Purification of procyanidins from *Kunlun Chrysanthemum* by macroporous resins combined with silica gel and evaluation of antioxidant activities *in vitro*

Siqun Jing[1,2], Xiaoming Zhang[1]* and Li Yue[2]

[1]State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Lihu Road, Wuxi, Jiangsu, China

[2]College of Life Sciences and Technology, Xinjiang University, Shengli Road 14, Urumqi, Xinjiang, China

Abstract: The objective of the present study was to evaluate an efficient purifying process of *Kunlun Chrysanthemum* procyanidins (KCPC) by combination of AB-8 macroporous adsorption resin and silica gel column through adsorption and desorption experiments under static and dynamic status with the purity of procyanidins and the antioxidant activity as indexes respectively. The optimum parameters for adsorption by AB-8 resin were as follows: Sample concentration 1mg/mL, pH6, injecting velocity 2 bed volume (BV) /h, with 3 BV 70% ethanol as eluting solvent, and elution flow rate 2BV /h. One cycle after treatment using AB-8 resin, the purity of KCPC obviously enhanced 3 times compared with that of not prepurified, that is from 22.68 to 63.76%. The optimum parameters for adsorption by silica gel column were as follows: The concentration of procyanidins in a sample solution of 1.2mg/mL (pH 6) with a speed of 2BV/h. Concentration for desorption, with 5 BV of 80% ethanol as an eluent at a flow rate of 2 BV/h. After one-run treatment with silica gel column, the purity of KCPC increased from 63.76 to 81.97%. The antioxidant activities of the purified KCPC *in vitro* were further investigated. The results indicated that the purification method of combination of AB-8 resin and silica gel column was superior to AB-8 adsorption resin used alone in term of antioxidant activities. Moreover, the 1,1-diphenyl-2-picrylhydrazyl (DPPH•) scavenging ability, the scavenging activity of hydroxyl radicals and the reducing ability appeared to be dose-dependent of KCPC. The novel purification method of combination of AB-8 resin and silica gel column will offer a promising way to purify KCPC for wider application.

Keywords: *Kunlun chrysanthemum*, procyanidins, purification, antioxidant activity.

INTRODUCTION

Kunlun Chrysanthemum (*Coreopsis tinctoria*) is a plant belonging to Coreopsis grown at an altitude of 3000 meters above on the north of Karakoram Mountains in Xinjiang, China. The flower buds of *Kunlun Chrysanthemum* were proved to be rich in procyanidins and flavonoids. *Coreopsis ticntoria* can be used as Chinese herbal medicine in the treatment of various diseases such as hypertension, palpitation and gastrointestinal discomfort (Yan *et al.,* 2010). In recent years, a number of pharmaceutical studies also showed that the extracts from C. ticntoria have the significant effects on antioxidtion, lowering blood pressure and improving circulation (Liang *et al.*, 2009; Liu *et al.*, 2009). Recently, more attentions have been paid to the extraction of some active ingredients from *Coreopsis tinctoria* such as chlorogenic acid (Dias *et al.*, 2010), flavonoids and es sential oils and their potential healthy benefits in antioxidant (Sha *et al.*, 2013; Dilnur *et al.*, 2013).Guk *et al* found that Coreopsis tinctoria Nutt. flower extracts have ACE inhibitory activity (Guk *et al.*, 2010). To our knowledge, little information about the purification and bioactivity of procyanidins from *Kunlun Chrysanthemum* grown in Xinjiang.

Procyanidins are polyphenol compounds from the flavonoid group which constitute oligomers or polymers of flavan-3-ols which exclusively consist of catechin and/or epicatechin units They are widely found in a variety of vegetables, nuts, seeds, flowers and bark (Yamakoshi *et al.,* 2002; Packer *et al.,* 1999). Furthermore, the well documented antioxidant capacity, pharmacological and chemo protective properties against free radicals and oxidative stress, such as antibacterial, antiviral, anti-inflammatory, anti-allergic and protect the cardiovascular (Ariga *et al.*, 1988; Landrault *et al.*, 2001; Pinent *et al.*, 2006; Yilmaz *et al.*, 2004). In the present paper, a novel process which was AB-8 macro porous resins followed by silica gel column was used to purify KCPC, and the adsorption and desorption properties of KCPC were investigated. In addition, The antioxidant activities *in vitro* of pre-KCPC purified by AB-8 macro porous resins and post-KCPC purified by combination of AB-8 macro porous resins and silica gel were evaluated respectively, including DPPH• free radical, hydroxyl free radical scavenging effects and reducing power test. We hope that this study will be useful to promote further exploit and utilize KCPC.

**Corresponding author:* e-mail: xmzhang168@hotmail.com

MATERIALS AND METHODS

Chemical reagent

Procyanidins standard substance, Tianjin Dingfeng biological; AB-8 resin was purchased from Tianjin Bohong Resin Technology Co., Ltd. (Tianjin, China); Other reagents were of analytical grade, double distilled water was used during whole experiment process.

Plant material

The sample made from the flower buds of Xinjiang Kunlun Chrysanthemum was kindly provided by the Xinjiang Hetian Desert Rose Limited Liability Company (Xinjiang, China) and was identified by phytology prof. Abudula Abbas who is a plant classification and identification experts belongs to Xinjiang University.

Preparation KCPC

Kunlun Chrysanthemum was air dried at room temperature for 2 weeks to get consistent weight. The dried flower buds were ground to powder, sieved through a 60 mesh screen and defatted with petroleum ether. Then using ultrasonic assisted extraction in 60 % ethanol for 33 min in a KQ-400KDE ultrasonic cleaner bath (Kunshan Ultrasound Instrument Co., Ltd., Jiangsu, China) at 44□. Extracts was centrifuged at 3500r/min for 10 min. The liquid supernatant was evaporated and concentrated under reduced pressure at 30□by a low temperature rotary evaporator (Yarong biochemistry instrument factory, Shanghai, China). The Concentrate was liophilized using a freeze-dryer (LGJ05, Beijing in China) , and KCPC was obtained, then stored at -20□ until use. Under the optimized conditions, the extraction ratio of KCPC was 13.29% and the purity was 22.68±1.02%.

Pretreatment of AB-8 macroporous resin

AB-8 resin was resined with 95% ethanol and 24 hours to fully infiltrate, and washed with 95% ethanol until no white turbidity. The same amount of 5% HCl and 2% NaOH passed through the column respectively to remove impurities. Finally, to make the effluent neutral, the column was washed with deionized water, then dried and placed in a desiccator for experiment behind.

Static adsorption and desorption tests on the macro porous resin

The adsorption tests were performed using the method reported by Toor *et al*. (Toor and Jin, 2012) with some modifications. Briefly, 3g hydrated test resin was placed in a 250mL flask, together with 20mL crude extracts with concentration of KCPC determined by the colorimetric method. The loaded flask was then constantly shaken at 90 rpm at 30□ in a shaker, measured the concentration of the KCPC solution every 2h and interval time were as following: 2h, 4h, 6h, 8h, 10h, 12h until the macro porous resin absolutely adsorption saturation, the adsorption amount was in accordance with the following equations, three replicates of static adsorption experiment were conducted from each resin.

Adsorption amount: $$Q = \frac{(C_1 - C_2) \times V}{W}$$

Adsorption rate: $$A(\%) = \frac{C_1 - C_2}{C_1} \times 100$$

Where: Q-adsorption amount (mg/g dry resin); C_1-before adsorption procyanidins solution concentration (mg/mL); C_2-solution after adsorption procyanidins concentration (mg/mL); V-volume of solution (mL); W-AB-8 macro porous resin weight (g).

Static desorption process was carried out as following: after adsorption reached equilibrium, test resin first washed with distilled water, then desorbed with 50mL of different concentrations of ethanol. The flask loaded with desorption solution was continually shaken at 90 rpm at 4□ for 12h till equilibrium, then measured the desorption solution concentration (C_3), and the desorption ratio was calculated according to the following equation:

$$D(\%) = \frac{V \times C_3}{W \times Q} \times 100$$

Where: Q-Adsorption amount (mg/g dry resin); C_3-The desorption solution after procyanidins concentration (mg/mL); V-The desorption solution volume (mL); W-Weight of resin (g).

Dynamic adsorption and desorption tests on the AB-8 macroporous resin

Dynamic adsorption and desorption tests (Yan *et al*., 2013) were performed on a glass column (20 × 300mm) wet-packed with selected AB-8 resin. The bed volume (BV) of resin was 30mL, then a certain concentration of KCPC extracted liquid through a macro porous resin. The effect of pH value of sample solution and the dynamic adsorption leak tests were carried out firstly. After the adsorption equilibrium, used deionized water to wash the column, and then eluted with 70% ethanol at appropriate eluting velocity. The elution volume of 70% ethanol was adjusted according to the rate of adsorption. All the results were average values of triplicate determinations. Thus, pre-KCPC was obtained and reserved.

Static adsorption on the silica gel column

Dynamic adsorption experiment was conducted in a glass column wet-packed with pretreated silica gel. Configuration 1.2mg/mL 200mL KCPC solution, adjust the appropriate speed on liquid. Prepare 15 colorimetric tubes, each 5mL effluent was collected in a colorimetric tube. Hydrochloric acid-vanillin method was determined for procyanidins content of each tube, get the leak, calculate the maximum amount of adsorption.

$$\text{Adsorption capacity} = \frac{5 \times \text{colorimetric tube leak point number}}{\text{Silica weight}}$$

Determination of silica gel dynamic adsorption curve

Pretreated wet packed column of silica gel filling height 20cm, column volume 63mL. Configuration 0.4mg/mL, 0.8mg/mL, 1.2mg/mL solution of pre-KCPC 200mL. Sample with 2mL/min flow rate, step by step to collect effluent (5mL per tube), determination of procyanidins content. The absorption curve was obtained with effluent volume (mL) as abscissa, and pre-KCPC effluent concentration C and C_0 injection ratio of the concentration of pre-KCPC (C/C_0) as vertical axis.

Silica gel column desorption curve

When the effluent concentration of pre-KCPC reached 10% of sample concentration, indicating that the adsorption has reached the saturation point of the adsorption, the sample was stopped, the adsorption flux calculated. The desorption process was carried out as follows: After adsorption equilibrium, the silica gel was washed with deionized water and then desorbed with ethanol-water (80:20, v/v) solution at a flow rate of 2mL/min, the effluent was collected (15mL per tube), then the procyanidins content was determined. With elution volume (mL) as the abscissa, pre-KCPC eluent concentration (mg/mL) as the vertical axis, the desorption curve was gained. Desorption rate was calculated with the equation:

$$\text{Adsorption capacity} = \frac{\text{Total KCPC of the eluent}}{\text{Total KCPC adsorption by silica gel}} \times 100\%$$

Silica gel different ethanol concentration gradient curve

Silicone was adsorpted respectively with 60%, 70%, 80 %, 90% ethanol concentration gradient and reached an adsorption capacity, followed by an elution step to elute. The procyanidins content was determined. With elution volume (15mL/tube) as the abscissa, pre-KCPC eluent concentrations (mg/mL) as the ordinate, the ethanol concentration elution gradient curve was gained. To elution volume (15mL/tube step collection) as the abscissa to procyanidins eluent concentration (mg/mL) as the vertical axis, a different ethanol concentration gradient curve was drawn.

Determination of scavenging activity on DPPH·

The scavenging activity of DPPH• free radicals was measured by using the reported method of S. Gonc and R. Mo *et al*. (Gonc *et al.*, 2013; Ho *et al.*, 2012) with some modifications. Briefly, 2mL 2×10^{-4} mol/L DPPH• in ethanol in ethanol was mixed with equivalent aliquot of different concentrations of sample in a tube. Absorbance at 517 nm was measured at 2min intervals by a spectrophotometer (US-spectrum Instrument Co., Ltd., Shanghai, China). After standing in dark for 30 min when the absorbance reached a plateau, it was measured against ethanol. The DPPH• radical scavenging activity (%) of the sample was calculated by the following equation: $1–(A_i–A_j)/A_0\times100$

Where A0 is the Abs of the control (without sample), Ai is the Abs of the sample and Aj is the Abs of the blank. Results were expressed as the amount of sample necessary to scavenge 50% of DPPH• radical (IC_{50}). Moreover, in order to compare the radical scavenging efficiency of the extracts, IC_{50} value showing the concentration of KCPC that caused 50% scavenging of DPPH radical was calculated by use of univariate linear regression equation.

Assay of scavenging activity on hydroxyl radical

Assessment of the scavenging ability of the purified procyanidins on hydroxyl radicals was performed by the reported method with some modifications (Zhao *et al.*, 2009). The reaction mixture comprised of 1mL of test sample, 1.5mL of 5mmol/L Phenanthroline application solution, 4mL of pH7.4 sodium phosphate buffer, 1mL of 7.5mmol/L $FeSO4.7H_2O$, 1.5mL of distilled water and 1.0mL of 0.1% H_2O_2 solution. The absorbance of the final solutions was measured at 536 nm with a UV-visible spectrophotometer after incubation at 37□ for 60 min. Deionized water was used as the blank, and ascorbic acid as positive control, respectively. The clearance rate was obtained by the following equation: Scavenging activity (%) $=(A_0\text{-}A_1)/(A_2\text{-}A_1)\times100$

Where A_0 is the absorption value after adding the sample and the hydrogen peroxide; A_1 is the absorption value after adding hydrogen peroxide without sample; and A_2 is the absorption value without sample and hydrogen peroxide. Antioxidant value was expressed as IC_{50}, which represented the concentration of KCPC that caused 50% inhibition of hydroxyl radical formation.

Reducing power

Reducing power of procyanidins was determined on the basis of Jayaprakasha *et al* (Jayaprakasha *et al.*, 2002; Sheng *et al.*, 2009). Various concentrations of procyanidins in 1.0mL MeOH were mix-up with 2.5mL of phosphate buffer (0.2M, pH 6.6) and 2.5mL 1% potassium ferricyanide in test tubes. 20 minutes of incubation mixture at 50°C. In the last stage, 2.5mL of 10% trichloroacetic acid was injected to the mixtures and centrifuged at 3000rpm for 10min. The supernate (2.5 mL) was interfused with 2.5mL of distilled water and 0.5 mL of 0.1% ferric chloride, the absorbance, at 700nm was measured. The reducing power experiment was conducted in triplicate. Increment in absorbance of the reaction demonstrated the reducing power of the test samples. Ascorbic acid was used as control and was revealed in the similar manner.

STATISTICAL ANALYSIS

Each experiment was conducted in three times and

statistical analyses were presented, the data were shown as mean ±S.D. P values less than 0.05 were considered statistically significant. One-way ANOVA followed by Tukey test was used for statistical analysis using SPSS program version 14.0.

RESULTS

Static adsorption curve of KCPC using AB-8 macroporous resin

As can be seen in fig. 1, the adsorption capacities towards KCPC increased with the extension of adsorption time. In the first 2h the adsorption capacities increased rapidly, increased slowly after 2h and about 6 hours to reach equilibrium. Hence, choosen 6 hours as the best purification KCPC time, and used in subsequent experiment.

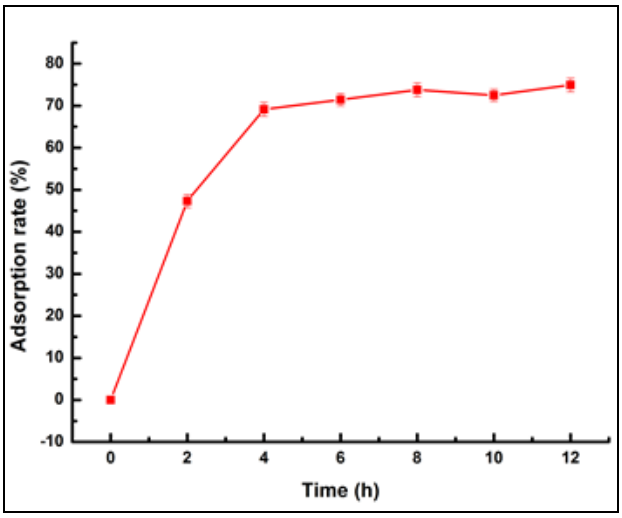

Fig. 1: Static adsorption curve

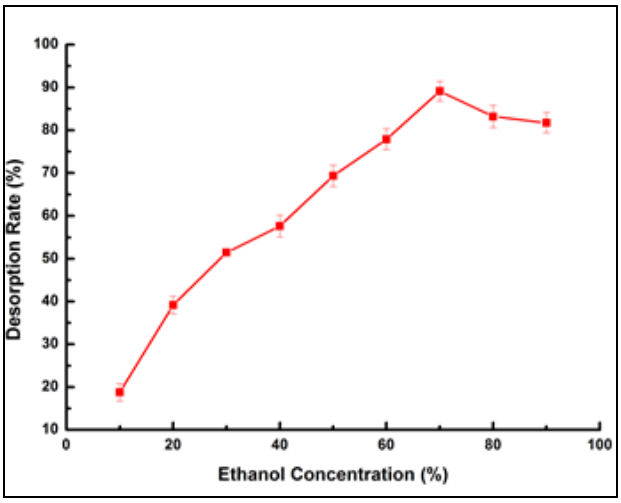

Fig. 2: Effect of ethanol concentration on desorption rate

AB-8 macro porous resin static desorption test results

Less toxic ethanol was chosen as eluent in view of KCPC as raw materials of pharmaceuticals and food. The effects of ethanol concentration on the stationary desorption rate of AB-8 macro porous resin was shown in fig. 2. With increasing of ethanol concentrations, the desorption ratio of KCPC increases accordingly. The maximum desorption ratio was up to 92.33% while using ethanol at a concentration of 70%. When ethanol concentration exceeded 70%, the desorption rate was decreased. Hence, 70% ethanol solution was selected as the proper desorption solution.

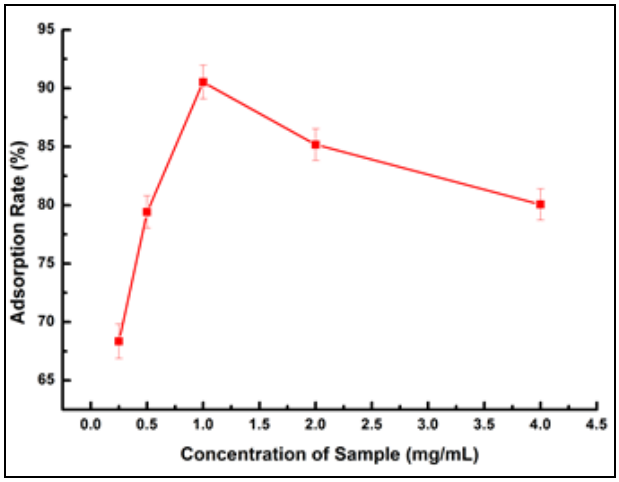

Fig. 3: Effect of sample concentration on absorption rate of KCPC

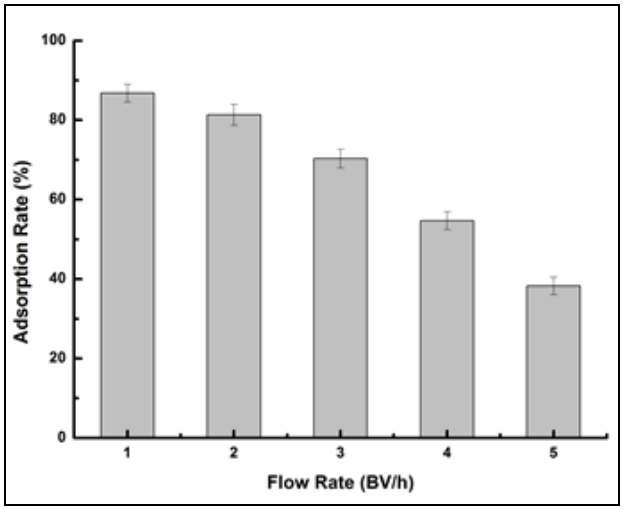

Fig. 4: Effect of flow rate on absorption rate of KCPC

Determination of the optimum conditions for dynamic adsorption

Effect of sample concentration on adsorption rate of KCPC using macroporous resin

The results showed in fig. 3, that when the KCPC concentration of the solution altered from 0.25 to 1 mg/mL, the adsorption capacity of the resin increased and the maximum adsorption rate reached 90.53% , when the sample concentration is higher than 1mg/mL, the adsorption rate would decrease. Thus, the best

concentration of the KCPC was 1mg/mL.

Effect of flow rate on adsorption rate of KCPC using macroporous resin

As can be seen in fig. 4, the best adsorption performance was obtained at the lowest flow rate (1.0 BV/h), which is likely due to better particle diffusion in sample solutions. An even lower flow rate prolonged the working period (Ma *et al.*, 2009). When the flow rate was quicker, it brought about incomplete adsorption on AB-8 resin. Taking a lower flow velocity extending the working hours for industrial production into consideration, 2.0 BV/h was elected as the suitable sample flow rate for further experiments.

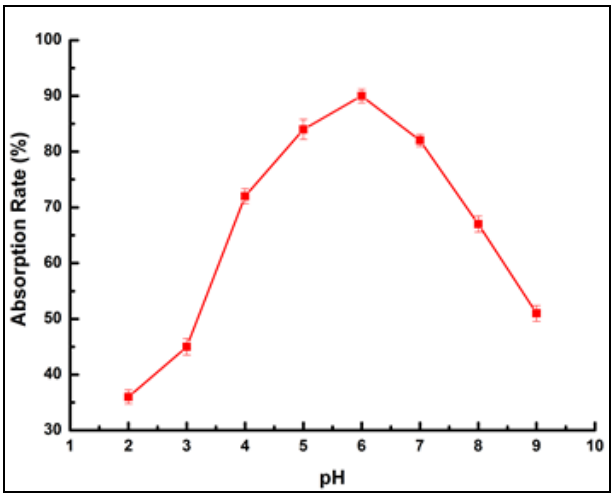

Fig. 5: Effect of different sample pH on absorption rate of KCPC

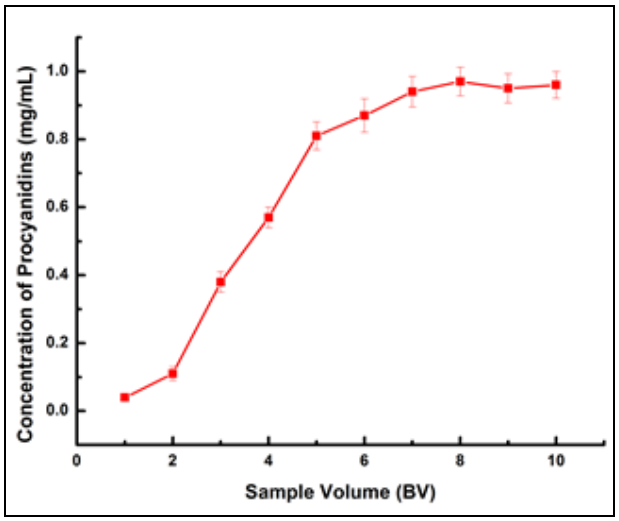

Fig. 6: Dynamic adsorption leak curve of KCPC

Effect of sample pH on macroporous resin dynamic adsorption of KCPC

Since the extent of ionization of KCPC is determined by pH value, the pH value of sample solution is extremely significant for the adsorption and desorption properties of resins, thus impacting their adsorption affinity (Fu *et al.*, 2006). The sample solution, pH is an important factor to influence the effect of the macroporous. As shown in fig. 5, the adsorption capacity increased firstly and attaining its crest value at the pH 6, then, it reduced slowly, which may be as KCPC as the weakly acidic polyhydroxy phenolic, according to the polarity compatible principle, must be adsorbed under weakly acidic conditions. Thus, the pH value of sample solution was modulated to 6 for all followed experiments taking account of the adsorption process.

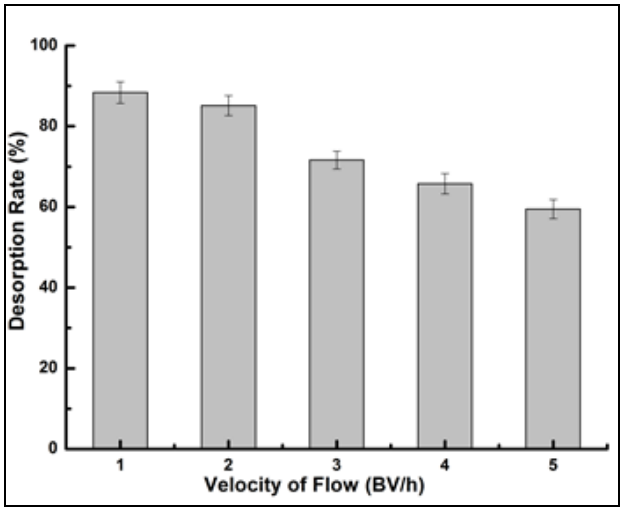

Fig. 7: Effect of flow rate on the content of desorption

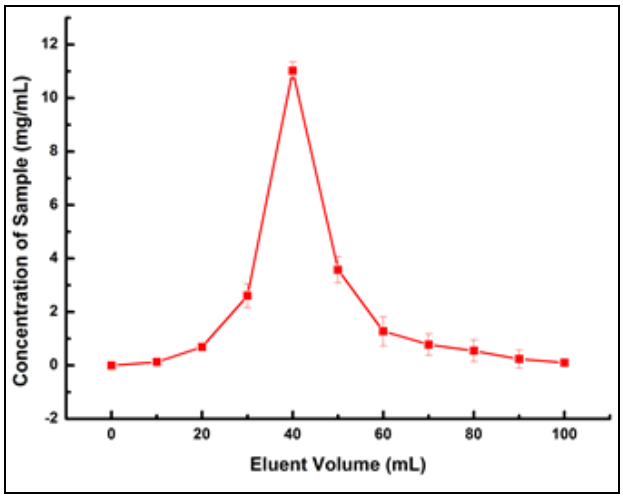

Fig. 8: Effect of the amount of eluent on the content of desorption

Dynamic leakage curves on AB-8 macroporous resin

The dynamic leakage curves on AB-8 macro porous resin were obtained (fig. 6) based on the concentrations of KCPC. The concentrations of KCPC increased at the outset and then back into equilibrium. With the increase of sample volume owing to competition in the active sites of AB-8 resin by the impurity in the KCPC extracts. The concentrations of KCPC clearly not likely to increase when the sample volume of sample was excess of 8 BV. In order to avoid the waste of raw materials, the

maximum sample volume was determined to be 150mL (5 BV).

Determination of the optimum process conditions for dynamic desorption

Effect of elution rate on adsorption rate of KCPC using macroporous resin

In order to reduce reagent consumption, make the desorption efficiency is higher, through dynamic desorption curves show that the influence of eluent velocity to the desorption ability. The results were shown in fig. 7. It shows that the increasing velocity of sample had an adverse effect on dynamic desorption capability of macro porous resin. When flow rate traffic is 1BV/h, the best desorption performance was obtained desorption to attain the best performance. In order to shorten the time and reduce the volume, the best desorption velocity is 2 BV/h.

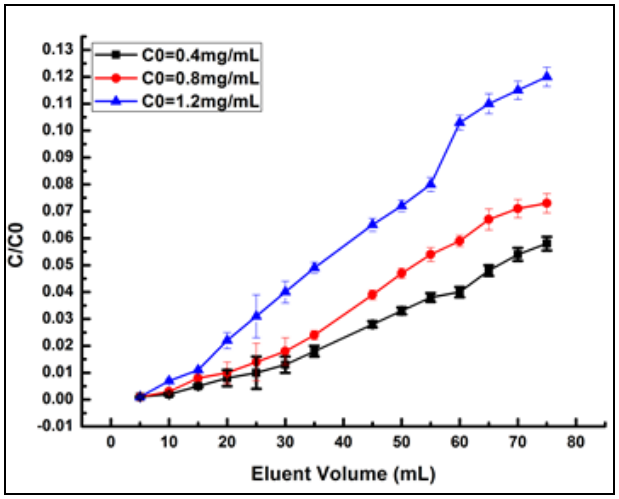

Fig. 9: The adsorption curve of pre-KCPC purified by silica gel column

Effect of the amount of eluent on adsorption rate of KCPC using macroporous resin

The elution curves were obtained as shown in fig. 8. The maximum concentration of the eluent was around 40mL, then decreased rapidly until 60mL and fell to less than 0.8mg/mL at 70mL. Therefore, eluent volume was selected to be 3BV because of the less volume consumption and shorter working time**.**

In summary, the results of static test and dynamic test showed that AB-8 macro porous resin have better purification effect on KCPC. After purification, the purity of KCPC was of 63.76±0.34% (w/w), but before purification the purity of procyanidin only 22.68±1.02% (w/w). Furthermore, the best purification conditions of KCPC using AB-8 macro porous resin were determined as follows: injecting concentration 1mg/mL, pH 6, injecting velocity 2BV/h, 3 BV of 70% (v/v) ethanol as desorption solvent, desorption velocity of flow 2BV/h.

Adsorption and desorption curve of pre-KCPC using Silica gel

Silica gel adsorption of KCPC solution is 2.0mL/g and desorption rate of KCPC reached 83.69%. The desorption curves using silica gel was acheived depending on the volume of desorption solution and the C/C0 (fig. 9). Choosing a proper injecting concentration is significant. As can be shown in fig. 9, in the desorption test, the desorption performance was better with higher desorption injecting concentration, the injecting concentration at 1.2mg/mL was the best. However, at this injecting concentration, the working was efficient. Therefore, 1.2mg/mL was choosen as the injecting concentration taking account of the lower volume consumption.

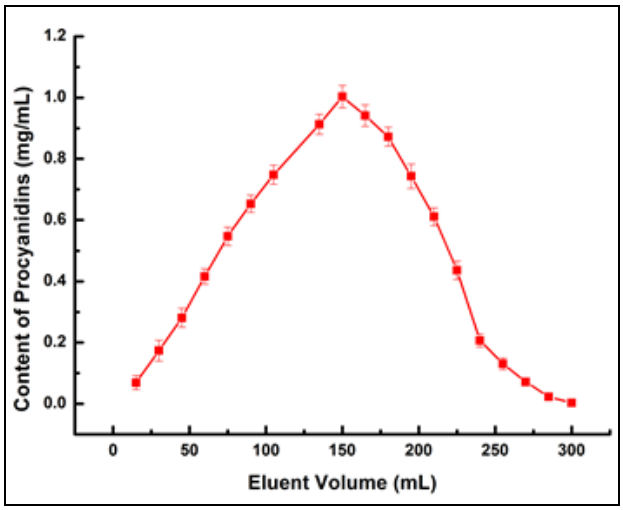

Fig. 10: The desorption curve of KCPC purified by silica gel column

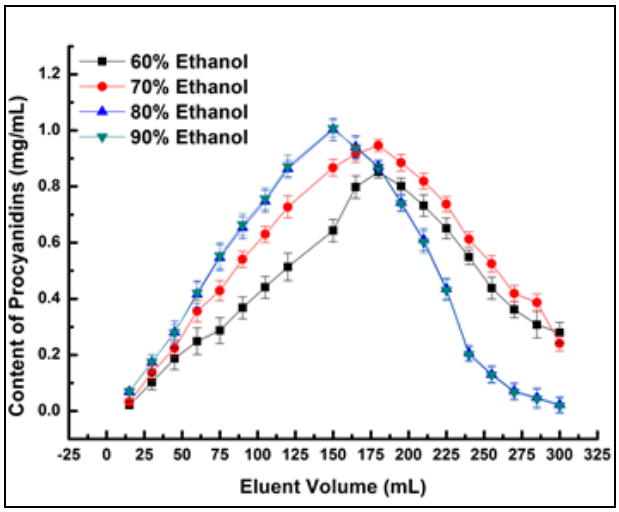

Fig. 11: Different ethanol concentration on procyanidins elution curves

Fig. 10 indicates the dynamic desorption curves of fully saturated columns on the silica gel based on the volume of desorption solution and the content of KCPC. With 5BV 80% ethanol solution eluted, when the eluent reached

150mL, the concentration of KCPC was up to maximum, and then gradually decreased. When the eluent reached 300mL, there is little KCPC in the elute. It indicated the desorption process of KCPC from the silica gel could be almost completed by near 300 mL(5 BV) of desorption solution.

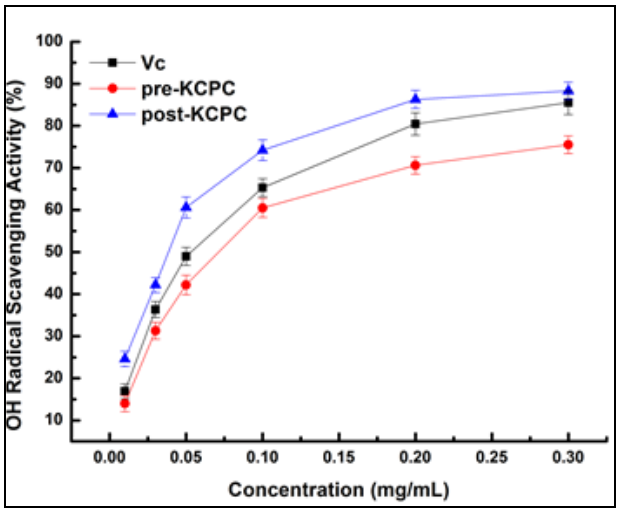

Fig. 12: Hydroxyl radical scavenging ability of KCPC

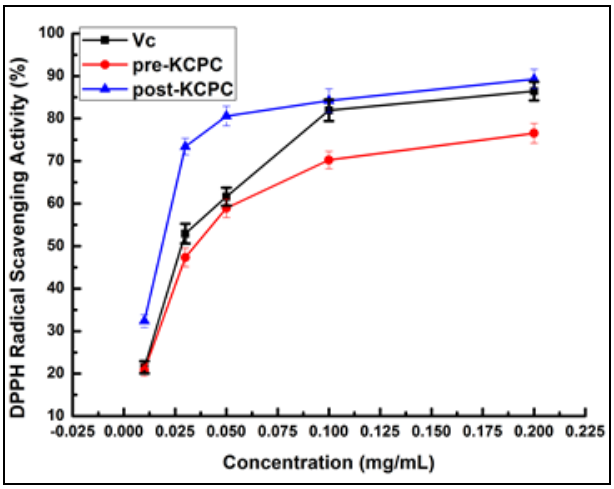

Fig. 13: DPPH· radical scavenging ability of KCPC

Different ethanol concentration gradient elution curves silica gel column

When the adsorption of KCPC on the silica gel resin was finished, it was very significant to select a proper reagent to desorbe KCPC from silica gel effectively. Taking the structure and property of KCPC into consideration, the diverse concentration of ethanol aqueous solutions was implemented. The KCPC content of desorption solution was determined. As shown in fig. 11,when the concentration of ethanol solution a*rrived* 80% or 90%,the results demostrated that the KCPC desorbed was no vital difference (P>0.05) and significantly better than the 60 and 70% ethanol. Thus, in desorption process, 80% ethanol-aqueous solution was appropriate for desorption of KCPC in the desorption process.

After purified by combination of AB-8 resin and silica gel column, the content of the KCPC reached to 81.97%.

Effect of purification process on antioxidant activity in vitro of KCPC

OH radical scavenging activity

Vc stand for vitamin C, Pre-KCPC stand for KCPC obtained by AB-8 macro porous resin (purity: 63.76±0.34 %), post- KCPC stand for KCPC obtained by combination of AB-8 macro porous resin and silica gel column (purity: 81.97±0.19%).

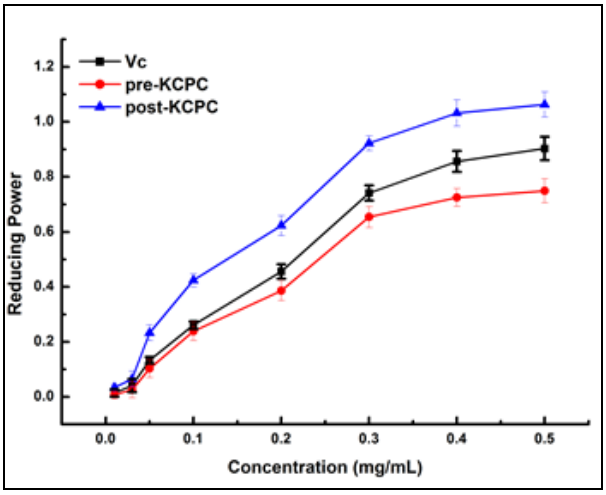

Fig. 14: Reducing power capacity of KCPC

OH which is well-known as the most reactive free radical. Hydroxyl radical could be produced in several methods, but the most significant mechanism was the Fenton reaction. The hydroxyl radical scavenging activities of pre- and post- purification KCPC were shown in fig. 12. All samples showed a scavenging ability on hydroxyl radicals in a dose dependent way (0.01 ~ 0.2mg/mL). Compared with the pre-KCPC, the post-KCPC exhibited stronger scavenging ability , and the scavenging ability was up to 74.21% at the concentration of 0.1mg/mL. The IC_{50} value of pre-KCPC and post- KCPC were of 0.073 mg/mL and 0.041 mg/mL, respectively. The result indicated that the purity of KCPC showed pronounced effect on scavenging the hydroxyl radical.

DPPH radical scavenging activity

The DPPH radical scavenging activity of the KCPC was tested and the results were compared with V_C (Vitamin C) (fig. 13). DPPH is generally used as a substrate to evaluate antioxidative activity of antioxidants. As shown in fig. 13, the procyanidins revealed a concentration-dependent antiradical activity by restraining the DPPH radical. Post-KCPC showed higher standard of free radical scavenging activity than V_C. The post-KCPC had a lower IC_{50} than the pre-KCPC. The results indicated that the post-KCPC had strong scavenging activity on DPPH

radical. The IC_{50} value of pre-KCPC and post- KCPC were of 0.036 mg/mL and 0.021 mg/mL, respectively.

In vitro reducing power capacity of KCPC

The decreasing capacity of a compound might act as a vital indicator of its potential antioxidant activity. As can be seen in fig. 14, all of pre-KCPC showed stronger reducing power than that of post-KCPC. It turned out to be that the antioxidant activity of various additive amount of procyanidins and V_C at a concentration-dependent manner. The result demonstrated that post-KCPC has stronger antioxidant activity than pre-KCPC.

DISCUSSION

In AB-8 macroporous resin static adsorption test, the reason why the desorption rate was decreased when ethanol concentration exceeded 70% is that the higher concentration of ethanol, the more conducive to cause the desorption of the other ingredients from the macro porous resin (fig. 2). It also probably because the concentration of ethanol was so high that the dissolution of other impurities was increased and competed solvent with procyanidins to make the desorption rate decline.

In AB-8 macroporous resin dynamic adsorption test, for a certain amount of solute, the increase of the concentration of the sample solution means increasing the area of contact of the solute and the surface of the resin, so the resin can enhancement the adsorption of solute, simultaneous adsorption effect is better. However, adsorption selectivity will decrease, and KCPC will leak earlier when the sample concentration is too high. Therefore, we must choose the appropriate concentration of the sample solution for assay of adsorption (fig. 3). Proanthocyanidins is a kind of phenolic compound, which diffuse more slowly, the sample flow rate is one of the main factors affecting KCPC diffusion rate in the macro porous resin, but also affect the adsorption of macro porous resin. Accelerate the sample flow rate, the adsorption rate will be significantly reduced, but the reduced sample flow rate will be extended production cycle. Therefore, the choice of sample flow rate should be comprehensively considerated (fig. 4). Proanthocyanidins is a kind of weakly acidic polyhydroxy phenols, weak acid conditions are favorable for the adsorption of macro porous resin according to the polarity of the compatibility principle. Thus, in practice, the sample solution was adjusted to a pH of 6.0 (fig. 5). Macro porous resin adsorption plays a major role in surface adsorption, form hydrogen bonds on surface or surface electrical resistance, when the liquid flows through the resin, solute in liquid is adsorped by resin. When the rate of adsorption is equal to desorption rate, that is named dynamic balance, then begin to leak. Obvious leaks of KCPC began to appear when the amount of sample was up to 5BV, which reached a dynamic equilibrium. In order to avoid wasting raw materials, the maximum sample volume was selected of 150mL, that is 5 BV (fig. 6).

In AB-8 macro porous resin dynamic desorption experiments, elution rate and desorption rate of KCPC show an inverse relationship, so the elution rate should be slow to increase the contact time of eluent with macro porous. But considering the too slow elution rate takes more time and will extend the production cycle, therefore, related influence factors should be considered for determining elution rate (fig. 7). The optimum amount of eluent is determined on condition that KCPC can basically be eluted from macro porous resin (fig. 8).

Studies have shown that polymerization of pro-anthocyanidins has stronger effect on its antioxidant. For the polymer, oligomers show higher bioactivity, and the antioxidant effect of proanthocyanidins on linoleic acid system and liposome systems decrease with the increase of the degree of polymerization (Yun S *et al*., 2007; Silvina *et al*., 2000). Researches on its fractionation characteristics show that relatively high content of oligomeric procyanidins component can be obtained from the low concentration of ethanol eluate using AB-8 resin adsorption of proanthocyanidins, while some proanthocyanidins with higher degree of polymerization of can irreversiblely adsorpt on a silica gel column (Yun S *et al*., 2007). Therefore, it is possible to obtain higher amounts of procyanidin oligomers with combination of AB-8 macro porous resin and silica gel column. The higher content of procyanidin oligomers, the stronger the antioxidant activity. Hence, the property of antioxidation of post-KCPC is stronger than pre-KCPC (fig. 12-fig.14).

CONCLUSION

The optimal purification conditions of KCPC through static and dynamic screening tests with AB-8 macroporous resin was as following: Concentration 1 mg/mL, the sample pH 6, the sample flow rate of 2 BV/h, with three times the bed volume 70% ethanol at a flow rate 2BV/h elution highest purity procyanidin. After purification, the purity of KCPC was up to 63.76±0.34% (w/w) while before purification was only 22.68±1.02% (w/w). The optimum purification conditions by silica gel column obtained are as follows: Concentration of 1.20 mg/mL, the sample pH 6, the sample flow rate 2mL/min, 5 column volumes of 80% ethanol at a flow rate of 2 mL/min. The purity of KCPC reached to 81.97±0.19%. The results indicated that the purification method of combination of AB-8 macro porous resin and silica gel column was superior to AB-8 macro porous adsorption resin used only in term of purity. Moreover, the KCPC purified by method of AB-8 macro porous resin followed by silica gel column has stronger antioxidant activity.

Further studies on separation of KCPC, structural and functional verification are in progress.

ACKNOWLEDGEMENTS

This project was supported by the Key Laboratory of Biological Resources Gene Engineering of Xinjiang (No.XJDX0201_2011_09).

REFERENCES

Ariga T, Koshiyama I and Fukushima D (1988). Antioxidative properties of procyanidins B-1 and B-3 from azuki beans in aqueous systems. *Agric. Biol. Chem.*, **52**: 2717-2722.

Teresa D, Bo L, Peter J, Peter JH, Helder MF and Alexandra P (2012). Cytoprotective effect of coreopsis tinctoria extracts and flavonoids on tBHP and cytokine-induced cell injury in pancreatic MIN6 cells. *J. Ethnopharmacol.*, **139**(2): 485-492.

Dilnur·abdurahman, Jing SQ and Wu S (2013). Composition analysis and antibacterial activity of essential oil from coreopsis oleoresin. *Food and Fermentation Industries*, **39**(12): 166-170.

Fu YJ, Zu YG, Liu W, Thomas E, Zhang NJ, Liu XN and Kong Y (2006). Optimization of luteolin separation from pigeonpea (*Cajanus cajan (L.) Millsp*.) leaves by macro porous resins. *J. Chromatogr. A.*, **1137**: 145-152.

InGuk H, HyunYoung K and SoLim S (2010). Biological activities of coreopsis tinctoria Nutt. flower extracts. *Hortl Sci & Tec*, **28**(5): 857-863.

Ho R, Violette A, Cressend D, Raharivelomanana P and Carrupt PA (2012). Hostettmann antioxidant potential and radical-scavenging effects of flavonoids from the leaves of Psidium *cattleianum* grown in French Polynesia. *Nat. Prod. Res.*, **26**(3): 274-277.

Jayaprakasha GK, Singh RE and Sakariah K (2002). Antioxidant activity of grape seed (vitis vinefera) extracts on peroxidation models *in vitro*. *Food Chem.*, **5**(73): 285-290.

Landrault N, Poucheret P, Ravel P, Gasc F, Cros G and Teissedre PL (2001). Antioxidant capacities and phenolics levels of French wines from different varieties and vintages. *J. Agric. Food Chem.*, **49**: 3341-3348.

Liang SH, Pang SB, Liu XY, L X and Sun YH (2009). Laboratory study of coreopsis extracts on reducing blood lipid in hyperlipemia model mice. *J Nongken Med*, **3**(6): 495-457.

Liu WX, Deng JH and Xu H (2009). Pharmacognostical studies on the flower of one species coreopsis sp. *J Med & Pharmacy of Chinese Minorities*, **1**(1): 24-25.

Ma C, Tao G, Lou Z, Wang H, Gu X and Hu L (2009). Preparative separati and purification of rosavin in Rhodiola rosea by macroporous adsorption resins. *Sep. Purif Technol.*, **69**: 22-28.

Packer L, Rimbach G and Virgili F (1999). Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (Pinus Maritima) bark, pycnogenol. *Free Radic. Biol. Med.*, **27**: 704-724.

Pinent M, Blade C, Salvadó MJ, Blay M, Pujadas G, Fernandez-Larrea J, Arola L and Ardévol A (2006). Procyanidin effects on adipocyte-related pathologies. *Crit. Rev. Food Sci. Nutr.*, **46**: 543-550.

Sha AL, Wu Y, Sheng HY, Yang XP, Wang W and Hao HY (2013). Effects of corepsis tinctoria flavonoids on cerebral index and viscera index in the aging model mice. *Progress inveterinary medicine*, **34**(7): 66-68.

Sheng W, Xue JP and Xie BJ (2009). Study on antioxidant activity of extracts from bulbil of Dioscorea opposite. *Food Science*, **3**(30): 92-93.

Silvina BL, Lucas AG, M.Lourdes R, Marina C, Dietrich R, Harold HS, Francene MS, Carl LK and Cesar GF (2000). Influence of oligomer chain length on the antioxidant activity of procaynidins. *Biochem. Biophys. Res.*, **276**(3): 945-951.

Sun Y, Xu BC, Gu WY and Xiong XH (2007). Relationship between the degree of polymerizati on and antioxidant activities of grape seed procyanidins. *Food and Fermentation Industries*, **32**(10): 41-46.

Sun Y, Xu BC, Gu WY and Xiong XH (2007). Study on the fractionation of grape seed procyanidins by AB-8 adsorbent resin. *Food sci. Technol.*, **32**(6):60-64.

Toor M and Jin B (2012). Adsorption characteristics, isotherm, kinetics, and diffusion of modified natural bentonite for removing diazo dye. *Chem. Eng. J.*, **187**: 79-88.

Yamakoshi J, Saito M and Kataoka S (2002). Safety evaluation of proanthocyanidins-rich extract from grape seeds. *Food Chem. Toxicol.*, **40**: 599-607.

Yan XX, Ren ZY, Xiang HX and Jing SQ (2010). Qualitative analysis of effective constituents of Cypems esculentus. *China Brewing*, **211**(8): 150-151.

Yan YJ, Zhang XY and Chen DW (2013). Enhanced catalysis of *Yarrowia lipolytica* lipase LIP2 immobilized on macroporous resin and its application in enrichment of polyunsaturated fatty acids. *Bioresour. Technol.*, **131**: 179-187.

Yilmaz Y and Toledo RT (2004). Health aspects of functional grape seed constituents. *Trends Food Sci. Technol.*, **15**: 422-433.

Zhao YH, Li JK, Zhao W and Wu XX (2009). Evaluation on antioxidant activities of extracts from common edible and medicinal plants *in vitro*. *Food Science*, **30**(3): 104-106.