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Polysaccharide from *Mesona chinensis*: Extraction optimization, physicochemical characterizations and antioxidant activities

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ABSTRACT

The optimization of extraction conditions, physicochemical characterizations and antioxidant activities of polysaccharide from *Mesona chinensis* (MCP) were investigated. Optimal extraction conditions of MCP with the highest yield of 7.05 ± 0.12% was obtained by response surface methodology (RSM). The physicochemical characterizations of MCP obtained at the optimal extraction conditions were detected by TU-1900 spectrophotometer, high performance gel permeation chromatography (HPGPC), high-performance anion exchange chromatography (HPAEC) and fourier transform infrared spectroscopy (FT-IR) as well, revealing that MCP was a heteropolysaccharide containing uronic acid (29.3 ± 1.3%) and protein (10.4 ± 0.8%), with an average molecular weight (Mw) of 1.45×10^6 Da, which mainly consisted of galactose (Gal) and glucose (Glc) in a molar ratio of 1.00:1.38. Furthermore, MCP exhibited considerable antioxidant potential on scavenging hydroxyl (53.87 ± 0.44%), superoxide anion (58.42 ± 1.17%) and DPPH radicals (55.59 ± 0.69%). Our results indicated that MCP might be a good candidate for further investigations of functional foods.

1. Introduction

Mesona chinensis also named Hsian-tsao, Herb Jelly and Mesona chinensis benth, is a yearly herbaceous plant containing a distinct flavor from *Lamiaceae* family [1]. It is generally planted in China and Southeast Asia like Indonesia, Vietnam and Burma [2], and has been extensively studied in the field of foods and pharmaceuticals for its biological functions like antioxidant, protective against heat-stroke, hepatoprotective and anti-hypertensive [3]. In China, it has been used as an important medical and edible plant resource, and usually employed as food ingredients in the productions of herbal tea and jam-type dessert and edible gel [4]. The main constituents isolated from *Mesona chinensis* are polysaccharides, flavonoids, terpenoids polyphenols, etc. [5].

As the main component of *Mesona chinensis*, *Mesona chinensis* polysaccharide (MCP) has attracted a great deal of attention for its various biological activities such as anti-oxidant, immunoregulation, and anti-diabetics [6,7]. The addition of *Mesona chinensis* gum to casein film showed better mechanical properties and stronger antioxidant capacities than pure casein film, and the anti-oxidative effect provided by *Mesona chinensis* leaf gum was strongly concentration dependent [8]. In recent years, work has been done on the crude polysaccharide as well as the purified polysaccharide from *Mesona chinensis* [9,10]. However, few studies have been conducted for the extraction and characterizations of MCP that might result in low yield of polysaccharide, and high consumption of raw material, which may impede their productions and developments.

There exist a variety of extraction methods to obtain polysaccharide, such as heating, boiling, or refluxing, and getting the best extraction conditions to gain the highest yield of polysaccharide is beneficial to the application and further study of plant resources [11]. Statistical technique and response surface methodology (RSM) are extensively employed for their superb combinations of extracting factors [12]. Compared with conventional approaches, Box–Behnken design (BBD) is more effective to conduct experiments which can simplify the intricacy of the experimental tests required to assess multiple variables and their mutual effects [13].

Oxygen-derived free radicals are occasionally produced, and act a part as mediators of inflammation injury which might damage a number of biomacromolecules, such as nucleic acids, lipid membranes and amino acids in living bodies, leading to

Abbreviations: MCP, polysaccharide from Mesona chinensis; HAE, hot alkali extraction; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FTIR, Fourier transform infrared spectroscopy; Mw, molecular weight; BBD, Box–Behnken design; RSM, response surface methodology; HPGPC, high performance gel permeation chromatography; HPAEC, high-performance anion exchange chromatography; •OH, hydroxyl radical; ANOVA, analysis of variance.

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multifarious sickness and maladjustment, like diabetes, hypertension, infarction, rheumatoid arthritis, and atherosclerosis, etc. [14]. Investigating natural and low cytotoxicity antioxidants has great potential to overcome the carcinogenicity of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [15]. However, the information on extraction optimization by RSM and physicochemical characterizations of polysaccharide from *Mesona chinensis* are not available till date. Accordingly, a systematic and comprehensive research of extraction, physicochemical characterizations and antioxidant activities of MCP will provide useful information on the highly produced fruits.

2. Materials and methods

2.1. Materials and reagents

Mesona chinensis were purchased from Xiaoshicheng, Ganzhou, Jiangxi, China. *Mesona chinensis* were air desiccated and milled into fine powder by a high speed disintegrator and passed through a 10-mesh sieve before extraction.

DPPH, ascorbic acid, bovine serum albumin and the standard monosaccharides including glucose (Glc), mannose (Man), xylose (Xyl), rhamnose (Rha), arabinose (Ara), galactose (Gal), fructose (Fru), ribose (Rib) and fucose (Fuc) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade and purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China). The ultra-pure water was utilized from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Preparation and determination of MCP

The dried *Mesona chinensis* were thoroughly sliced and ground into fine powder in a mill, then extracted with ten-fold 95% ethanol for 12 h to remove interference impurities and small lipophilic molecules like monosaccharide, disaccharide, oligosaccharide, fat acids and polyphenols [11]. The preprocessed samples were separated manually from the organic solvent through the nylon cloth (pore diameter: 38 μm), then dried. Each pretreated sample (5.0 g) and sodium carbonate solution at different liquid to solid ratios (5:1–30:1, mL/g) were put into 50 mL, 100 mL, 250 mL, 500 mL, 500 mL, 500 mL beaker, respectively. Then the extractive procedures were carried out at different temperatures (50 ~ 100 °C) in a JRY electric-heated thermostatic water bath at various sodium carbonate concentrations (1–6 mg/mL) for various time (0.5–3.0 h). The extracts were centrifuged at 4800 rpm for 10 min and then the supernatants were gathered. The supernatants were diluted with ultra-pure water and the polysaccharide content of MCP was determined by the phenol–sulfuric acid method [16]. The yield of polysaccharide (Y) in dried *Mesona chinensis* was calculated as:

$$Y\ (\%) = c \times v/w \times 100\% \quad (1)$$

Where c is the concentration of polysaccharide in the sample solution (mg/mL), v is the volume of sample solution (mL), and w is the mass of the dried sample (mg).

After identifying the best extraction conditions, extraction procedure was conducted to obtain the dried MCP for latter physicochemical characterizations and antioxidant activities assays. After extraction, the extraction solutions were separated from insoluble residues by centrifugation (4800 rpm for 10 min, at 20 °C), followed by collected, the supernatants were concentrated with a rotary evaporator (EYELA OSB-2100, Tokyo, Japan) at 60 °C under vacuum, then precipitation with alcohol to a concentration of 80% (v/v) and kept at 4 °C overnight. The precipitate was collected by centrifugation at 4800 rpm for 10 min, and washed using 95% ethanol, 100% ethanol and acetone, respectively. After filtering and centrifuging, the precipitate was re-dissolved in ultra-pure water, and centrifuged at 9000 rpm for 15 min, then, the supernatant was further dialyzed for 36 h in natural water and 12 h in ultra-pure water (MW cut-off 14 kDa) before concentration under vacuum evaporator at 55 °C. Lastly, the precipitate was frozen at −20 °C overnight and lyophilized in vacuum freeze dryer (model ALPHA 2–4, Christ, Germany) to obtain the dried MCP.

Table 1
Levels and code of variable used for Box–Behnken design (BBD), and the observed responses for the extraction yields of MCP.

Independent variables	Symbol	Range and level		
		−1	0	+1
Extraction time (h)	X_1	1.5	2.0	2.5
Extraction temperature (°C)	X_2	80	90	100
Sodium carbonate concentration (mg/mL)	X_3	2	3	4
Ratio of extraction solvent to raw material (mL/g)	X_4	15	20	25

Run	Coded variable levels				Extraction yield (%)	
	X_1	X_2	X_3	X_4	Experimental[a]	Predicted
1	2	80	4	20	5.58	5.43
2	2	90	3	20	6.85	6.76
3	2	90	4	15	5.46	5.53
4	1.5	90	3	15	5.43	5.60
5	2	90	4	25	5.72	5.89
6	2.5	90	4	20	5.69	5.60
7	2	90	2	15	5.39	5.26
8	2	90	3	20	6.85	6.76
9	1.5	80	3	20	5.39	5.35
10	2	80	2	20	5.09	5.02
11	2.5	90	2	20	5.31	5.58
12	2	90	2	25	5.56	5.53
13	2.5	100	3	20	5.82	5.90
14	2.5	90	3	15	5.22	5.26
15	2	100	3	15	5.86	5.64
16	2.5	80	3	20	5.12	5.18
17	2	100	2	20	5.39	5.59
18	2	90	3	20	6.60	6.76
19	2	100	4	20	5.70	5.82
20	2	90	3	20	6.83	6.76
21	2	90	3	20	6.68	6.76
22	2.5	90	3	25	6.12	6.00
23	2.5	90	2	20	5.82	5.58
24	2	100	3	25	6.01	5.85
25	2	80	3	15	4.98	5.05
26	2	80	3	25	5.36	5.48
27	1.5	90	3	25	5.48	5.50
28	1.5	90	4	20	5.94	5.82
29	1.5	100	3	20	5.60	5.58

[a] Experiments were performed in triplicate and the data were reported as means of three values.

2.3. Experimental design

To confirm the variation range of extraction parameters, single factor experiment were carried out. Every test was performed in triplicates.

Based on previous single factor experimental results, a four-variable, three-level experiments coded +1, 0 and −1 for high, middle and low value, respectively, and 29-run BBD were adopted for optimization, as shown in Table 1. The selected variables are numbered in accordance with the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} i = 1; 2; 3 \quad (2)$$

Where x_i is the numbered value of independent parameter; x_i is the practical value of independent parameter; x_0 is the real value of independent parameter at the center point; and $\triangle x$ is the step change value.