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Short communication

Effect of extraction methods on property and bioactivity of water-soluble polysaccharides from *Amomum villosum*

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ABSTRACT

In the present study, effect of different extraction methods on property and bioactivity of water-soluble polysaccharides (WSP) from the seeds of *Amomum villosum* were investigated. Firstly, four different extraction methods were used to extract WSP, which include hot water extraction (HWE), ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE) and enzyme-assisted extraction (EAE). As a result, four WSP samples, WSP_H, WSP_U, WSP_M and WSP_E were acquired. Then, the difference of four WSP samples in yield, characterization and antioxidant activities *in vitro* were further compared. Experimental results showed that the four WSP samples had the same monosaccharide composition, but mere difference in the content; they all had typical IR spectra characteristic of polysaccharides. WSP_U contained the highest contents of uronic acid and sulfate. The yield of WSP_U was the highest and its antioxidant activity was the best. These results suggested that ultrasonic-assisted extraction was the best extraction method for WSP.

1. Introduction

In recent decades, lots of interests have been generated in polysaccharides due to their diverse and potentially significant pharmacological activities (Li, Li, & Zhou, 2007; Raveendran Nair et al., 2004). The seeds of *Amomum villosum* (Zingiberaceae) has been used for treatment of gastrointestinal diseases in China for hundreds of years (Zhang, Liu, & Xu, 2005). It is rich in polysaccharides that may contribute to antioxidant and anticancer activities (Zhang, Li, Xiong, Jiang, & Lai, 2013).

Various novel techniques for extraction of polysaccharides have been developed currently, including microwave-assisted extraction (MAE) (Wang et al., 2010), enzyme-assisted extraction (EAE) (Yin, You, & Jiang, 2011) and ultrasonic-assisted extraction (UAE) (Hromadkova, Ebringerova, & Valachovic, 1999). Each extraction method has its own advantages and disadvantages. However, little attention has been devoted to comparison of different extraction methods to find the optimal one.

Therefore, in this study, effects of different extraction methods on yield, characterization and antioxidant activities of WSP were investigated to obtain optimal one. Firstly, the seeds of *A. villosum* were extracted to gain polysaccharides by using hot water extraction (HWE), UAE, MAE and EAE. Then the different characterizations of four WSP samples were analyzed by Fourier transform-infrared spectroscopy (FT-IR), gas chromatography (GC) and high-performance liquid chromatography (HPLC). Finally, antioxidant activities *in vitro* of crude WSP were compared.

2. Materials and methods

2.1. Reagents and materials

The seeds of *A. villosum* were purchased from Yangchun Amomum plantation in Guangdong, China. Cellulase (15,000 U/g), pectinase (20,000 U/g) and papain (6000 U/mg) were acquired from Sinopharm Chemical Reagent Beijing Co., Ltd. Nitroblue tetrazolium, reduced nicotinamide adenine dinucleotide and phenazine methosulfate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Other reagents were of analytical grade. All the experiments data were an average of three parallel replicates.

2.2. Pretreatment of the seeds of A. villosum

Pretreatment method was according to the reported method (Zhang et al., 2013).

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Table 1
Comparison of the yields and basic extraction conditions of polysaccharides of the four methods.

Method	HWE	UAE	MAE	EAE
Compound enzyme amount (%)				2
Extraction pH				4.3
Extraction time (min)	133	71	20	124
Extraction temperature (°C)	95	79	80	54
Ratio of water to raw material (ml/g)	33	40	10	52
WSP yield (%)				
Predicted	10.2	13.2	11.3	11.6
Experimental	10.3 ± 0.26^{a}	13.1 ± 0.3^{b}	11.4 ± 0.3^{c}	11.7 ± 0.3^{c}

Different alphabets (a–c) in superscript for each yield denote significant difference ($P < 0.05$).

2.3. Extraction of WSP by various methods

Extraction by HWE was based on the reported method with some modifications (Jiang, Wang, Liu, Gan, & Zeng, 2011). The pretreated dry powder was extracted using designed ratio of water to raw material, extraction time and extraction temperature. Experimental conditions were optimized by Box–Behnken Design based on single-factor experiment. Protein was removed by Sevag Reagent (Yin et al., 2011), the polysaccharide content of the crude WSP was measured by the phenol–sulfuric acid method using glucose as a standard substance to obtain the polysaccharide yield (Zhang et al., 2013).

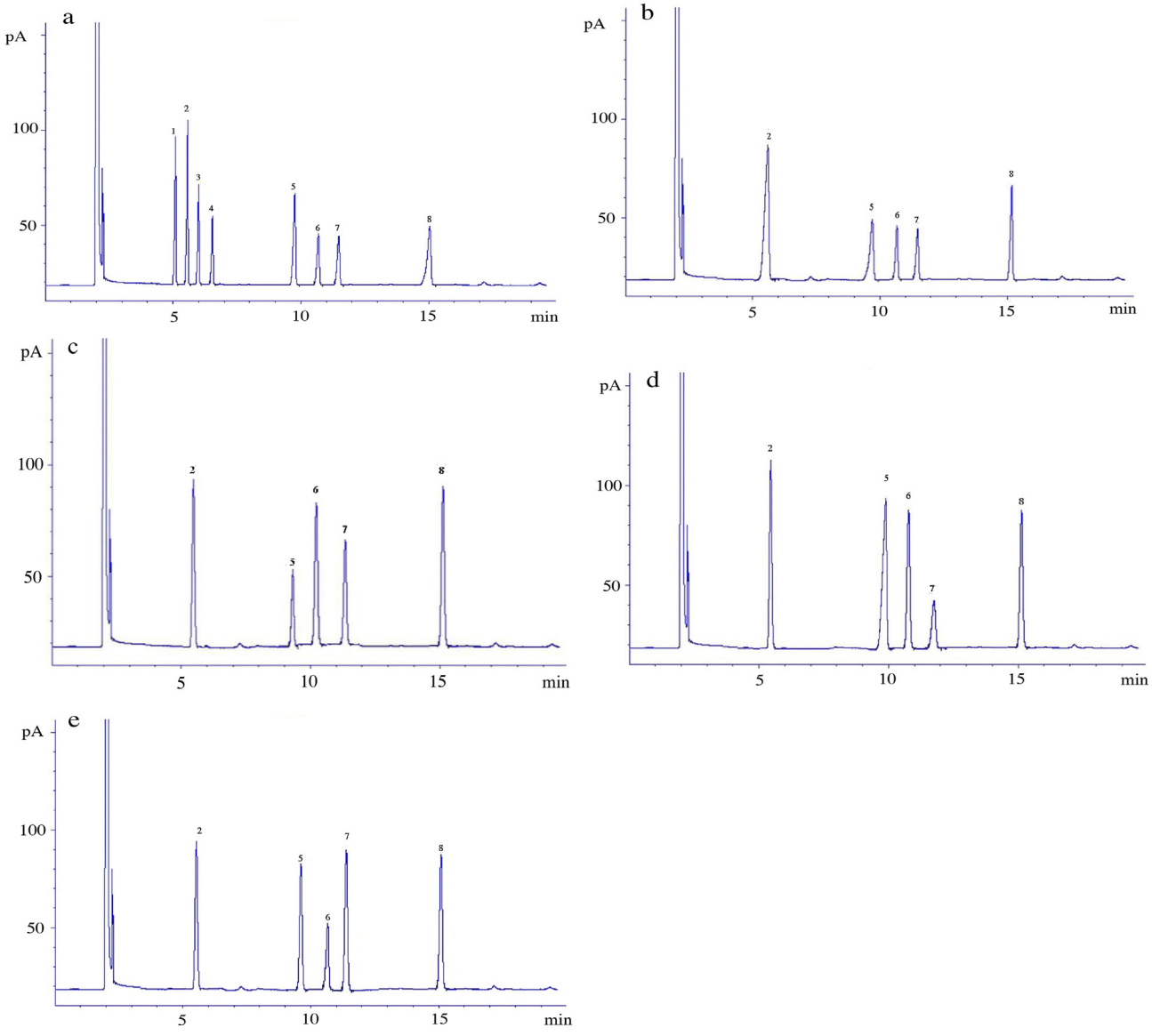

Fig. 1. GC spectra of sample reference (a), WSP_H (b), WSP_U (c), WSP_M (d) and WSP_E (e) (1, Rhamnose; 2, Arabinose; 3, Fucose; 4, Xylose; 5, Mannose; 6, Glucose; 7, Galactose; 8, Inositol.).

Table 2
Monosaccharide content of WSP_H, WSP_U, WSP_M and WSP_E.

Samples	Sugar component				Carbohydrate (%)	Protein (%)	Uronic acid (%)	Sulfuric radical (%)
	Arabinose	Mannose	Galactose	Glucose				
WSP_H	32.35	21.61	22.18	23.86	82.45	2.17	1.69	1.17
WSP_U	25.28	15.92	27.31	31.49	76.38	3.63	2.23	2.41
WSP_M	25.39	26.75	15.54	32.32	67.82	3.16	1.94	2.33
WSP_E	23.43	23.43	31.38	21.76	73.50	3.08	2.17	2.35

EAE, MAE and UAE were operated according to the reported method (Wang et al., 2010; Yin et al., 2011; Zhang et al., 2013), respectively. Their following procedures were consulted for HWE.

2.4. Preliminary characterization of different WSP

The content of protein was determined by the method of Bradford using bovine serum albumin as the standard, the content of uronic acid was determined according to the method of Blumenkrantz and Asboe-Hansen by using D-glucuronic acid as the standard, the content of sulfate radical was determined according to the reported methods (Zhang et al., 2013). The molecular weight of WSP was determined by HPLC, monosaccharide compositions of WSP was analyzed by GC and infrared spectroscopy analysis were executed as the reported method (Zhang et al., 2013)

2.5. Determination of antioxidant activities in vitro of WSP

The superoxide radical scavenging activity, chelating of ferrous ions and reductive potential of WSP samples were determined in accordance with the previous reported methods (Li et al., 2007; Liu, Wang, Xu, & Wang, 2007; Zhang et al., 2013).

3. Results and discussion

3.1. Effect of extraction methods on yield of WSP

The WSP yield and optimized extraction conditions of HWE, UAE, MAE and EAE were shown in (Table 1). WSP_U yield was highest. WSP_H showed the lowest yield with the longest extraction time. MAE had higher extraction efficiency, but it requires higher equipment cost and operator skill.

3.2. Preliminary characterization of different WSP

As shown in (Table 2), WSP_U contained the highest contents of uronic acid and sulfate; these may indicate that WSP_U has good antioxidant activity.

The monosaccharide composition of WSP samples were determined by GC, and the monosaccharides in WSP hydrolyte were identified by comparing the retention times with those of standards (Fig. 1a–e) and the results were presented in Table 2. Four WSP samples have the same monosaccharide composition; only slight different in content.

As shown in (Fig. 2), there was a strong and broad peak around the 3400 cm^{-1} of WSP infrared spectroscopy, which belongs to the hydroxyl groups stretching vibration. The weak peaks around 2930 cm^{-1} and 1320 cm^{-1} were assigned to the C—H asymmetric stretching vibration. The bands around 1612 cm^{-1} was the C=O asymmetric stretching vibration absorption peak, the bands at 1300–1400 cm^{-1} was the C=O symmetry stretching vibration absorption peak, these mean four WSP samples contained carboxyl group (Cerna et al., 2003). This result was consistent with the results of chemical methods (four WSP samples contain uronic acid). All these showed that four WSP samples were polysaccharides.

The calibration curve of dextrans was plotted as the molecular weights on a log scale versus the retention time, then got a standard regression curve equation, $\log Mw = -0.41t + 7.263$, $r = 0.9945$ (t was the retention time, min). The average molecular weights for WSP_H, WSP_U, WSP_M and WSP_E were determined to be 64.7, 55.8, 60.6 and 49.3 kDa, respectively.

3.3. Antioxidant activity in vitro of different WSP

3.3.1. Superoxide radical scavenging activity of WSP

Superoxide radical is a reduced form of molecular oxygen created by receiving one electron from mitochondrial electron transport systems. It can be decomposed to some reactive oxygen species. Then formation of superoxide radical can induce oxidative damage in lipids, proteins and DNA. Thus, the scavenging of superoxide radical is very important to antioxidant work.

The scavenging activities of WSP on superoxide radical were shown in Fig. 3a WSP_U showed stronger superoxide radical scavenging effect than WSP_H, WSP_M and WSP_E. The strong scavenging activity of WSP_U might be attributed to its high contents of uronic acid and sulfuric radical.

3.3.2. Chelating ability of ferrous ion

Fig. 3b shows chelating activity of WSP to Fe^{2+}. At a concentration of 0.05–2 mg/ml, ferrous metalions chelating activity of WSP_U was significantly stronger than others.

3.3.3. Reductive potential of WSP

Fig. 3c shows the reductive activity of WSP. When the concentration exceeded 2 mg/ml, reductive power of WSP_U was significantly higher than that of WSP_H, WSP_M and WSP_E.

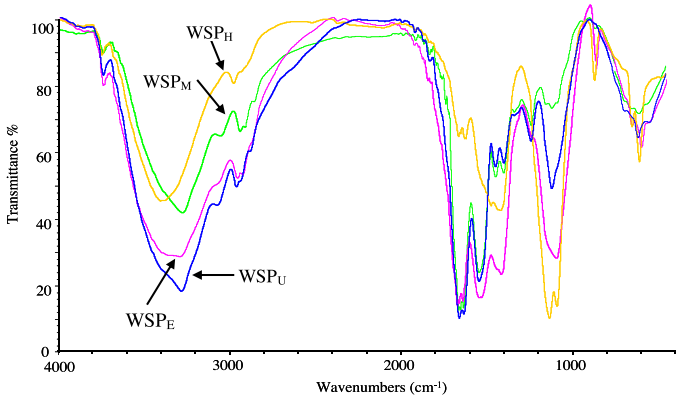

Fig. 2. FT-IR spectra of WSP.

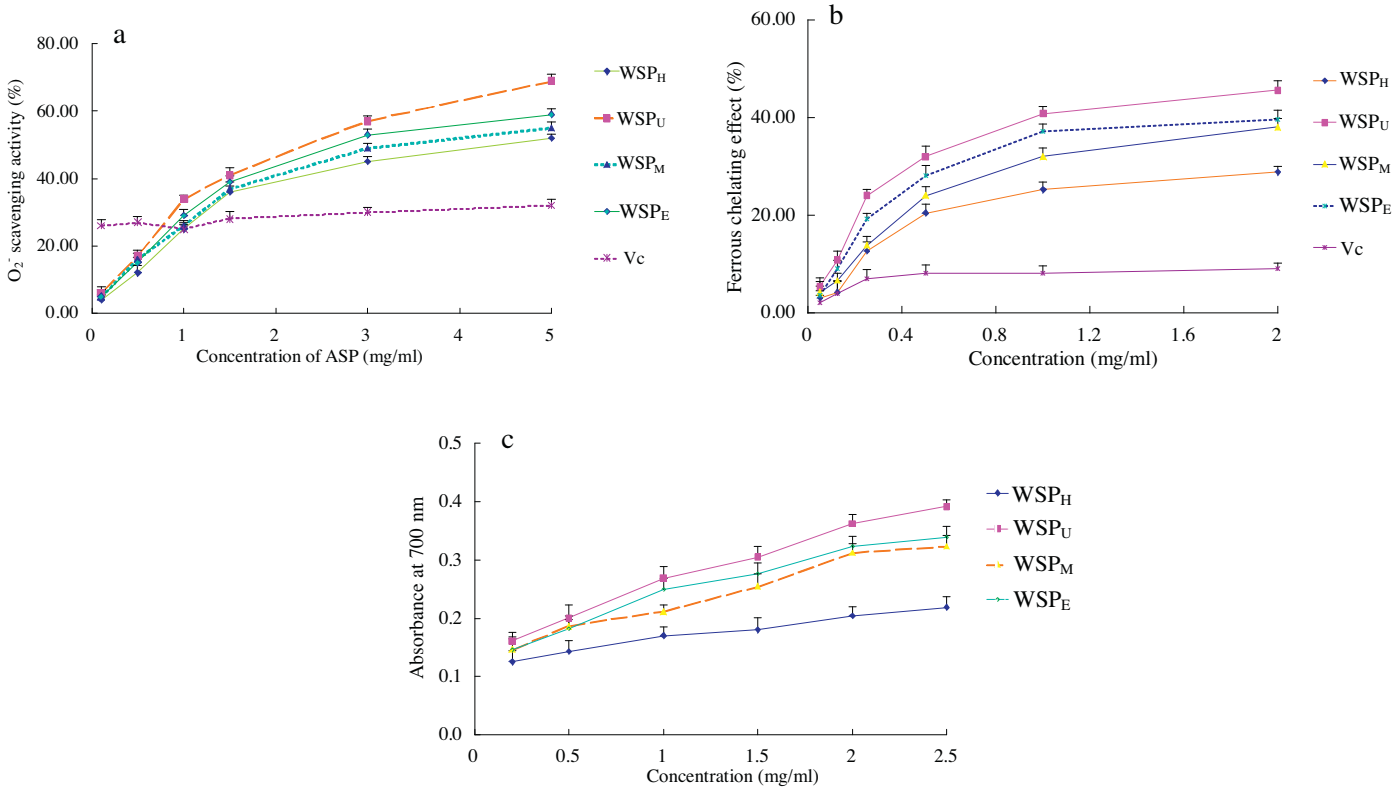

Fig. 3. Scavenging effects on superoxide radical (a), chelating activity on Fe^{2+} (b) and total reductive potential (c) of WSP_H, WSP_U, WSP_M and WSP_E.

4. Conclusion

In the present study, effect of four different extraction methods on yield, characterization and antioxidant activities of WSP were investigated to obtain an optimal extraction method. Experimental results showed that, four WSP samples had the same monosaccharide composition, but mere difference in the content; they all had typical IR spectra characteristic of polysaccharides. WSP_H had the lowest yield and worst antioxidant activity, but the molecular weight of it was the largest. WSP_U had the highest yield and less molecular weight, and its antioxidant activity was the best; this might be attributed to its high contents of uronic acid and sulfuric radical. The yield and antioxidant activity of WSP_M were higher than WSP_H, and the molecular weight slightly less than WSP_H. The yield of WSP_E was close to WSP_M, the molecular weight of it was smallest, and its antioxidant activity was second only to the WSP_U. All these showed that ultrasonic-assisted extraction should be the best extraction method for WSP.

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