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Molecule Characteristics of *Eucalyptus* Hemicelluloses for Medical Microbiology

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Eucalyptus **biomass was used as the fine nutritive material for medical microbiology. However, although single composition of woody biomass has been succeed in preparation for medical microbiology, but green and high efficient separation of woody biomass was still a very difficult. Therefore, the molecule characteristics of Eucalyptus hemicelluloses were investigated and analyzed by NMR and FTIR. The result showed that the three Eucalyptus hemicelluloses had a structure composed of the (1→4)-linked β-D-xylopyranosyl backbone with 4-O-methyl-R-D-glucuronic acid attached to O-2 of the xylose residues. In** ***Eucalyptus urophydis***, ***Eucalyptus urophynis*** **and** ***Eucalyptus camaldulensis*** **hemicellulosic samples, xylose was an extremely predominant component sugar which relative percent were 80.06, 77.80% and 77.54%, respectively. And** ***Eucalyptus camaldulensis*** **hemicelluloses had a higher molecular weights than ones of other two** ***Eucalyptus*****.**

Key words: *Eucalyptus urophydis; Eucalyptus urophynis*; *Eucalyptus camaldulensis*; Hemicelluloses; Medical microbiology.

Traditional Chinese medicine, which originated from China for a long history of several thousand years, had formed a unique system to diagnose and cure illness by clinical practice, master dictate, constant awareness and gradual accumulation, and had made significant contributions on treatment of diseases, protection of human health and prosperity of the Chinese nation[1]. Currently, there are about 12,000 kinds of medicinal plants in China which were used to develop new drug and lead compounds with novel structure, high efficiency and low toxicity[2]. Some natural medicine has been found in poisonous plants.

According to historical records, there were more than 900 species of poisonous plants in China, some toxic components coexist with the active ingredient, and some toxic ingredients is the active ingredient, and the proper dosage could treat many severe diseases[3]. Many novel structure and strongly active compounds such as Camptothecin, vincristine and taxol were found in poisonous plants such as acuminata, periwinkle and yew[4]. Traditional Chinese medicine came mainly from herbaceous. But herbaceous were too few to meet the current demand for traditional chinese medicine. However, plantations have been planted extensively in China to provide resources for woody medicine. Extraction industries of woody medicine cross four edges between pharmaceuticals, fine chemicals, agriculture and forestry is emerging.

Hemicelluloses were defined as being polysaccharides that could be extracted by water

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or aqueous alkali from plant tissue[5]. They were associated with various other cell-wall components such as cellulose, lignin, and other phenolic compounds by covalent and hydrogen bonds, and by ionic and hydrophobic interactions[6]. Hemicelluloses had a very wide variety of direct food and nonfood applications, and could be converted to chemicals as feed, such as furfural, erythritol, xylitol, ethanol, or lactic acid[7]. The plant hemicelluloses were used as viscosity modifiers, gelling agents, tablet binders, or wet strength additives[8]. What's more, xylans also have been studied for their possible medical use as antitussive, ulcer protective, antitumor agents, and immunostimulatory[9-12]. Subfractionation of hemicelluloses could produce low-branched xylans and further obtain the xylose, an intermediate for the production of xylitol which had already been used in food applications such as chewing gum or tooth paste and could provide an alternative sweetener for diabetics[13-15]. And molecular identification of hemicelluloses was a crucial perquisite to applications, in particular on a larger industrial scale. However, the papermaking focused on the extraction and fractionation of hemicelluloses in order to obtain different substituted hemicellulosic subfractions for various industrial uses. Therefore, elucidation of the physicochemical properties and structural characteristics of the *Eucalyptus* hemicelluloses were investigated and analyzed by NMR and FTIR.

MATERIALS AND METHODS

Materials and Reagents

The 5-year-old *Eucalyptus urophynis* (*Eucalyptus urophylla* ×*Eucalyptus tereticornis*) and *Eucalyptus urophydis* (*Eucalyptus urophylla*×*Eucalyptus camaldulensis*) were collected from Yangjiang Forest Farm, Guangdong province, P. R. China. The 18-year-old *Eucalyptus camaldulensis* was collected from the Forest Farm of Central South University of Forestry and Technology, P. R. China. Their sample chips were processed from fresh material, and dried to absolute dry with rotary evaporator in 55°C and negative 0.01MPa. About 200 mesh powder was sieved out using AS200 Sieving Instrument (Made in America). Lignocellulosic biomass was obtained by benzene/ethanol, methanol and acetic ether extractions, and dried to absolute dry with rotary evaporator in 55°C and negative 0.01MPa. The benzene-ethanol solution was mixed according to $V_{ethanol}$ /$V_{benzene}$ 2 double. KOH, Acetic acid, 30% hydrogen peroxide (analytically pure grade) and deionized water were prepared for the subsequent experiments.

Experiment methods

Hemicelluloses Separation

The 5g above-mentioned powders of *Eucalyptus urophynis* and *Eucalyptus urophydis* lignocellulosic biomass were treated in 100 mL 17.5%KOH solution under 25°C for 3h, 6 h, 9 h, 12 h, 16 h, 20 h, 24 h, 36 h, respectively. After treated, the samples were filtered, then dried in the air, and dried to absolute dry in an oven, finally reserved in the dryer.

Spectroscopic Characterization

FT-IR spectra of hemicellulosic samples were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disk containing 1% finely ground samples. Thirty-two scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. The solution-state 1H NMR spectra were recorded on a Bruker NMR spectrometer at 400 Mz using 15 mg of hemicelluloses in 1.0 mL of D_2O. The chemical shifts reported were calibrated relative to the signals fromD_2O, used as an internal standard, at 4.7 ppm for the 1H NMR spectra.

Table 1. Uronic Acids and Neutral Sugars (Relative Percent Hemicellulosic Sample, w/w) and Uro/Xyl Ratios of Hemicellulosic Subfractions

Sugars	Arabinose	Galactose	Glucose	Xylose	Uronic acid	X/U (Uro/Xyl)
Eucalyptus urophydis	0.75	0.92	3.53	80.06	14.74	5.43(0.18)
Eucalyptus urophynis	1.39	2.63	3.01	77.80	15.18	5.12(0.20)
Eucalyptus camaldulensis	0.92	1.49	3.84	77.54	16.18	0.21

Note: Uro/Xyl, Uronic acids/ Xylose.

RESULTS AND DISCUSSION

The content of uronic acid and neutral sugar composition of three hemicellulosic subfractions were listed in Table 1. The neutral sugar analysis of the hydrolysates showed that xylose (77.54-80.06%) was an extremely predominant component sugar in three hemicellulosic subfractions. Uronic acid (14.74-16.18%), mainly glucuronic acid (GlcpA) or 4-O-methyl-glucuronic acid (4-O-Me-R-D-GlcpA), was present as a substantial amount. Arabinose (0.75-1.39%), galactose (0.92-2.63%), and glucose (3.01-3.84%) were present in smaller amounts. The predominance of xylose and the substantial amount of uronic acids indicated that the 17.5% alkali-soluble hemicelluloses of three *Eucalyptus* probably consisted mainly of glucuronoxylans. Xylose relative percent of *Eucalyptus urophydis*, *Eucalyptus urophynis* and *Eucalyptus camaldulensis* hemicellulosic samples were 80.06, 77.80% and 77.54%, respectively. Uronic acid relative percent of *Eucalyptus urophydis*, *Eucalyptus urophynis* and *Eucalyptus camaldulensis* hemicellulosic samples were 14.74%, 15.18% and 16.18%, respectively. From the ratio of uronic acid to xylose (0.18-0.21) in the two hemicellulosic subfractions, it could be concluded that the alkali-soluble hemicelluloses from Populus gansuensis consist of relatively low substituted xylans.

Table 2 shown the weight-average (M_w) and number-average (M_n) and polydispersity (M_w/M_n) of hemicellulosic subfractions. As shown in the data in Table 2, the M_w of hemicelluloses

Table 2. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersity (M_w/M_n) of the Hemicellulosic Subfractions

Index	*Eucalyptus urophydis*	*Eucalyptus urophynis*	*Eucalyptus camaldulensis*
Uro/Xyl	0.18	0.20	0.21
M_w	91530	99960	79420
M_n	43240	42950	33430
M_w/M_n	2.12	2.32	2.37

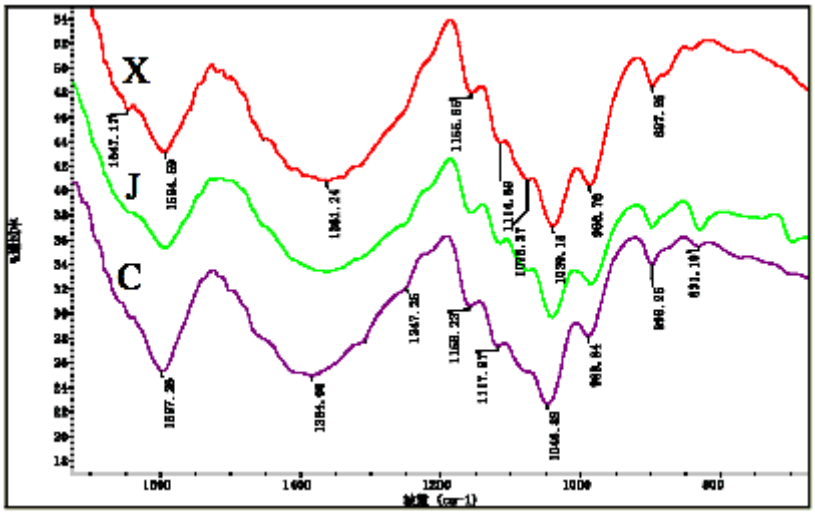

Fig. 1. FT-IR spectra of hemicellulosic subfractions X(*Eucalyptus urophynis*), J (*Eucalyptus urophydis*) and C (*Eucalyptus camaldulensis*)

followed the order *Eucalyptus urophynis* hemicelluloses > *Eucalyptus urophydis* hemicelluloses > *Eucalyptus camaldulensis* hemicelluloses, and the M_w/M_n of hemicelluloses followed the order *Eucalyptus camaldulensis* hemicelluloses > *Eucalyptus urophynis* hemicelluloses > *Eucalyptus urophydis* hemicelluloses. Among three *Eucalyptus,* Uro/Xyl ratio of *Eucalyptus urophydis* hemicelluloses was the lowest, one of *Eucalyptus camaldulensis*

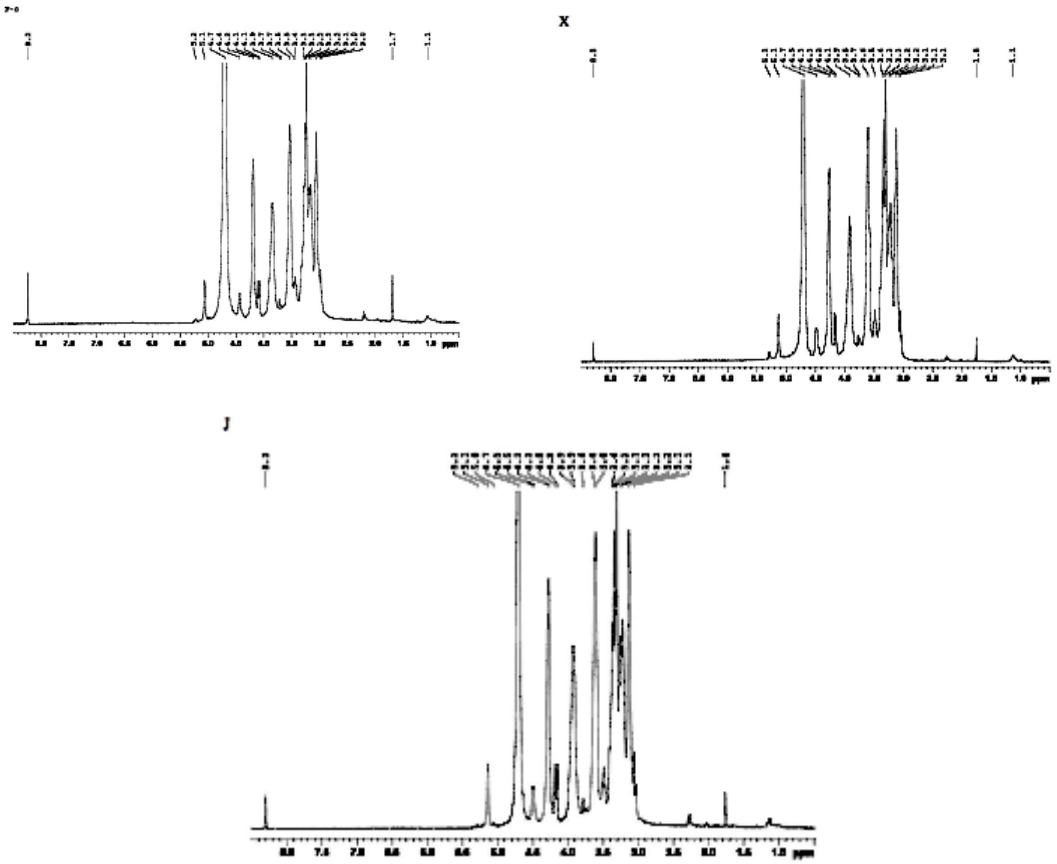

Fig. 2. ^{1}H NMR spectra (in D_2O) of hemicellulosic subfractions X(*Eucalyptus urophynis*), J (*Eucalyptus urophydis*) and C (*Eucalyptus camaldulensis*)

hemicelluloses was the largest, resulting that *Eucalyptus camaldulensis* hemicelluloses had a higher molecular weights.

FT-IR spectra of hemicellulosic subfractions J (*Eucalyptus urophydis*) and C (*Eucalyptus urophynis*) X(*Eucalyptus urophynis*), J (*Eucalyptus urophydis*) and C (*Eucalyptus camaldulensis*) were shown in Figure 1. From FT-IR spectra of three *Eucalyptus* hemicelluloses, the major absorbance at 1039-1047 cm^{-1} was assigned to the C-O-C stretching of glycosidic linkages, which was typical of xylans (25), the band at 1156-1158 $cm^{-1}$was assigned to the C-O-C stretching of glycosidic linkages from arabinoxylans, while the small sharp band at 898 cm^{-1} was characteristic of dominant â-glycosidic linkages between the sugar units in the hemcellulosic subfractions. FT-IR spectra of hemicellulosic subfractions theree *Eucalyptus* were similar, their peak intensities were different resulting that there were groups of *Eucalyptus urophynis* and *Eucalyptus urophydis* hemicelluloses.

As could be seen from Figures 2, the three hemicellulosic sufractions showed very similar spectra, indicating a similar structure of hemicelluloses. The anomeric ^{1}H NMR signals of three *Eucalyptus* hemicelluloses were found in the spectral region of 3.1-4.7 ppm (Figure 2) . The signals of R-anomeric protons were seen in the spectral region of 5.0-5.3 ppm and â-anomeric protons at 4.4-4.6 ppm. Examination of data relative to 1H NMR analysis revealed three important groups of protons: the unsubstitued (1→4) linked β-D-xylopyranosyl ((1→4)-β-D-Xylp, X), the substituted β- D-xylopyranosyl ((1→4)-β-D-Xylp-2-O-GlcpA, XU), and (1→2) linked 4-O-methyl-R-D-glucopyranosyl uronic acid (4-O-Me-R-DGlcpA, U) residues

CONCLUSION

Xylose was an extremely predominant component sugar in three *Eucalyptus* hemicellulosic subfractions, and their relative percent were 80.06, 77.80% and 77.54%, respectively. Uronic acid, which were mainly glucuronic acid (GlcpA) or 4-O-methyl-glucuronic acid (4-O-Me-R-D-GlcpA), were 14.74%, 15.18% and 16.18%, respectively. And M_w of hemicelluloses followed the order *Eucalyptus urophynis* hemicelluloses > *Eucalyptus urophydis* hemicelluloses > *Eucalyptus camaldulensis* hemicelluloses, and the M_w/M_n of hemicelluloses followed the order *Eucalyptus camaldulensis* hemicelluloses > *Eucalyptus urophynis* hemicelluloses > *Eucalyptus urophydis* hemicelluloses, resulting that *Eucalyptus camaldulensis* hemicelluloses had a higher molecular weights. FT-IR spectra and ^{1}H NMR spectroscopy analysis indicated that the three *Eucalyptus* hemicelluloses had a structure composed of the (1→4)-linked β-D-xylopyranosyl backbone with 4-O-methyl-R-D-glucuronic acid attached to O-2 of the xylose residues.

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