Extraction Salidroside with Aqueous Two-phase System

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Abstract. Extraction salidroside with the aqueous two-phase system. Through several single-factor tests: Polyethylene glycol (PEG) molecular weight, the mass fraction of PEG, the kinds of salt, the salt mass fraction and ionic strength. Get changes Salidroside extraction rate. And, obtain the optimum conditions by orthogonal experimental design. When the aqueous two-phase system is 16%PEG600, 20% (NH4)2SO4, 1% KCl and the salidroside initial concentration is 0.02g/l, the extracting effect is best, the extracting rate is 95.32%. This method was more is environmentally and more cost effective.

Introduction

Rhodiola rosea, pharmacological studies have shown that, containing several active ingredients such as salidroside, tryosol and rhodionin, whole plant can medicine [1], the salidroside ($C_{14}H_{20}O_7$,) is the main medicinal active ingredients. In recent years, Salidroside get the wide attention of people, because it can play the role of adaptogen, can help body restore homeostasis, and has high medicinal value. Have effects such as anti-tumor, anti-fatigue, anti-anoxia [2-7]. So, in the field of military medicine, aviation medicine, sports health care medicine and others has important application value. However, rhodiola rosea grow at the altitude of 1800-2700 m in the zone in harsh environments and growing conditions [8]. Therefore, it is very important to determine salidroside accurately in rhodiola rosea for its quality control.

Aqueous two-phase system (ATPS) might be an alternative for extraction salidroside of from rhodiola rosea. Recently, ATPS composed of two incompatible water-soluble polymers, or a kind of polymer and inorganic salt or organic salt such as sodium citrate [9], potassium sodium tartrate[10]and ionic liquid[11,12]; and also use cationic and anionic surfactants[13,14]. And the ATP technique is widely used in biological separation process because of more environmentally benign without using harmful volatile organic solvents in the whole process. In the aqueous two-phase system is 70-90% water, has a mild environment, can keep struggling to physical activity, commonly used in separation of biomolecules (protein, antibody, enzyme, RNA and DNA), can also be isolated viruses and cells [15-18]. ATPS technique is characteristics of easy separation and purification, mild conditions, easy preparation and recycling are widely used. The purpose of this study was to develop a simple and environmentally benign extraction method based on PEG–salt ATPS, as a new pretreatment strategy for the analysis of salidroside in rhodiola rosea.

Experimental Section

Reagents: The entire Poly (ethylene glycols) (PEG600, PEG1000, PEG2000 and PEG6000), and all salts were used were in analytical reagent grade, and were purchased from Tianjin Guangfu Fine Chemical Research Institute (China), were used without further purification. The reagents Standard of salidroside was obtained from Aladdin Chemistry Co Ltd.

Analysis: The concentration of salidroside in the bottom phase was determined by UV (UV1901) at 275 nm. Draw the curve graph of standard solution of salidroside, concentration (C) and

absorbance (A) have a good linear relationship, the equation of linear regression: A= 4.459586C +0.004151. Linear correlation R^2=0.999313. The extraction rate (Y) was determined by calculating the ratio of the mass of bottom phase (M) to the total mass of phase (M_t).

Experimental Procedure

Preparation of phase diagrams: The determination of the bimodal curve of phase diagrams was carried out by a turbidimetric titration method [14]. Stocked 40 % PEG of different molecular weight and 30 % $(NH_4)_2SO_4$. 40% PEG solution was placed into a 20 mL test tube. Then 30% $(NH_4)_2SO_4$ solution was added dropwise to the tube, until turbidity and a two-phase system was formed. The composition of this mixture was noted, and this mixture was weighed. Then, water was added dropwise to the tube to get a clear one-phase system and then the mixture was weighed. And more salt solution was added again to afford a two-phase system. The cycles for formation of two-phase system were repeated to obtain the final phase diagram.

Extract the crude extract of salidroside: Water bath circulation reflux with 80% ethanol in 80℃, and 80 min as the extracted time, three times. And further purification use Petroleum ether, chloroform, ethyl acetate and n-butanol, respectively. Obtain relatively pure salidroside solution.

Effect of the molecular weight and mass fraction of PEG on extraction efficiency of salidroside: Use different PEG with salt ($(NH_4)_2SO_4$) and a known volume standards salidroside solution of known concentration into a 10 ml centrifuge tube. ATPS formed with mass fraction of 12%, 16%, 20%, 24%, 28% PEG and the mass fraction of 16% $(NH_4)_2SO_4$. Centrifuge 10 min, top to bottom phase volume is read, and the phase absorbance value was measured.

Effect of different salts on extraction efficiency of salidroside: $(NH_4)_2SO_4$, K_2HPO_4, sodium citrate and sodium tartrate was used. 16% different salts with different mass fraction (12%, 16%, 20%, 24%, 28%) of PEG1000 and a known volume standards salidroside solution of known formed the ATPS.

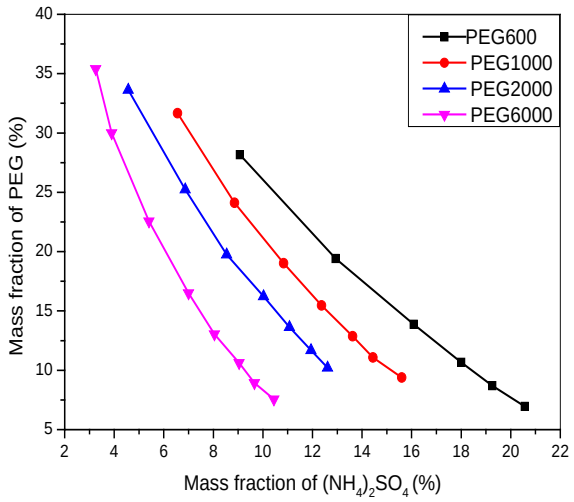
Effect of different mass fraction of salt on extraction efficiency of salidroside: Stock 12%, 16%, 20%, 24%, 28% $(NH_4)_2SO_4$ and 20% PEG1000 ATPS.

Effect of Ionic strength on extraction efficiency of salidroside: 12% PEG1000 and 16% $(NH_4)_2SO_4$ ATPS, the different mass fraction (0, 1%, 2%, 3%, 4%, 5%) of NaCl and KCl was added into the ATPS, respectively.

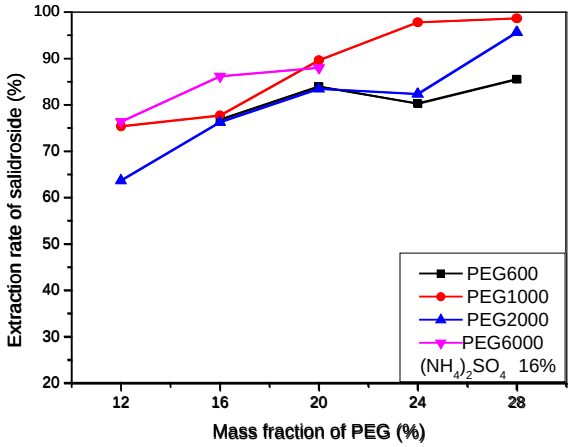
Results and Discussion

PEG/ $(NH_4)_2SO_4$ phase diagrams: As shown in Fig.1 (a), above the curve is the two-phase area; while below the curve is the homogeneous phase area. Results showed, at the same concentration of PEG, that the higher the PEG molecular weight was, the lower the salt concentration was required for phase separation; And at the same mass fraction of PEG and salt, the higher molecular weight of PEG, the easier the two-phase was formed. When the molecular weight of PEG increases, the more hydrophobic groups containing, the incompatibility between the water structures in the hydration shells of ions and PEG macromolecules will increase, the larger of hydration membrane area, between PEG membrane hydration and salt ions are incompatible, the easier formation of biphasic.

Effect of the molecular weight and mass fraction of PEG on extraction efficiency of salidroside: As shown in Fig.1 (b), with the increase of the mass fraction of the polymer, the extraction rate will increase. When PEG1000 is 24%, the extraction capability is best. Hydrophobic groups and hydrogen are two important factors that affect the extraction rate of salidroside. With the increase of mass fraction of PEG, the hydrogen will increase in system, salidroside containing hydrogen bonds, similar compatible principle, it will increase the extraction rate of salidroside. With the molecular weight of increase, the concentration of hydrophobic chains in system became higher, salidroside dissolve easily in water, so the extraction rate decreased.



(a) PEG-(NH4)2SO4 phase diagrams



(b) Effect of the . molecular weight and mass fraction of PEG on extraction efficiency of salidroside

Fig.1

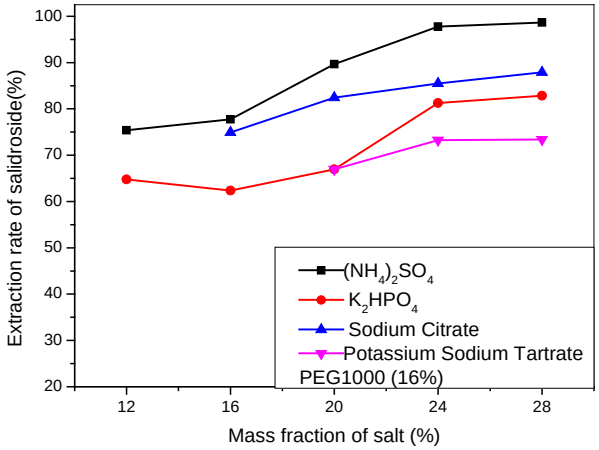
Effect of different salts and salt mass fraction on extraction efficiency of salidroside: Seen from the Fig.2 (a), the extraction efficiency of $(NH_4)_2SO_4$ and PEG1000 ATPS is best. The hydration of Gibbs free energy (ΔG) of ions, the effect of NH_4^+ are better than K^+, the hydration of ion is greater, and its ability to compete for water molecules is stronger in the solution. According to the incompatibility between the hydration shells of ions and PEG macromolecules, hydration membrane of the polymer becomes large, hydrogen is increased, salidroside easily soluble in water and increasing the extraction rate. With the changes of the salt mass fraction, the extraction rate of salidroside first increased and then decreased. When 28% $(NH_4)_2SO_4$and 16% PEG1000 the extraction rate of salidroside is highest, 98.67%.

Effect of Ionic strength on extraction efficiency of salidroside: The results are shown in Fig.2 (b).The extraction rate of Salidroside increase with NaCl added. When add KCl, its first increase and then decrease and then tends to a constant. This is because of the salting-in effect of KCl and the salting-out effect of NaCl. The difference in the distribution coefficient of positive and negative ions between top and bottom phases can affect the interphase potential difference and the interfacial tension, which change the mass transfer, and affect the extraction rate of Salidroside.

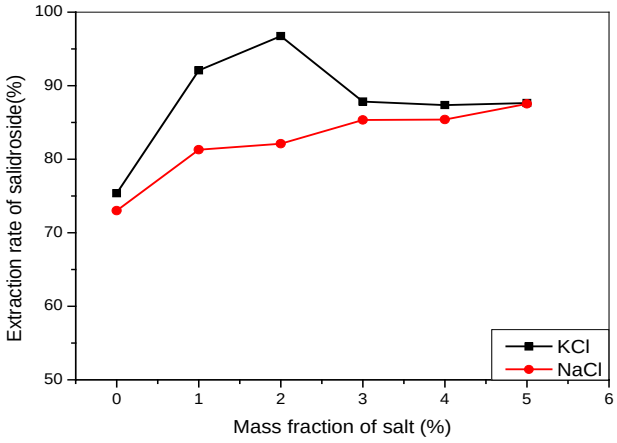
Optimal extraction conditions

According to the analysis of above various factors, design the orthogonal test. The influence factors are PEG molecular weight(PEG600,PEG1000,PEG2000), PEG mass fraction(16%,20%,24%), $(NH_4)_2SO_4$ (16%,20%,24%), KCl(0,1%,2%). An orthogonal test table L9 (3-4) was used. The

extraction rate of salidroside Rhodiola followed by 91.81%, 95.32%, 98.27%, 91.57%, 77.32%, 91.50%, 76.19%, 85.42%, and 92.37%. When the mass fraction of PEG600 is 20% and the mass fraction of $(NH_4)_2SO_4$ is 20%, the KCl mass fraction is 1%, the extraction rate of salidroside Rhodiola is 95.32%; 24% PEG600, 24% $(NH_4)_2SO_4$ and 2% KCl, the extraction rate of salidroside Rhodiola is 98.27%, but this group occur crystallization and cannot be used. So 95.32% is the best extraction rate.



(a) Effect of different salts on extraction efficiency of salidroside



(b) Effect of ionic strength on extraction efficiency of salidroside

Fig .2

Conclusions

A novel ATP extraction technique was applied to separation and purification salidroside from Rhodiola. In this study, we studied the PEG molecular weight and the mass fraction, the kinds of salt, the ionic strength and the salt mass fraction, which influenced the salidroside extraction rate. The results show, when the extracting rate is 98.27%, appeared salt-out. So when the aqueous two-phase system is 24% PEG1000, 16% $(NH_4)_2SO_4$ and the salidroside initial concentration is 0.02g/l, the extracting effect is best, the extracting rate is 96.08%. Therefore, this technology has good prospects for industrial development.

Acknowledgements

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