE were integrated to extract piceid, resveratrol and emodin directly from raw herb of *Polygonum cuspidatum*.

Effect of extraction strategies on yield of piceid, resveratrol and emodin

Comparing the four strategies, the yield of the three compounds was the highest by MAATPE and the lowest when $(NH_4)_2SO_4$ solution was used as solvent and ethanol was added after extraction (see Fig. 4). The phenomenon is related to phase separation occurring during microwave irradiation in MAATPE, and the raw herb is suspended between the top and bottom phase, so high concentrations of ethanol in the top phase facilitates the extraction of compounds. In contrast, in the other three strategies, it takes 5 min to form an aqueous two-phase after mixing which attenuates the diffusion of compounds from herb to solvents. MAATPE is the optimal extraction strategy because of its high extraction efficiency and time-saving operation.

Effect of phase composition on yield of piceid, resveratrol and emodin in MAATPE

Based on the results above, MAATPE with different ethanol and $(NH_4)_2SO_4$ concentrations were performed. The results in Table 1 indicated that phase ratio increased with the increasing concentration of

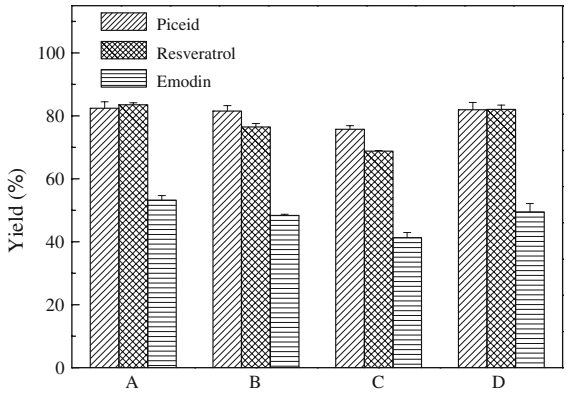

Fig. 4 Comparison of different extraction strategies. (A) Microwave-assisted aqueous two-phase extraction from raw herb; (B) Microwave-assisted extraction from raw herb by ethanol solution and then solid $(NH_4)_2SO_4$ was added to form aqueous two-phase system; (C) Microwave-assisted extraction from raw herb by $(NH_4)_2SO_4$ solution and then ethanol was added to form aqueous two-phase system; (D) Microwave-assisted extraction from raw herb by water and then ethanol and solid $(NH_4)_2SO_4$ were added to form aqueous two-phase system

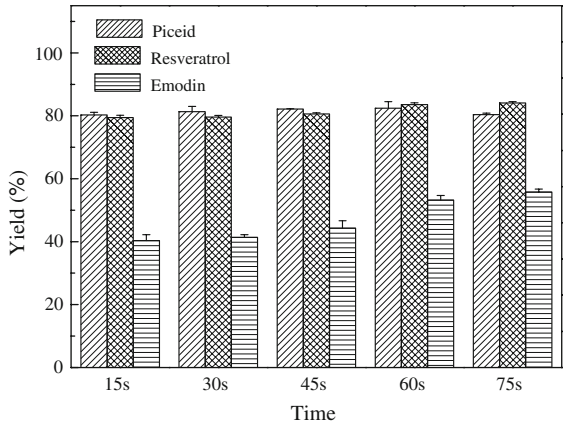

Fig. 5 Effect of extraction time on yield of piceid, resveratrol and emodin in MAATPE

ethanol in the system. Comparing the three compounds, yield of emodin was the lowest, with the highest yield of 55%, whereas the highest yield of both piceid and resveratrol got to 86%. That is because emodin is much more hydrophobic than piceid and resveratrol, and its solubility is much lower in ethanol. Considering phase ratio and yield, the system composed of 25% (w/w) ethanol and 21% (w/w) $(NH_4)_2SO_4$ was selected as the optimum system.

Effect of extraction time on yield of piceid, resveratrol and emodin in MAATPE

To optimize MAATPE, the effect of extraction time on yield of piceid, resveratrol and emodin was investigated (see Fig. 5). Yields of piceid and resveratrol increased slightly with the increasing extraction time, while that of emodin increased obviously until 60 s, when the mixture boiled, and stayed constant afterwards. The solubility of piceid and resveratrol is high and temperature hardly affects their extraction efficiency at high concentration of ethanol; however, the solubility of emodin in ethanol is much lower, so high temperature facilitates its extraction efficiency. Therefore, an extraction time of 60 s was optimum to achieve the highest yield.

Comparison of MAATPE, MAE and heat-reflux extraction

Comparison of the three extraction methods was depicted in Fig. 6. Yield of piceid was equal to that by MAE and heat reflux extraction, whereas that of resveratrol and emodin was 1.1- and 1.9-times higher, respectively. Emodin is more hydrophobic and it cannot be fully extracted with ethanol of low concentration. However, in $(NH_4)_2SO_4$/ethanol aqueous two phase systems, ethanol is enriched in the top phase and its concentration is much higher, which facilitates the extraction of emodin.

Table 1 Effect of phase composition on yield of piceid, resveratrol and emodin in MAATPE

System no.	1	2	3	4	5
Ethanol (%, w/w)	15	20	25	30	35
$(NH_4)_2SO_4$ (%, w/w)	28	25	21	18	15
Phase ratio	0.20	0.44	0.69	1.2	1.7
Yield of piceid	62 ± 3	76 ± 3	82 ± 2	85 ± 1	86 ± 1
Yield of resveratrol	66 ± 1	72 ± 2	84 ± 1	84 ± 1	86 ± 0
Yield of emodin	45 ± 2	52 ± 1	53 ± 2	55 ± 2	55 ± 1

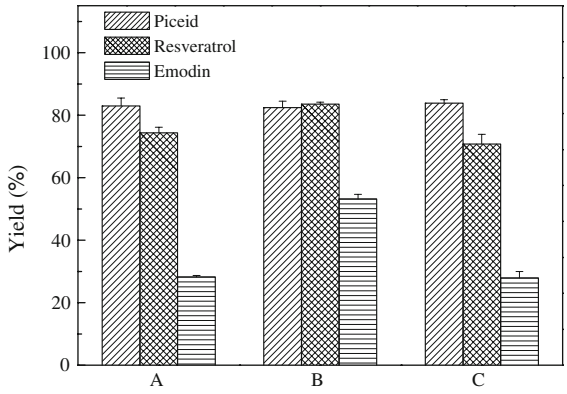

Fig. 6 Comparison of different extraction methods. (A) Microwave-assisted extraction by ethanol solution (25 g absolute ethanol and 54 g water) for 1 min at 650 W; (B) Microwave-assisted aqueous two-phase extraction (25 g absolute ethanol, 54 g water and 21 g solid $(NH_4)_2SO_4$) for 1 min; (C) Heat reflux extraction by ethanol solution (25 g absolute ethanol and 54 g water) for 1 h

Traditionally, heat-reflux extraction is always used in the extraction of the three compounds from *Polygonum cuspidatum*, but 15-fold of 70% (v/v) ethanol as well as 6 h (Liu et al. 2007) were consumed. In addition, filtration and concentration are needed before purification by column chromatography (Xiang et al. 2005). MAE has the advantages of a short time, less solvent and high efficiency (Pan et al. 2000); however, filtration and concentration are also needed, which decreases yield of the compounds and increases the cost. MAATPE, however, integrates MAE and ATPE into a single step to extract compounds directly from the raw herb and during which phase separation occurs and raw herb is suspended between the top and bottom phase. The top phase is clear after extraction which greatly reduces the filtration load. MAATPE possesses the advantages of both MAE and ATPE and simplifies the operation to get higher yield of the products.

Besides components of herbs, MAE has also been employed for the extraction of pollutants from environmental matrices (Shu et al. 2003), and ATPE has been widely used for the purification and enrichment of proteins (Balasubramaniam et al. 2003), antibiotics (Mokhtarani et al. 2008) and metal ions (Shibukawa et al. 2001). MAATPE is the integration of the two methods, so it is expected to be an alternative method for the extraction and enrichment of target compounds directly from fermentation broth, plant materials, environmental matrices and so on.

Conclusion

MAATPE of effective constituents in *Polygonum cuspidatum* was performed by an ethanol/$(NH_4)_2SO_4$ system. Ethanol/$(NH_4)_2SO_4$ aqueous two-phase system consisting of 25% (w/w) ethanol and 21% (w/w) $(NH_4)_2SO_4$ was selected for MAATPE from *Polygonum cuspidatum* with an equal yield of piceid and yields of resveratrol and emodin 1.1- and 1.9-times higher, respectively, than that by MAE and heat-reflux extraction. MAATPE possesses the advantages of MAE and ATPE and integrates the two operations into a single step to provide a simple method. Therefore, it is a potential method for the extraction and subsequent purification of target products.

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