Industrial Crops & Products 121 (2018) 18–25

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop

Effects of different extraction techniques on physicochemical properties and activities of polysaccharides from comfrey (*Symphytum officinale* L.) root☆

Shilun Chen[a], Hongmei Shang[a,b,*], Junyan Yang[a], Ran Li[a], Hongxin Wu[c]

[a] College of Animal Science and Technology, Jilin Agricultural University, Changchun 130118, PR China
[b] Key Laboratory of Animal Nutrition and Feed Science of Jilin Province, Jilin Agricultural University, Changchun 130118, PR China
[c] Grassland Research Institute of CAAS, Hohhot 010010, PR China

ARTICLE INFO

Keywords:
Symphytum officinale L. polysaccharides
Extraction techniques
Antioxidant activity
Antidiabetic activity

ABSTRACT

Comfrey (*Symphytum officinale* L.) has long been used in folk medicine due to its effects of anti-inflammatory and acesodyne. The present study was designed to evaluate the effects of extraction techniques on the physicochemical properties and activities of polysaccharides from comfrey root (CRPs). CRPs were extracted by using four methods including hot water extraction (HW), ultrasonic assistance extraction (UA), enzyme assistance extraction (EA) and enzyme-ultrasonic assistance extraction (EUA). The results showed that CRPs extracted by EUA method (EUA-CRPs) had the highest extraction yield of 24.51%. HPLC analysis presented that the monosaccharide compositions of the four CRPs were indentical, but the monosaccharide content was significantly different. EUA-CRPs had better antioxidant activity, which might be related to its smaller molecular weight and higher content of uronic acid. EUA-CRPs exhibited notable α-glucosidase inhibition activity. The results suggested that enzyme-ultrasonic assistance technology was a good way to extract polysaccharides from comfrey root.

1. Introduction

Polysaccharides are one of the biological macromolecules in the activities of life. Recently, polysaccharides had been attracted widely attention of scientific researchers due to their pharmacological activities, such as antioxidant activity, antiviral activity, antidiabetic activity, antitumor, anti-inflammation, immunoregulation, antilipidemic effect and so on (Han et al., 2016; Kang et al., 2014; Wu et al., 2014).

The extraction techniques had significant effects on the yield, physical properties, chemical properties and biological activities of polysaccharides. At present, the techniques in common use to extract polysaccharides including hot water extraction (HW), ultrasonic assistance extraction (UA), enzyme assistance extraction (EA) and enzyme-ultrasonic assistance extraction (EUA). Each technique has its own advantages and disadvantages when considering the convenience, cost, time consumption, environmental impact, as well as extraction efficiency of polysaccharides. HW is a traditional and environmental safe method, and possesses the advantage of convenience during operation. However, the time consumption and extraction temperature of HW method are usually higher, and polysaccharides extraction yield of HW method is lower than some emerging extraction techniques, such as UA and EA. The extraction yield of polysaccharides can be improved by UA method through generating cavitation effect and more energy during the collapsing process of ultrasonic bubbles (Li and Wang, 2016). EA is considered to be a mild and efficient method to extract polysaccharides, enzymes such as cellulase, papain and pectinase can effectively degrade the cell wall of plant materials, and then promote the release of polysaccharides in the cell (Cheng et al., 2015). In addition, various extracting methods of polyaccharides can cause the difference of biological activities (Wang et al., 2016a). Therefore, it is necessary to compare the polysaccharides extraction techniques for a plant.

Comfrey (*Symphytum officinale* L.) is a perennial herb belonging to Boraginacae. It has long been utilized in folk medicine for the treatment of traumatism, bedsore, sprain and catagma, and has the potent pharmacological actions of anti-inflammatory, acesodyne, granulation promoting and anti-exudation (Grube et al., 2007; Smith and Jacobson,

Abbreviations: ABTS, 2,2-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid); CRPs, polysaccharides from comfrey root; DPPH, 1,1-diphenyl-2-picrylhydrazyl; EA, enzyme assistance extracting technology; EA-CRPs, CRPs extracted by EA method; EUA, enzyme-ultrasonic assistance extracting technology; EUA-CRPs, CRPs extracted by EUA method; HPLC, high performance liquid chromatographic; HW, hot water extracting technology; HW-CRPs, CRPs extracted by HW method; UA, ultrasonic assistance extracting technology; UA-CRPs, CRPs extracted by UA method

☆ The work was performed in College of Animal Science and Technology, Jilin Agricultural University. Address: No. 2888 Xin-Cheng Street, Changchun 130118, Jilin, PR China.

* Corresponding author at: College of Animal Science and Technology, Jilin Agricultural University, No. 2888 Xin-Cheng Street, Changchun 130118, Jilin, PR China.
E-mail address: shangmei2000@163.com (H. Shang).

https://doi.org/10.1016/j.indcrop.2018.04.063
Received 6 February 2018; Accepted 21 April 2018

2011). The pharmacological function of comfrey is connected with the critical chemical components in its root, such as ureidohydantoin, rosmarinic acid and polysaccharides (Andres et al., 1989). The extraction efficiency of polysaccharides from comfrey root (CRPs) has great effects on the utilization of comfrey. Nevertheless, there is little report related to the extraction methods of CRPs.

The present study was designed to evaluate the extraction yields, physicochemical properties and activities of CRPs extracting by four techniques including HW, UA, EA and EUA. The physicochemical characteristics of CRPs were determined on the base of chemical composition, pH, solubility, molecular weight and monosaccharide composition. The activities of the four CRPs were evaluated based on the determination of antioxidant activity and antihyperglycemic activity.

2. Materials and methods

2.1. Materials and chemicals

Comfrey in bloom stage was harvested to obtain the fresh root in Changchun (Jilin Province, China). Roots were dried at 50 °C after being cut into 5 mm slices, and then ground into powders (1 mm). The lipids of the powders were extracted with 85% ethanol (v/v) for a day. The residue was dehydrated at 50 °C and prepared for extracting CRPs. Chemical reagents including 2,2-azino-bis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), cellulase, papain, pectinase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), vitamin C and monosaccharide standards were purchased from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade.

2.2. CRPs extraction

Comfrey root powder (25 g) was mixed with distilled water (375 mL) and extracted by using four techniques (HW, UA, EA and EUA), and the corresponding polysaccharides obtained were named as HW-CRPs, UA-CRPs, EA-CRPs and EUA-CRPs, respectively. Each method was performed in triplicate. The extraction of HW-CRPs was performed in a water-bath at 90 °C for 70 min. The extraction of UA-CRPs was performed in an ultrasonic bath (KQ-100KDE, Kunshan Ultrasonic Instrument co., LTD, Kunshan, Jiangsu, China) with a power of 100 W at 50 °C for 70 min. The complex enzyme used to extract EA-CRPs and EUA-CRPs was composed of cellulose, papain and pectinase in the ratio of 1:1:1, and the quality fraction of each enzyme in the extraction solvent was 1%. The extraction of EA-CRPs was carried out with the complex enzyme mentioned above at 50 °C for 70 min. The extraction of EUA-CRPs was firstly performed with the complex enzyme at 50 °C for 35 min, then in an ultrasonic bath with a power of 100 W at 50 °C for 35 min.

After extraction, the extracting solution of HW-CRPs, UA-CRPs, EA-CRPs and EUA-CRPs was centrifuged at 3000 rpm for 15 min. Then one-quarter volume of the primary supernatant was obtained by vacuum concentration at 60 °C. The starch fraction in the concentrated solution was removed by α-amylase at 60 °C. After the starch removal process, the concentrated solution was added into a few drops of hydrogen peroxide to decolorization. Four times volume of ethanol was mixed with the concentrated extraction solution to precipitate CRPs (4 °C, 12 h). Subsequently, the precipitates were obtained by centrifugalization at 3000 rpm for 15 min, and washed successively with ether, absolute ethanol and acetone. The extracts were dissolved with distilled water and got rid of protein by Sevag reagent (chloroform: normal butanol, 4:1, v/v) method. After dialysis (MWCO 1400 Da, Union Carbide) the extraction solution, the four CRPs were obtained by freeze drying. The CRPs yield of the four extraction techniques was calculated by using the following Eq. (1):

$$\text{CRPs yield}(\%) = \frac{W_{CRPs}}{W_{\text{sample}}} \times 100 \quad (1)$$

where W_{CRPs} and W_{sample} are the weights of CRPs and comfrey root powder used for extracting CRPs, respectively.

2.3. Preliminary purification of CRPs

Before determination the physicochemical properties and biological activities of CRPs obtained by four extraction methods, the CRPs was preliminarily purified by using DEAE-52 cellulose (3.5 cm × 20 cm) column, and eluted first with distilled water, then with a linear gradient (0–1 mol/L) of sodium chloride solution at a flow rate of 2 mL/min. The eluent (8 mL/tube) was collected automatically, and the polysaccharides content of each tube was monitored by the phenol-sulfuric acid method (Dubois et al., 1956). The fraction (one fraction eluting with distilled water) containing carbohydrates were collected and concentrated to evaluate the physicochemical properties and biological activities of CRPs.

2.4. Physicochemical properties of CRPs

2.4.1. Chemical composition analysis of CRPs

The chemical compositions, such as the content of total polysaccharides, uronic acid and protein of CRPs were analyzed by colorimetry method. The content of total polysaccharides in CRPs was measured using phenol-sulfuric acid method (Dubois et al., 1956). The content of uronic acid in CRPs was determined using m-hydroxybiphenyl method (Blumenkrantz and Asboe-Hansen, 1973). Protein content in CRPs was determined using Bradford's method (Bradford, 1976). The content of moisture in CRPs was determined based on the report of Kong et al. (2015).

2.4.2. pH and solubility determinations of CRPs

A pH meter (PHS-3C-02, Quzhou Aipu Metering Instrument co., LTD, Quzhou, China) was used to determine the pH values of CRPs at the concentration of 2 mg/mL. The solubility of CRPs was measured at 20, 40, 60, 80, 100 °C according to the method as described by Shang et al. (2017).

2.4.3. Molecular weight distribution determination of CRPs

The molecular weight of CRPs was determined by gel filtration chromatography on Sepharose CL–6B column (2.6 cm × 100 cm) using distilled water as the eluant at a flow rate of 0.9 mL/min. The eluent (4.05 mL/tube) was collected and monitored for carbohydrate content using phenol-sulfuric acid method (Dubois et al., 1956). Series of dextran (T-10, T-40, T-70 and T-500) were used as standards for calibration.

2.4.4. Monosaccharide composition analysis of CRPs

The monosaccharide composition of CRPs obtained by four extraction methods was analyzed using high performance liquid chromatographic (HPLC) by following methods as described previously (Chai and Zhao, 2016; Ye et al., 2016) with some modifications. The CRPs sample (2 mg) was hydrolyzed with 0.5 mL trifluoroacetic acid (TFA, 2 mol/L) in a sealed flask fulfilled with N_2 at 120 °C for 2 h. After hydrolysis, the excess TFA in the system was removed by repeated co-evaporation with ethanol at 45 °C. Subsequently, dry hydrolysate samples of CRPs or monosaccharide standard were added 0.5 mL methanol solution of 1-phenyl-3-methyl-5-pyrazolone (PMP, 0.5 mol/L) and 0.5 mL aqueous solution of NaOH (0.3 mol/L) for derivatization at 70 °C for 30 min. Then, the mixture solution was centrifuged at 10 000 rpm for 5 min. The supernatant was mixeded with 0.05 mL HCl (0.3 mol/L), the reaction mixture was extracted with chloroform for three times to remove the excess PMP. The aqueous layer was filtered through a 0.22 μm membrane and analyzed by a Shimadzu 2010AHT HPLC system (SHIMADZU, Kyoto, Japan) equipped with an UV detector (245 nm). Amethyst C18 column (4.6 mm × 250 mm, 5 μm, Sepax, Delaware, USA) was used and the column oven was kept at 25 °C. The injection