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Analysis of chemical composition of polysaccharides from Poria cocos Wolf and its anti-tumor activity by NMR spectroscopy

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ABSTRACT

In this study, we extracted the polysaccharide from Poria cocos Wolf and examined its chemical components using gas chromatography–mass spectrophotometer (GC–MS) and fourier transform infrared (FT-IR) spectra. Then its anti-tumor effects was investigated via in vivo assays. Chemical analysis revealed that the polysaccharide was composed of ribose, arabinose, xylose, mannose, glucose, and galactose in the molar contents of 1.49, 1.17, 0.62, 10.34, 86.39 and 1.31 μM, respectively, and their corresponding mole percentages were 1.6%, 1.09%, 0.54%, 11.3%, 85.9%, and 1.01%, respectively. After 7 weeks of feeding, the polysaccharide significantly inhibited tumor cells growth and increased serum superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities in rats. These results indicate that the polysaccharide possessed strong anti-tumor activity.

1. Introduction

Poria cocos Wolf (P. cocos Wolf) is a well-known traditional East-Asian medicinal plant that grows around the roots of pine trees in China, Japan, Korea, and North America (Lee & Jeon, 2003). The dried sclerotia of P. cocos Wolf are used in the Chinese drug, Hoelen, and are also used in combination with other herbal medicines. P. cocos Wolf is used to treat chronic gastritis, edema, nephrosis, gastric atony, acute gastroenteric catarrh, dizziness, nausea, and emesis (Okui et al., 1996). P. cocos Wolf consists of 90% β-glucan and 10% various terpenes by dry weight. The accumulating data revealed that polysaccharides of P. cocos showed anti-tumor activities.

The present study investigated the chemical composition and anti-tumor potential of the Polysaccharides from Poria cocos Wolf in order to obtain more information about the pharmacological properties of the polysaccharides. The anti-oxidant activity of the compound was further evaluated.

2. Materials and methods

2.1. Preparation of plant materials

Poria cocos Wolf were purchased from a local herb market of Shantou city (Guangzhou Province, China). The prepared drugs were then dried at 60 °C for 3 h. The dried drugs were ground and stored at room temperature in a vacuum-packed container before use.

2.2. Extraction of polysaccharides from Poria cocos Wolf

Sample were immersed into 100 ml of distilled water. The extraction process was performed at 100 °C for 3 h. The extract was filtered through a Whatman No. 1 filter paper and the filtrate was then concentrated to 25 ml with a rotary evaporator at 80 °C under vacuum. The proteins in the extract were removed using the Sevag reagent (Wang, Luo, Zha, & Feng, 2010). After removal of the Sevag reagent, 100 ml of anhydrate ethanol was added, then the mixture was kept in a beaker overnight at 4 °C to precipitate polysaccharides. Polysaccharides was obtained by centrifugation at 3860*g* for 15 min. The content of polysaccharides was determined by the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.3. Monosaccharide composition analysis

Samples were prepared as described previously (Kim, Laskowich, Michon, Kaiser, & Arumugham, 2006). Briefly, dried samples were methanolyzed with 3 N HCl in MeOH for 2 h at 121 °C, followed by re-*N*-acetylation and trimethylsilylation. The system used was an Agilent 6890 gas chromatograph/5973 mass selective detector with a 30-m HP-5 capillary column. Helium was used as

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the carrier gas at a constant flow rate of 1 ml/min. The oven conditions included an initial temperature of 50 °C and an initial time of 2 min, 30 °C/min to 150 °C, 3 °C/min to 220 °C, and finally 30 °C/min to 300 °C for a 10-min bakeout. The inlet temperature was kept constant at 250 °C, and the MS transfer line was set at 300 °C. MS acquisition parameters included scanning from m/z 50–550 in the electron impact (EI) mode for routine analysis.

2.4. FT-IR spectra

FT-IR spectra were recorded with different resolutions. The polysaccharides samples were incorporated into KBr (spectroscopic grade) and pressed into a 1-mm pellet. The spectra were scanned between 4000 and 400 cm^{-1}, by averaging 64 scans for each spectrum with a resolution of 4 cm^{-1} (data point resolution/interval 1 cm^{-1}) and with a resolution of 8 cm^{-1} (data point resolution/interval 2 cm^{-1}), respectively.

2.5. Animals and treatment

Male Wistar rats, l0–12 weeks old, 200–218 g b.w., were housed in steel cages. They were allowed free access to drinking water and normal diet during the experiment. The rats were acclimatized for 7 days and randomly allotted into four groups (I, II, III and IV). All animal experiments were performed under the guidelines of the Laboratory Animal Experiment Committee of China.

Liver cancae rats (group II, III and IV) were induced according to the reference. In the normal group (I), rats were orally fed with physiological saline. Tumor animals were distributed into model group (II) and polysaccharides-fed group. polysaccharides-fed animals were allotted into group III (100 mg/kg) and group IV (200 mg/kg) according to the amounts of polysaccharides supplementation. After 7 weeks, all animals were sacrificed and blood samples were taken from the abdominal aorta using heparin-coated syringes for plasma and regular syringes for serum. Plasma and serum were obtained by centrifuging the blood at 3000 rpm for 15 min at 4 °C. All samples were stored at −70 °C until analyzed.

2.6. Nuclear magnetic resonance (NMR) spectroscopy

NMR experiments were performed with a Varian INOVA UNITY 7.0 T/18 cm horizontal bore small animal imaging spectrometer (Towner, Foley, & Painter, 2005). Animals were anesthetized with 2% isoflurane via an inhalation rate of 0.5 l O_2/min. The adequacy of anesthesia was verified by noting the presence or absence of the paw-withdrawal reflex. The rats were then secured into position on a cradle and placed into a Varian saddle coil probe. A SEMS (spin-echo multiple slice) sequence was used for imaging. The parameters used were as follows: The matrix dimensions were 128 × 128, there were 2 steps/acquisition, and a field of view (FOV) of 8 × 8 cm^2 and the slice thickness was 2 mm. T1-weighted images were obtained in both the transverse and sagittal planes with a repetition time (TR) of 800 ms and an echo time (TE) of 30 ms. Sagittal images were used to plan the position of multiple slices in the transverse plane. Transverse MR image co-ordinates were used to obtain ^1H MR spectra of control and tumor tissue by the positioning of a voxel within the liver tissue. A water-suppressed (CHESS)-stimulated echo acquisition mode (STEAM) localization sequence was used for the spectroscopy, with a voxel size of 125 mm^3 and 512 averages, a TE of 24 ms, a TM (mixing time) of 80 ms and a TR of 2000 ms. Respiratory gating was used to acquisition of the phase-encoding steps to reduce motion artifacts. Localized shimming was used to optimize magnet field homogeneity, and a line width of <80 Hz was used as a criteria for obtaining localized spectra.

2.7. Anti-oxidant enzyme activities

The hepatic superoxide dismutase (SOD) activity was determined using Marklund and Marklund's method (Marklund & Marklund, 1974). The hepatic catalase (CAT) activity was measured

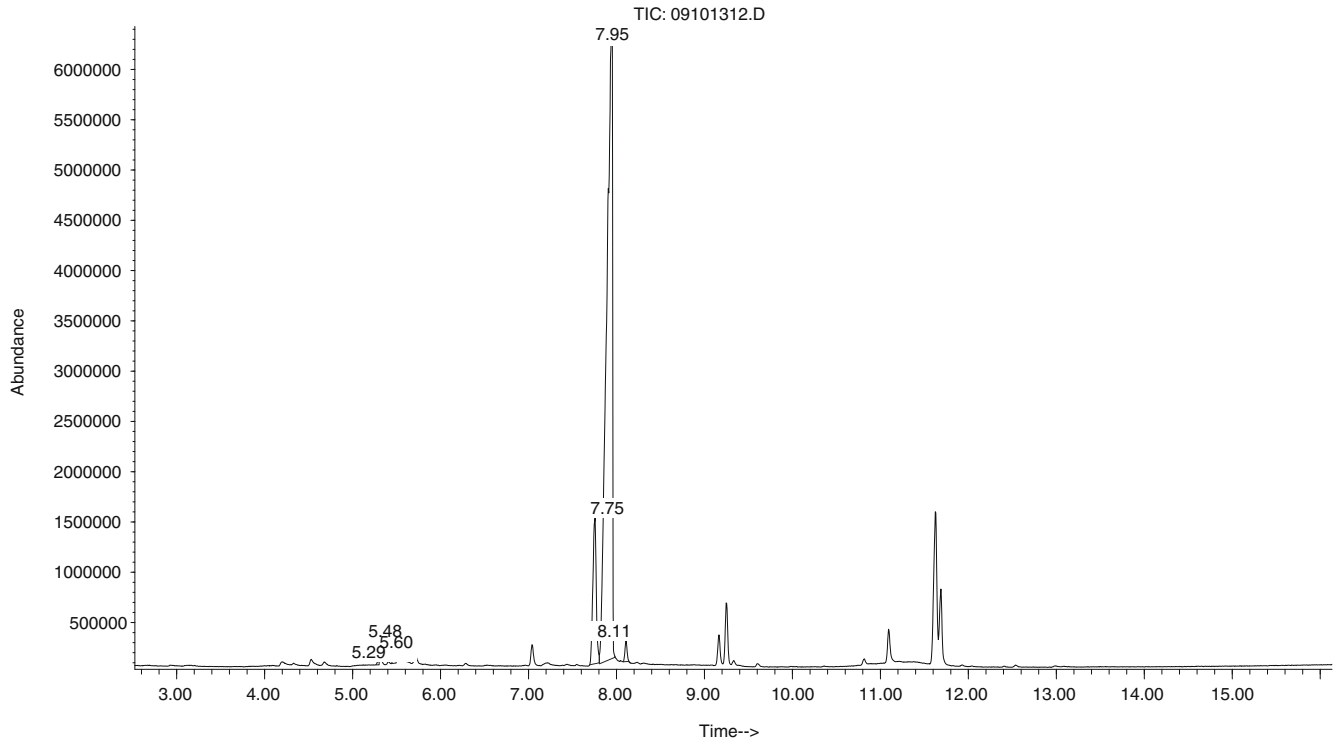

Fig. 1. GC–MS analysis of polysaccharides from Poria cocos Wolf.

Table 1
Chemical composition of polysaccharides from Poria cocos Wolf.

Mannosaccharides composition	Relative content (%)
Ribose	0.16
Arabinose	1.09
Xylose	0.54
Mannose	11.3
Glucose	85.9
Galactose	1.01

using Abei's method (Abei, 1984). The activity of hepatic glutathione peroxidase (GSH-Px) was measured using Paglia and Valentine's method (Paglia & Valentine, 1967).

2.8. Statistical analyses

All data are presented as the mean ± SD. The data were evaluated by a one-way ANOVA using the SPSS program, and the differences between the means assessed using Duncan's multiple range test. Statistical significance was considered at $p < 0.05$.

3. Results and discussion

3.1. Analysis of the polysaccharide from Poria cocos Wolf

This experiment was designed to develop a rapid, repeatable and accurate analysis method for the quantification of the component carbohydrates in the polysaccharide. In order to evaluate the applicability of the proposed method, the isolated polysaccharide was methanolyzed with 3 N HCl in MeOH for 2 h at 121 °C, followed by re-*N*-acetylation and trimethylsilylation and the released monosaccharide derivatives were analyzed by the described GC–MS method under the optimized conditions using six mannosaccharides (ribose, arabinose, xylose, mannose, glucose, and galactose) standards as internal standard. Typical chromatogram of the polysaccharide sample showed that the derivatives of the component monosaccharides released from the polysaccharide sample could be still baseline separated and the component monosaccharides could be identified by comparing with the chromatogram of the mixture of standard monosaccharides (Fig. 1). The results showed that the polysaccharide was a typical heteropolysaccharide and was composed of ribose, arabinose, xylose, mannose, glucose, and galactose in the molar contents of 1.49, 1.17, 0.62, 10.34, 86.39 and 1.31 μM, respectively, and their corresponding mole percentages were 1.6%, 1.09%, 0.54%, 11.3%, 85.9%, and 1.01%, respectively (Fig. 1 and Table 1). It was clear that the predominantly composition monosaccharides in the polysaccharide were neutral glucose and mannose up to 98.11% (mol.%) of total carbohydrates (Fig. 1 and Table 1).

3.2. FT-IR of polysaccharides from Poria cocos Wolf

The FT-IR spectra of carbohydrates are used for determination of their structural features. The wave number between 950 and 1200 cm^{-1} is often called the fingerprint of molecules because it allows the identification of major chemical groups in polysaccharides: the position and intensity of the bands that are specific for each polysaccharide (Mao et al., 2009; Fellah, Anjukandi, Waterland, & Williams, 2009; Popescu et al, 2009). Since monosaccharide analysis revealed that the polysaccharides were mainly composed

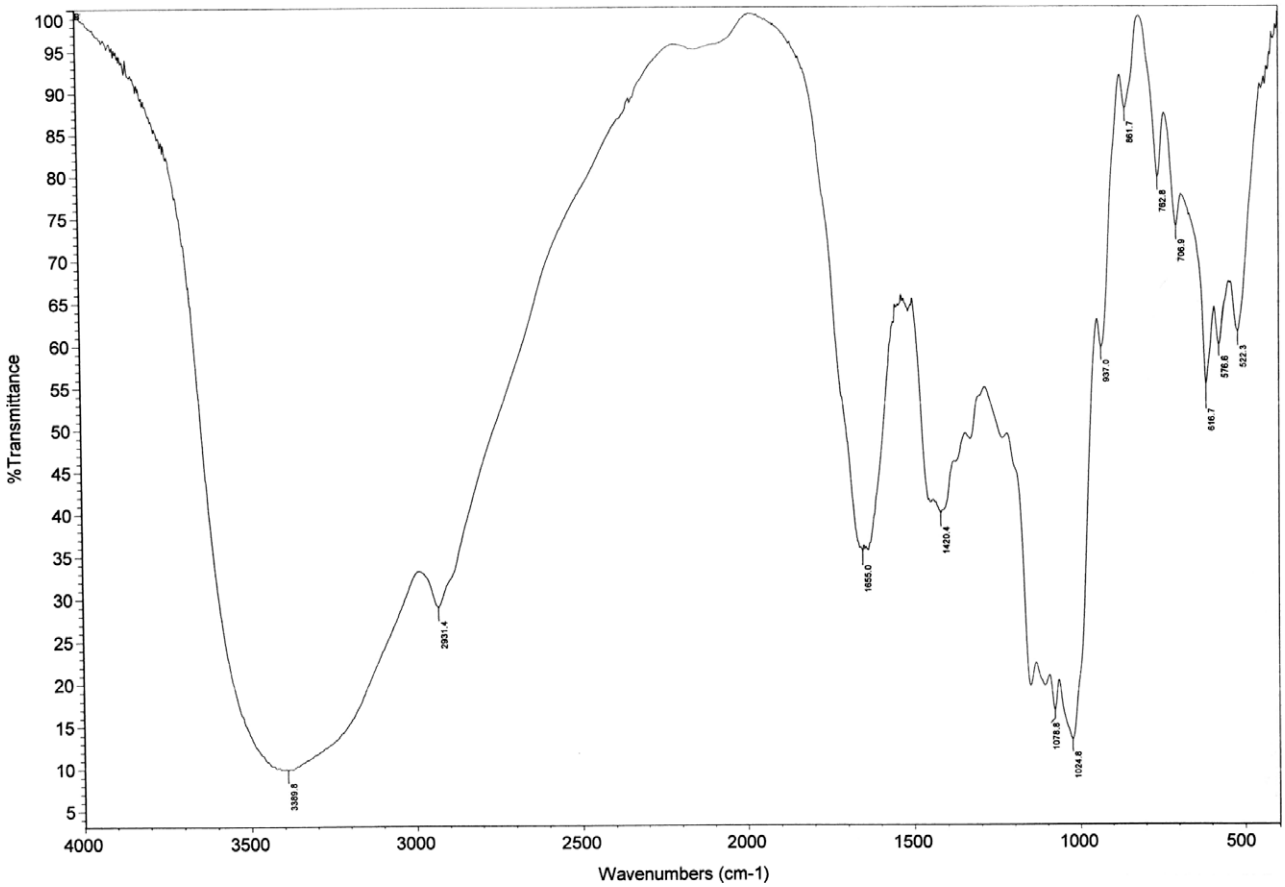

Fig. 2. FT-IR spectroscopy of polysaccharides from Poria cocos Wolf.

Table 2
Effects of polysaccharides from Poria cocos Wolf on body weight of rats.

Groups	Initial body weight (g)	Final body weight (g)
I (control)	204.3 ± 24.8	227.2 ± 39.5
II (model)	201.9 ± 20.7	218.3 ± 26.1
III (polysaccharides)	203.8 ± 30.5	222.6 ± 17.9
IV (polysaccharides)	205.1 ± 29.9	224.1 ± 33.5

Table 3
Effects of polysaccharides from Poria cocos Wolf on tumor cells growth.

Groups	Tumor weight (g)	Tumor inhibition rate (%)
I (control)	–	
II (model)	3.14 ± 0.11	
III (polysaccharides)	2.78 ± 0.08	11.5
IV (polysaccharides)	2.56 ± 0.06	18.5

Table 4
Effects of polysaccharides from Poria cocos Wolf on anti-oxidant enzymes activities.

Groups	SOD	CAT	GPx
I (control)	211.8 ± 16.8	21.62 ± 1.57	18.65 ± 0.68
II (model)	131.5 ± 11.4[a]	10.58 ± 0.96 [a]	8.79 ± 0.73 [a]
III (polysaccharides)	169.3 ± 15.4[b]	17.40 ± 0.57 [b]	14.83 ± 1.22 [b]
IV (polysaccharides)	231.7 ± 17.9 [b]	22.48 ± 1.42 [b]	19.35 ± 1.11 [b]

[a] $p < 0.01$, compared with group I.
[b] $p < 0.01$, compared with group II.

of glucose, FT-IR spectra of polysaccharides were compared against three commercial glucose standards (Fig. 2). It was found that the FT-IR spectra of polysaccharides exhibited similarities in absorption pattern to glucose, confirming the preliminary conclusion derived from chemical composition analysis that the polysaccharide is a heteropolysaccharide. The broader band of absorption between 3300 and 2500 cm^{-1} was due to O–H stretching whereas an intense ring and (COH) side group band at ~1047 cm^{-1} dominated the spectrum of mannose with β (1 → 4) backbone, whilst in the β (1 → 3) linked xylan two partially overlapping bands at 1066 and 1030 cm^{-1} were found instead. These bands of polysaccharides were broad, which could be due to mixed β (1 → 4) linked with β (1 → 3) linked xylose. The FT-IR spectrum of polysaccharides is usually overlapped by the carboxyl and carboxylate vibrations at around 1730 and 1600 cm^{-1} (Qian, Chen, Zhang, & Zhang, 2009). The high intensity of band around 1730 cm^{-1} indicated that the glucose content in the polysaccharides was higher (Fig. 2).

3.3. Effects of polysaccharides on body weight of rats

Table 2 summarizes the effects of polysaccharides administration on body weight of rats relative to control rats (group I) that were not drug treated. After 7 weeks. decreased body weight was observed in model rats (group II) in comparison to normal rats. Polysaccharides administration enhanced body weight of rats (group III and IV). No significant differences were found between the body weights of untreated and drug-treated rats at the end of the drug treatment period.

3.4. Effects of polysaccharides on tumor cells growth

Table 3 show the effects of polysaccharides on tumor cells growth in rats. The results of two-way ANOVA on the data on a group-by-drug basis are shown in Table 2. Daily administrations of polysaccharides (100 and 200 mg/kg b.w.) for 7 weeks were effective in fully inhibiting tumor cells growth as detected in groups III and IV. In livers in both the groups III and IV rats, tumor weight were effectively reduced by the administration of polysaccharides in a dose dependent manner.

3.5. Effects of polysaccharides on anti-oxidant enzymes activities

The result of polysaccharides effects on enhancing serum anti-oxidant enzymes activities in rats is presented in Table 4. Serum anti-oxidant enzymes (SOD, CAT, GPx) activities in model rats (group II) were significantly decreased ($p < 0.01$) in comparison with the control rats (group I). There was significant difference in serum anti-oxidant enzymes activities between groups after 7 weeks of polysaccharides supplementation. In both the groups III and IV rats, polysaccharides supplementation significantly ($p < 0.01$) enhanced serum anti-oxidant enzymes activities.

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