Purification and identification of flavonoids in *Portualca olearcea* by macroporous adsorption resin and HPLC

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Abstract. *Portulaca oleracea* is a kind of food and medicinal plant in China. Flavonoids are the main active ingredients of the medicinal function of this plant. In this study, the macroporous adsorption resins (MARs) were employed to purify flavonoids from the crude extract of *P. oleracea*. The properties of six kinds of common used MARs were evaluated. The results showed that HPD600 had higher separation efficiency. The separation parameters were also optimized according to dynamic adsorption and desorption experiments. In addition, a high-performance liquid chromatography (HPLC) method had been performed and validated for flavonoids analysis in *P. oleracea*. The results showed that quercetin, kaempferol, luteolin, and apigenin were the main component of flavonoids compounds of *P. oleracea*. This study is expected to provide useful help for the purification and application of flavonoids of *P. oleracea*.
Keywords: flavonoids; *Portulaca oleracea*; Macroporous adsorption resin; HPLC.

1 Introduction

Portulaca oleracea (common purslane, also known as verdolaga, pigweed, little hogweed, pursley, and longevity vegetables) is an annual succulent in the family of the Portulacaceae [1]. It is widely distributed in temperate and tropical region of the world [2]. *P. oleracea* is one of the drug and edible wild plant identified by National Health Ministry. It has been used in traditional Chinese medicine for the treatment of various diseases for thousands of years in China [3-4]. In recent years, scientific research has found that *P. oleracea* contains not only abundant nutrition with contents of fatty acids, vitamins, and other nutrients, but also a variety of bioactive compounds, including flavonoids, alkaloids, polysaccharides, sterols, glycoside, and phenolic compounds [5].

   It is these active ingredients that endow *P. oleracea* with a wide range of biological and pharmacological activities. Among these effective ingredients, flavonoids have received more attention and have become a hotspot in the field of development and application of natural products [6]. Flavonoids have been proved to have a variety of biological and pharmacological activities including anti-microbial (antibacterial, antifungal, and antiviral), antioxidant, anti-inflammatory, anti-cancer, anti-diarrheal activities, etc. in in vitro studies [7-8]. Flavonoids will have a broad prospect in the medical and food industry. The extraction and identification of components is the basis of the application of flavonoids in industry. In this paper, the macroporous adsorption resins (MARs) and high-performance liquid chromatography (HPLC) were carried out to purify and identify flavonoids from *P. oleracea*.

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2 Materials and methods

2.1 Materials and Reagents.

The *P. oleracea* samples were collected from the suburb of Xi'an city. The crude extract of *P. oleracea* flavonoids was obtained by ultrasonic-microwave assisted extraction process [9]. The macroporous adsorption resins, including D101, AB-8, D3520, HPD300, HPD600, Polyamide resins were purchased from Tianjin Bohong Resin Technology Co., Ltd. Corresponding physical and chemical properties were summarized in Table 1. Before use, all resins were soaked with 95 % ethanol for 24 h and then washed with 5 % HCl, 5 % NaOH solution, and double-distilled water successively. The standards of quercetin, kaempferol, luteolin, and apigenin were purchased from Shanghai Jinsui (polyene) Biotechnology Co., Ltd. Methanol used for HPLC was chromatographic pure grade and bought from Tedia (Fairfield, USA). All other reagents used in this study were of analytical grade and purchased from Xi'an Xinglongda Chemical Reagent Co., Ltd.

Table 1. The physical and chemical properties of MARs used in this study

MARs	Polarity	Particle size / nm	Specific surface Area / $m^2\,g^{-1}$)	Pore size / nm	Moisture content / %
D101	Weak	0.25-0.84	500-550	9-10	65-75
AB-8	Weak	0.3-1.25	480-520	13-14	65-75
D3520	None	0.3-1.25	480-520	8.5-9.0	70-80
HPD300	None	0.3-1.2	800-870	5.0-5.5	65-75
HPD600	Strong	0.3-1.2	550-600	8.0	65-75
Polyamide	Strong	-[a]	5-10	-[a]	-[a]

[a] Means not to be collected

2.2 Instruments

The major instruments and their manufacturers used in this study were as follows: Stand-Drying and Air Circulation Oven (DGF-1AB, Tianjin Taisite Instruments Co., Ltd.), Electronic balance (AL204, Shanghai Mettler Toledo instruments Co., Ltd), UV-Vis spectrophotometer (752N, Shanghai Precision Scientific Instrument Co., Ltd.), Rotary Evaporator (R-205, Shanghai Shenshun Biotechnology Co., Ltd.), Water Circulating Multi-Purpose Vacuum Pump (SHZ-D III, Gongyi Yingyu Yuhua Instrument Factory), Ultrasonic Cleaner (SK5200LH, Shanghai Kedao Instrument Co., Ltd.), Multi-function Crusher (XS-04, Shanghai Zhaoshen Technology Co., Ltd.), and High Performance Liquid Chromatography (TechonLogies-1200series, Agilent Technologies Inc.), etc.

2.3 Determination of Flavonoids

Ten microgrammes of rutins were added to a 10 mL volumetric flask, followed by addition of 70% ethanol to the scale. The mixture was shaken and left to stand for 2 hours to be prepared into the standard solution. Using sodium nitrite colorimetry and 510 nm as determination wavelength, the standard curve was obtained and the regression equation was $C=0.0925A+0.0002$ ($R^2=0.9998$). The yield of total flavonoids was calculated according to the formula $Y=10 \times C \times V/m$. Above, Y is the total flavonoids yield (mg/g). C is the concentration of sample solution (g/L) calculated by the standard curve equation. V is the volume of the sample solution (mL) and m is the quality of the sample.

2.4 Static adsorption and desorption experiments

Six kinds of macroporous resins including D101, AB-8, D3520, HPD300, HPD600, Polyamide resins were used respectively in the experiments. Purslane flavonoid extracted solution and 2 g pretreated macroporous resins were added into a 100 mL Erlenmeyer flask. The flask was shaken in a shaker (120 rpm) at room temperature for 24 hours. After adsorption, the solutions were filtered and analyzed. Subsequently, the resins in the flasks were soaked with 100 mL 80% ethanol and shaken for another 24 hours. Then, the desorption solutions were filtered and analyzed. The adsorption and desorption ratios were calculated by the following formula.

Adsorption ratio= $(A_0-A_1)/A_0 \times 100\%$
Desorption ratio= $A_2/(A_0-A_1) \times 100\%$

Where A_0 is the initial solution absorbance value; A_1 is the absorbance value of absorption solution; A_2 is the absorbance value of desorption solution. All adsorption and desorption experiments were carried out in triple and the results were expressed at mean.

2.5 Dynamic adsorption and desorption experiments

Dynamic adsorption and desorption experiments were performed in a glass column (1 cm × 20 cm) packed with the selected optimal resin. Several variables which have possible influences on the adsorption and desorption ratio of purslane flavonoids, such as the sample volume, the concentrations of purslane flavonoid solutions, the pH value, the concentration of elution agent and the dosage of elution agent were systematically investigated.

2.6 Identification and quantification of flavonoid in *P. oleracea* by HPLC

Samples of purslane flavonoids were dried at 60°C in a vacuum drying oven. Samples (1 g, accurately weighed) were dissolved in 10 mL 50% methanol and treated for 35 minutes using ultrasonic waves. The extract was isolated by membrane filtration and the filtrate was evaporated to dryness at 60°C under reduced pressure in a rotary evaporator. The dry extract was dissolved in methanol (10 mL) and filtered through a 0.25 μm syringe filter before injection. 1.4 mg of the quercetin (1.5 mg kaempferol, 1.2 mg luteolin, and 1.5 mg apigenin) was added to 10 mL volumetric flask respectively, followed by addition of methanol to the scale. Then it was diluted to 140 μg/mL, 93.33μg/mL, 70μg/mL, 46.67μg/mL, 18.68μg/mL, respectively to form mixed standard solution. High-performance liquid chromatography (HPLC) was carried out on a C18 column (SHIMADZU-C18, 5 μm, 150mm×4mm) at the temperature of 30°C. The mobile phase was methanol: 0.2% phosphoric acid (45:55). The flow rate was set at 1.0 mL/min, the injection volume was 10 μL, and the UV detector was monitored at 360 nm.

3 Results and discussion

3.1 Optimization of MARs

In the static adsorption and desorption experiments, the adsorption and desorption properties of six kinds of MARs to separate and purify purslane flavonoid were studied at ambient temperature, as shown in Table 2. It could be clearly seen that the adsorption ratio of purslane flavonoid on D101, HPD300, HPD600 and Polyamide resins were considerably higher which were all above 70%, while the desorption ratio of D3520, HPD300, and HPD600 were more than 70%.

Table 2. The results of static adsorption and desorption experiments

MARs	C_0/mg·mL^{-1}	C_1/mg·mL^{-1}	C_2/mg·mL^{-1}	Adsorption ratio /%	Desorption ratio /%
D101	0.04867	0.01353	0.01943	72.20	55.29
AB-8	0.04867	0.01473	0.02128	69.73	62.70
D3520	0.04867	0.01938	0.02220	59.87	75.80
HPD300	0.04867	0.00991	0.03217	79.64	82.99
HPD600	0.04867	0.00954	0.03197	80.40	81.70
Polyamide	0.04867	0.01164	0.02592	76.08	69.99

The chemical characterization and physical properties of MARs such as polarities, specific surface areas, and pore sizes were the important factors for outstanding adsorption/desorption efficiency [10]. The MAR with strong polarity usually displayed excellent performance in purification of flavonoids in the published literatures [11]. The experimental results indicated that the MAR with larger specific surface area (the specific surface areas of HPD300, and HPD600 were 800-870, and 550-600 m^2 g^{-1}, respectively) and smaller pore size (the pore sizes of HPD300, and HPD600 were 5.0-5.5, and 8.0 nm, respectively) would be suitable to separate and purify purslane flavonoid. Generally, it is favorable for the adsorption/desorption process on MAR with larger specific surface area due to more exposed adsorption sites. However, it is disadvantageous for the adsorption on MAR with smaller pore size, because smaller pore size prevents adsorbates entering the pore of MARs. Therefore, taking those factors, the adsorption ratio, and the desorption ratio into considerations, HPD600 was selected as the suitable MAR to purify purslane flavonoid for further research.

In the dynamic adsorption and desorption experiments, the effects of the concentrations of purslane flavonoid solutions, the sample volume, the pH value, the concentration of elution agent and the dosage of elution agent on the adsorption and desorption ratio of purslane flavonoids were carried out in a glass column packed with the selected optimal resin HPD600. The results were shown separately in Fig.1 to Fig.5.
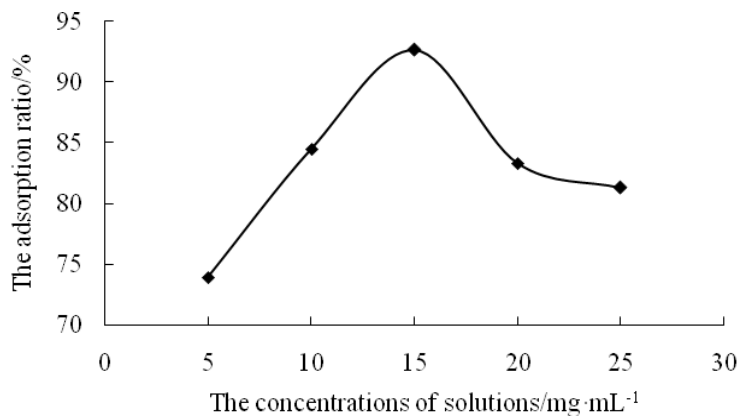
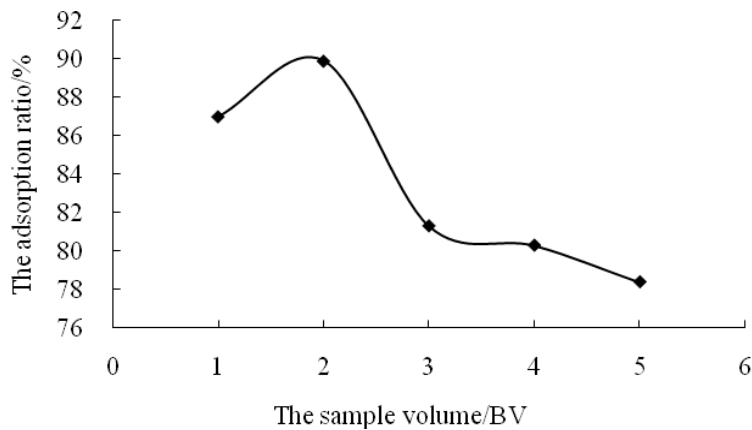**Figure 1.** The effect of the concentrations of solutions on the adsorption ratio

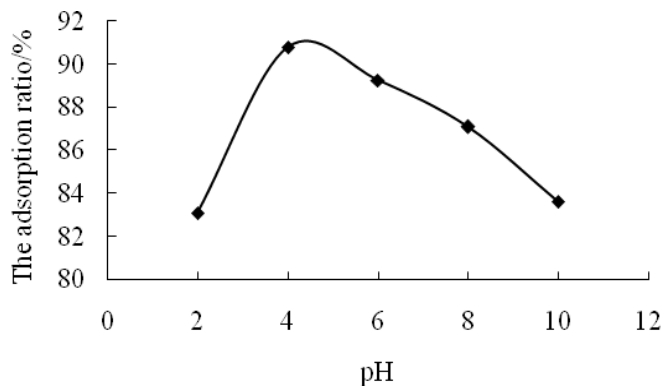**Figure 2.** The effect of the sample volume on the adsorption ratio

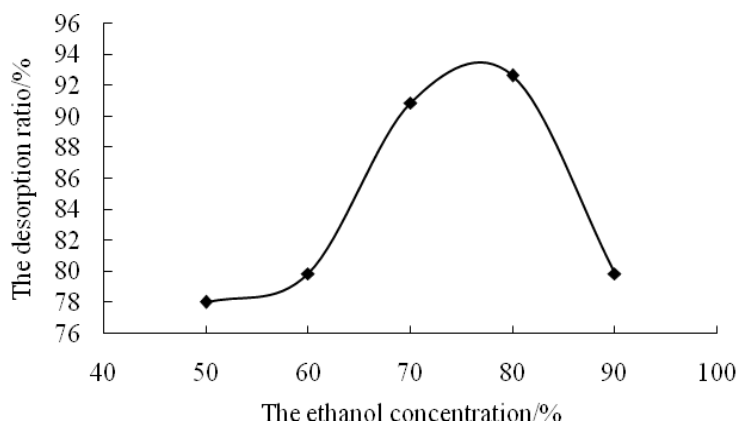**Figure 3.** The effect of the pH value on the adsorption ratio

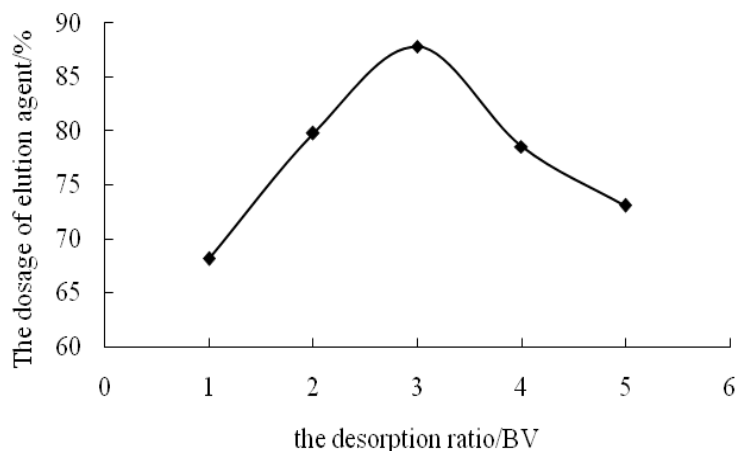**Figure 4.** The effect of the concentration of elution agent on the desorption ratio

Figure 5. The effect of The dosage of elution agent on the desorption ratio

The results of dynamic adsorption and desorption experiments showed that the purslane flavonoid with optimal purity were obtained when the concentration of purslane flavonoid solutions was 15 mg/mL, the sample volume was 2 BV (the bed volume), pH value was 4, the MAR was eluted by 80% ethanol, and the dosage of elution agent was 3 BV. Under this condition, the purity of the total flavonoids was 29.35%, which was about 4 times of that of the former 7.33%.

3.2 Identification and quantitative analysis of flavonoids using HPLC

Chromatographic separation of standard quercetin, kaempferol, luteolin, and apigenin was achieved on the SHIMADZU-C18 column under the conditions described above. The same method was used for the chromatographic separation of the samples extracts. The chromatograms of the standard mixture of the flavonoids and the extract of *P. oleracea* showed in Fig.6 and Fig.7, respectively. It was obvious from these figures that good separation can be achieved within 60 min. Symmetrical, sharp, and well-resolved peaks were observed for the four flavonoids. Retention times for standard quercetin, kaempferol, luteolin, and apigenin were 20.8, 28.0, 39.3, and 47.7 min, respectively. Components identified by direct comparison with standards are indicated.
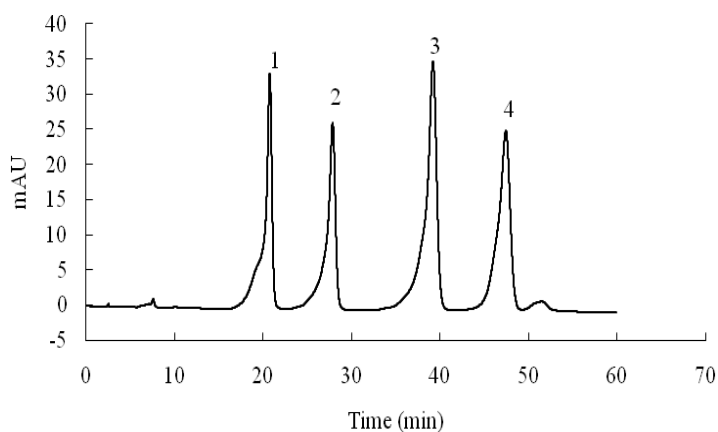
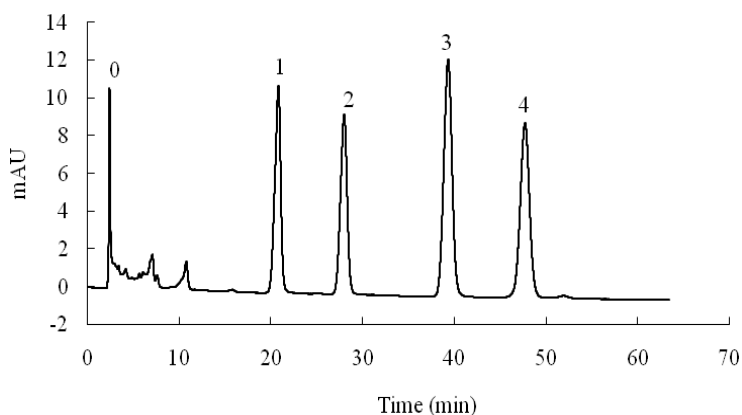**Figure 6.** HPLC chromatograms obtained from a mixed reference standard

Figure 7. HPLC chromatograms obtained from sample extract

Table.3 Quantitative analysis of flavonoids from *P. oleracea* using HPLC

Compound	Retention time (min)	Calibration curves	R^2	Content (μg/mg)
1	20.8	y=5.9672x-55.521	0.9911	0.7799
2	28.0	y=6.0640x-31.667	0.9912	0.7189
3	39.3	y=6.6307x+31.698	0.9911	1.0240
4	47.7	y=6.1454x+23.678	0.9926	0.8668

The flavonoid contents and compositions from *P. oleracea* were determined using HPLC. Separation of four flavonoids on a chromatogram was shown in Fig.6. A calibration curve for HPLC analysis was established using five different concentrations of each flavonoid. The R^2 values ranged from 0.9911-0.9926 (Table 3). Concentrations of flavonoids in *P. oleracea* were determined based on

peak chromatogram areas and a corresponding calibration curve. Quantitative analysis of four flavonoids from *P. oleracea* showed in Table 3. Luteolin (compound 3, 1.0240 µg/mg) was the major flavonoid, followed by apigenin (compound 4, 0.8668 µg/mg), quercetin (compound 1, 0.7799µg/mg), and kaempferol (compound 2, 0.7189 µg/mg).

4 Conclusions

In recent years, there has been a growing interest in extracting bioactive compounds from diverse natural sources. Flavonoid is an ideal natural compound, which has many medical and health function, and has broad application foreground and market demand. In the present study, Purification of purslane flavonoid was performed by Macroporous Adsorption Resin, and the HPLC method had been established to identify and quantify flavonoids in *P. olearca*. The static adsorption and desorption experiments showed that HPD600 resin was quite qualified for the purification of the purslane flavonoids. The optimum purifying process confirmed by the dynamic adsorption and desorption experiments were as follows: the concentration of the purslane flavonoid sample were 15 mg/mL, the sample volume was 2 BV, pH value was 4, the elution agent was 80% ethanol, and the elution volume was 3BV. HPLC analysis results showed that the main components of purslane flavonoid were quercetin, kaempferol, luteolin, and apigenin, whose content were 0.7799µg/mg, 0.7189µg/mg, 1.0240µg/mg, and 0.8668µg/mg respectively. This method is of high accuracy, good separation effect, can be used as an ideal method for the determination of flavonoid components of *P. olearca*. This study will be helpful for the extraction and quality control of purslane flavonoid compound.

Acknowledgements

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