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SEPARATION AND ENRICHMENT OF TOTAL PHYTOSTERONE IN *Achyranthes bidentata* BY SOLVENT SUBLATION

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Achyranthes bidentata is a perennial herbaceous plant widely distributed and grown in tropical areas of Asia and Africa, particularly China, Korea, and Vietnam. Its root is a well-known traditional Chinese medicine, and it has been proved that the total phytosterone in *Achyranthes bidentata* has great efficacy in certain medications [1–3]. The most common methods of total phytosterone separation and enrichment are water extraction [4], adsorption with resin [5], solvent extraction [6], ultrasonic extraction [7], and so on. Solvent sublation was originally introduced by Sebba [8] as an auxiliary method to ion flotation. It is a kind of adsorptive bubble separation technique, in which the hydrophobic compounds in water are adsorbed on the bubble surfaces of an ascending gas stream and then extracted into an immiscible liquid layer (usually an organic solvent lighter than water) placed on top of the water column. This method, with its advantages of simultaneous separation and enrichment, has attracted much attention in the fields of environmental analysis and wastewater treatment [9–13] recently. In the solvent sublation process of *Achyranthes bidentata*, total phytosterone can be separated and enriched simultaneously, from which we obtain the total phytosterone after vacuum desiccation.

Selection of Sublation Solvent. According to the "like dissolves like" rule and the effect of hydrogen bonding, *n*-butanol was used as the sublation solvent in this work.

Effect of pH. Satisfactory efficiency of *Achyranthes bidentata* solvent sublation was achieved at pH 5. At pH 5, total phytosterone is easily sublated; with increasing pH, the hydroxyl group in total phytosterone can be ionized and the efficiency of solvent sublation decreases; however, when pH is below 5, those hydroxyl groups would react with hydrochloric acid in solution, which may increase the hydrophilicity of total phytosterone. So, in all further work, the pH 5 was used for *Achyranthes bidentata* solvent sublation.

Effects of Nitrogen Flow Rate and Sublation Time. The experimental results show that the efficiency of *Achyranthes bidentata* solvent sublation increases with increasing nitrogen flow rate and sublation time. Generally, too high a gas flow rate can result in turbulent mixing at the solvent–aqueous solution interface, and such mixing can promote the re-dissolution of the floated product in the aqueous phase. Moreover, when the sublation time was more than 80 min, the efficiency did not markedly increase, so the nitrogen flow rate can be fixed at 40 mL/min and the sublation time at 80 min in this work.

Efficiency of *Achyranthes bidentata* Solvent Sublation. The efficiency of solvent sublation can be calculated by use of the following equation. In the formula, E is the efficiency of solvent sublation, C_{out} is concentration of the aqueous phase after sublation, and C_{in} is the concentration of the aqueous phase before sublation. The sublation results in the selected optimum conditions are as follows: efficiency (%), 80.0–82.4, average (%) 81.4, RSD (%) 1.53.

$$E = (1 - C_{out}/C_{in})100\%$$

Method of Comparison. Traditional solvent extraction was also used to prepare total phytosterone from *Achyranthes bidentata* for the purpose of comparison with solvent sublation. Table 1 shows the comparison results.

As shown in Table 1, the efficiency of *Achyranthes bidentata* solvent sublation is far better than solvent extraction. Hence, it is clear that the solvent sublation method is better than the traditional solvent extraction method.

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TABLE 1. The Comparison Result between Solvent Sublation and Solvent Extraction

Method	Recovery, %	Time/h	Enrichment times
Solvent sublation	81.41 (n = 3)	1.5	20
Solvent extraction	16.53 (n = 3)	2.5	1

Structure Analysis of the Floated Product. In the UV spectrum of the floated product there are two bands at 210 and 247 nm; the former originates from the conjugate $\pi \rightarrow \pi^*$ transition of the double bond and carbonyl group in the total phytosterone, termed the K band, and the latter originates from an $n \rightarrow \pi^*$ transition of the carbonyl group in the total phytosterone, termed the R band. In the IR spectrum of the floated product, the peaks at 2955, 2922, 2852, 1454, and 1377 cm^{-1} are the characteristic absorbance of methyl and methylene originating from the total phytosterone. The peaks at 3392 cm^{-1} (from the O-H stretching vibration) and 1072 cm^{-1} (from the C-O stretching vibration) are the characteristic absorbance of the hydroxyl group, the peak at 1726 cm^{-1} (from the C=O stretching vibration) is the characteristic absorbance of the carbonyl group, and the peaks at 1658 cm^{-1} (from the C=C stretching vibration) and 798 cm^{-1} (from the out-of-plane bending stretching vibration of the single hydrogen in a trisubstituted C=C) are the characteristic absorbance of trisubstituted C=C. The hydroxyl group, carbonyl group, and trisubstituted C=C observed above originated from the total phytosterone. According to the analysis above, it can be proved that the floated product is total phytosterone.

Procedures. About 5.0 g *Achyranthes bidentata* was accurately weighed into a 500 mL beaker, and 50 times the amount of water was added, with boiling at 80~100°C twice, 2 h each time. After filtration, the solution was extracted with ether 3 times in order to remove pigment and fat, then transferred to a 1000 mL volumetric flask and diluted with deionized water. The result was used as stock solution; 20 mL of the stock solution was transferred to a 300-mL beaker and diluted with deionized water to 200 mL; then the pH of the solution was adjusted to 5 with hydrochloric acid solution and sodium hydroxide solution on a Mettler Toledo 320-S pH meter. This solution was transferred to a flotation cell [14]. The total phytosterone was floated by bubbling nitrogen gas at a flow rate of 40 mL/min from the bottom of the cell for 80 min, and extracted into 10 mL of *n*-butanol on the surface of the aqueous solution. The organic phase was transferred to a 25 mL beaker and dried in vacuum. After the organic solvent evaporated, the dry floated product was obtained.

Procedure for Structure Analysis of the Floated Product. The floated product was dissolved in *n*-butanol and identified by a Hitachi U-3010 ultraviolet-visible spectrophotometer in 1 cm quartz cuvettes for absorption spectrum (200–350 nm). Moreover, the IR spectrum of the floated product was also determined by a Nicolet 210-FTIR spectrophotometer.

Conclusion. A novel and more effective method for separating and enriching total phytosterone was established. By solvent sublation, the total phytosterone was separated from *Achyranthes bidentata* and subsequently enriched; it could then be positively identified by UV and IR spectroscopy. A comparison showed that the efficiency of the *Achyranthes bidentata* solvent sublation method was better than that of the traditional solvent extraction method.

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