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Research Article

Fractionation of DMSO-Extracted and NaOH-Extracted Hemicelluloses by Gradient Ethanol Precipitation from *Neosinocalamus affinis*

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Neosinocalamus affinis hemicelluloses were extracted with pure DMSO and 3% NaOH in sequence. The DMSO- and NaOH-extracted hemicelluloses were then successively fractionated by gradient ethanol precipitation. NaOH-extracted hemicellulosic fractions with different branch degree could be separated by gradient ethanol precipitation, while DMSO-extracted hemicellulosic fractions could not. FT-IR spectra showed that DMSO-extracted fractions have more complete structure, while NaOH-extracted fractions have no acetyl at all. The FT-IR and NMR revealed that the DMSO-extracted *Neosinocalamus affinis* hemicelluloses were 4-*O*-methyl-glucuronoarabinoxylans consisting of a linear (1→4)-*β*-D-xylopyranosyl backbone with branches at *O*-2,3 of acetyl, *O*-2 of 4-*O*-methyl-*a*-D glucuronic acid, and *O*-3 of arabinose.

1. Introduction

The sharp shrink in the range of available petroleum resource and the gradually severe environmental pollution have led the research of biomass to a hot topic. The utilization of diverse lignocellulosic biomass is indeed worth exploring for sustainable development. Bamboo is a kind of potential lignocellulosic feedstock with desirable advantages such as high productivity and fast growing, which includes 1250 species within 75 genera [1]. There is a wide distribution of it in China, and it is convenient to utilize the biomass resource in papermaking, fabricating bamboo products, textiles, activated carbon, and food industry as well as bioenergy applications [1–4]. *Neosinocalamus affinis* is one-timing flowering perennial plants that belong to Bambusoideae, a subfamily of Gramineae, which takes a big proportion in economic bamboo species in our country [5]. Up to now, the novel utilization and development of research on *Neosinocalamus affinis* are still on the way.

Hemicelluloses, the second abundant lignocellulosic resource in nature, are prevailing in botanical materials. This kind of relatively low molecular weight polysaccharides associates with cellulose and lignin through hydrogen bonds and various covalent bonds in plant cell walls. Different botanical origins lead to differences in composition and structures of various hemicelluloses [6]. Hemicelluloses account for 22–35% of the bamboo composition, and the dominant species is xylan [1]. Due to its excellent property, a great growing trend in utilization of hemicelluloses in chemicals, medicine, and biomaterials has been generated in recent decades [7, 8]. Chemical and biological methods are the predominant extraction processes for bamboo hemicelluloses. Today, plenty of chemical methods have been well investigated, for example, alkaline extraction, alkaline peroxide extraction, organic solvent extraction, and the biological method mainly which includes enzyme for hydrolysis of bamboo cell walls [9, 10]. In addition, treatments such as ultrasonic irradiation, steam explosion, ultrafiltration, and autohydrolysis treatment are used for effective fractionation of hemicelluloses [11–14]. Alkaline extraction is one of the most effective methods employed for the isolation of hemicelluloses, but this method has the disadvantage of deacetylating

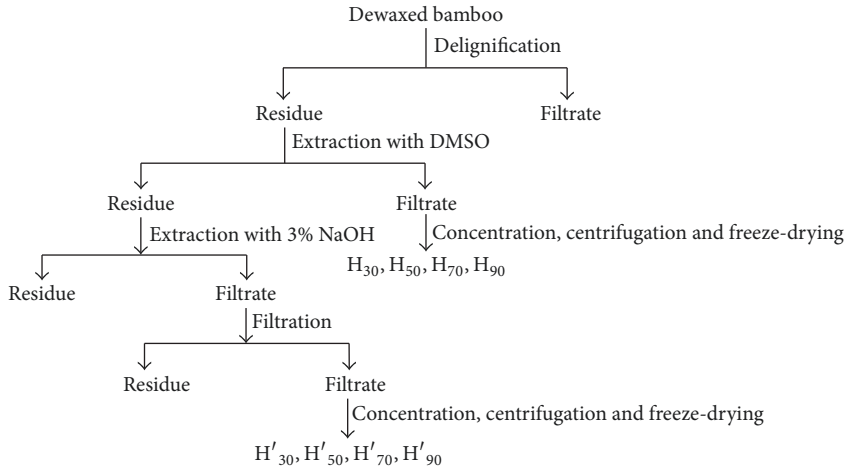


Figure 1: Scheme for extraction and fractionation of hemicelluloses from *Neosinocalamus affinis*.

hemicelluloses [15]. Compared with alkali, DMSO is an ideal solvent for extraction of polysaccharides from plant, which can well remain both *O*-acetyl group and glycosidic linkages of hemicelluloses [16]. Hemicelluloses with various structures are also extracted by DMSO/LiCl system successfully from some plant materials [17, 18]. The isolated hemicelluloses generally consist of various populations of polysaccharide molecules which vary in structural characteristics, and several fractionation techniques such as sodium sulfite precipitation and gradient ethanol precipitation have been employed in order to obtain more homogeneous fractions [15].

In our group, the gradient ethanol precipitation technique has been used for fractionating alkali-extracted hemicelluloses. It was found that, with an increment of ethanol concentration, the arabinose/xylose (Ara/Xyl) and glucuronic acid/xylose (GlcA/Xyl) ratios of the alkali-extracted hemicellulosic fractions increased. The two ratios reflect the branch degree of xylan from gramineous plants. Namely, the less branched alkali-extracted hemicelluloses are likely to be precipitated by ethanol with low concentration, while the highly substituted alkali-extracted hemicelluloses are preferred to be fractionated by ethanol with high concentration [19]. The purpose of the current work is to investigate the condition that DMSO-extracted hemicelluloses are precipitated by ethanol with gradient concentration, and a comparison will be made with the result of NaOH-extracted hemicelluloses. Furthermore, high performance anion exchange chromatography (HPAEC), Fourier-transform infrared (FT-IR) spectroscopy, and nuclear magnetic resonance spectroscopy (NMR) are employed to obtain structure characteristic of the hemicellulosic fractions.

2. Experimental

2.1. Materials. *Neosinocalamus affinis* was harvested in Sichuan province, China. They were dried in sunlight, cut into small pieces (1–3 cm), and then ground to pass through a 0.8 mm screen. The chemical components of the bamboo culms were determined according to the standard of National Renewable Energy Laboratory (NREL) [20], and the results were expressed on a percentage basis of the oven-dry raw material. The chemical composition of the dewaxed bamboo was as follows: 50.8% glucose, 22.9% xylose, 1.1% arabinose, 19.5% galactose, 17.0% Klason lignin, 2.5% acid-soluble lignin, and 2.5% ash. To remove the non-cell wall components, such as wax and chlorophyll, the powder was extracted with toluene/ethanol (2 : 1, v/v) in a Soxhlet apparatus for 6 h and then overdried at 60°C for 16 h. All chemicals used were of analytical or reagent grade.

2.2. Methods

2.2.1. Extraction and Fractionation. The process of extraction and fractionation of hemicelluloses from *Neosinocalamus affinis* is illustrated in Figure 1. The dewaxed bamboo powder was delignified with 6% sodium chlorite at pH 3.6–3.8, adjusted with aqueous chlorine hydride, at 78°C for 2 h. The residue obtained after delignification is holocellulose, which needs to be subsequently washed with distilled water and dried at 60°C overnight. Next, the holocellulose powder was extracted by DMSO at 80°C for 7 h, using a solid to liquid ratio of 1 : 25 (g/ml). After the extraction, the residue was separated from the filtrate with a nylon cloth, washed with distilled water, and further dried in a cabinet oven under air circulation at 60°C, waiting for following alkali extraction. Next, the filtrate needed to be concentrated at reduced pressure to around 20 ml. Ethanol was carefully added to the concentrated filtrate up to 30% saturation. After a night, the precipitation was centrifuged (3400 rpm, 10 min), freeze-dried, and finally labelled as H_{30}. Then the supernatant was subsequently adjusted stepwise to 50, 70, and 90% (v/v). The corresponding precipitated hemicelluloses fractions were labelled as H_{50}, H_{70}, and H_{90}, respectively. Total dried residue was weighted and extracted by 3% NaOH (w/v) at 60°C for 6 h, using a solid to liquid ratio of 1 : 25 (g/ml). After the extraction, the residue was separated, washed, and dried. Then, the obtained filtrate would go through a dialysis process for a week. Following treatment is as described

in DMSO-extraction above, including concentration, precipitation by ethanol with certain concentration, and freeze drying. The final four alkali-extracted samples were named as H'_{30}, H'_{50}, H'_{70}, and H'_{90}, respectively.

2.2.2. Analytical Methods. The constituent neutral sugar in the isolated hemicellulosic fractions was determined by high performance anion exchange chromatography (HPAEC). The hemicellulosic fraction (~5 mg) in a sealed tube was hydrolyzed by 1 M H_2SO_4 at 105°C for 2.5 h, and the neutral sugar was obtained. Then, the hydrolyzed sample was diluted 50-fold, filtered, and injected into the HPEAC system (Dionex ISC 3000) with an amperometric detector, an AS 50 autosampler, and a CarbopacTM PA20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex) [21]. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-mannose, D-galactose, glucuronic acid, and galacturonic acids. FT-IR experiments were conducted using a TensorII Sample Compartment RT-DLaTGS. Dried samples were ground and pelletized using KBr, and their spectra were recorded from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} and 16 scans per sample. The solution-state ^1H and ^{13}C NMR spectra were recorded on a Bruker NMR spectrometer at 400 MHz. The samples (15 mg in 0.55 ml of DMSO-d_6 for ^1H, 65 mg in 0.55 ml of DMSO-d_6 or ^{13}C) were prepared as required. The degree of acetylation (DS_{AC}) was determined from the relative intensities of signals of the acetyl group at 2.1 ppm and those of all carbohydrate signals in ^1H NMR spectra. The following equation was used:

$$DS_{AC} = \frac{(\text{sum of integrals for acetyl groups at 2.1 ppm})/3}{(\text{sum of integrals for carbohydrate signals at 3.0–5.5 ppm})/6}. \quad (1)$$

The proton-detected heteronuclear single quantum correlation (HSQC) spectra were acquired by the HSQCGE experiment mode [19], over a t_1 spectral width of 10,000 Hz and a t_2 width of 1800 Hz, and the acquired time (AQ) was 0.1163 s. The number of scans (NS) was 32. The delay between transients was 2.6 s, and the delay for polarization transfer was set to correspond to an estimated average ^1H-^{13}C coupling constant of 150 Hz. Data processing was performed using standard Bruker Topspin-NMR software.

3. Result and Discussion

3.1. Yield and Sugar Composition. A wide variation in content and chemical structures of large amounts of hemicelluloses exists in plant cell walls, and hemicelluloses are generally composed of several populations of polysaccharides molecules which vary in structural characteristics. Hemicelluloses are formed with lignin by covalent bonds and with acetyl units and hydroxycinnamic acids by ester linkages; in addition, extensive hydrogen bonding among the individual polysaccharides in the cell wall is also formed. These complicated structures remain obstacles for liberation of hemicelluloses from cell wall matrix [21]. In the present work, hemicelluloses from *Neosinocalamus affinis* are extracted with DMSO at 80°C for 6 h and precipitated stepwise in ethanol with different concentrations. After DMSO-extraction, around 9.65% of the starting holocellulose was recovered (Table 1). Stepwise addition of ethanol to the DMSO-extracted solution resulted in four fractions, named as H_{30}, H_{50}, H_{70}, and H_{90}, respectively. Table 1 shows the yields and composition of the four fractions extracted with DMSO and NaOH. Obviously, for DMSO-extraction, the major hemicellulosic fraction (H_{50}) was obtained at the ethanol concentration of 50%, which accounted for 6.23% of the delignified materials. The yield of H_{70} (0.82%) is a little higher than that of H_{30} (0.56%) and H_{90} (0.45%). The total yield of the four fractions is 8.06%, indicating that there are some small amounts of hemicelluloses, mainly degraded oligosaccharides or other low molecule substances, that are not recovered. Counterpart information of NaOH-soluble hemicelluloses was also included in Table 1. After the 3% NaOH extraction, about 11.03% of the starting holocellulose was obtained (Table 1), and this is a little higher than that of DMSO-extraction. Four fractions were named as H'_{30}, H'_{50}, H'_{70}, and H'_{90} after gradient ethanol precipitation. The most recovered group is H'_{50} (1.69%), and the yield of H'_{70} (1.64%) is a little less, while the yield of H'_{90} (0.04%) was really less compared with the other three fractions. The sum of the four yields is 4.15% which is much less than that of DMSO-extracted hemicellulose. This can be explained that the alkali extraction led to severe degradation of hemicelluloses; thus parts of oligo- or monosaccharides could not be recovered in this circumstance.

Table 1: Yields of hemicelluloses during extraction with DMSO and NaOH based on starting holocellulose.

Fraction	Yield (% delignified material)
Extracted with DMSO	9.65
Extracted with NaOH	11.03

The composition of the hemicellulosic fractions obtained from the DMSO-soluble hemicelluloses of *Neosinocalamus affinis* is shown in Table 2. Whatever fraction it is, xylose is the predominant composition accounting for 93.6–95.4% of the total sugar. Arabinose and glucuronic acid are the other two main components, which account for 2.8–4.0% and 1.1–1.5%, respectively. This result suggests that glucuronoarabinoxylans are the substantial proportion of hemicelluloses in the cell wall of *Neosinocalamus affinis*, and this is in accordance with those found in other bamboo species [22]. Less galactose (0.3–0.5%) and glucose (0.5–1.0%) are also present in the fractions. The small portion of galactose may come from arabinogalactan, and the glucose is probably due to β-D-glucan, which is a group of polysaccharides found in the cell wall of grain, including grasses and cereals, and this needs to be further proved [23, 24]. Arabinoxylan and glucuronoarabinoxylan with various botanic resources own the same basic chemical structure; they differ in the manner of the xylan backbone. The main differences were found in the ratio of arabinose to xylose (Ara/Xyl) and glucuronic acid to xylose (GlcA/Xyl), in the relative proportions and sequence of various linkages between these sugars, and in the presence of other substituents [25, 26]. The ratios of Ara/Xyl

Table 2: The contents of neutral sugars and uronic acid (relative%, w/w) of hemicellulosic fractions obtained from DMSO-extracted and NaOH-extracted hemicelluloses.

Subfraction[a]	Yield[b] (%)	Molar composition[c] (mol%)					GalA	Molar ratio[d]		DSAC[e]
		Ara	Gal	Glu	Xyl	GlcA		Ara/Xyl	GluA/Xyl	
H_{30}	5.8	3.6	0.5	0.7	93.7	1.5		0.038	0.016	0.13
H_{50}	64.5	4	0.4	0.5	93.7	1.5		0.043	0.016	0.23
H_{70}	8.5	2.8	0.3	0.5	95.4	1		0.029	0.011	0.28
H_{90}	4.7	3.8	0.5	1	93.6	1.2		0.04	0.013	0.25
H'_{30}	7	8.5	0.3	0.3	87.5	3.4	0.1	0.097	0.039	
H'_{50}	15.3	10.3	0.8	0.2	93.2	5.3	0.2	0.124	0.063	
H'_{70}	14.9	23.1	4.6	0.4	62.8	8.7	0.4	0.367	0.139	
H'_{90}	0.4	29.8	5.8	2.6	56.2	4.8	0.9	0.53	0.086	

[a]H_{30}, H_{50}, H_{70}, and H_{90} represent the hemicellulosic fractions obtained from DMSO-extracted hemicelluloses and H'_{30}, H'_{50}, H'_{70}, and H'_{90} represent the hemicellulosic fractions obtained from NaOH-extracted hemicelluloses. [b]Based on DMSO-extracted and NaOH-extracted hemicelluloses (w/w). [c]Expressed in relative molar percentages, Ara, arabinose; Gal, galactose, Glc, glucose, Xyl, xylose, GlcA, glucuronic acid, and GalA, galacturonic acid. [d]Represented the ratio of arabinose to xylose and glucuronic acid to xylose. [e]The degree of acetyl substitution (DS_{AC}) of the four hemicellulosic fractions which are determined by integration of the signals of acetyl groups at 2.01 ppm and those of all carbohydrate signals.

and GlcA/Xyl reflect the degree of linearity or branching of hemicelluloses [27]. Based on the data in Table 1, little differences exist in composition of sugars among H_{30}, H_{50}, and H_{90}. With increasing the concentration of ethanol, there is also no apparent variation in Ara/Xyl and GlcA/Xyl, and this means the three fractions almost have the same degree of branching. The composition of H_{70} shows an obvious different result; it has the highest xylose component (95.4%) and the contents of arabinose and glucose as well as glucuronic acid are much lower compared with the other three fractions. The two molar ratios, Ara/Xyl (0.029) and GlcA/Xyl (0.011), are also much less than that of the others. This suggests that the DMSO-soluble hemicellulosic fraction H_{70} has more linear chemical structure.

Under the treatment of NaOH extraction, the molar composition of sugar units is listed. Xylose is the most predominant portion (56.2–93.2%). Arabinose (8.5–29.8%) and glucuronic acid (3.4–8.7%) are two main components, and this is similar to the result of DMSO-extraction. From Table 2, it is obvious that the molar percentage of arabinose and glucuronic acid increased as the concentration of ethanol increases in the precipitation process. Thus, elevating trend of the two molar ratios (Ara/Xyl and GlcA/Xyl) appeared, which is in accordance with our previous studies [28]. As for the DMSO-extracted hemicelluloses, no striking differences among the values of Ara/Xyl and GlcA/Xyl ratios were found, which was probably due to the fact that the DMSO-soluble hemicellulosic fractions possess acetyl groups in structures and the branch degrees of them are too similar to be distinguished by gradient ethanol precipitation. From the above, when compared with DMSO-soluble hemicellulosic fractions, NaOH-soluble hemicellulosic fractions with different degrees of branch could be well distinguished by gradient ethanol precipitation. Namely, there are significant discrepancy of branch degree among the NaOH-soluble hemicellulosic fractions, and gradient ethanol precipitation seems like a satisfied fractionation method for them. For DMSO-soluble hemicellulosic fractions, which own similar branch degrees, gradient ethanol precipitation could not separate them well. A conclusion could be drawn that gradient ethanol precipitation is suitable for fractionation of hemicellulosic fractions with significant discrepancy of branching degree.

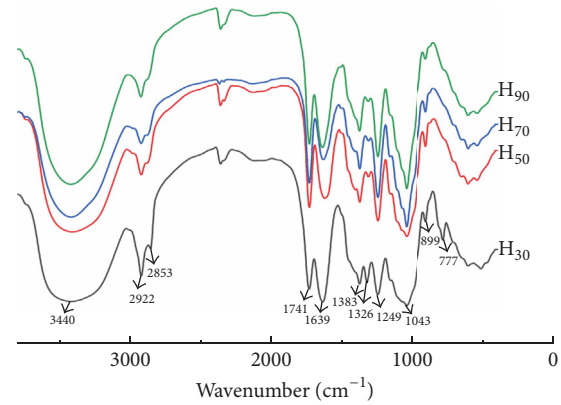


Figure 2: FT-IR spectra of DMSO-extracted fractions H_{30}, H_{50}, H_{70}, and H_{90}.

3.2. FT-IR Spectra. The spectra of the four DMSO-extracted hemicellulosic fractions, H_{30}, H_{50}, H_{70}, and H_{90}, which are precipitated by ethanol with concentration of 30%, 50%, 70%, and 90%, respectively, can be seen in Figure 2. The striking peaks around 3440 cm^{-1} are due to strong hydrogen-bonded O-H stretching and two absorptions at 2922 and 2853 cm^{-1} are results of C-H stretching [29]. A sharp peak at 1741 cm^{-1} is attributed to the characteristic feature of C=O stretching of carbonyl and acetyl groups in the hemicelluloses, which indicated that the acetyl ester bond could be preserved under the DMSO treatment [30]. Figure 2 clearly shows that the signals at 1741 cm^{-1} in spectra of H_{50} and H_{70} are stronger than those of H_{30} and H_{90}; this is finely consistent with the

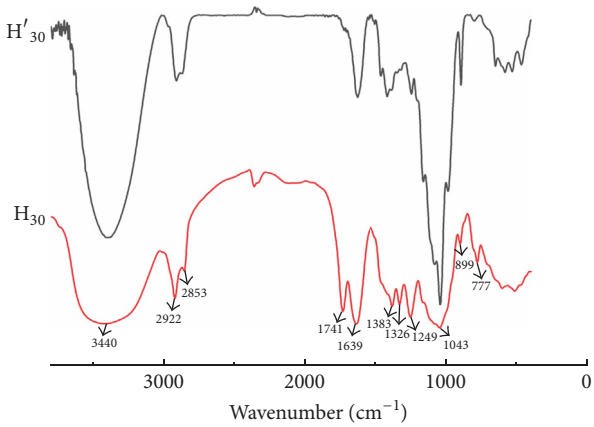


Figure 3: FT-IR spectra of DMSO-extracted fraction H_{30} and NaOH-extracted fractions H'_{30}.

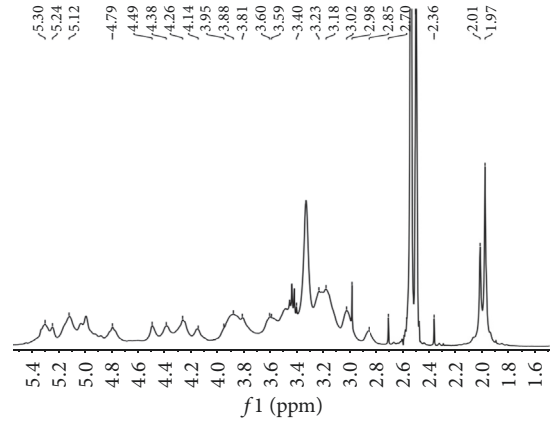


Figure 4: ^{1}H NMR spectrum of hemicellulosic fraction H_{50}.

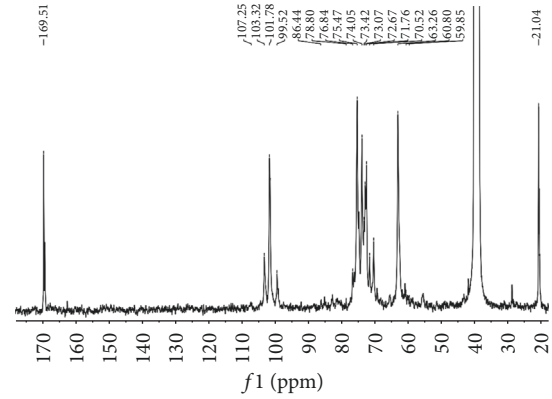


Figure 5: ^{13}C NMR spectrum of hemicellulosic fraction H_{50}.

result of sugar analysis (Table 1). The signals at 1414 cm^{-1} are assigned to CH or OH bending [31]. Bands between 1166 and 1000 cm^{-1} are the typical evidences of xylan. A really weak signal around 1166 cm^{-1} is characteristic of the dominant C-O stretching in C-O-C glycosidic linkages, and the contribution of C-OH bending from arabinoxylan and the variation of the signal intensity reflect the degree of substitution by arabinose residues [30]. The absorption at 1043 cm^{-1} might be assigned to C-OH bending [32], and the band at 897 cm^{-1}, which corresponds to the C_1 group frequency or ring frequency, is characteristic of β-glycosidic linkages between the sugar units [33]. The signal at 777 cm^{-1} indicates that the arabinose, glucose, and glucuronic acid are probably linked by α-glycosidic linkages [34]. The remaining bands at 1248 and 1383 cm^{-1} represent O-H in-plane and C-H bending vibrations, respectively [35]. The four spectra of NaOH-extracted fractions are also similar, but they are different from that of DMSO-extracted fractions. For instance, the comparison of the two spectra H_{30} and H'_{30} is exhibited in Figure 3. The sharp peak at 1741 cm^{-1} which is characteristic of C=O stretching of carbonyl and acetyl groups can be only seen in spectrum of H_{30}, while in NaOH-extracted fractions, it was not found.

3.3. NMR Spectra. Many NaOH-extracted hemicellulosic fractions have been characterized by NMR in our previous experiments; therefore, only structure elucidation of DMSO-extracted hemicellulosic fractions is determined by means of 1D (^{1}H and ^{13}C) and 2D (HSQC) NMR spectrometry now. Only spectrum of H_{50} is displayed here because the hemicellulosic fractions have similar structures based on the results of FT-IR spectra. Both the ^{1}H NMR and ^{13}C NMR spectra are compared with data in literatures and this allows the assignments of major peaks to be made. In Figure 4, signals of arabinofuranosyl and 4-*O*-methylglucuronic acid units appear in the 5.0–5.4 ppm region, while those from the xylopyranosyl residues are at 4.4–4.6 ppm region. Signals at 4.26 (H-1), 2.98 (H-2), 3.18 (H-3), 3.44 (H-4), 3.02 (H-5_{ax}), and 3.88 (H-5_{eq}) ppm correspond to 1→4 linked β-D-xylose residues [36]. The peak that appeared at 5.30 ppm is characteristic of terminal arabinofuranosyl residues linked to *O*-3 in H_{50}, and peaks at 3.81, 3.59, 3.95, and 3.44 are attributed to H-2, H-3, H-4, and H-5 of arabinofuranosyl residues, respectively [37, 38]. Other minor signals at 5.24, 3.02, 3.40, and 3.23 ppm are attributed to C-1, C-2, C-3, and the methoxyl group of 4-*O*-methyl-*a*-D-glucuronic acid residues, respectively [37, 39]. The total balance of acetyl group was checked by integration of its CH_3- moieties at 2.05–2.30 ppm [40]. The signal at 2.01 ppm apparently indicates that the hemicellulosic fraction in H_{50} is highly acetylated, and the degree of acetyl substitution (DS_{AC}) of the hemicellulosic fraction H_{50} was 0.23 (Table 1), which was determined by integration of the signals of acetyl groups at 2.01 ppm and those of all carbohydrate signals. The peak at 5.12 ppm indicates the 2-3-di-*O*-acetylated (H-3 integral at δ_H 5.11–5.15) structure [37]. The DS_{AC} of fractions H_{50}, H_{70}, and H_{90} are similar, while that of H_{30} is relatively lower, only about 0.13.

The ^{13}C NMR spectrum (Figure 5) contains five major signals corresponding to those of (1→4) linked β-D-xylose residues. The signal at 101.78 ppm is assigned to the anomeric region in a β-configuration of β-D-xylose. Signals at 72.67, 73.42, 75.47, and 63.26 ppm are characteristic for C-2, C-3, C-4, and C-5 of β-D-xylose residues, respectively [41].

Table 3: ^{1}H and ^{13}C chemical shift (ppm) assignments for hemicellulosic fractions H_{50}.

Sugar residue	Chem shift (ppm) H/C							
	1	2	3	4	5ax	5eq	6	OMe
→4)-β-Xylp(1→	101.78/4.26	72.67/2.98	73.42/3.18	75.47/3.44	63.26/3.02	63.26/3.88		
α-GlcAp-(1→2	99.52/5.24	70.52/3.02	71.76/3.40					58.82/3.23
α-Araf-(1→2,3	107.25/5.30	78.80/3.81	76.84/3.59	86.44/3.95	61.12/3.44			
→4)-β-Xylp(1→, 2-O-Ac		73.07/4.38						
→4)-β-Xylp(1→, 3-O-Ac			74.05/4.79					

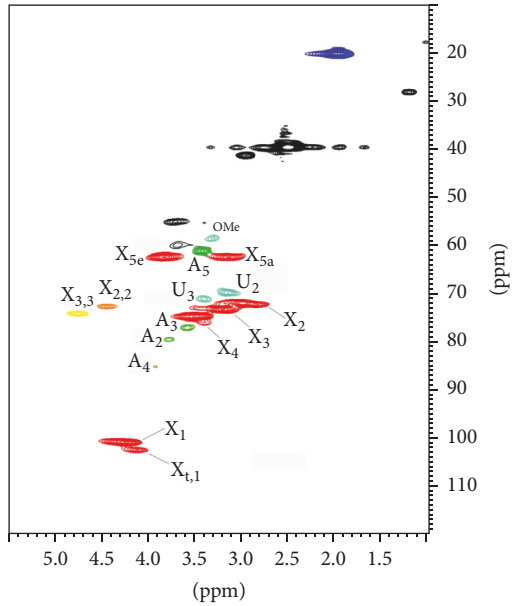


Figure 6: HSQC spectrum of hemicellulosic fraction H_{50}. Designations are as follows: X, Xyl*p*; A, Ara*f*; U, 4-*O*-Me-α-Glc*p*A; X_2, 2-*O*-acetylated Xyl*p*; X_3, 3-*O*-acetylated Xyl*p*; Xt, nonacetylated xylose with terminal end.

A weak signal at 107.25 ppm is assigned to C-1 of arabinofuranosyl residues. Peaks around 99.52, 70.52, 71.76, and 58.82 ppm are original from C-1, C-2, C-3, and -OCH_3 in 4-*O*-methylglucuronic acid residues [42]. Signals in the high-field resonance at 21.04 ppm suggest the presence of *O*-acetyl groups in the hemicellulosic fraction, which is in accord with the results of FT-IR and ^{1}H NMR [43].

The chemical shift assignments of HSQC ^{13}C/^{1}H cross peaks for hemicellulosic fractions H_{50} are provided in detail by Table 3. The dominating five signals in Figure 6 are at 101.78/4.26, 72.67/2.98, 73.42/3.18, 75.47/3.44, and 63.26/(3.02, 3.88) ppm, corresponding to C-1/H-1~C-5/H-5 of 4-linked β-xylose residues, respectively [44]. The cross peaks at 70.52/3.02, 71.76/3.40, and 58.82/3.23 ppm are unambiguously assigned to C-2/H-2, C-3/H-3, and -OCH_3 of 4-*O*-methylglucuronic acid residues substituted at *O*-2 of xylose [42, 45]. Two cross peaks at 73.07/4.38 and 74.05/4.79 ppm are due to the resonance of acetylation at positions 2 and 3 of 1, 4-linked β-Xyl*p* residues, respectively [46]. Three relative weak signals that appear at 107.25/5.30, 78.80/3.81, and 76.84/3.59 are attributed to C-1/H-1, C-2/H-2, and C-3/H-3 of arabinofuranosyl residues [47]. The 2D integrals of H-2/C-2 and H-3/C-3 cross peaks indicate that *O*-acetyl groups at C-2 and C-3 are in proportion of 0.87/1.00. The cross peak at 103.32/4.14 is assigned to nonacetylated Xyl with terminal end [48]. On the basis of the NMR analysis, it can be inferred that the fraction H_{50} mainly consists of arabinose linked on position 3 of xylan, 4-*O*-methylglucuronic acid residues linked on position 2 of the xylan bone and acetyl groups linked on positions 2 and 3. The other three hemicellulosic fractions have similar structures.

4. Conclusions

In this study, hemicelluloses from *Neosinocalamus affinis* were extracted by DMSO and 3% NaOH, sequentially precipitated by ethanol with gradient concentration. In gradient ethanol precipitation, results of sugar analysis suggest that Ara/Xyl and GlcA/Xyl of NaOH-extracted fractions are ascending as the concentration of ethanol increases, while this trend was not found in DMSO-extracted fractions with different branch degree obtained by gradient ethanol precipitation. Through the characterization of GPC, FT-IR, and NMR, it can be inferred that DMSO is a kind of mild solvent which could preserve important structures of hemicelluloses such as acetyl groups. The DMSO-extracted *Neosinocalamus affinis* hemicelluloses mainly consist of highly acetylated arabino-4-*O*-methylglucurono-(1→4)-β-D-xylan, in which acetyl residues are linked at positions *O*-2 and *O*-3, 4-*O*-Me-β-D-GlcA-(1→2) units are linked at position *O*-2, and arabinofuranosyl residues are linked at *O*-3.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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