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Bioactivities of water-soluble polysaccharides from fruit shell of *Camellia oleifera* Abel: Antitumor and antioxidant activities

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ABSTRACT

Polysaccharides were extracted from fruit shell of *Camellia oleifera* Abel. Fruit shell of *Camellia oleifera* Abel polysaccharide (WEP2) was a water-soluble compound. Its molecular weight was about 362 kDa. HPLC analysis showed that this polysaccharide was composed of rhamnose, fucose, arabinose, mannose, galactose and glucose in the molecular ratio of 4.05, 11.62, 1.78, 3.91, 8.76 and 27.06, respectively. The broad intense characteristic peak around 3463 cm^{-1} due to the hydroxyl stretching vibration of the polysaccharide was observed in the polysaccharide. The characteristic absorption bands at 852 cm^{-1} and 893 cm^{-1} indicated that WEP2 contained both α-glycosidic and β-glycosidic linkages. WEP2 exhibited remarkable antitumor activity against Sarcoma180 cell compared to the negative control group. At the highest dose 40 mg/kg days, the tumor inhibition rate reached 65.2%. The scavenging effects of WEP2 to hydroxyl radical and superoxide radical anion were 72.5% and 86.3% at a concentration of 1.0 mg/ml, respectively.

1. Introduction

Camellia oleifera Abel is an evergreen shrub or small tree in *Camellia* family. China is not only the most widely distributed country of *Camellia* plant in the world, but also the world's largest *Camellia oleifera* Abel production bases, in addition to Southeast Asia, Japan and other State-owned very little distribution (Fu & Zhou, 2003). There is 4 million hectare of *Camellia oleifera* Abel forest in China and the fruit shell of *Camellia oleifera* Abel is about 1 million ton every year (Shen & Jiang, 2006). Fruit shell of *Camellia oleifera* Abel can be used to extract tannin, furfural and xylitol and can also be used to produce activated carbon and potassium carbonate (Liu, 1997). According to the dictionary of Chinese materia medica records the whole plant of *Camellia oleifera* Abel has some biological activity. In the past several years, medicinal plant polysaccharides have been widely studied for their chemical properties and biological activities (Chen, Zhang, Qu, & Xie, 2008), including antitumor (Wasser, 2002), immunostimulation (Li et al., 2003), and antioxidation (Li et al., 2003). However, little information is available about polysaccharide extracted from the fruit shell of *Camellia oleifera* Abel (Kang, Chen, Chen, & Shen, 2010; Shen, Kang, Chen, & Chen, 2010). The present study reports isolation and biological activity of a water extracted polysaccharide isolated from fruit shell of *Camellia oleifera* Abel. To determine the biological activity of the polysaccharides, its antioxidation and anti sarcoma180 tumor cell was investigated.

2. Materials and methods

2.1. Samples

Fruit shell of *Camellia oleifera* Abel was collected from Hunan Province, China, during fruit ripening stage (October 2009). The samples were cut into small pieces and further ground into a fine powder in a high speed disintegrator after discarding the rotted ones and dried at 65 °C (Model DFY-500, DaDe Chinese Traditional Medicine Machine Co., Ltd., Zhejiang, China), and passed through a 40 mesh sieve.

2.2. Extraction of polysaccharide

The samples were defatted in a Soxhlet apparatus with petroleum ether at 60–90 °C, and soaked with 80% ethanol twice to remove some colored materials, oligosaccharides and some small molecules. The organic solvent was volatilized, and pretreated powder was obtained, as described previously (Chen, Cao, & Song, 1996).

Extraction of polysaccharide was conducted by stirring the pretreated powder (10.0 g) in deionized water at a solute to solvent ratio of 1:15 (w/v) at 90 ± 1 °C for an hour. Separation of the residue from the extract was performed by filtration through a glass filter (G-3). The insoluble material was extracted twice more under

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similar condition at a solute to solvent ratio of 1:20 (w/v). The combined liquid extract was dialyzed extensively against water and lyophilized. The recovered material was dissolved in water, precipitated by the addition of ethanol until the concentration of ethanol reached 75% (v/v) and then collected by centrifugation (repeated three times). The final pellet was dissolved in water and lyophilized to yield the water extracted polysaccharide, named WEP.

2.3. Determination of molecular weight

The molecular weight of WEP was determined by HPLC on an Agilent-LC 1100 instrument (Agilent, USA), equipped with a TSK gel 4000 PWXL column and eluted with 0.05 M Na_2SO_4 solution at a flow rate of 0.8 ml/min. Elution was monitored by an Agilent refractive index detector.

A series of solutions made from standard dextrans were run under the same conditions and a standard curve linear over a wide range (10–1000 kDa) was obtained by plotting the elution volume versus the logarithm of the corresponding molecular weight.

2.4. Chemical structure of WEP

2.4.1. Infrared spectra

Infrared analysis of the sample was obtained by grinding a mixture of polysaccharide with dry KBr and then pressing in a mold. IR spectra were recorded on a Bruker-VERTEX 70 Fourier transform infrared spectrophotometer in a range of 4000–400 cm^{-1}.

2.4.2. Chemical properties

The yield of crude polysaccharides was calculated as a percentage of the total weight of sample used. The neutral carbohydrate content was determined by the phenol–sulfuric acid method (Chaplin & Kennedy, 1986). The uronic acid content was measured by a modified hydroxydiphenyl assay (Huang, Lin, Tian, & Ji, 1998) with glucuronic acid as the standard. Protein was measured with the Bradford method (Spector, 1978) using bovine serum albumin as a standard. The analysis of monosaccharide compositions was performed by gas chromatography (GC, Agilent 6890, USA) equipped with a DB-1701 capillary column (30 m × 0.32 mm, film thickness 0.25 μm) (Yin, Nie, Zhou, Wan, & Xie, 2009). The polysaccharides from fruit shell of *Camellia oleifera* Abel were hydrolyzed by 2 M trifluoroacetic acid at 100 °C for 12 h into monosaccharides, and were detected with a flame ionization detector (FID).

2.5. Measurement of hydroxyl radical scavenging activity

The scavenging activity of hydroxyl radical was determined based on the method described by Sun, Wang, Shi, and Ma (2009). Test sample (0.2–1.0 mg/ml, 1 ml) was incubated with a solution containing phenanthroline (5 mM, 1 ml), phosphate buffer (50 mM, pH 7.4), $FeSO_4$ (7.5 mM, 0.5 ml) and H_2O_2 (0.1%, 0.5 ml) at 37 °C for 1 h. The absorbance measured at 510 nm using UV-2550 spectrophotometer (Shimadzu, Kyoto, Japan) was designated A_1. The absorbance when sample was instead of deionized water was A_2, and when H_2O_2 and sample were replaced with distilled water was A_3. The ability to scavenge hydroxyl radicals was calculated with the following equation:

$$\text{Scavenging effect}\,(\%) = \frac{A_1 - A_2}{A_3 - A_2} \times 100.$$

2.6. Measurement of superoxide anion scavenging activity

The scavenging activity of superoxide radical anion was determined by the method of Wang and Luo (2007). WEP samples (0.1–5.0 mg/ml, 0.2 ml) with different concentrations were added with a Tris–HCl buffer solution (16 mM, pH 8.0, 5.4 ml) and 1,2,3-phentriol (0.6 mM, 0.3 ml) at 25 °C, ascorbic acid (5%, 0.1 ml) was used to terminate the reaction after 3 min. The absorbance (A_2) was read at 325 nm. Self-oxidation system without any inhibitory agents was performed as negative control. The absorbance was designated as A_1. The ability to scavenge superoxide radical anion was expressed with following equation:

$$\text{Scavenging effect}\,(\%) = \frac{A_1 - A_2}{A_1} \times 100.$$

2.7. In vivo antitumor test

Animal care and handling were according to the Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines after research project approval by the Institutional Animal Ethics Committee.

BALB/C mice (male, 6–8 weeks old, weighing 20 ± 2 g) were purchased from the Animal Center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China.

Each of polysaccharide sample as well as control agents were tested on individual group of animals consisting of 6 BALB/C mice.

Sarcoma180 tumor cells (5×10^6 cells ml^{-1}) were transplanted subcutaneously into the right groin of the mice. After 24 h of tumor inoculation, WEP samples were dissolved in 0.9% aqueous NaCl, then injected intraperitoneally to the mice in experiment group every other day for 20 days. The equivalent volume of 0.9% aqueous NaCl was injected intraperitoneally in the negative control group. The mice were killed on the next day of the last injection, and the tumors were removed and weighed.

The inhibition ratio ξ and enhancement ratio of bodyweight f were calculated as follows:

$$\xi = \frac{W_c - W_t}{W_c} \times 100\%$$

$$f = \frac{W_a - W_b}{W_b} \times 100\%$$

where W_c is the average tumor weight of the negative control group, W_t is the average tumor weight of WEP group; W_b and W_a are the body weight of mice before and after the assay.

2.8. Statistical analysis

Data are expressed as mean obtained from a minimum three separate experiments. Differences between various groups were analysed by performing one-way analysis of variance (ANOVA) and Student–Newman–Keuls test. Results were considered statistically significant if the two-tailed P value was <0.05.

3. Results and discussion

3.1. Isolation and purification of WEP2

The crude polysaccharide WEP was gained from the fruit shell of *Camellia oleifera* Abel that contained 98% carbohydrate, and the yield was about 6.5%. WEP was further purified by DEAE-cellulose column and Sephadex G-200 column chromatography. The main fraction (WEP2) was collected and lyophilized for further study on structure and antitumor bioactivity. WEP2 appeared as a white powder and showed negative iodine–potassium iodide reactions, indicating that it did not contain starch-type polysaccharide. The homogeneity of WEP2 was confirmed by a single symmetrical peak appeared in HPLC.

Table 1
Monosaccharide compositions of WEP2.

	Monosaccharide					
	Rhamnose	Fucose	Arabinose	Mannose	Galactose	Glucose
Content (wt.%)	6.69	19.03	2.65	7.07	15.72	48.82
Molecular ratio	4.05	11.62	1.78	3.91	8.76	27.06

Table 2
Antitumor activities of WEP2 against Sarcoma180 solid tumor grown in BALB/C mice.

Sample	Dose (mg/kg days)	Enhanced ratio of body weight (%)	Tumor weight (g)	Tumor inhibitory rate (%)
Negative control		35.2	1.15 ± 0.36	–
WEP2	10 × 20	43.8	0.55 ± 0.10	52.1
	20 × 20	45.2	0.42 ± 0.06	63.4
	40 × 20	44.8	0.40 ± 0.07	65.2

Based on the equation of the standard curve made by different dextran standards and the retention time of WEP2, the molecular weight of WEP2 was estimated to be 362 kDa.

3.2. Composition and physical property of WEP2

WEP2 was a water-soluble compound. The results showed that the polysaccharide was composed of rhamnose, fucose, arabinose, mannose, galactose and glucose at a molecular ratio of 4.05:11.62:1.78:3.91:8.76:27.06 as shown in Table 1.

From the infrared spectrum analysis, it displayed a strong absorption in the range of 1200–1000 cm^{-1} that showed the monosaccharide in WEP2 had a pyranose ring. The broad intense characteristic peak around 3463 cm^{-1} was due to the hydroxyl stretching vibration of the polysaccharide. The bands in the region of 2933 cm^{-1} and 1648 cm^{-1} were due to C–H stretching vibration and associated water, respectively (Luo, Xu, Yu, Yang & Zheng, 2008). Furthermore, the characteristic absorption bands at 852 cm^{-1} and 893 cm^{-1} indicated that WEP2 contained both α-glycosidic and β-glycosidic linkages (Zhang et al., 2010). The bands at 875 cm^{-1} and 813 cm^{-1} demonstrated that there was mannose in WEP2 (Frank, 1971; Synytsya, Copikova, Matejka & Machovic, 2003).

3.3. Antioxidant activity of WEP2

The scavenging effects of the WEP2 on hydroxyl radical and super-oxide radical anion are shown in Fig. 1. As shown in Fig. 1 both the scavenging effect of hydroxyl radical and superoxide anion increased with the WEP2 concentration increased. WEP2 represented obviously better activity for scavenging superoxide radical anion than hydroxyl radical. The scavenging effects of WEP2 to hydroxyl radical and superoxide radical anion were 72.5% and 86.3% at a concentration of 1.0 mg/ml, respectively. These results showed that the scavenging ability of both hydroxyl radical and superoxide anion of WEP2 was higher than that of VC at the same concentration (Xing et al., 2005; Zhang, Wang, & Dong, 2011).

3.4. In vivo antitumor activity

A variety of polysaccharides from different biological sources has been shown to enhance the immune system. The immunostimulating potential of these biopolymers is described as mainly promoting the activity of phagocytosis of the respective macrophages and granulocytes, inducing the production of TNFα and interleukins, and, finally, acting on the complement system. The most active polymers appear to be branched (1→3) β-D-glucans, which possess randomly dispersed single β-D-glucopyranosyl units attached to C6. A great variety of other neutral and acidic polymers of different molecular dimensions have been proposed as so called "anti-tumor polysaccharides". The corresponding activities were mostly shown on allogeneic tumors such as Sarcoma180. However, clinical experience has only been documented for the fungal β1→3 glucans, such as lentinan and schizophyllan (Franz, 1989). According to reports, more than 100 kinds of plant polysaccharides were extracted. Due to the wide source and no cytotoxicity being applied to organisms the research on the plant polysaccharides has become a hot domain in medical field (Franz, 1989).

The inhibition ratio of WEP2 *in vivo* assay was summarized in Table 2. WEP2 exhibited remarkable antitumor activity against Sarcoma180 cell compared to the negative control group. As shown in Table 2, the tumor inhibition ratio of WEP2 increased as the dose increased. At the highest dose 40 mg/kg days, the tumor inhibition rate reached 65.2%. Many investigators reported that polysaccharides with antitumor action differ greatly in their chemical composition, configuration and physical properties. The high antitumor activity against Sarcoma180 cell of WEP2 might be attributed to its molecular weight, configuration and chemical composition different from other polysaccharides. In some reported cases, minor changes in structure, molecular weight or chain conformation may have dramatic effects on potency.

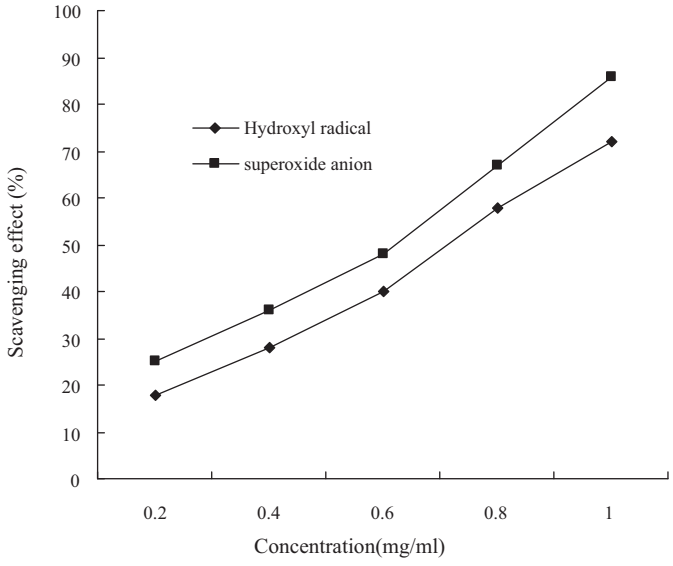

Fig. 1. Free-radical scavenging of WEP2.

Antitumor activity assays implied that WEP2 seemed to have powerful tumor-fighting properties *in vivo*, furthermore, the enhancement ratios of body weight indicated that the polysaccharide might not have cytotoxicity, which kills normal cells as well as cancer cells, which would greatly promote the development of antitumor polysaccharides from *Camellia oleifera* Abel.

4. Conclusion

This study represents the first account on the *in vivo* antitumor activity of the water extracted polysaccharide from fruit shell of *Camellia oleifera* Abel (WEP2) and its structural features. Biological investigations indicated that WEP2 displayed promising activity in the antitumor assay and antioxidant assays. Furthermore, the enhancement ratios of body weight indicated that the polysaccharide might not have cytotoxicity, it can be assumed to be potentially useful as a safe antitumor agent for industries. In addition, as the isolation of these polysaccharides involves a few inexpensive and easy steps it will be of an added advantage. Finally, the biological activity observed in WEP2 provides a scientific basis for the use of the plant in traditional medicines.

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