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MICROWAVE–ASSISTED EXTRACTION AND IDENTIFICATION OF POLYSACCHARIDE FROM *Lycoris aurea*

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Three polysaccharide fractions (LAP-1, LAP-2, and LAP-3) were separated from the extract of Lycoris aurea *using DEAE-52 and Sephadex G-150 column chromatography. FT-IR spectroscopy revealed the typical characteristics of polysaccharide fractions. The molecular weights of three polysaccharides were characterized by HPGFC, which were 31.9 kDa, 35.7 kDa, and 72.7 kDa, respectively. Their chemical compositions were also studied. The conditions for microwave-assisted extraction (MAE) of polysaccharide from* L. aurea *were investigated through an orthogonal design of* L_9 (3^3) *in this work.*

Key words: *Lycoris aurea*, polysaccharide, microwave-assisted extraction, chemical composition.

Lycoris aurea is a perennial herbaceous plant. It is a member of the Amaryllidaceace family, possesses highly decorative characteristics, and has been used from ancient times in floriculture and medicine. Amaryllidaceace is rich in alkaloids, especially galanthamine, which is a long-acting, selective, reversible, and competitive inhibitor of acetylcholinesterase; therefore, it is a reasonable approximation of the ideal concept of symptomatic Alzheimer′s disease therapy [1].

Polysaccharides, another important components, are also present in large amounts in the Amaryllidaceace family, especially in the bulb. Our laboratory found that the bulb of *L. aurea* contained, depending on the seasons, from 10.87% to 37.44% polysaccharides (per fresh weight). Some studies have been conducted on the extraction, purification, structural analysis, and biological activity of polysaccharides from *Narcissus*, e.g., *Narcissus tazetta* L. [2–6]. However, to the best of our knowledge, there are no published reports on the extraction and phytochemical composition of polysaccharides from *L. aurea*. Microwave-assisted extraction (MAE) is an extraction procedure that, compared to conventional techniques, is fast and uses very little solvent [7]. Therefore, we focused on the conditions for microwave-assisted extracting of polysaccharides from *L. aurea*, which ubiquitously improved polysaccharide production and shortened procedure time, providing a foundation theory for applying the technology to mass production. To learn more about the identification of *L. aurea* polysaccharides, a pectic polysaccharide-rich extract from the bulb was purified, and the molecular weights (M_w) and chemical compositions of purified polysaccharides were also measured.

Table 1 shows the yields of polysaccharides under the indicated conditions. An orthogonal design [$L_9(3^3)$] was applied to optimize the solide-liquid ratio, temperature, and microwave time. Generally, a higher solid-liquid ratio for a longer time and higher temperature of incubation gave a higher yield of polysaccharide. A significant yield was observed at temperatures from 90°C to 100°C. A longer microwave time produced a higher yield. The solid-liquid ratio also had a significant impact on the overall yield of polysaccharide. The order of impact of the different factors on the yield was: microwave temperature > time of extraction > solid-liquid ratio. A comparison of polysaccharide yield and purity between microwave-assisted and traditional hot-water immersed methods under optimal extracting condition is shown in Table 2. It shows that the microwave-assisted technique compared favorably with the classical hot-water immersion extraction; it has several advantages compared to the traditional method, mainly rapidity, which makes it suitable for the extraction of polysaccharides from the bulb of *L. aurea*.

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TABLE 1. Polysaccharide Yields under the Condition Indicated Using $L_9(3^3)$ Orthogonal Design

Programs	Solid-liquid ratio	Temperature, °C	Time, min	Yield (w/w), %
1	1:60	80	10	12.12
2	1:60	90	15	21.14
3	1:60	100	20	30.35
4	1:80	80	15	19.16
5	1:80	90	20	24.42
6	1:80	100	10	25.20
7	1:100	80	20	24.76
8	1:100	90	10	21.62
9	1:100	100	15	33.19
k_i[a]	21.20	18.68	19.65	
k_{ii}[a]	22.92	22.39	24.50	
k_{iii}[a]	26.52	29.58	26.51	
R[b]	5.32	10.90	6.86	

[a]Average responses (k_i, k_{ii}, k_{iii}) of each level with respect to extraction yield.
[b]*R* value means range between three average responses of each level with respect to extraction yield.

TABLE 2. Comparison of MAE and Traditional Method during Extraction of Polysaccharide from *L. aurea*

Extraction method	Solid-liquid ratio	Temperature, °C	Time, min	Yield (w/w), %	Purity, %
MAE method	1:100	100	20	32.14*	70.82*
Traditional method	1:100	100	240	25.73	63.54

*Mean significant differences were determined at $p<0.05$.

TABLE 3. Physicochemical Properties of Polysaccharide Fractions from *L. aurea*

Polysaccharide fraction	Molecular weight, kDa	Monosaccharide composition
LAP-1	31.9	Mannose (45.7%), glucose (52.7%), galactose (1.7%)
LAP-2	35.7	Glucose (79.1%), galactose (18.4%), mannose (2.5%)
LAP-3	72.7	Galactose (100%)

The polysaccharides were obtained using MAE from the bulb of *L. aurea*, designated LAP. LAP contains 2% (w/w) protein, so it was treated with 5% (w/v) TCA to remove the protein. The result showed LAP contained no protein after TCA treatment.

Three fractions of polysaccharides, obtained from DEAE-52 anion-exchange chromatography and Sephadex G-150 column chromatography, were detected by HPGFC for their homogeneities. The homogeneous polysaccharide fraction showed a symmetrical peak in HPGFC, which was also used to elucidate the M_w of the polysaccharide fraction (Table 3). Three independent peaks were identified, and the average M_w values were 31.9 kDa (LAP-1), 35.7 kDa (LAP-2), and 72.7 kDa (LAP-3), respectively, according to the calibration curve with standard dextrans. We investigated the IR spectra of the fractions at various stages of separation. All samples exhibited a broad intense peak at around 3400 cm^{-1}, which was the characteristic absorption of hydroxyl groups and a weak C-H band at around 2920 cm^{-1}. The relatively strong absorption peak at around 1640 cm^{-1} and the weak ones from 1400 cm^{-1} to 1200 cm^{-1} also indicated the characteristic IR absorption of the polysaccharide. LAP-1 and LAP-2 showed IR absorption peak (844 cm^{-1}), which was characteristic of α-pyranoses, whereas no absorption was observed in LAP-3. Furthermore, LAP-3 showed an absorption peak near 1742 cm^{-1}, indicating the presence of a carboxyl group, which was not detected in both LAP-1 and LAP-2.

GC-MS traces of the polysaccharide hydrolyzates, compared with standard monosaccharides, showed the monosaccharide components of the samples. The results indicated that LAP-1 was composed mainly of mannose and glucose (relative mass 45.7% and 52.7%, respectively) with low levels of galactose (relative mass 1.7%). The monosaccharide of LAP-2 was glucose (relative mass 79.1%) with low levels of galactose and mannose (relative mass 18.4% and 2.5%, respectively). In additional to LAP-3, which was eluted at 0.1 M NaCl concentration, there was absolute galactose (relative mass 100%).

In this work, the extraction conditions of *L. aurea* polysaccharides were optimized as 1:100 solid-liquid ratio for 20 min at 100°C. Compared with the traditional method of polysaccharide extraction, MAE was effective in polysaccharide extraction because of its shorter time and higher yield and may be potentially useful for mass production of Amaryllidaceace polysaccharides. Three different polysaccharides were isolated, and their physicochemical properties were determined. Further studies will focus on determination of the structure and bioactivity of the purified *L. aurea* polysaccharide and the relationship between its structure and bioactivity, which are already under way in our laboratory.

EXPERIMENTAL

L. aurea was collected from Chengdu, Sichuan, P. R. China, in July 2005 and identified by Herbarium Specimen Search of Xiamen University. The bulb part was dried at 50°C in oven. The voucher specimens were deposited in the School of Life Sciences, Xiamen University.

Preparation of Crude Polysaccharide. MAE was performed in an MSP-1000 microwave (CEM Corporation, USA), equipped with pressurized 120 mL vessels. The polysaccharide extraction parameters are shown in Table 1. The defatted *L. aurea* powder was mixed with distilled water, and programs were run according to Table 1; then it was centrifuged at 4800 rpm for 15 min and the pellet removed. The supernatant was concentrated in vacuo to 25% of original volume and treated with 5% TCA for 30 min at 4°C for protein removal, then neutralized with 10% NaOH (w/v). The crude polysaccharide part precipitated from the 4-fold 95% alcoholic liquor during its subsequent standing at 4°C overnight. The ethanol precipitation step was repeated three times. The precipitate that formed was collected by centrifugation at 4800 rpm and repeatedly washed sequentially with smaller amounts of ethanol, acetone, and ether, then dried in vacuum, yielding the water-extractable crude polysaccharide, LAP.

Purification of Polysaccharide. The refined LAP was dissolved in deionized water. Ion exchange chromatography was performed in a column (2.5 cm × 30 cm) of DEAE-52 (Whatman, UK). Crude polysaccharide (500 mg) was loaded onto the column each time. Then the column was eluted stepwise with deionized water, 0.05 M NaCl, and 0.1 M NaCl at a flow rate of 1 mL min^{-1}. The fractions were combined according to the total carbohydrate content measured by the phenol–sulfuric acid method at 485 nm [8]. The three fractions obtained were concentrated and dialyzed against reversely flowing distilled water for 72 h with dialysis tubing (molecular weight cut-off, 12 kDa) to remove low-molecular-weight matter (e.g. chromones and anthranoids), and then concentrated and precipitated with a 4-fold volume of 95% ethanol for three times to obtain polysaccharide-enriched fractions. The fractions corresponding to the major peak were further chromatographed on a column (2.0 cm × 120 cm) of Sephadex G-150 (Pharmacia, USA) with 0.01 M NaCl at a flow rate of 0.2 mL min^{-1}. The afforded more pure products were further precipitated in the same manner to yield finally homogeneous polysaccharides, named LAP-1, LAP-2, and LAP-3, respectively.

Purity and Molecular Weight Determinations. The purity and molecular weight of LAP-1, LAP-2, and LAP-3 were measured using HPGFC. The eluent was 0.1 M $NaNO_3$ in HPLC-grade water, with a flow rate of 0.9 mL min^{-1} and detected by a Waters 2410 Refractive Index (IR) Detector. The samples were dissolved in 0.1 M $NaNO_3$ and filtered through a 0.22 μm filter before injection. The system was calibrated using linear dextran with molecular weights 2,000,000, 70,000, 40,000, and 10,000 (Sigma), and then the retention times were plotted against the logarithms of their corresponding average molecular weights. The retention times of three samples were also plotted in the same graph, and the average molecular weights were determined [9].

Physicochemical Properties Analysis. Infrared analysis of the samples was obtained by grinding a mixture of polysaccharide with dry KBr and then pressing in a mold. IR spectra were recorded with a Nicolet FT-IR 200 spectrometer in the range 4000–400 cm^{-1} [10].

Two milligrams of a polysaccharide fraction sample was hydrolyzed in 1 mL of 4 M TFA at 100°C for 5 h in a sealed glass tube and evaporated under a stream of N_2. After reduction with 10 mg of sodium borohydride, AcOH was added after 18 h, and the mixture was evaporated under N_2. The resulting boric acid was removed as trimethyl borate by co-evaporation with

MeOH. Acetylation was carried out with Ac_2O–pyridine (1:1, v/v; 1 mL) at room temperature for 12 h. To the solution was added excess ice-water, which was extracted with $CHCl_2$ and then evaporated under a stream of N_2 [11, 12]. The monosaccharide composition was analyzed by GC-MS on a Varian 1200L instrument using a CP-Sil 24 CB Lowbleed/MS (30 m × 0.25 mm), a split injector (split ratio 1:100), and a FID detector. Helium was used as the carried gas at a constant rate of 1 mL min^{-1}. The oven conditions included an initial temperature of 100°C and an initial time of 3 min, 10°C/min to 230°C, and finally 5°C/min to 260°C and held at this temperature for 5 min. The inlet temperature was kept constant at 280°C. As reference, the following monosaccharides were converted to their acetylated derivatives and analyzed: ribose, rhamnose, arabinose, fucose, xylose, mannose, glucose, galactose, and fructose. Peaks were identified and estimated using *myo*-inositol as the internal standard. The quantity of fractions was determined from the peak area using response factors.

Statistical Analysis. The data were presented as means of three determinations. Statistical analysis was performed using the One-Way ANOVA and General Linear Models (SPSS 11.0). A probability value of <0.05 was considered significant.

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REFERENCES

1. M. Rainer, *Drugs Today*, **33**, 273 (1997).
2. M. Tomoda, M. Yokoi, A. Torigoe, and K. Maru, *Chem. Pharm. Bull.*, **28**, 3251 (1980).
3. K. S. Zhauynbaeva, M. K. Malikova, D. A. Rakhimov, and Z. A. Khushbaktova, *Chem. Nat. Comp.*, **39**, 520 (2003).
4. M. K. Malikova, D. A. Rakhimov, and K. S. Zhauynbaeva, *Chem. Nat. Comp.*, **38**, 190 (2002).
5. D. A. Rakhimov and K. S. Zhauynbaeva, *Chem. Nat. Comp.*, **33**, 208 (1997).
6. K. S. Zhauynbaeva, M. K. Malikova, and D. A. Rakhimov, *Chem. Nat. Comp.*, **39**, 240 (2003).
7. A. M. Carro, R. A. Lorenzo, F. Fernandez, R. Phan-Tan-Luu, and R. Cela, *Anal. Bioanal. Chem.*, **388**, 1021 (2007).
8. G. Cuesta, N. Suarez, M. I. Bessio, F. Ferreira, and H. Massaldi, *J. Microbiol. Meth.*, **52**, 69 (2003).
9. S. P. Nie, M. Y. Xie, and Y. X. Wang, *Anal. Bioanal. Chem.*, **383**, 680 (2005).
10. Y. Wang, M. Zhang, D. Ruan, A. S. Shashkov, M. Kilcoyne, A. V. Savage, and L. Zhang, *Carbohydr. Res.*, **339**, 327 (2004).
11. M. L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, **2**, 65 (1963).
12. M. L. Wolfrom and A. Thompson, *Methods Carbohydr. Chem.*, **2**, 211 (1963).