Characterization of a novel polysaccharide purified from a herb of Cynomorium songaricum Rupr.

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ABSTRACT

Cynomorium songaricum Rupr. (C. songaricum, Cynomoriaceae) is a food for local people and also a tonic in traditional Chinese medicine. As polysaccharide is an active component in it, it is great significant to study the structure. This research studied a new polysaccharide fraction purified from C. songaricum, named CSP-DS1 (Mw of 48.1 × 10^4 Da). The CSP-DS1 was investigated by chemical and instrumental analysis, including acid hydrolysis and methylation analysis, infrared spectra (IR), gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR) (1H, 13C, 1H-1H COSY, HMBC and HMQC). The results demonstrate that the CSP-DS1 is comprised of three kinds of monosaccharide: Man, Glc and Gal, in approximately mole ratios of 5.01: 89.17: 5.82. Based on characterization and analysis, its possible glucosidic linkage of the CSP-DS1 is also established.

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1. Introduction

Throughout history of traditional Chinese medicine, many natural products have been widely used as medicines for preventing and treating different types of diseases due to the nature of both food and medicine (Chu, Tlan, Lin, & Ye, 2006; Li, Jiang & Chen, 2008; Shi, Jiang, Cai, & Sui, 2011; Li et al., 2014; Avi, Yanai, Gal, & Yoav, 2014). Cynomorium songaricum Rupr. (C. songaricum, Cynomoriaceae) is a good example of that. The species C. songaricum, which is a non-photosynthetic plant, found in Mediterranean countries (Rosa et al., 2012), also known as “suo yang” in China, is a wild plant grows in the Gobi desert. As a special plant, the C. songaricum was recorded as early as in China’s pre-Qin. It is a perennial herb and known to parasitize the plant roots of Nitraria spp. The roots and inflorescences of C. songaricum are extremely tiny and the rust-colored edible fleshy stems are as rigid as bamboo shoot. It also resembles a male penis, perhaps that is why the C. songaricum is referred to as suo yang in Chinese that has the meaning of “Vigra”. The stem of C. songaricum is generally used to increase sexual capability and to treat lumbar weakness in oriental countries (Cui et al., 2013; Shi et al., 2011; Zhang et al., 2012). It also has been widely used as health food for centuries because of its taste, strong tonic effects and non-toxic (Meng & Ma, 2013; Ma, Sato, Li, Nakamura, & Hattori, 2010).

Undoubtedly, its fine properties are related to the chemical components. A more recent study has shown that numerous chemical ingredients exist that affect multiple pharmacological effects of C. songaricum, including condensed tannins, steroids, triterpenes, butyl fructosides, flavanoids, lignan glycosides, alkaloids, etc. Among them, polysaccharide stands out as an important one because it is safe, replenishable and biodegradable (Ma, Nakamura & Hattori, 2001; Nwokocha & Williams, 2014). Actually, the studies on the C. songaricum polysaccharide have been reported in the last decades and discovered they play special roles, such as anti-diabetes (Wang et al., 2010), anti-aging effects by increasing telomere length of senescence mice (Liu et al., 2011; Meng, Wang,
Li, Kuang, & Ma, 2013) and scavenging free radicals. These suggest that the C. songaricum polysaccharide may be well applied to medical health care or fit by the food industry like other active polysaccharide. For example, numerous chemical synthetic anti-oxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used in food products (Karre, Lopez, & Getty, 2013), but they have fallen under scrutiny due to potential toxicological effects (Vijayabaskar & Vaseela, 2012). Therefore, attention has been directed toward the isolation of natural antioxidants from botanical sources, and polysaccharide is one. However, the information on the purified C. songaricum polysaccharides of the pharmacological functions and the structure activity relationship is lacking. Most of these researches focus on the crude polysaccharides of the C. songaricum, which contain other ingredients such as proteins and make its composition is too complex to determine the effect of polysaccharides. In addition, it proves that the different fractions from the crude polysaccharide have distinct biological activities (Chi, Chen, Wang, Xiong, & Li, 2008).

For fine polysaccharides from food or herb have been used widely, it is valuable to isolate and characterize the fractions from crude polysaccharide of the C. songaricum. In this study, a polysaccharide named CSP-D1 was purified from the C. songaricum, and the chemical structure was characterized, as a prelude to establish the structure-activity relationships.

2. Materials and methods

2.1. Materials and reagents

The C. songaricum was obtained from yellow river medicine market, Lanzhou, Gansu province, China (Dry fleshy stem, identified by Dr. Yifeng Wang, professor of botany at northwest normal university). DEAE-52 Cellulose and Sepharose CL-6B were purchased from Solarbio Co., Ltd., Beijing, China. Papain was obtained from Sigma-Aldrich, Shanghai, China. All kinds of pure monosaccharide standards (L-ribose (Amr), L-rhamnose (Rha), D-lyxose (Lxy), D-arabinose (Ara), D-xyllose (Xyl), D-mannose (Man), D-glucose (Glc), D-galactose (Gal)) were obtained from Tianjin chemical reagent factory, Tianjin, China. The other chemicals used were of reagent grade from commercial sources.

2.2. Preparation and quality control of the CSP-D1

As shown in Fig. 1, the separation procedures of the crude polysaccharide (CSP) were based on a previous work (Zhang et al., 2009). Briefly, the dried C. songaricum was crushed in a grinder to make fine powder and per-extracted with ethanol to remove lipids and pigments. The filtered residues were air dried and extracted three times with boiled water in a ratio of 1:10 (w/v) for 1.5 h. The extract was concentrated in a rotary evaporator under reduced pressure, precipitated by 95% (v/v) ethanol at room temperature for 48 h, then centrifugation and vacuum freeze-dried to obtain the CSP.

The protein of CSP was removed by the Sevage method combined with papain (Zhang, Huang, Hou, & Wang, 2006), again further used hydrogen peroxide to decolorize, and then was dialyzed with dialysis bags with a molecular weight cut off 8000–12,000 Da, then obtained the more pure polysaccharide after alcohol precipitating and drying.

The 200 mg above mentioned polysaccharide was dissolved in distilled water and loaded onto a column (1.6 cm × 50 cm) of DEAE-52 Cellulose. The column was sequentially eluted with distilled water and gradient eluted with NaOH (0 – 0.2 M) at a flow rate of 0.6 ml min⁻¹ and each fraction of 4.8 ml of eluate was collected (Peng et al., 2010; Zhang, Tian, Jiang, Miao, & Mu, 2014). The fractions were combined according to the carbohydrate content, as determined by the phenol-sulfuric acid method. Water eluate (named CSP-D1) was further purified by a Sepharose CL-6B column (2.5 cm × 90 cm). The column was eluted with distilled water at a flow rate of 0.6 ml min⁻¹ and each fraction of 6 ml of eluate was collected (Lin et al., 2012). The corresponding fractions were pooled together, dialyzed and lyophilized, generated the purity of polysaccharide collection named CSP-D1.

The quality control of the CSP-D1 was performed. Total contents of carbohydrate and uronic acid were determined by colorimetric methods of phenol-sulphuric acid and m-hydroxydiphenol using glucose and glucuronic acid as reference, respectively (Blumenkrantz & Asboe, 1973; Dubois, Gilles, Hamilton, Roberts, & Smith, 1956). The content of protein was determined by Lowry’s method with bovine serum albumin (BSA) as a standard (Kumar, Joo, Choi, Koo, & Chang, 2004). UV absorption spectrum was recorded with a UV spectrophotometer (Labtech UV1000).

2.3. Homogeneity and molecular weight determination

The homogeneity and molecular weight of the CSP-D1 were evaluated and determined by size-exclusion chromatography combined with multi-angle laser light scattering (SEC-LLS). SEC-LLS measurements were carried out on a multi-angle laser photometer (MALLS, DAWN® HELEOS, Wyatt Technology Co., Santa Barbara, CA, USA) at 690.0 nm and an Ultrahydrogel™ column (7.8 × 300 mm, Waters, USA) was used as the SEC instrument. An Optilab
refractometer (Dawn®) was simultaneously connected. The samples were dissolved in ultrapure water overnight with stirring. All solution were filtered through a 0.45 μm filter before use and achieved by filtration into a scattering cell. The injection volume was 200 μl for each sample and the flow rate was 0.5 ml min⁻¹. The refractive index increment (dn/dc) value of the sample was determined by using an OptiLab refractometer at 690 nm and 25 °C, to be 0.147 ml g⁻¹. As the column separates the polymer according to molecular weight, each fraction was led to the light scattering detector for instantaneous measurement of the scattering intensities. The refractive index detector, connected in series, gave the estimate of the polymer concentration based on the peak area, according to glucon standards and Astra software (Version 5.3.1) was utilized for data acquisition and analysis. In chromatography mode, there was a single and sufficiently low concentration at a particular slice because of the further dilution by the SEC column of the already diluted injected solutions.

2.4. Structural characterizations

2.4.1. Monosaccharide composition

The CSP-DS1 (10 mg) was hydrolyzed with 4 M trifluoroacetic acid (TFA) ml at 120 °C for 10 h under the atmosphere of nitrogen. After boiled away water, ammonium hydrochloride and pyridine were added and allowed to react at 90 °C for 30 min. Next, acetic anhydride was added to the flask, and the mixture was incubated at 90 °C for a further 30 min to allow the acetylation reaction to occur (Zhang et al., 2007). The acetylated derivatives were dissolved in chloroform analyzed by GC–MS (Agilent Technologies Inc., USA) with an HP-5 column (0.25 mm × 30 m × 0.25 μm). The temperature program was set at 160 °C and maintained for 3 min, then increased to 210 °C at an increment of 2 °C min⁻¹. This temperature was held for 1 min, injection and detector temperatures was 250 °C and the loading quantity of sample was 0.2 μl. Standard monosaccharides were measured following the same procedure.

2.4.2. Infrared spectrum analysis

IR spectrum of the CSP-DS1 was determined using a Fourier transform infrared spectrophotometer (FT-IR FTS3000, PE, Co., Ltd., USA). The purified polysaccharide was grounded with KBr powder and pressed into a 1 mm pellet for IR measurement between 400 and 4000 cm⁻¹ (Dong, Yao, Yang, & Fang, 2002).

2.4.3. Methylation analysis

Methylation analysis was carried out according to the method of Needs and Selvendran (Ciucanu & Kerek, 1984). Briefly, 10 mg of CSP-DS1 was dissolved in 5 ml of anhydrous dimethylsulfoxide (DMSO) in a three-necked flask at room temperature (25 °C) under the atmosphere of nitrogen. Then, 10 mg of NaOH was added, after intensive mixing, the mixture was stirred for 1 h under the atmosphere of nitrogen. Methyl iodide (3.7 ml) was added and maintained for 1 h at room temperature (25 °C), finally added 1 ml distilled water to terminate reaction. The product was then extracted with frequent, small amounts of chloroform. The chloroform layer was washed twice with 2 ml of distilled water, and the chloroform was then dried in a rotary evaporator at room temperature. Repeated the methylation reaction until exhaustive methylation could be detected by infrared examination. The residues were further hydrolyzed with 4 M trifluoroacetic acid (TFA) at 120 °C for 10 h in protection of nitrogen. Excess trifluoroacetic acid was removed by evaporation under reduced pressure. Next, 20 mg of NaBH₄ and 1 ml NaOH (0.05 M) were added and reacted for 20 min at room temperature. As the residues cooled, with glacial acetic acid adjust pH value. The residues were dissolved in methanol and evaporated to dryness three times. The partially methylated residues were acetylated by 0.6 ml of acetic anhydride at 120 °C for 2 h. The corresponding acetate derivatives were dissolved in chloroform and analyzed with a GC–MS. The CSP-DS1 was identified by its fragment ions in according to the MS spectrums and the relative retention times on GC profile, as well as, the mole ratios of each sugar was calibrated using the peak areas.

2.4.4. NMR analysis

The CSP-DS1 sample was dissolved in D₂O to make a 15 mg ml⁻¹ solution, freeze-dried and redissolved in 0.5 ml of D₂O at room temperature for 3 h before NMR analysis. For 1D and 2D NMR analysis, spectrometers were obtained on Bruker Advance DRX-400 spectrometer (Bruker, Germany), equipped with a process controller, operating on 399.95 MHz and 100.58 MHz, using a 5 mm broad-band probe. Heteronuclear multiple quantum coherence (HMOC) was recorded with carbon decoupling. For the ¹³C assignment, a heteronuclear multiple bond correlation (HMBC) spectrum was recorded with a J-evolution time of 80 ms, the chemical shift was expressed in ppm.

3. Results and discussion

3.1. Basic properties of the CSP-DS1

The CSP was isolated from the hot-water extract of C. songaricum with a yield of 9.1%, and separated on a DEAE-S2 cellulose column. Two main fractions were obtained, the water elute fraction and the NaOH elute fraction, named CSP-D1 and CSP-D2, respectively (Fig. 2a). The CSP-D1 was further purified on Sepharose CL-6B column, and obtained a main fraction, with a yield of 0.03% and named CSP-DS1 (Fig. 2b), the result indicates that only one symmetrical peak on chromatography. Likewise, it has no typical absorption of nucleic acid or protein by ultraviolet (UV) scanning at 200 – 400 nm (the concentration of CSP-DS1: 1 mg ml⁻¹) and has higher carbohydrate content (89.2% w/w) without uronic acid. This study was only concerned with the CSP-DS1 as the CSP-D2 is low in carbohydrate content for doing polysaccharide research.

Light laser scattering (SEC-LLS) was applied to determine the range and the distribution of molecular weight of the CSP-DS1. The SEC-LLS chromatogram of the CSP-DS1 is shown in Fig. 3. The weight-average molecular weight (Mw), polydispersity (Mw/Mn) and radius of gyration (Rg) are measured to be 48.1 × 10⁴, 3.19, 31.8 nm, respectively. The chromatogram of the CSP-DS1 exhibits a good symmetrical peak, indicating the CSP-DS1 is homogenous.

3.2. Monosaccharide composition and linkage features of the CSP-DS1

The CSP-DS1 was decomposed by acid hydrolysis, and its products were subjected to GC–MS after derivatization. Compared with the monosaccharide standards, the Man, Glc and Gal are detected (Fig. 4) and the peak of every monosaccharide is sharp and symmetrical. These peaks of the CSP-DS1 with mole ratios of 5.01: 89.17: 5.82, which indicates that the CSP-DS1 is heterogenous. The results also indicates that Glc is the major monosaccharide component.

The GC–MS was also used to determine and separate the fractions of the CSP-DS1 after methylation, hydrolysis and acetylation. The IR spectrum of the CSP-DS1 displays a broad stretching intense characteristic peak near 3388 cm⁻¹ for the hydroxyl group. After repeated methylation, this peak disappears, identifies the complete methylation of the CSP-DS1 (Fig. 5). Based on methylation analysis, the linkage patterns of CSP-DS1 are shown in Table 1.
The data from the methylation analysis shows the presence of five components, namely 2, 4, 6-Me$_3$-Glc, 2, 3, 4, 6-Me$_4$-Glc, 4, 6-Me$_2$-Glc, 2, 4, 6-Me$_3$-Gal and 2, 3, 4- Me$_3$-Man. The results indicate that the CSP-DS1 is a polysaccharide with numerous single sugar unit branch points. The proportion of the non-reducing terminal- $\alpha$-glucopyranosyl residue is 19.8%. Both results of acid hydrolysis and methylation linkage analysis of the CSP-DS1 indicate that Glc is the main composition, and the CSP-DS1 is mainly composed of a Glcp backbone linked 1/$\beta$ bonds. Some 1/$\alpha$ bonds of Galp and 1 → 6 bonds of Manp could be in the backbone or the side chains. The relative amounts of (1 → 2, 3)-linked-Glc indicates that approximate branch ratios may theoretically is 4.8%.

The IR spectra of the CSP-DS1 is shown in Fig. 5, the strong absorption is attributed to the $\varepsilon$OH stretching vibration appears around 3388.7 cm$^{-1}$ as a broad peak and the weaker bands at 2933.08 cm$^{-1}$ are due to the C–H stretching. The two peaks describes above are attributed to the hydroxyl stretching vibration of the polysaccharides (Geresh, Mamontov, & Weinstein, 2002). The $\alpha$-anomer and $\beta$-anomer of sugar is caused by C–H bending vibrations of end group. The wavenumber at 846.6 cm$^{-1}$ is corresponding to the $\alpha$-anomer bond, due to the C–H is equatorial bond that distinguishes it against the axial bond of $\beta$-anomer. In addition, the CSP-DS1 has no absorption bond in the range of terminal- $\alpha$-glucopyranosyl residue.

### Table 1

<table>
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<tr>
<th>Methylated sugar</th>
<th>Deduced linkage</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,5,6-O-Ac$_2$-2,3,4-O-Me$_1$-mannitol</td>
<td>1,6-Manp</td>
<td>6.2%</td>
</tr>
<tr>
<td>1,3,5-O-Ac$_2$-2,4,6-Me$_3$-glucitol</td>
<td>1,3-Glcp</td>
<td>75.6%</td>
</tr>
<tr>
<td>1,5-O-Ac$_2$-2,3,4,6-Me$_3$-glucitol</td>
<td>t-Glcp</td>
<td>5.1%</td>
</tr>
<tr>
<td>1,2,3,5-O-Ac$_4$-4,6-Me$_2$-glucitol</td>
<td>1,2,3-Glcq</td>
<td>4.8%</td>
</tr>
<tr>
<td>1,3,5-O-Ac$_2$-2,4,6-Me$_3$-galactitol</td>
<td>1,3-Galp</td>
<td>8.3%</td>
</tr>
</tbody>
</table>
846.62 – 936.8 cm⁻¹. These data suggest that the CSP-DS1 mainly contains α-anomer, and the β-anomer is scanty or non-existent (Miao et al., 2014). The peaks in the range of 936.8 ~ 1241.4 cm⁻¹ are attributed to the stretching vibration of C–O–C group and hydroxyl of pyranose ring, it indicates the presence of pyranose. The absorption bond gives rise to peaks at 846.6 cm⁻¹ corresponded to α-D-glucopyranose (pyranose), and has no specific absorbing peak of furan ring in the range of 763.2 ~ 846.6 cm⁻¹ (Kacurakova, Capek, Sasinkova, Wellner, & Ebringerova, 2000; Wang et al., 2012).

### 3.3. Determination of the sugar residues and their sequence by NMR spectroscopy

The ¹H, ¹³C, ¹H–¹H COSY, HMQC and HMBC NMR experiments were carried out and the structural features of the CSP-DS1 were further elucidated. The completely assigned spectrum of the CSP-DS1 is given in Table 2, which is derived from the 1D and 2D NMR spectras.

*Fig. 6a* shows the ¹H NMR spectrum of the CSP-DS1, the signals at 5.0 – 5.4 ppm and 4.4 – 5.0 ppm are assigned as the anomic protons of the α-glycosidical configuration and β-glycosidical configuration (Zhao et al., 2014). The ¹H NMR spectrum of the CSP-DS1 shows only one high intensity signal could find at 5.34 ppm, other signals at 4.8 – 5.2 ppm are weak. Since they has no enough information about the anomic configuration, the ¹H–¹H COSY (Fig. 7a), HMBC (Fig. 7b), HMQC (Fig. 7c) NMR experiments were further carried out. The five glycosyl moieties are designated as A, B, C, D and E according to their increasing anomeric shifts (Table 2). No chemical shift appearing around 5.65 ppm, indicates the CSP-DS1 without uronic acid again (Yu & Yang, 1999).

*Fig. 6b* shows the ¹³C NMR spectrum of CSP-DS1. The carbohydrate carbon signals are distributed in the range of 58 ~ 102 ppm. Some weak signals could also be observed from the spectrum at 83.8 ppm, 83.9 ppm and 69.2 ppm, respectively. It indicates the existence of C-2, C-3, C-6 substituted glycosyl. However, the signals are too weak to the continued identification of these residues.

The A has been confirmed to be an α-glycosidical residue. The proton chemical shift of the A is assigned from H-1 to H-6 by ¹H–¹H COSY spectra (Fig. 7a). The anomeric signals for the moiety A at 5.34 ppm and the C-1 signal of the A at 99.47 ppm, indicate that the A could be assigned to anomic protons of the α-D-pyranose (Gutiérrez de, Martínez, Sanabria, de Pinto & Igartuburu, 2005). These observations also are supported by the IR data. The downfield shifts of C-3 (83.9 ppm) signals indicate that the A is

### Table 2

<table>
<thead>
<tr>
<th>Residues</th>
<th>¹H/¹³C</th>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>→3→α-D-GlcP→</td>
<td>5.34</td>
</tr>
<tr>
<td>A</td>
<td>99.47</td>
</tr>
<tr>
<td>T→α-D-GlcP→</td>
<td>5.16</td>
</tr>
<tr>
<td>B</td>
<td>97.57</td>
</tr>
<tr>
<td>→6→α-D-Man-→</td>
<td>5.23</td>
</tr>
<tr>
<td>C</td>
<td>95.77</td>
</tr>
<tr>
<td>→3→β-D-GalP→</td>
<td>4.91</td>
</tr>
<tr>
<td>D</td>
<td>98.47</td>
</tr>
<tr>
<td>→2,3→β-D-GlcP→</td>
<td>4.10</td>
</tr>
<tr>
<td>E</td>
<td>97.95</td>
</tr>
</tbody>
</table>

The A has been confirmed to be an α-glycosidical residue. The proton chemical shift of the A is assigned from H-1 to H-6 by ¹H–¹H COSY spectra (Fig. 7a). The anomeric signals for the moiety A at 5.34 ppm and the C-1 signal of the A at 99.47 ppm, indicate that the A could be assigned to anomic protons of the α-D-pyranose (Gutiérrez de, Martínez, Sanabria, de Pinto & Igartuburu, 2005). These observations also are supported by the IR data. The downfield shifts of C-3 (83.9 ppm) signals indicate that the A is
substituted at C-3. Thus, considering the results of methylation and NMR analysis, it may be concluded that the A is 1, 3-linked-α-D-Glc.

The proton chemical shift of the B (H-1, H-4, Table 2) is identified from 1H-1H COSY spectra (Fig. 7a). It has an anomeric chemical shift at 5.16 ppm indicates an α-linked residue (Wu, Cui, Eskin, Goff, & Nikiforuk, 2011). The 13C NMR spectrum of the CSP-DS1 exhibits the C-1 signal at 98.47 ppm and the downfield shift of the C-6 peak at 69.61 ppm is due to the effect of glycosylation (Charles, 2010; Dong et al., 2002).

The C has an anomeric chemical shift at 5.23 ppm indicate an α-linked residue, the proton signal of C from H-1 to H-6 are assigned to α-D-Man. The carbon signals from C-2 to C-6 for the C are identified from the HMQC (Fig. 7c). The downfield shift of C-6 (62.9 ppm), with standard values, indicates that the C is 1, 6-linked-α-D-Man (Charles, 2010).

The anomeric proton chemical shifts of the D and E, exhibited at 4.91 ppm and 4.80 ppm, respectively. It indicates a β-linked moiety. The proton signals of the D and E from H-1 to H-6 are assigned to the 1, 3-linked-β-D-Galp, 1, 2, 3-linked-β-D-Glc, respectively (Charles, 2010).

Long-range 1H/13C correlations were obtained from an HMBC. The cross-peaks of both anomeric protons and carbons of each of the sugar moieties were examined, and intra- and inter-residual
connectivity were observed from the HMBC experiment. The HMBC spectrum (Fig. 7c) shows the distinct cross peak in the anomic region of glycosyl moieties. The C-1 signals at 99.47, 98.47, 97.95, 97.57 and 95.76 ppm could be assigned to the 1, 3-linked-α-D-Glc, 1, 3-linked-β-D-Galp, 1, 2, 3-linked-β-D-GlcP, terminal-α-D-GlcP and 1, 6-linked-α-D-Manp, respectively. These signals cross link of the proton signals at chemical shifts 5.34, 4.91, 4.80, 5.16 and 5.23 ppm, respectively. The HMBC spectrum (Fig. 7b) indicates that the H-1 signals of 1, 3-linked-α-D-GlcP correlate with the C-3 of 1, 3-linked-β-D-Galp. It is also observed that H-1 of 1, 3-linked-α-D-GlcP correlates with the C-3 of 1, 2, 3-linked-α-D-GlcP. Reversely, the cross peak of C-1 of 1, 3-linked-α-D-GlcP correlates with the H-3 of 1, 3-linked-β-D-Galp (Benie & Sorensen, 2006).

Therefore, based on the chemical and spectroscopic findings, the predicted primary structure of the CSP-DS1 repeating unit present in polysaccharide, CSP-DS1 is assigned as:

\[ \text{R} \rightarrow 3\text{-α-D-GlcP}(1→6)-\text{d-A-Manp}(1→2)-\text{β-D-GlcP}(1→3)-\text{α-D-GlcP}(1→3)-\text{β-D-Galp}(1→3)-\text{α-D-GlcP}(1→3) \]

\[ \rightarrow 3\text{-α-D-GlcP}(1→6)-\text{d-A-Manp}(1→2)-\text{β-D-GlcP}(1→3)-\text{α-D-GlcP}(1→3)-\text{β-D-Galp}(1→3)-\text{α-D-GlcP}(1→3) \]

4. Conclusions

A novel polysaccharide, named CSP-DS1, was separated and purified from C. songaricum, by DEAE-52 column chromatography and a Sepharose CL-6B column chromatography. The molecular weight of the CSP-DS1 is about 48.1 × 10^4 Da. Monosaccharide analysis reveals that the CSP-DS1 is a heteropolysaccharide composed of Man, Glc and Gal residues in mole ratio of 5.01:89.17:5.82. Based on the analysis of methylation, IR, 1D and 2D NMR, the backbone structure of the CSP-DS1 is assigned as:

\[ \rightarrow 3\text{-α-D-GlcP}(1→6)-\text{d-A-Manp}(1→2)-\text{β-D-GlcP}(1→3)-\text{α-D-GlcP}(1→3)-\text{ β-D-Galp}(1→3)-\text{α-D-GlcP}(1→3) \]

\[ \rightarrow 3\text{-α-D-GlcP}(1→6)-\text{d-A-Manp}(1→2)-\text{β-D-GlcP}(1→3)-\text{α-D-GlcP}(1→3)-\text{ β-D-Galp}(1→3)-\text{α-D-GlcP}(1→3) \]

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