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ORIGINAL PAPER

Extraction and purification of anthraquinones derivatives from *Aloe vera* L. using alcohol/salt aqueous two-phase system

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Abstract An alcohol/salt aqueous two-phase system (ATPS) composed of 1-propanol and $(NH_4)_2SO_4$ was employed to purify anthraquinones (AQs) extracted from *Aloe vera* L. The main influencing system parameters such as type of alcohol, type and concentration of salt, temperature and pH were investigated in detail. Under the optimal extraction conditions, AQs can be extracted into alcohol-rich phase with high extraction efficiency, meanwhile majority polysaccharides, proteins, mineral substances and other impurities were extracted into salt-rich phase. Partitioning of AQs is dependent on hydrophobic interaction, hydrogen bond interaction, and salting-out effect in ATPS. Temperature also played a great role in the partitioning. After ATPS extraction, alcohol can be recycled by evaporation; moreover, salt can be recycled by dilution crystallization method. Compared with other liquid–liquid extractions, this alcohol/salt system is much simpler, lower in cost with easier recovery of phase-forming components, which has the potential scale-up in down-processing of active ingredients in plant.

Keywords Alcohol/salt system · Aqueous two-phase system · Anthraquinones derivatives · Purification

Abbreviations

ATPS	Aqueous two-phase system
Anthraquinones	AQs
Triton X-114	Octylphenoxypolyethoxyethanol
PEG	Polyethylene glycol
MW	Molecular weight
rpm	Revolutions per minute
UPLC	Ultra performance liquid chromatography
B-R buffer	Britton-Robinson buffer
R	Phase ratio
K_d	Partition coefficient
E	Extraction efficiency
ΔG	Gibbs energy change
ΔH	Enthalpy change
ΔS	Entropy change

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Introduction

Aqueous two-phase system was first introduced by Albertsson [1]. It has gained increased attention for purification and separation purposes particularly in the biotechnology field because of its biocompatibility and high capacity [2]. Aqueous two-phase systems (ATPSs) are usually composed of two or more polymers (e.g. PEG and dextran [3]), a polymer and a salt (e.g. PEG and Na_2SO_4 [4]), and a non-ionic surfactant micellar system (e.g. Triton X-114 [5]). There are some disadvantages using ATPS polymer/polymer, polymer/salt and surfactant, such as the high cost, high viscosity, slow segregation, and complication in isolating the extracted molecules from the polymer phase or micellar phase by re-extraction [6, 7].

In recent years, as a novel liquid–liquid extraction technique, alcohol/salt ATPS has many advantages such as low cost, low viscosity, easy recovery of alcohol by evaporation and easy to scale-up in industrial production [8, 9]. Short-chain alcohols (methanol, ethanol, 1-propanol, etc.)

can form ATPS with inorganic salts, which might be due to salting-out effect and the low solubility of inorganic salts in alcohols [10]. Alcohol/salt ATPS was applied to extract and purify lots of compounds in a single-step procedure, such as enzyme of serine protease [9], antibiotics of tetracycline hydrochloride [11], active ingredient from natural plant of lithospermic acid B [12] and natural pigment of crocins [13].

In this paper, we developed a simple, efficient and cheap technique for the purification of AQs from *Aloe vera* L. The AQ derivatives (whose structures were shown in Fig. 1) are one kind of major active ingredients in aloe leaves, which have multiple pharmacological activities including laxative, anti-bacterial, anti-oxidant, hemostatic and anti-cancer [14–16]. When the alcohol/salt ATPSs reached phase equilibrium, the influencing parameters such as type of alcohol, type and concentration of salt, extraction temperature and pH were investigated in detail. Under the optimal conditions, majority aloe AQs had been extracted into the alcohol-rich phase, while the impurities of aloe polysaccharides, proteins and minerals had migrated into the salt-rich phase. After aloe AQs being extracted into alcohol-rich phase, the alcohol can be recycled by evaporation. Furthermore, the salt in salt-rich phase can be crystallized through dilution crystallization by methanol. The whole flow chart of extraction, separation, purification of aloe AQs and recovery of alcohol and salt is shown in Fig. 2.

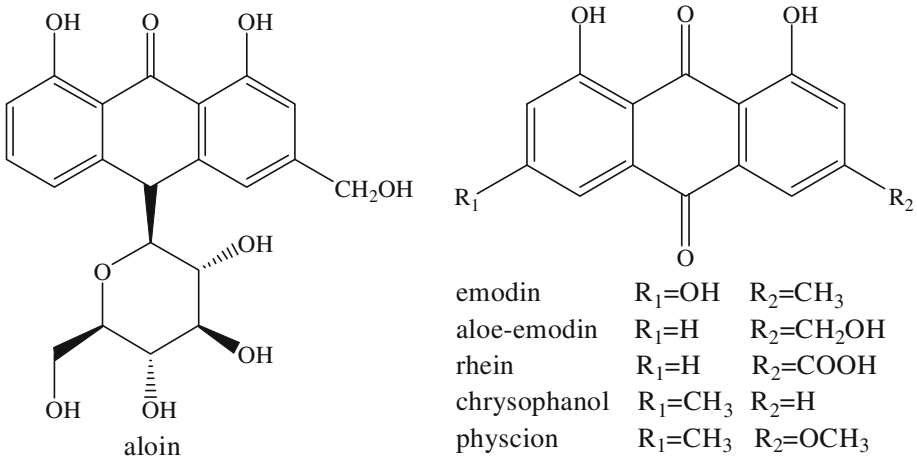

Fig. 1 Chemical structures for major AQs derivatives extracted from *Aloe vera* L.

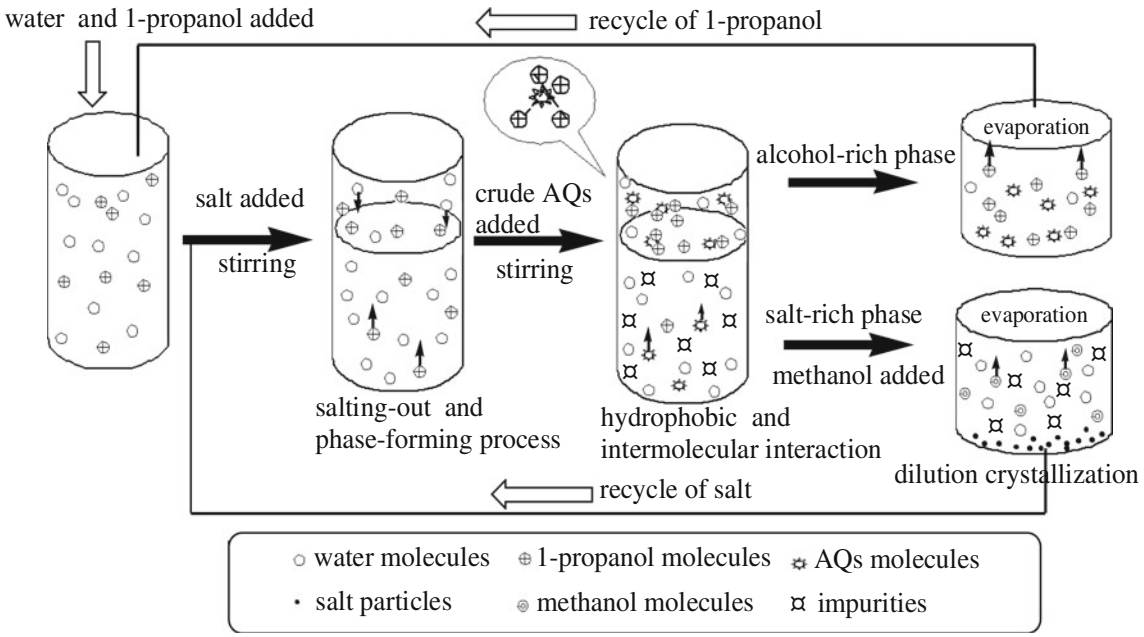

Fig. 2 Flow chart for purification of aloe AQs and recycle of phase-forming components

Materials and methods

Materials

Aloe peel powder was obtained from NanTong DeFu Aloe Products Co., Ltd. (Nantong, China). Ethanol, 1-propanol, 2-propanol, PEG (MW 4,000, 6,000 and 10,000), dextran (MW 40,000), NaH_2PO_4 and $(NH_4)_2SO_4$ were all purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Triton X-114 was purchased from Sunshine Biotechnology Co., Ltd. (Nanjing, China). The standards of aloe-emodin, chrysophanol, aloin, rheiin, emodin and physcion (purity >98 % by HPLC) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Other reagents were all analytical grade and used without further treatment. De-ionized water was used to prepare the sample solutions.

Preparation of crude AQ extract

This procedure was reported in our previous work [17]. Aloe peel powder was soaked in 60 % ethanol solution for 24 h. The solution and residue were isolated by a centrifuge (800B, Changsha, China) at a rolling speed of 4,000 rpm. After ethanol and water were removed, a colloidal aloe extract was obtained. Sulfuric acid solution and chloroform were added to the extract and then was refluxed. The chloroform extract was removed by a separating funnel; a yellowish-brown colloid was the crude extract obtained after evaporating the chloroform, and then dissolved in methanol for further studies.

Preparation of alcohol/salt ATPS

To a 15 mL centrifuge tube, 5.0 mL distilled water, 2.0 mL alcohol, a given amount of salt and 0.1 mL aloe AQs solution were added. Another tube with the same phase-forming components but without aloe AQs was prepared as a blank to avoid interference. The mixture was stirred well to make the salt dissolve completely. The phase separation can be achieved during a few seconds, two clear phases formed. The top phase was mainly composed of alcohol and AQs with a small volume, and the bottom phase was the salt-rich solution containing impurities with large volume. The volume of each phase was noted down. The alcohol-rich phase was withdrawn to another tube using a syringe, diluted using 0.5 % magnesium acetate–methanol solution, the AQ solution turns to red color under this weakly basic condition. The total AQ concentration was determined by colorimetric method using a UV–Vis spectrophotometer (Spectrumlab 755s, Lengguang, Shanghai). The AQs concentration in the salt-rich phase was determined by mass balance.

The phase ratio (R) is defined in Eq. (1):

$$R = \frac{V_t}{V_b} \tag{1}$$

where V_t and V_b are the volumes of alcohol-rich phase and salt-rich phase, respectively. The partition coefficient (K_d) is defined in Eq. (2):

$$K_d = \frac{C_t}{C_b} \tag{2}$$

where C_t and C_b are the AQs concentrations in the alcohol-rich phase and salt-rich phase, respectively. The extraction efficiency (E) of AQs in the alcohol-rich phase is determined from Eq. (3).

$$E = \frac{K_d}{\left(K_d + \frac{1}{R}\right)} \times 100\,\%. \tag{3}$$

Phase diagram

The phase diagram was prepared by a turbidimetric titration method [18]. The phase diagram could provide information about the concentration of phase-forming components required to form an ATPS. Firstly, alcohol of known mass was added into a 10 mL graduated cylinder. A solution of known mass fraction of a salt was added dropwise and then the mixture was well mixed. The solution was clear at first, but after a certain amount of the salt solution was added, one further drop made the mixture turbid, which then separated into two phases. The composition of each component was noted down. Then, a few drops of water was added to make the mixture clear again, and the above procedure was repeated to obtain sufficient data to construct the phase diagram.

Results and discussion

Selection of the optimal alcohol/salt ATPS

Three short-chain alcohols of ethanol, 1-propanol and 2-propanol were chosen as the phase-forming alcohols. Phosphate, sulfate, carbonate and citrate were considered as the phase-forming salt. The phase diagram of the alcohol/salt system is shown in Fig. 3. The abilities of the alcohol and salt studied for phase separation were in an order of 1-propanol > 2-propanol > ethanol and $(NH_4)_2SO_4$ > NaH_2PO_4, respectively.

There are three reasons for choosing $(NH_4)_2SO_4$ as the phase-forming salt for further studies: firstly, it has good solubility and stronger phase-forming ability, was widely used as phase-forming salt in different ATPSs; secondly, it can result in an appropriate pH range below 7.0, which is more suitable for the existence of acidic aloe AQs, while

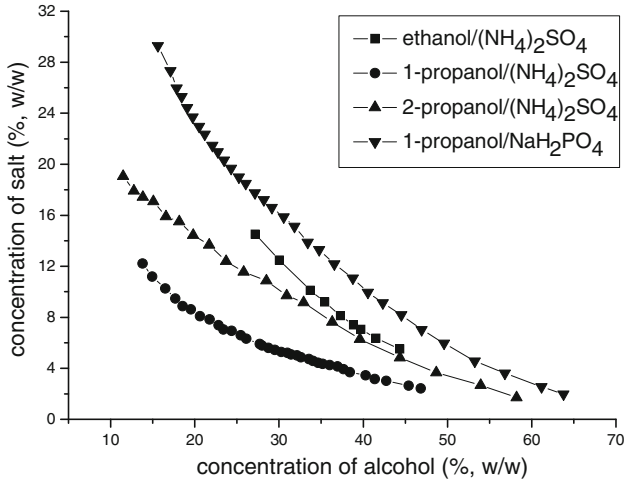

Fig. 3 Binodal *curves* for the alcohol/salt ATPSs at 298.15 K

the carbonate and citrate ATPSs are all basic; thirdly, the alcohol/$(NH_4)_2SO_4$ system has better extraction of AQs than alcohol/NaH_2PO_4 system in this study.

The extraction efficiencies of different alcohol/salt ATPSs are shown in Table 1, it can be seen that 1-propanol/$(NH_4)_2SO_4$ system has the highest extraction efficiency than other alcohol/salt systems. To optimize the $(NH_4)_2SO_4$ concentration, 1.5–3.0 g (18.50–31.23 %, w/w) $(NH_4)_2SO_4$ was added into the system. The alcohol/salt system with 17.84 % 1-propanol and 26.66 % $(NH_4)_2SO_4$ has the best extraction ability, so it was chosen for further studies. The extraction efficiency increased with $(NH_4)_2SO_4$ concentration, it can be explained that the increase of $(NH_4)_2SO_4$ will strengthen the salting-out effect and lead to increase of the 1-propanol concentration in the alcohol-rich phase. The phase separation of ATPS is widely regarded as the result of the salting-out effect of salts in the alcohol/salt system [11]. When the salt concentration reaches saturation, the continuous addition of salt will be precipitated out and the extraction efficiency will not be increased.

Moreover, due to the hydrophobicity of AQs, the hydrophobic interactions between AQs and alcohol-rich phase probably are the main driving forces for the extraction [19]. To summarize the results above, 1-propanol/$(NH_4)_2SO_4$ system was chosen in the following studies.

Effect of temperature

The partitioning of aloe AQs in 1-propanol/$(NH_4)_2SO_4$ system at the temperature range of 25–45 °C was investigated. As shown in Fig. 4, the partition coefficient and extraction efficiency decreased with increase of temperature. The alcohol will easily volatilize at higher temperature resulting in the decrease of alcohol concentration in alcohol-rich phase. In addition, the thermal-sensitive aloe AQs are more stable at lower temperature.

To investigate the thermodynamics, extraction of aloe AQs can be regarded as a transfer process of the AQs from the salt-rich phase to the alcohol-rich phase. The Gibbs

Table 1 Extraction of aloe AQs in different alcohol/salt ATPSs (the system contains 2.0 mL alcohol, 5.0 mL water, a given amount of salt and 0.1 mL crude AQs extract solution)

Alcohol/ salt system	Concentration of alcohol/salt (%, w/w)	Phase ratio (R)	Partition coefficient (K_d)	Extraction efficiency (E, %)
Ethanol/$(NH_4)_2SO_4$	18.84/21.48	0.448	1.68 ± 0.02	42.97 ± 0.47
	18.40/23.31	0.441	2.51 ± 0.13	52.95 ± 0.19
	17.98/25.06	0.433	3.77 ± 0.08	62.06 ± 0.12
	17.58/26.73	0.419	5.39 ± 0.16	69.34 ± 0.22
1-Propanol/$(NH_4)_2SO_4$	19.82/18.50	0.383	8.83 ± 0.24	77.19 ± 0.05
	19.12/21.41	0.393	9.25 ± 0.08	78.45 ± 0.25
	18.46/24.12	0.344	11.85 ± 0.07	80.29 ± 0.13
	17.84/26.66	0.338	64.35 ± 0.33	95.61 ± 0.07
	17.27/29.01	0.324	66.84 ± 0.15	95.58 ± 0.09
	16.73/31.23	0.300	65.25 ± 0.13	95.14 ± 0.15
2-Propanol/$(NH_4)_2SO_4$	19.45/18.59	0.389	8.77 ± 0.03	77.38 ± 0.14
	18.76/21.51	0.393	21.04 ± 0.08	89.22 ± 0.06
	18.11/24.22	0.344	18.05 ± 0.18	86.12 ± 0.24
	17.50/26.76	0.323	22.57 ± 0.17	87.94 ± 0.33
	16.94/29.13	0.322	14.30 ± 0.12	82.23 ± 0.35
1-Propanol/NaH_2PO_4	18.04/25.82	0.328	3.25 ± 0.09	51.59 ± 0.21
	17.27/27.93	0.354	4.28 ± 0.24	60.22 ± 0.07
	16.73/30.18	0.328	5.02 ± 0.07	62.25 ± 0.15
	16.22/32.30	0.338	8.99 ± 0.24	75.25 ± 0.14
	15.75/34.29	0.304	20.87 ± 0.08	86.40 ± 0.09

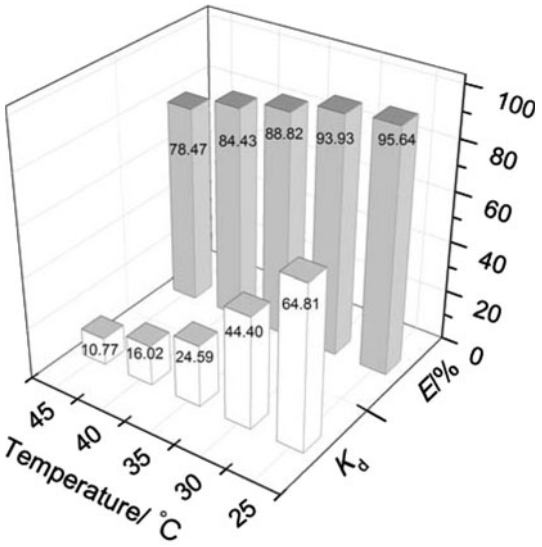

Fig. 4 Effect of temperature on partition coefficient (K_d) and extraction efficiency (E) for AQs [the system contains 2.0 mL 1-propanol, 5.0 mL water, 2.4 g $(NH_4)_2SO_4$ and 0.1 mL crude AQs extract solution, pH = 4.75]

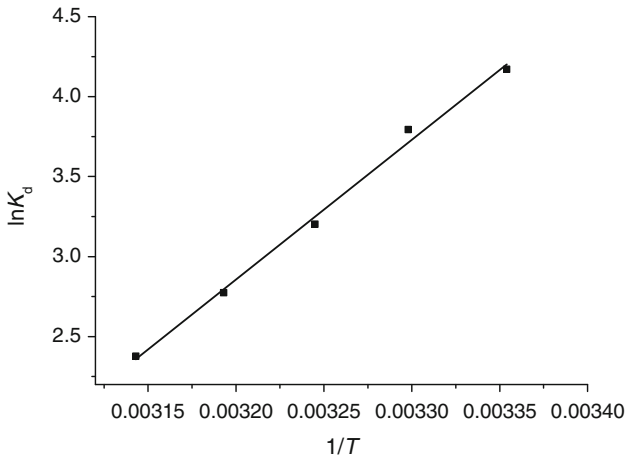

Fig. 5 Van't Hoff plot of ln K_d versus 1/T produces a *straight line* [the system contains 5.0 mL water, 2.0 mL 1-propanol, 2.4 g $(NH_4)_2SO_4$ and 0.1 mL crude AQs extract solution, pH = 4.75]

energy change (ΔG) is related to the partition coefficient (K_d) and can be calculated by Eq. (4). The enthalpy change (ΔH) and entropy change (ΔS) were obtained from the slope and intercept of the linear Eq. (5) which plots ln K_d versus 1/T. The effect of temperature on the partition coefficient expressed through Van't Hoff approach is shown in Fig. 5.

$$\Delta G = -\mathrm{RT}\ln K_d \tag{4}$$

$$\ln K_d = \frac{-\Delta H}{\mathrm{RT}} + \frac{\Delta S}{R} \tag{5}$$

The calculated ΔG, ΔH and ΔS values obtained by linear least-square analysis are shown in Table 2. The ΔG values

Table 2 The transfer thermodynamic properties for aloe AQs from the salt-rich phase to the alcohol-rich phase in ATPS

T (K)	K_d	ΔG (KJ mol^{-1})	$T\Delta S$ (KJ mol^{-1})	ΔH (KJ mol^{-1})
298.15	64.81	−10.34	−62.33	−72.67
303.15	44.40	−9.56	−63.21	
308.15	24.59	−8.20	−64.47	
313.15	16.02	−7.22	−65.55	
318.15	10.77	−6.29	−66.38	

are all negative, reflecting that the extraction of AQs is spontaneous and preferential partitioning in the alcohol-rich phase as indicated by the $K_d > 1$. Partitioning of AQs in ATPS is marked by negative values for ΔH and $T\Delta S$ with the enthalpy change being greater in value than the entropy change. Thus it can be concluded that extraction of AQs form salt-rich phase to alcohol-rich phase is an exothermic, spontaneous process [20], the extraction of AQs using alcohol-based ATPSs is highly dependent on temperature. Some other reports also proclaimed that temperature can greatly influence the partition of target materials in ATPS [21, 22]. The following partitioning experiments were done at room temperature in this study.

Effect of pH

B-R (Britton-Robinson) buffer comprising 0.04 M phosphoric acid, 0.04 M acetic acid, 0.04 M boric acid, and 0.20 M sodium hydroxide was used to adjust the pH of the ATPSs. The effect of pH on extraction efficiency in the range of 1.98–6.80 was investigated. As shown in Fig. 6, the pH had little influence on extraction efficiency, but

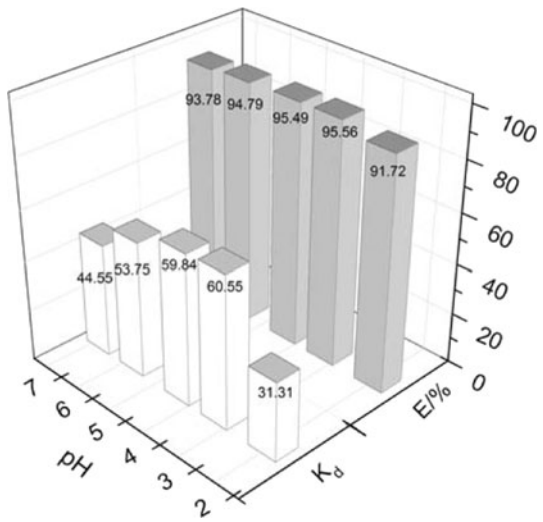

Fig. 6 Effect of pH on partition coefficient (K_d) and extraction efficiency (E) for AQs [the system contains 2.0 mL 1-propanol, 5.0 mL water, 2.4 g $(NH_4)_2SO_4$ and 0.1 mL crude AQs extract solution at room temperature]

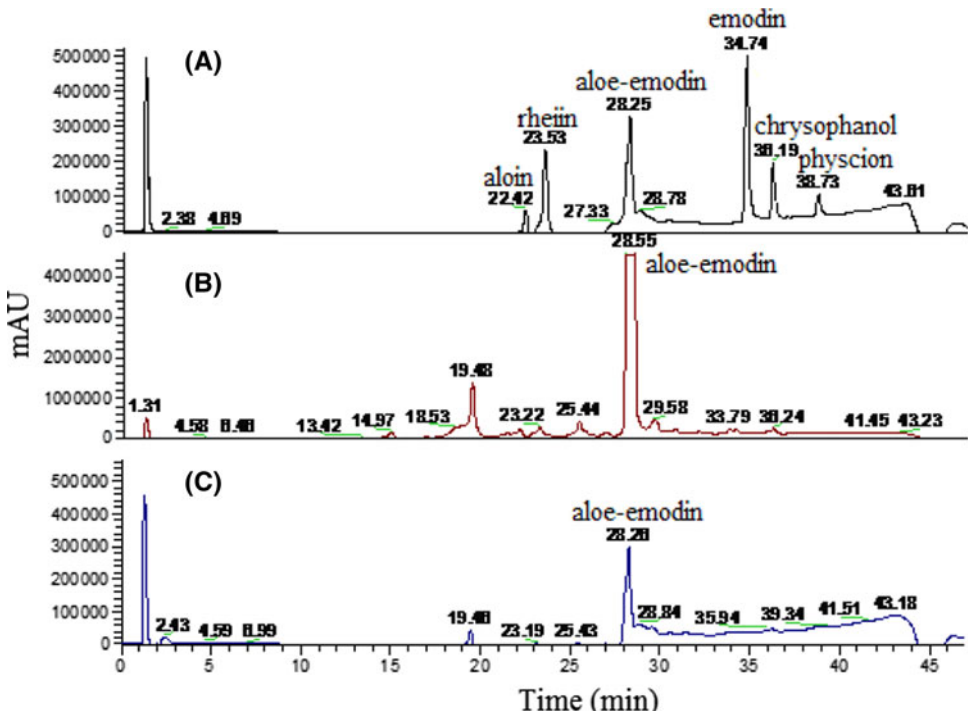

Fig. 7 LC chromatograms for **a** six standard AQs derivatives; **b** crude AQs extract; **c** AQs sample withdraw from alcohol-rich phase after 1-propanol/$(NH_4)_2SO_4$ ATPS extraction

acidic condition was more suitable for the stability of acidic AQ derivatives. The 1-propanol/$(NH_4)_2SO_4$ system (salt concentration was 26.66 %) has a proper pH value of 4.75, so the pH of the system was not adjusted in the following experiments.

Analysis of the aloe AQ constitutes

A Waters AcQuity™ UPLC system (Waters, USA) and a Waters XTerra MS C_{18} (2.1 mm × 150 mm, i.d. 5 μm) column were used in analysis. Mobile phase consisted of (A) methanol and 10 mM (B) ammonium formate solution. The gradient elution was as follows: 0–2 min (20 % A, 80 % B); 2–29 min (20–80 % A, 80–20 % B); 29–38 min (80–95 % A, 20–5 % B); 38–40 min (95–20 % A, 5–80 % B); 40–50 min (20 % A, 80 % B). The flow-rate was 0.3 mL min^{-1} and the UV detection wavelength was 254 nm. The column temperature was maintained at room temperature. The injection volume was 10 μL. The LC chromatograms for six standard AQ derivatives and the samples before and after ATPS extraction were shown in Fig. 7. The results demonstrated that the major constitutes of aloe AQs are aloe-emodin and few other kinds of AQ derivatives.

Partitioning mechanism of AQs in ATPS

The major constitutes of aloe AQs are aloe-emodin, aloin, emodin, physcion, chrysophanol, etc. All of them have two phenolic hydroxyl groups, so we consider the aloe AQs as a weak dibasic acid (H_2A) to neglect the minor constitutes. This extraction model is similar to the extraction of metal ions using alcohol/salt ATPS reported by Gao [23, 24]. The ionization equilibrium balance occurred in Eqs. (6) and (7), the mass balance is shown in Eq. (8):

$$H_2A + H_2O \overset{K_{a1}}{\leftrightarrow} HA^- + H_3O^+ \tag{6}$$

$$HA^- + H_2O \overset{K_{a2}}{\leftrightarrow} A^{2-} + H_3O^+ \tag{7}$$

$$C_0 = [H_2A] + [HA^-] + [A^{2-}] \tag{8}$$

where C_0 is the total AQs concentration, $[H_2A]$, $[HA^-]$ and $[A^{2-}]$ are AQs equilibrium concentrations in molecular and ionic states. The 1-propanol/$(NH_4)_2SO_4$ system contains one alcohol-rich top phase with higher polarity and the other salt-rich bottom phase with lower polarity, the hydrophobic AQs preferentially migrate into alcohol-rich phase. Because the concentrations of HA^- and A^{2-} are very low, their extraction can be neglected. The extraction equilibrium is shown in Eqs. (9) and (10):

$$H_2A_w + PrOH \overset{K}{\leftrightarrow} H_2A \cdot PrOH \tag{9}$$

$$K = \frac{[H_2A \cdot PrOH]_o}{[H_2A]_w [PrOH]_o} \tag{10}$$

where K is the equilibrium constant, PrOH and H_2A·PrOH stand for 1-propanol and complex formation of H_2A and PrOH, respectively. $[H_2A]_w$ is the concentration of H_2A in salt-rich phase, $[PrOH]_o$ and $[H_2A \cdot PrOH]_o$ are the concentrations of 1-propanol and H_2A·PrOH, respectively, in

Table 3 The extraction efficiencies of AQs and viscosity values in different ATPSs

Component 1	Component 2	Extraction efficiency (%)	Viscosity in top phase (mpa s)	Viscosity in bottom phase (mpa s)
PEG4000 (8 %, w/w)	$(NH_4)_2SO_4$ (12 %, w/w)	84.8 ± 0.3 (PEG-rich phase)	13.5 ± 0.3 (PEG-rich phase)	2.4 ± 0.2 (salt-rich phase)
PEG6000 (8 %, w/w)	$(NH_4)_2SO_4$ (12 %, w/w)	86.3 ± 0.1 (PEG-rich phase)	19.5 ± 0.4 (PEG-rich phase)	2.6 ± 0.1 (salt-rich phase)
PEG10000 (8 %, w/w)	$(NH_4)_2SO_4$ (12 %, w/w)	82.5 ± 0.2 (PEG-rich phase)	47.0 ± 0.2 (PEG-rich phase)	2.8 ± 0.2 (salt-rich phase)
PEG6000 (13.16 %, w/w)	Dextran 40000 (7.89 %, w/w)	80.7 ± 0.2 (PEG-rich phase)	13.4 ± 0.2 (PEG-rich phase)	130.6 ± 0.6 (salt-rich phase)
Triton X-114 (4.76 %, w/w)	Nil	94.8 ± 0.4 (surfactant-rich phase)	2.1 ± 0.3 (aqueous phase)	40.2 ± 0.4 (surfactant-rich phase)
1-Propanol (7.69 %, w/w)	$(NH_4)_2SO_4$ (15.38 %, w/w)	92.5 ± 0.5 (alcohol-rich phase)	2.4 ± 0.2 (alcohol-rich phase)	1.2 ± 0.1 (salt-rich phase)

alcohol-rich phase. The concentration of H_2A in alcohol-rich phase is $C_0' = [H_2A \cdot PrOH]_o$.

The concentration of H_2A was expressed by the distribution factor δ_{H2A} in Eqs. (11)–(14):

$$[H_2A] = C_0\delta_{H_2A} = \frac{C_0[H^+]^2}{[H^+]^2 + [H^+]K_{a1} + K_{a1}K_{a2}} \quad (11)$$

$$K = \frac{C_0'}{C_0\delta_{H_2A}[PrOH]_o} = \frac{D}{\delta_{H_2A}[PrOH]_o} \quad (12)$$

$$D = K\delta_{H_2A}[PrOH]_o \quad (13)$$

$$\lg D = \lg K + \lg \delta_{H_2A} + \lg[PrOH]_o \quad (14)$$

where D is the distribution ratio, which is dependent on the $[PrOH]_o$ when the pH is constant. $[PrOH]_o$ is influenced by the concentration of $(NH_4)_2SO_4$ (W_{salt}) and the initial 1-propanol volume (V_i). The values of $[PrOH]_o$ and D will increase with increase in W_{salt} and V_i. As to the Eq. (9), K is influenced by the interaction such as electrostatic interaction, molecular steric exclusion interaction, hydrophobic interaction, intermolecular interaction, etc. between AQs molecules and the ATPS components. The electrostatic interaction and molecular steric exclusion interaction can be neglected in this ATPS. The hydrophobic interaction plays great role in the extraction of AQs. The intermolecular interactions such as the hydrogen bond between the hydroxyl groups in 1-propanol and phenolic hydroxyl groups in AQs can also make AQs easily enter into alcohol-rich phase. So the equilibrium constant of AQs in ATPS can be expressed in Eq. (15):

$$\ln K = \ln K_h + \ln K_b + \ln K_s \quad (15)$$

where K_h is hydrophobic interaction, K_b is hydrogen bond interaction, K_s is the salting-out effect and other factors. It is well known that equilibrium constant K is also influenced by temperature, which is consistent with the conclusion that temperature can greatly influence the partition of AQs as mentioned in "Effect of temperature".

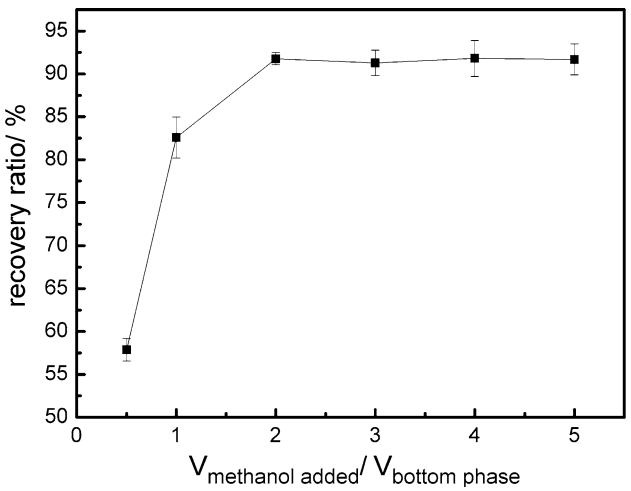

Fig. 8 Effect of volume ratio (methanol added/bottom phase) on the recovery ratio of $(NH_4)_2SO_4$

Comparison of different ATPSs extraction of AQs and recycle of phase-forming components

The extraction efficiencies of the PEG-based, surfactant-based and alcohol-based ATPSs were studied. The viscosity of each ATPS was determined by a rotary viscosimeter (NDJ-5S, Hengping, Shanghai). As shown in Table 3, the alcohol/salt and surfactant systems have relatively higher extraction efficiencies. Alcohol/salt system has smaller viscosity, shorter phase-separation time and lower cost than other ATPSs. So, it is more suitable for scale-up in industrial production.

After ATPS extraction, 1-propanol in alcohol-rich phase can be recycled by evaporation. Furthermore, $(NH_4)_2SO_4$ in salt-rich phase can be crystallized through dilution crystallization by addition of methanol [25]. As shown in Fig. 8,

when the volume ratio of methanol to bottom phase is 2.0, the recovery of salt almost reached maximum; further addition of methanol will not result in the increase of salt amount. Methanol has low boiling point and was easily recovered than other alcohols, it cannot form azeotrope with water and lower the energy cost [25].

Conclusion

Aloe AQs derivatives from *Aloe vera* L. was successfully purified using 1-propanol/$(NH_4)_2SO_4$ ATPS. Under the optimal conditions, 95.56 % AQs can be extracted into alcohol-rich phase with the system comprising 17.84 % (w/w) 1-propanol and 26.66 % (w/w) $(NH_4)_2SO_4$ at 25 °C and with pH not being adjusted. The LC analysis results demonstrated that the major constitutes of aloe AQs are aloe-emodin and a few other derivatives. By investigating the probable partitioning mechanism of AQs in ATPS, it demonstrates that the partitioning was influenced by many factors. The hydrophobicity, salting-out effect and hydrogen bond interaction are the probable driving forces in influencing the distribution behavior of AQs, temperature also plays a great role in 1-propanol/$(NH_4)_2SO_4$ system by studying the thermodynamics. After the ATPS extraction, 1-propanol can be recycled by evaporation, the highest recovery of $(NH_4)_2SO_4$ in salt-rich phase can be achieved by addition of 2.0 times volume of methanol to make $(NH_4)_2SO_4$ precipitate out. The alcohol/salt system has low cost and viscosity, efficient extraction ability, and it can be used in purification of other active compounds in natural plant or biomolecules.

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