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 K2HPO4/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% K2HPO4, 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 (NH4)2 SO4 in the ATPS because of it is harmful to human health and found K2HPO4 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 anserina 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.
Aqueous two-phase extraction: The phase diagram of ethanol and K₂HPO₄ were designed on the basis of a turbidity titration method described by Jiang et al. (2009). Different amounts of K₂HPO₄ 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 K₂HPO₄ were calculated. At different turbid points, the concentrations of K₂HPO₄ 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 K₂HPO₄, respectively. \(m_1\), \(m_2\) and \(m_3\) were the amount of ethanol, K₂HPO₄ and water, respectively.

Partition behaviors of polysaccharides in ATPS: To investigate the effects of ethanol and K₂HPO₄ on partition behaviors of polysaccharides, the extracts of Potentilla anserine L. roots were mixed with ATPS. A certain weight of K₂HPO₄ was mixed in 15 mL graduated tubes with ethanol and 0.1 g of the powders 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):

\[
K = \frac{C_k}{C_l}
\]

\[
R = \frac{C_l V_k}{C_l V_k + C_l V_l} \times 100\%
\]

where,

\(C_l\) and \(C_k\): The concentrations of the upper and lower phases

\(V_l\) and \(V_k\): The volumes of the upper and lower phases

RESULTS AND DISCUSSION

Phase diagram of ethanol/K₂HPO₄: In order to select the appropriate ratio of phase composition, the phase diagram of the ethanol/K₂HPO₄ 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 K₂HPO₄ 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 K₂HPO₄. Because excessive K₂HPO₄ 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
Partition behavior of polysaccharides in ATPS: Figure 3 shows the effects of ethanol concentration and K₂HPO₄ concentration on the $R$ and $K$ of polysaccharides. Partition behavior of polysaccharides in ATPS is important in the whole extraction process. When the K₂HPO₄ concentration was 25% and the ethanol concentration was less than 16%, no two-aqueous phase could be formed. However, when the K₂HPO₄ 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 K₂HPO₄ on the $K$ and $R$ of polysaccharides in ATPS. Four of K₂HPO₄ (19, 21, 23 and 25%, respectively) concentration was used. When the concentration of K₂HPO₄ 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 K₂HPO₄ 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

<table>
<thead>
<tr>
<th>Extraction technology</th>
<th>Polysaccharides</th>
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<tr>
<td></td>
<td>Yield (%)</td>
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<tr>
<td>ATPS$^a$</td>
<td>12.47</td>
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<tr>
<td>Conventional extraction$^b$</td>
<td>10.30</td>
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</tbody>
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$^a$: ATPS, extracted with 27% (w/w) ethanol/21% (w/w) K$_2$HPO$_4$; $^b$: Conventional extraction, under condition: solid-liquid ratio 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 *Potentilla 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 anserina*. On the different types of ATPS, K$_2$HPO$_4$ offers the advantage such as no harmful, the K$_2$HPO$_4$/ethanol were selected as the separation system. 27% ethanol, 21% K$_2$HPO$_4$, 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 anserina*.

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REFERENCES


