Effects of Extraction Methods on Antioxidant Activities of Polysaccharides from the *Curcuma phaeocaulis* Rhizomes

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ABSTRACT — ***The polysaccharides were extracted from the Curcuma phaeocaulis rhizomes by hot water extraction, ultrasonic-assisted extraction, microwave-assisted extraction and enzyme extraction. The physicochemical properties of C. phaeocaulis polysaccharides were determined by chemical composition analysis. Further, the antioxidant activities were studied via different methods in vitro, including DPPH assay, hydroxyl radical assay and superoxide anion radical assay. The results of the physicochemical property assay showed that the carbohydrate content and uronic acid content of CPP-H, CPP-U, CPP-M, and CPP-E were different, and the protein content was similar. Antioxidant assay indicated that four polysaccharides exhibited significant antioxidant activities in a dose-dependent manner.***

Key words — *Curcuma phaeocaulis* Velton, Polysaccharides, Extraction, Antioxidant activities

1. INTRODUCTION

Rhizomes associated with the root system of the perennial herb *Curcuma phaeocaulis* Valeton (Family: Zingiberaceae) are recorded officially in the Chinese Pharmacopoeia (2010) because of their medicinal properties. They are one of the most common folk herbal remedies known by the popular name Rhizoma Curcumae, and have been used to reduce pain and help digestion prescribed in the BenCaoGangMu (Li, 1975). This plant is a congener of the herb whose rhizomes are the source of turmeric (*C. longa*), also well known for its health benefits (Ravindran et al., 2007). In recent clinical research, Rhizoma Curcumae has been prescribed regularly for anti-inflammation and tumor therapy because of its bioactive constituents (Makabe et al., 2006; Chen et al., 2011; Gonda et al., 1992), such as curcumins, volatile oil, and polysaccharides.

Polysaccharides play important roles in the growth and development of living organisms, and those found in extracts from animals, plants, and microorganisms have been studied widely in recent years for their unique biological, chemical and physical properties (Schepetkin and Quinn, 2006). To date, several studies have reported that plant polysaccharides show strong antioxidant abilities, which gives them great potential for use in combating free radicals. For example, water-soluble polysaccharides obtained from *Psidium guajava*, *Litchi chinensis*, and *Acanthopanax senticosu* exhibit strong activity for scavenging free radical (Chen and Yen, 2007; Kong et al., 2010; Chen et al., 2011).

The most commonly used method for polysaccharides extraction is hot water extraction (HWE). It is the classical approach for extraction polysaccharides and it has been the main method used in recent research. The yield of HWE largely depends on extraction time and temperature (Bendjeddou et al., 2003). In order to increase the yield, other new methods are employed to extract polysaccharides, ultrasonic-assisted extraction (UAE) has been proved to be a desirable method of extraction with many advantages, such as increasing extraction yield, reducing solvent usage, economizing power consumption and shortening extraction duration (Zhao et al., 2013). Microwave-assisted extraction (MAE) are composed of electric and magnetic fields and thus represent electromagnetic energy with the main advantages of reducing both extraction time and solvent consumption (Wang et al., 2010). Enzyme-assisted extraction (EAE) possesses the advantage of being environmentally friendly, highly efficient, and easily operated owing to the relatively mild reaction conditions (Zhu et al., 2014). The better extraction efficiency by UAE, MAE, and EAE is mainly attributed to the mechanical effects or catalytic action, which may influence the structure of polysaccharides.

In this paper, we extracted four polysaccharides from *C. phaeocaulis* via the method of HWE, UAE, MAE, and EAE. The preliminary structural characterization and antioxidant activities of four polysaccharides were estimated by chemical composition analysis and antioxidant assays, which include DPPH assay, hydroxyl radical assay, and superoxide anion radical assay. The aim of this research was to investigate the influence of different extraction methods on the physicochemical properties and antioxidant activities of polysaccharides from *C. phaeocaulis.*

2. MATERIALS AND METHODS

2.1 Materials

Rhizomes were obtained from roots of *C. phaeocaulis* plants growing in Shuangliu, Sichuan Province, China and identified by Prof. R Yang of Sichuan Agricultural University, China. The materials were washed thoroughly with water, dried at 60℃, pulverized in a powerful mill (FW177, Taisite Instrument Co., Ltd., Tianjin, China), and screened through an 80 mesh sieve. The powder of the materials was stored in a desiccator at room temperature.

Ethanol, phenol and sulphuric acid were purchased from the Chengdu Kelong Chemical Factory (Chengdu, China). 2,2-Diphenyl-1-picryl-hydrazy (DPPH), 1,10-phenanthroline, dihydro nicotineamide adenine dinucleotide (NADH), and nitroblue tetrazolium (NBT) were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Ascorbic acid (Vc) was purchased from the Sinopharm Chemical Reagent Co. (Beijing, China). All other chemicals were analytical regents.

2.2. Extraction

The dried *C. phaeocaulis* powder was extracted in a Soxhlet apparatus with petroleum ether at 60–90℃ and ethanol for 5 h to fully defat and remove some small molecules. After the powder dried, the crude polysaccharides were extracted by different methods as described previously with minor modifications, including HWE, UAE, MAE, and EAE, and the polysaccharides extracted from *C. phaeocaulis* were designated as CPP-H, CPP-U, CPP-M, and CPP-E, respectively. CPP-H was extracted with hot water in a ratio (material to water) of 1:30 at 85℃ for 3 h. CPP-U was extracted by the ultrasonic-assisted method in a ratio of 1:30 with a power of 250 W in an ultrasonic bath (KQ-400GKDV, Kunshan Ultrasonic Instrument Co., Ltd., China) and extraction time of 30 min. CPP-M was extracted by the microwave-assisted method in a ratio of 1:30 for 1 h. The extraction process was performed in an oven model MDS-2000 (CEM Corporation, Matthews, NC). CPP-E was extracted by the enzyme method in a ratio of 1:30 with cellulase at 60℃ for 1 h. After extraction, all the extraction solutions were isolated by centrifugation (5 000 rpm, 10 min). The supernatants were collected and concentrated in a rotary evaporator, respectively. The protein in the concentrate was removed using the Sevage method (DuBois et al., 1956). Anhydrous ethanol was added to reach a final concentration of 80% (*v/v*) and the mixture was kept overnight at 4℃. The precipitate was collected and lyophilized. Then, four polysaccharides (CPP-H, CPP-U, CPP-M, and CPP-E) were obtained.

2.3. Physicochemical property analysis

The physical characteristics were analyzed by color and texture observation. The carbohydrate content was determined by phenol–sulfuric acid colorimetric method using d-glucose as the standard (DuBois et al., 1956). The uronic acid content was measured by vitriol-carbazole method using glucuronic acidas the standard (Bitter and Muir, 1962). The protein compounds content was estimated by the Coomassie Brilliant Blue reaction (Bradford, 1976).

2.4 Antioxidant activity

2.4.1 Scavenging activity of DPPH radical

The DPPH free radical scavenging activity was determined based on the method described in the literature (Yang et al., 2009) with minor modifications. Polysaccharide samples were dissolved in distilled water to form sample solution in final concentrations of 0, 0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL, respectively. 2 mL of the sample solution was mixed with 2 mL of 0.2 mmol/L DPPH ethanol solution. The reaction solution was incubated for 60 min at room temperature, and the absorbance of the mixture was measured at 517 nm using the spectrophotometer (UV-1750, Shimadzu). The scavenging activity on DPPH radical was calculated by the following equation:

$$\text{Scavenging activity (\%)} = (1\text{-}A_{\text{sample}}/A_{\text{DPPH}}) \times 100$$

Where A_{sample} was the absorbance of a mixture of sample and DPPH solutions, and A_{DPPH} was the absorbance of the control reaction in which the sample was replaced by ethanol.

2.4.2 Scavenging activity of hydroxyl radical

The antioxidant activity of scavenging hydroxyl radical was determined according to the Fenton reaction (Wu et al., 2012). The samples were firstly dissolved in deionized water for a series of different concentration solutions (0, 0.25, 0.5, 0.75, 1.0 and 1.25 mg/mL). Then, the sample (1 mL) mixed with phenanthroline (5 mM, 1 mL), phosphate buffer (50 mM, PH 7.4), ferrous sulfate (7.5 mM, 0.5 mL), and H_2O_2 (0.1 %, 0.5 mL) at 37℃ for 1 h. The absorbance of the mixture was measured at 510 nm with a spectrometer (UV-1750; Shimadzu, Kyoto, Japan). The antioxidant activity of hydroxyl radical scavenging effect was calculated with the following equation:

$$\text{Scavenging activity (\%)} = (A_{sample} - A_{blank})/(A_{control} - A_{blank}) \times 100$$

Where $A_{control}$ was the absorbance of the control (blank, without of H_2O_2), A_{blank} was the absorbance in the absence of

sample, and A_{sample} was the absorbance in the presence of sample.

2.4.3 Scavenging activity of superoxide anion radical

The superoxide radical scavenging activity was conducted according to the previously described method (Liu et al., 2009) with some modifications. The reaction solution consisted of 1.0 mL Tris–HCl (16 mmol/L, containing 468 μmol/L NADH, pH 8.0), 1.0mL Tris–HCl (16 mmol/L, containing 468 μmol/L NBT, pH 8.0), and 1.0 mL Tris–HCl (16 mmol/L, containing 60 μmol/L PMS, pH 8.0). Before addition of 1.0 mL sample solution (0, 0.25, 0.5, 0.75, 1.0, and 1.25 mg/mL), the mixture was incubated at 20℃ water bath for 20 min. Absorbance at 560 nm was measured after 5 min at 25℃. Vc was used as a control. The capability to scavenge the superoxide radical was calculated by using the following equation:

Scavenging activity (%) = $(1\text{-}A_{sample}/A_{blank}) \times 100$

Where A_{sample} was the average absorbance value of the sample, and A_{blank} was the average absorbance in the absence of sample.

3. RESULTS AND DICUSSION

3.1 Chemical compositions

Table 1 lists the physicochemical properties of the four polysaccharides. The colors of the four polysaccharides were both brown. The texture of the four polysaccharides also showed different characteristics. CPP-H, CPP-U, and CPP-M were both tight, whereas CPP-E was loose.

CPP-H and CPP-E showed significantly higher carbohydrate contents than CPP-U and CPP-M. There was no significant difference in the protein contents among four polysaccharides. But the uronic acid contents were significant difference followed the order: CPP-H > CPP-E > CPP-U > CPP-M. The differences may be related to the type of extraction method.

Table 1 Physicochemical properties of CPP-H, CPP-U, CPP-M and CPP-E

Samples	CPP-H	CPP-U	CPP-M	CPP-E
Color	Brown	Brown	Brown	Brown
Texture	Tight	Tight	Tight	Loose
Carbohydrate (%)	59.74±1.02	19.55±0.86	17.18±1.12	43.84±1.15
Protein (%)	2.96±0.32	2.55±0.19	1.94±0.15	3.31±0.28
Uronic acid (%)	6.84±0.24	4.06±0.13	3.18±0.16	4.36±0.22

Each value is expressed as mean means ± standard deviation (n = 3).

3.2 Antioxidant activity

3.2.1 Scavenging activity of DPPH radical

DPPH, a stable free radical, is used to evaluate free radical scavenging effect of natural compounds. DPPH shows maximum absorption at 517 nm in ethanol (Kedare and Singh, 2011). The activity to act as a donor of hydrogen atoms in the transformation of the DPPH radical to its reduced form was investigated for four polysaccharides (CPP-H, CPP-U, CPP-M, and CPP-E). The results were shown in Fig. 1, CPP-H, CPP-U, CPP-M, CPP-E and V_C possessed DPPH radical scavenging activities in a concentration dependent manner, and the abilities were followed by Vc, CPP-U, CPP-H, CPP-M, and CPP-E. At 1 mg/mL, the scavenging activities were 83.32%, 86.64%, 79.14% and 74.45% for the CPP-H, CPP-U, CPP-M, and CPP-E, respectively. The IC_{50} values were 0.15, 0.19, 0.21, and 0.24 mg/mL for CPP-H, CPP-U, CPP-M, and CPP-E. The results indicated that CPP-U could supply more hydrogen atoms than other polysaccharides and had a strong scavenging effect on DPPH radical.

3.2.1 Scavenging activity of hydroxyl radical

The hydroxyl radical is one of the important reactive oxygen species, which can be formed in biological cells via the Fenton reaction and cause the general processes of aging and tissue damage (Zhong et al., 2010; Mårtensson et al., 1990). As shown in Fig. 2, all samples exhibited obvious scavenging activity on hydroxyl radical in a dose-dependent pattern at all concentrations. CPP-H had the stronger activity than CPP-U, CPP-M, and CPP-E. At the 1.25 mg/mL, the scavenging activities of four polysaccharides were 82.13%, 77.58%, 66.73% and 44.39%, respectively. The IC_{50} values were 0.35, 0.45, 0.42, and 0.56 mg/mL, respectively for CPP-H, CPP-U, CPP-M, and CPP-E.

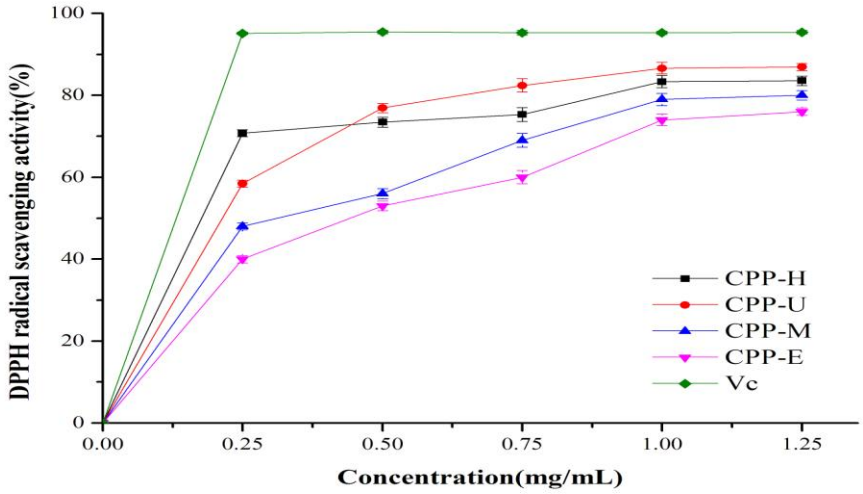

Fig. 1 DPPH Radical Scavenging Activities of CPP-H, CPP-U, CPP-M, CPP-E, and Vc.

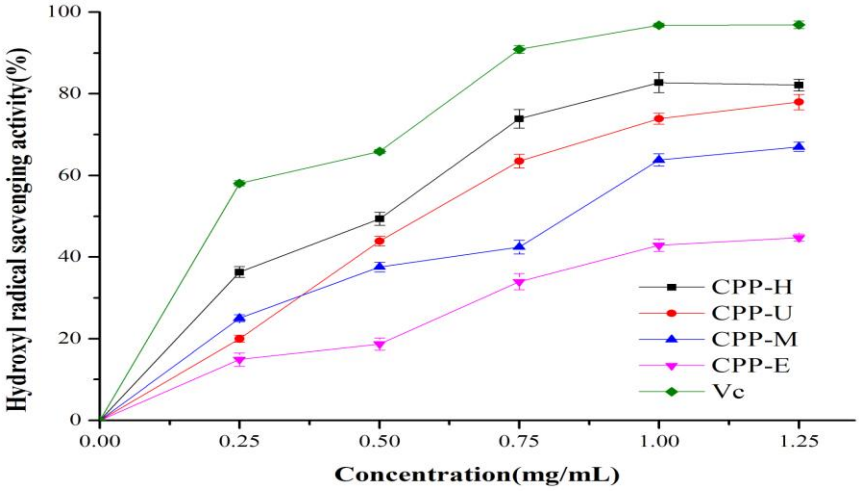

Fig .2 Hydroxyl Radical Scavenging Activities of CPP-H, CPP-U, CPP-M, CPP-E, and Vc.

3.2.3 Scavenging activity of Superoxide anion radical

Superoxide radicals are an initial free radical and one of the precursors of singlet-oxygen and hydroxyl radicals, which possess even greater oxidative and oleophilic ability. Superoxide radicals are highly toxic and generated by numerous biological and photochemical reactions (Gan and Latiff, 2011), so their removal is essential. Four polysaccharides are capable of scavenging superoxide radical in a dose-dependent manner. As shown in Fig. 3, the scavenging effects of CPP-H, CPP-U, CPP-M, and CPP-E were 84.33, 71.23, 55.63, and 38.29%, respectively at a concentration of 1.25 mg/ml. IC_{50} values of CPP-H, CPP-U, CPP-M, and CPP-E were 0.36, 0.51, 0.46, and 0.43 mg/ml, respectively. The result proved that four polysaccharides had significant effect on scavenging superoxide radical and the CPP-H had the highest activity among the four polysaccharides.

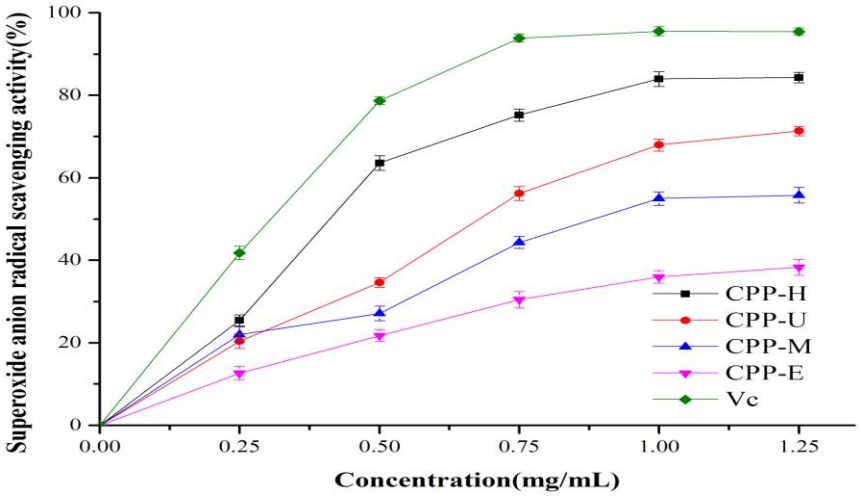

Fig.3 Superoxide Anion Radical Scavenging Activities of CPP-H, CPP-U, CPP-M, CPP-E, and Vc

4. CONCLUSION

In this study, four polysaccharides (CPP-H, CPP-U, CPP-M, and CPP-E) from the herbs rhizomes of *C. phaeocaulis* were obtained using hot water extraction, ultrasonic-assisted extraction, microwave-assisted extraction, and enzyme extraction, respectively. Structural characterizations were conducted using physicochemical property. The physicochemical property indicated that the carbohydrate content and uronic acid content of CPP-H, CPP-U, CPP-M, and CPP-E were different, and the protein content was similar. Moreover, *C. phaeocaulis* polysaccharides showed significant free radical scavenging activities in a dose-dependent manner. CPP-H and CPP-U showed significantly higher scavenging activity on DPPH assay, hydroxyl radical assay, and superoxide anion radical assay than CPP-M, and CPP-E. All the differences should be attributed to the different extraction methods. In conclusion, polysaccharide from *C. phaeocaulis* could be explored as potential natural antioxidant in functional foods and medicine industry.

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