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Research Article

Aqueous Two-phase Extraction of Polysaccharide from *Potentilla anserine* L.

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Abstract: Aqueous two-phase system is a useful method for purifying bio-logical materials in biochemistry and biotechnology. In this study the potential use of a K_2HPO_4/ethanol aqueous two-phase system to extract polysaccharides from *Potentilla anserine* L. The effects of salt, ethanol, NaCl concentrate and pH on the partitioning of polysaccharides were studied. The ATPS with 27% ethanol, 21% K_2HPO_4, 0.4 mol/L NaCl and pH 7 were selected as the optimal process system according to the partition coefficient and recovery. Furthermore, a comparison between the ATPS and the conventional extraction was conducted. For ATPS, the extraction yield and purity were 12.47 and 16.18%; while the extraction yield and purity were 10.3 and 6.14% by conventional extraction, respectively. ATPS showed a higher the extraction yield and the purity was around 2-fold with conventional method. So the ATPS can be employed to become an efficient and prospective process for the extraction.

Keywords: Aqueous two-phase system, polysaccharides, *Potentilla anserina*

INTRODUCTION

Potentilla anserina L. is distributed far-reaching in the western areas of China, especially in Qinghai-Tibetan Plateau. For thousands of years, it has been used frequently as a crude substance, such as taken as a functional food and folk medicine. It is documented that the rhizomes of *P. anserina* contain a large number of carbohydrates, protein, tannin, flavonoids, lysine, histidine, etc., (Chen *et al*., 2000). In recent years, the isolated polysaccharides have been found to play an important role in the biomedical field, because of their antioxidant, immunostimulatory and antitumor effects.

During the last few years, environmentally friendly techniques have become more important. Maceration, mechanical rabbling and heat reflux are the traditional extraction methods of polysaccharides. Usually, it requires long extraction time and high extraction temperature with low extraction yield. However, alternative extraction techniques such as Ultrasonic Assisted Extraction (UAE) with lower temperature and enhanced yields had been also reported (Li *et al*., 2007). The Aqueous Two Phase System (ATPS) is a new extraction technology and offer several advantages over traditional methods, including shorter process time, lower costs, easier separation from the system constituents, higher purity and higher extraction capacity. It based on short chain alcohols (Minami and Kilikian, 1998) and inorganic salts and has been widely used in the separation of proteins, enzymes and several other biomolecules (Patil and Raghavarao, 2007). In this study, a certain mass of root powder was placed into the ATPS solvent system (an upper ethanol-rich phase and a lower salt-rich phase). When the mixture because an emulsion, the ethanol-rich phase forms tiny droplets and disperse into the salt-rich. The polysaccharides transferred into the salt-rich (Fig. 1). Based on previous research, we don't chose $(NH_4)_2SO_4$ in the ATPS because of it is harmful to human health and found K_2HPO_4 is the better inorganic salt to form ATPS (Zhang *et al*., 2013).

The present study reports the extraction of polysaccharides from *P. anserina* by using ATPS. The influence of various process parameters such as salt, ethanol, NaCl concentration and pH on the partition coefficient (K) and the Recovery (R) were studied. The aim was to demonstrate that this environmentally friendly technique can be successfully applied to the extraction of polysaccharides.

MATERIALS AND METHODS

Materials: *Potentilla anserine* L. roots were obtained from the Lanzou, Gansu province, China. The samples were dried, powered, sieved (40-80 mesh) and then stored at room temperature until used. All other chemicals were of analytical-reagent grade.

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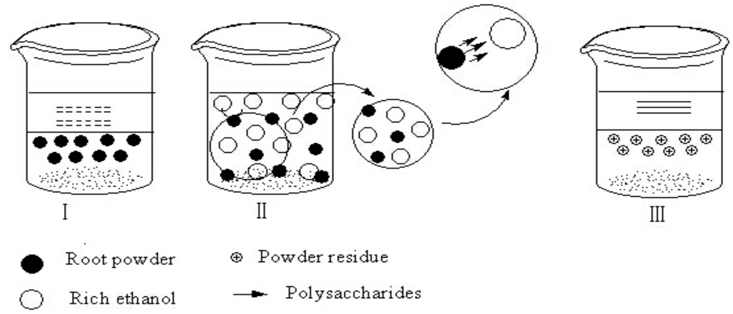

Fig. 1: Schematic diagram of the ATPS process used to extract polysaccharides from root powder

Aqueous two-phase extraction: The phase diagram of ethanol and K_2HPO_4 were designed on the basis of a turbidity titration method described by Jiang *et al.* (2009). Different amounts of K_2HPO_4 added to a series of tubes. Then, deionized water was added and mixed until the turbidity disappeared. Before the bimodal curve was plotted, the concentrations of ethanol and K_2HPO_4 were calculated. At different turbid points, the concentrations of K_2HPO_4 and ethanol were calculated according to the following equations:

$$W_1 = m_1 / (m_1 + m_2 + m_3)$$

$$W_2 = m_2 / (m_1 + m_2 + m_3)$$

where, W_1 and W_2 were the mass fraction of ethanol and K_2HPO_4, respectively. m_1, m_2 and m_3 were the amount of ethanol, K_2HPO_4 and water, respectively.

Partition behaviors of polysaccharides in ATPS: To investigate the effects of ethanol and K_2HPO_4 on partition behaviors of polysaccharides, the extracts of *Potentilla anserine* L. roots were mixed with ATPS. A certain weight of K_2HPO_4 was mixed in 15 mL graduated tubes with ethanol and 0.1 g of the powers extract. Deionized water was added to give a final mass of 10.0 g. After standing for 40 min at 80°C, the upper and lower phases were separately withdrawn using pipettes and the phase volumes were determined.

The partition coefficient (K) and the Recovery (R) of polysaccharides were calculated as the following equations (Guo *et al.*, 2013):

$$K = \frac{C_b}{C_t}$$

$$R = \frac{C_b V_b}{C_b V_b + C_t V_t} \times 100\%$$

where,

C_t and C_b : The concentrations of the upper and lower phases

V_t and V_b : The volumes of the upper and lower phases

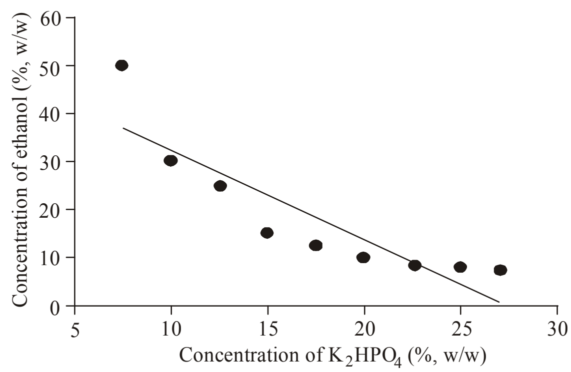

Fig. 2: Phase diagram of ethanol/K_2HPO_4

Determination of polysaccharides: The top phase and the bottom phase were withdrawn for analysis of polysaccharides by the phenol-H_2SO_4 method. The whole procedure was as follows: 0.2 mL solution was added to a 10 mL colorimeter tube. Then 1.0 mL 5% phenol and 5.0 mL of concentrated sulfuric acid were added. The tube was well shaken and kept for 20 min before analysis. The absorbance was measured at the wavelength of 490 nm by a spectrophotometer (721-Vis, Shanghai). Using the glucose as the standard solution, where *Y* is the absorbance and *X* is the concentration of glucose in the range of 0.02-0.12 mg/mL.

RESULTS AND DISCUSSION

Phase diagram of ethanol/K_2HPO_4: In order to select the appropriate ratio of phase composition, the phase diagram of the ethanol/K_2HPO_4 system was designed (Fig. 2). A curve delineated had two zones. In this zone, the top phase was the ethanol denser phase and the bottom phase was the K_2HPO_4 rich aqueous phase. When the concentration of ethanol increased, the curve would result in the extreme points that formed ATPS, specially, at higher concentration of K_2HPO_4. Because excessive K_2HPO_4 would separate out as solid, as the ethanol concentration increasing. The solvent dilution crystallization that recovered the salt after extraction could explain this phenomenon. There are two situations (high concentration of ethanol and low

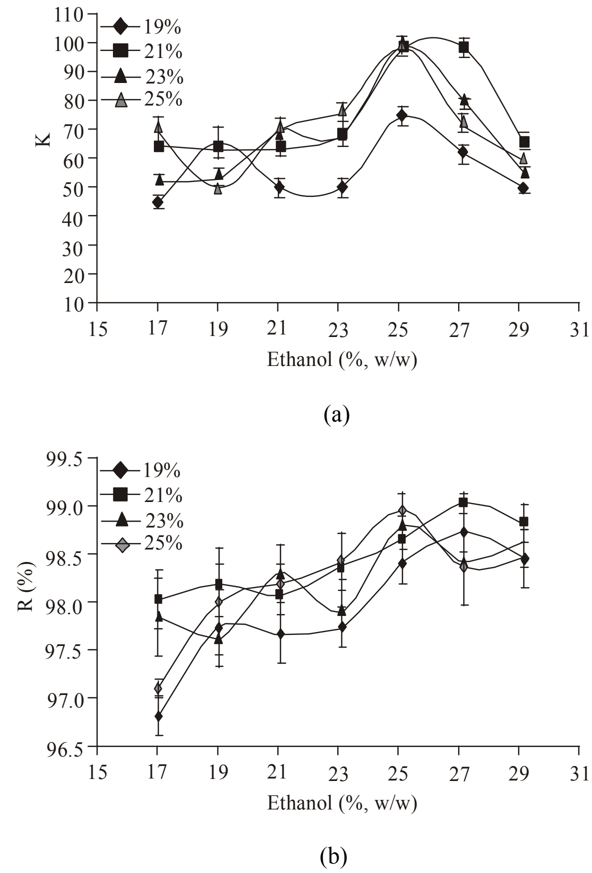

Fig. 3: Effects of the K_2HPO_4 concentration on; (a): The partition coefficient; (b): The recovery

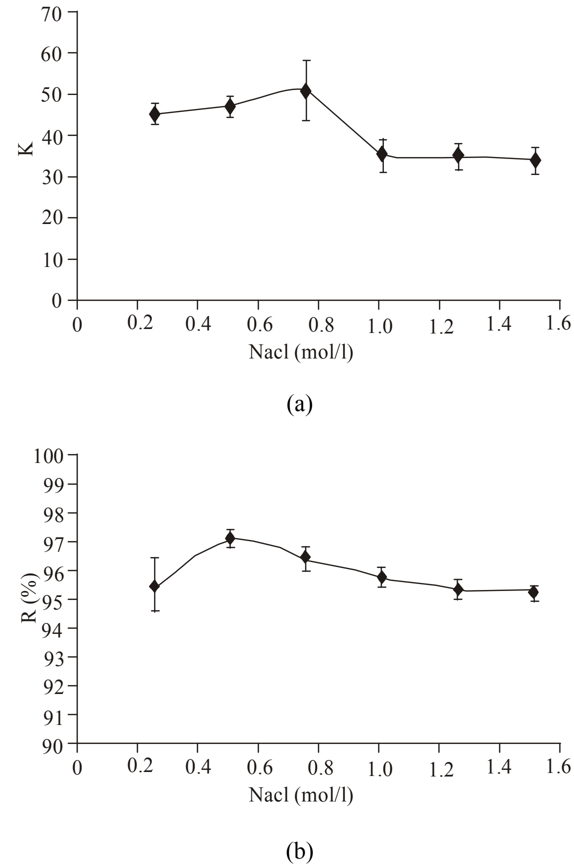

Fig. 4: Effect of additives on; (a): The partition coefficient; (b): The recovery of polysaccharides

concentration of K_2HPO_4) that it will be no ATPS formed. So, the concentration of ethanol and K_2HPO_4 should be chosen appropriate in the zone above the cave.

Partition behavior of polysaccharides in ATPS: Figure 3 shows the effects of ethanol concentration and K_2HPO_4 concentration on the *R* and *K* of polysaccharides. Partition behavior of polysaccharides in ATPS is important of the whole extraction process. When the K_2HPO_4 concentration was 25% and the ethanol concentration was less than 16%, no two-aqueous phase could be formed. However, when the K_2HPO_4 concentration was above 25%, it would precipitate in the system. With the ethanol concentration increasing from 17 to 29%, the *K* increased from 43.91 to 97.32. The reason was that the polysaccharides entered into the bottom phase and the increase of ethanol concentration resulted in the volume of the top phase increase. So, the *K* of polysaccharides increased. At the ethanol concentration of 27%, the highest *K* (97.32) and R (99.02%) were achieved, so, the ethanol concentration of 27% was chosen.

The experiment studied the influences of K_2HPO_4 on the *K* and *R* of polysaccharides in ATPS. Four of K_2HPO_4 (19, 21, 23 and 25%, respectively) concentration was used. When the concentration of K_2HPO_4 increased, the water would enter the bottom phase. Hence, the *K* (97.32) and *R* (99.02) of 21% were higher than those of 25% (70.93, 98.36). Thus, the concentration of K_2HPO_4 of 21% was used in further study.

Effect of additives on the partition coefficient and the recovery of polysaccharides in ATPS: The effect of NaCl on the partition coefficient and the recovery of polysaccharides were investigated (Fig. 4). The results show that the *R* and *K* increased when a small amount of NaCl was added in the aqueous two-phase system. With increased NaCl, the recovery and the partition coefficient of polysaccharides were decreased because of salting-out. If the content was more than 0.4 mol/L, the *R* and *K* decreased with the increase in NaCl. So, the concentration of NaCl of 0.4 mol/L was selected.

Effect of pH on the partition coefficient and the recovery of polysaccharides in ATPS: The effect of pH (6-11) on the *R* and *K* was measured and reported (Fig. 5). High *R* (>60) and *K* (>92%) were indicated at all tested pHs, but the *K* and *R* had a minor change with

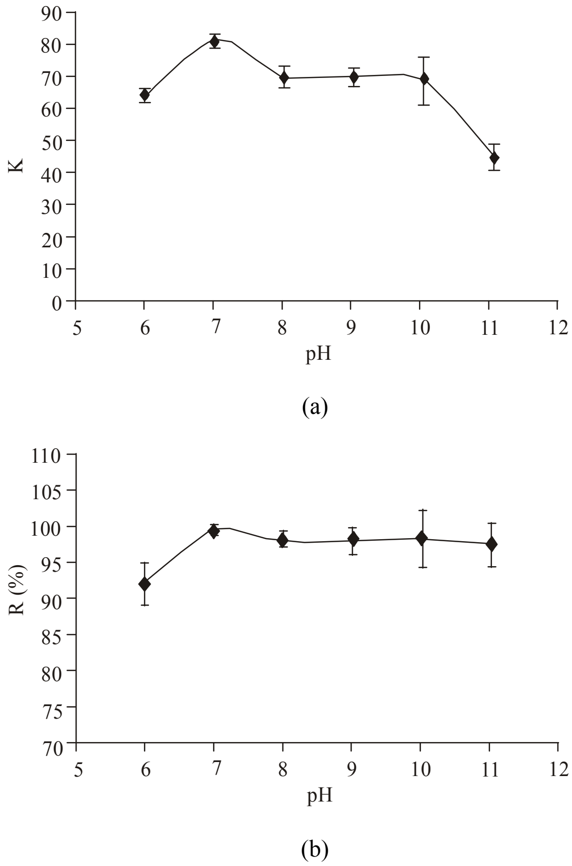

Fig. 5: Effect of pH on; (a): The partition coefficient; (b): The recovery of polysaccharides in ATPS

Table 1: Comparison of ATPS and conventional extraction method

	Polysaccharides	
Extraction technology	Yield (%)	Purity (%)
ATPS[a]	12.47	16.18
Conventional extraction[b]	10.30	6.14

[a]: ATPS, extracted with 27% (w/w) ethanol/21% (w/w) K_2HPO_4; [b]: Conventional extraction, under condition: solid-liquid radio of 1/20, 100°C and 60 min

an increase in pH from 7 to 10. This means that the change in the surface charge of polysaccharides was not enough to alter their hydrophilicity. The maximum activity was found at pH 7.0. For the present result, it should be noticed that pH 7.0 should be selected (Pakhale *et al*., 2013).

Comparison of ATPS and conventional extraction: The aim of the study was to develop a method for the high efficiency extraction of polysaccharides from *P. anserina*. A comparison between conventional extraction with ATPS was conducted. The different extraction methods were used and the results are shown in Table 1. For ATPS, the extraction yield and purity were 12.47 and 16.18%, for conventional extraction, the extraction yield and purity were 10.3 and 6.14%, respectively. Therefore, as we can see, the extraction yield and purity with ATPS was higher than conventional extraction. In general, ATPS is a promising process as an extraction technology.

CONCLUSION

The study has demonstrated the feasibility of using an ATPS for the extraction of polysaccharides from *Potentilla anserine* L. On the different types of ATPS, K_2HPO_4 offers the advantage such as no harmful, the K_2HPO_4/ethanol were selected as the separation system. 27% ethanol, 21% K_2HPO_4, 0.4 mol/L NaCl and pH 7 indicated the greatest potential to be used for extraction. The concentrations of salt and ethanol produced a significant effect on the partition coefficient and the recovery. However, the effect of NaCl and pH was moderate. A comparison between ATPS with conventional extraction was conducted, ATPS showed a higher extraction purity of polysaccharides. The findings reported ATPS was an efficient and prospective process for the extraction of polysaccharides from *Potentilla anserine* L.

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