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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).

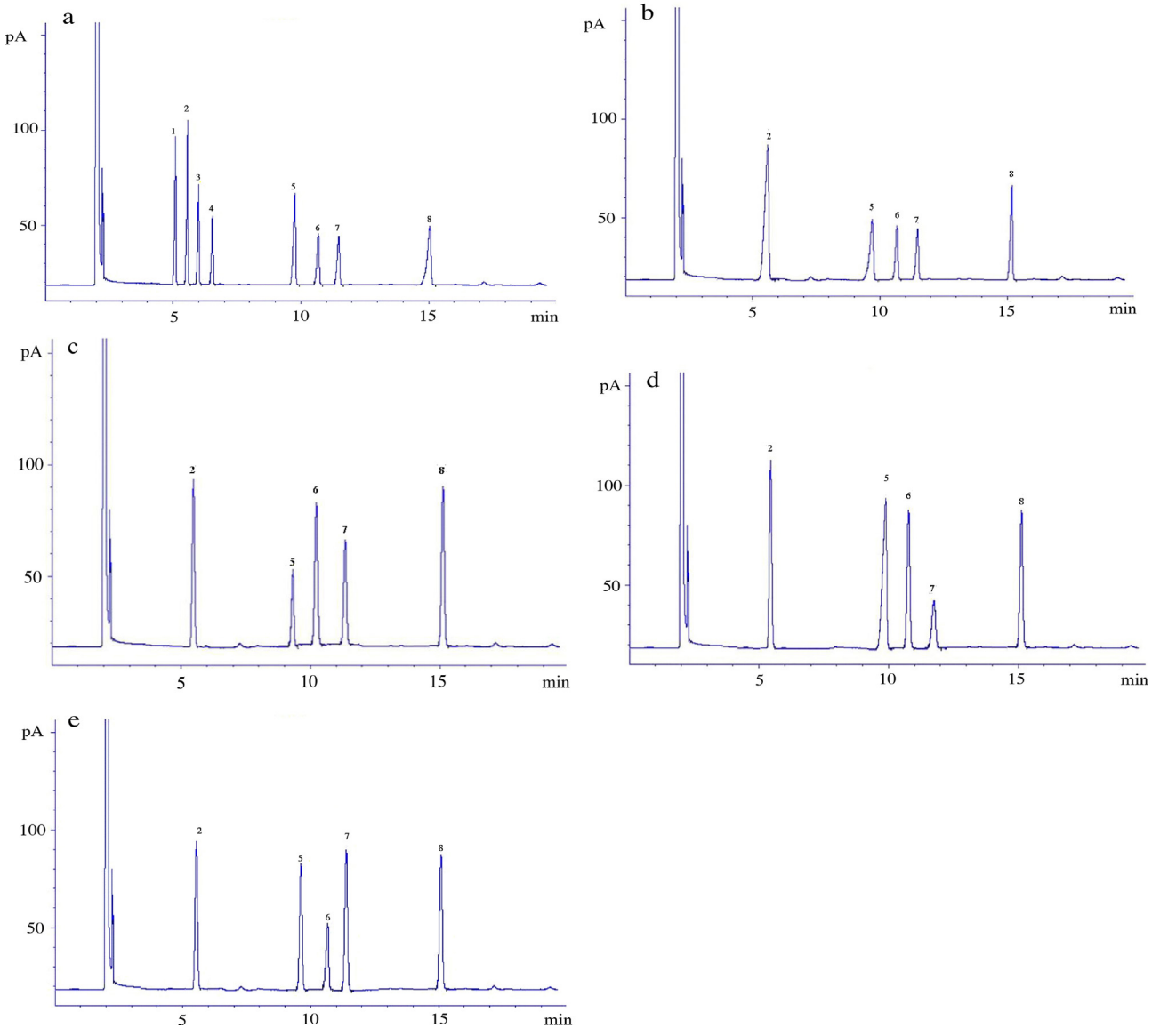

Fig. 1. GC spectra of sample reference (a), WSP_H (b), WSP_U (c), WSP_M (d) and WSP_E (e) (1, Rhamnose; 2, Arabinose; 3, Fucose; 4, Xylose; 5, Mannose; 6, Glucose; 7, Galactose; 8, Inositol.).