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Extraction of resveratrol and emondin from *Polygonum cuspidatum* by supercritical CO\(_2\) with different solubilizers

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In order to improve the extraction yields of resveratrol and emondin obtained by supercritical CO\(_2\) extraction (SCE), different solubilizers were added in the supercritical CO\(_2\) extraction process. Resveratrol and emondin were extracted from *Polygonum cuspidatum* by SCE with different solubilizers, including bis(2-ethylhexyl) sodium sulfosuccinate (AOT), povidone k-90 (PVP k-90), Poloxamer 188 and Tween 80, at extraction pressure 25 MPa and extraction temperature 50°C. The results show that resveratrol was hardly extracted by SCE with ethanol as modifier, but AOT, PVP k-90, Poloxamer 188 and Tween 80 could enhance the extraction yield of resveratrol significantly, while the yield of emodin was influenced gently by using these solubilizers. Based on the comparison of SCE and heat reflux extraction, the advantage of SCE is less extraction time at lower operating temperature without using large amount of organic solvent. The yields of resveratrol and emodin extraction from *P. cuspidatum* by SCE can be improved by introducing AOT, PVP k-90 and Poloxamer 188 into supercritical CO\(_2\).

**Key words:** *Polygonum cuspidatum*, resveratrol, emodin, supercritical CO\(_2\) extraction, solubilizer.

INTRODUCTION

*Polygonum cuspidatum* (*P. povidone*) is a member of the Polygonaceae family, which is widely distributed in Asia and North America. The dried root of *P. povidone* is a well-known Traditional Chinese Medicine (called Huzhang) officially listed in the Chinese Pharmacopoeia (The State Pharmacopoeia Committee of the People's 2010) and also used as folk medicine remedies for the treatment of cuts, burns and abscesses in Korea and Japan (Bralley et al., 2008; Wu et al., 2012). Resveratrol and emodin were the major polyphenols in *P. povidone* extracts, having antibacterial, antioxidant and antimutagenic properties (Shan et al., 2008; Pandit et al., 2012). Several studies have evaluated the anti-tumor effect of *P. povidone* extracts (Lin et al., 2010; Shin et al., 2011).

*P. povidone* is used to produce resveratrol supplements because it grows easily and quickly. Because of the wide range of possible health benefits of resveratrol and emodin, a huge effort has been made towards the development of extraction, isolation, purification and
quantification methodologies (Wang et al., 2008; Mantegna et al., 2012).

The conventional extraction method is heat reflux extraction with ethanol, followed by filtration, concentration and purification. This procedure is time consuming and requires a large amount of solvent (Xiang et al., 2005; Liu et al., 2007). Due to its environmental friendliness and unique physical and chemical properties, SCE has attracted much attention (Melo et al., 2014; Ruttaranamongkol et al., 2014). However, supercritical CO2 (SC) is not a good solvent for polar molecules such as saponins, flavones and alkaloids. Therefore improvements in polar compound solubility and mass transfer are required for SCE. By introducing polar solvent into SC can improve CO2 dissolution of polar molecules. Ethanol and acetonitrile are always added in SC as modifier to improve CO2 dissolution of polar molecules. By introducing surfactants or solubilizers into SC to form reverse micelles or microemulsion can improve CO2 dissolution of polar molecules too. The aim of this paper is to explore the effects of different solubilizers on the extraction yields of resveratrol and emodin from *P. povidone* by SCE.

### MATERIALS AND METHODS

**Chemicals and reagents**

*P. povidone* was purchased from Guangzhou Qingping Market for Traditional Chinese Medicine. Resveratrol and emodin standard (pharmaceutical grade) were purchased from National Institute for Control of Pharmaceutical and Biological Products. Bis(2-ethylhexyl) sodium sulfo succinate (AOT) of analytical reagent grade was obtained from Aladdin Chemistry Co. Ltd. PVP k-90 and Poloxamer 188 (pharmaceutical grade) were supplied by BASF (Germany). Tween-80 (analytical grade) was obtained from Guangzhou Chemicals Company. Ethanol (HPLC grade) was purchased from Tianjin Chemicals Company.

**Supercritical CO2 extraction**

The SCE equipment (capacity 100 ml) was manufactured by Guangzhou Hanwei Co. Ltd. 30 g of the milled powder of dried *P. povidone* roots, particle size 0.5 to 0.2 mm, was placed in the extractor. The solubilizer (30 ml) was added via the high-pressure pump. The powder was statically extracted for 45 min followed by circularly extraction for 15 min at extraction pressure 25 MPa, extraction temperature 50°C, separation pressure 2 MPa, separation temperature 25°C. The process was repeated 2 times. The extracts were collected and analyzed.

**Heat reflux extraction**

The heat reflux extraction was performed as follows (Xiang et al., 2005): 30 g of the milled powder of dried *P. povidone* roots was mixed with 150 ml of 70% ethanol in water, boiled at boiling point for 4 h. Then, the mixture was filtrated through filter paper. The extracts were collected. The residue was mixed with 150 ml 70% ethanol in water, boiled at boiling point for 4 h. This extraction process was repeated 2 times. The total extraction yields of resveratrol and emodin were calculated.

**HPLC analysis**

The high performance liquid chromatography (HPLC) system consisted of a pump, a column (Hypersil ODS, 5 μm, 4.6 mm × 150 mm), an auto-injector and a UV-detector (Agilent 1100, USA). The gradient elution HPLC method was used for determining the contents of resveratrol and emodin in extracts. The gradient elution time was shown in Table 1. HPLC was performed at a flow-rate of 1.0 ml/min at 30, using a mobile phase of acetonitrile and water, and a UV detector at 306 nm. The SCE sample was put into a 100 ml volumetric flask and reached the scale accurately with methanol. 5 ml sample solution was pipetted into a 25 ml volumetric flask and diluted to the scale accurately with methanol. 20 μl diluted solution was detected by HPLC. The chromatograms of the reference substances and samples are shown in Figure 1.

### RESULTS

**Optimization of supercritical fluid extraction**

In this study, parameters of SCE such as type of solubilizer, concentration of solubilizer, pressure and extraction times were optimized. Each experiment was performed with 30 g of dried and milled *P. povidone* roots.

**Effect of solubilizer**

Type of solubilizer was selected at the beginning of the whole optimization process. Tested solubilizers were as follows: Ethanol, AOT, PVP k-90, Poloxamer 188 and Tween 80. The samples obtained by SCE with these solubilizers were analyzed and the results are shown in Figure 2. The highest extraction yield of resveratrol was 0.304 mg/g when AOT was used. The highest extraction yield of emodin was 1.008 mg/g when PVP k-90 was used. The lowest extraction yield of resveratrol was 0.015 mg/g when ethanol was used. The highest extraction yield of resveratrol was 20 times more than the lowest yield.

**Effect of AOT concentration**

Five different concentrations of AOT in 95% ethanol (that is, 0.02, 0.05, 0.1, 0.2 and 0.5 mol/l) were investigated.

### Table 1. The gradient elution time for determining the contents of resveratrol and emodin in extracts.

<table>
<thead>
<tr>
<th>T/min</th>
<th>Water % (v/v)</th>
<th>Acetonitrile % (v/v)</th>
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<tbody>
<tr>
<td>0</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>50</td>
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<tr>
<td>12</td>
<td>28</td>
<td>72</td>
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<tr>
<td>14</td>
<td>28</td>
<td>72</td>
</tr>
<tr>
<td>20</td>
<td>82</td>
<td>18</td>
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</tbody>
</table>
Figure 1. Chromatogram of standard solution (A), typical chromatogram of SC extracts of P. povidone (B) and blank sample (C). The blank sample solution contained 0.5 mg/ml PVP K-90, Poloxamer 188, Tween 80 and AOT. As shown in chromatograms, the determination of target compounds (resveratrol and emodin) was not interfered by solubilizers or other compounds in the sample.
while the other parameters were fixed: 50 L, extraction 1 time and 20 MPa. As seen in Figure 3, the extraction yields of resveratrol and emodin were greatly influenced by the AOT concentration in 95% ethanol from 0.02 through 0.5 mol/L. The best results were achieved at the concentration 0.05 mol/L. The extraction yield was increased as AOT concentration increased to 0.05 mol/L. When the concentration was higher than 0.05 mol/L, the extraction yield decreased with the increase in AOT concentration. This is because the concentration of AOT is too high to completely dissolve in the SC phase, so excess AOT deposits on the material surface and limits mass transfer.

**Effect of pressure**

Four different pressures (that is, 10, 15, 20 and 25 MPa) were investigated while the other parameters were fixed: 50 L, extraction 1 time and 0.05 mol/L AOT as solubilizer. As shown in Figure 4, it is evident that the extraction yield of resveratrol was increased gently with increasing pressure, while the increase of extraction yield of emodin is more significant. The extraction yield of resveratrol and emodin is highest when applying the pressure 25 MPa.
Effect of extraction times

Four different extraction times (that is, 1, 2, 3 and 4 times) were investigated while the other parameters were fixed: 50 L, 25 MPa and 0.05 mol/L AOT as solubilizer. As shown in Figure 5, the extraction yields of resveratrol and emodin increased gently with the increase of extraction times. The increase of extraction yield is noticeable to the extraction 3 times: the extraction yield stays at the same level even the extraction more than 3 times. The results demonstrated that 3 times was enough to extract resveratrol and emodin from *P. cuspidatum* by SCE.

Comparison of SCE and Heat reflux extraction

Heat reflux extraction is a conventional extraction technique, but this procedure is time consuming and requires
a large amount of organic solvent. SCE is a new extraction technique, which primarily uses CO2 as its extraction medium, has been widely used in food preparation and natural herbs extraction at low operating temperature without using large amount organic solvent. These two techniques were compared as shown in the Table 2. The extraction yield of resveratrol by heat reflux extraction was approximately 5.5 times higher than by SCE method, while the extraction yield of emodin by heat reflux extraction was approximately 2 times lower than by SCE.

DISCUSSION

CO2 is a non-polar solvent, it is hardly polarised by ethanol. Resveratrol is hardly extracted by SC under normal condition. However, AOT, PVP k-90, Poloxamer 188, and Tween 80 could increase the extraction yield of resveratrol significantly, because they could induce to form micelles or microemulsion in SC. The extraction yield of resveratrol obtained by SCE could be improved significantly by introducing AOT, PVP k-90 and Poloxamer 188 into SC, while the extraction yield of emodin was influenced gently by using these solubilizers. SCE for 3 h gave higher extraction yield of emodin than the heat reflux extraction for 12 h at 90 L using 450 ml 70% ethanol. From time point of view the SCE is advantageous than heat reflux extraction and SCE also do not require large amount of solvent. Based on the comparison of SCE and heat reflux extraction, SCE is more suitable for emodin than for resveratrol. These results are compatible with Blanka B’s research (Beňová et al., 2010). But in Blanka B’s research the extraction yield of resveratrol is 0.1 mg/g by SCE with ethanol as modifier while the extraction yield is only 0.015 mg/g in our research. This phenomenon may be due to the difference of medicinal materials breeds.

ACKNOWLEDGEMENTS

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**Table 2.** Comparison of the extraction yields of resveratrol and emodin by SCE and heat reflux extraction.

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Extraction condition</th>
<th>The extraction yield (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resveratrol</td>
</tr>
<tr>
<td>SCE</td>
<td>30 g sample, 50 L, 1 h, 25 MPa, AOT (0.05 mol/L) as solubilizer, three replicate extraction</td>
<td>0.31±0.03</td>
</tr>
<tr>
<td>Heat reflux extraction</td>
<td>30 g sample, 90 L, 4 h, 150 ml 70% ethanol as extraction solvent, three replicate extraction</td>
<td>1.68±0.07</td>
</tr>
</tbody>
</table>

Standard deviations are given from three replicate experiments.

**Conflict of interest**

The author(s) have not declared any conflict of interests.

**REFERENCES**


