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Chemical analysis and antioxidant activities *in vitro* of polysaccharide extracted from *Opuntia ficus indica* Mill. cultivated in China

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ABSTRACT

The polysaccharide of *Opuntia ficus indica* Mill. was isolated by water, and purified by Sephacryl S-400 HR column. The purity of polysaccharide was evaluated by HPLC coupled with gel permeation chromatography. Results showed that the neutral monosaccharide composition of *O. ficus indica* polysaccharide was rhamnose, arabinose, and glucose in a molar ratio of 1.00:2.98:2.57, with an average molecular weight of about 172,591 Da. Further, its antioxidant capacities *in vitro* were respectively evaluated by the assays of inhibition of pyrogallol autoxidation, scavenging hydroxyl radical, and liver lipid peroxidation inhibiting. The crude water-soluble polysaccharide and purified polysaccharide showed notable free radical scavenging activity and hydroxyl radical-induced liver lipid peroxidation inhibiting ability, suggesting that *O. ficus indica* polysaccharide may be considered as a potential candidate for the natural antioxidants and possible health-promoting functional foods.

1. Introduction

Opuntia ficus indica Mill., a member of the cactaceae family, is a tropical or subtropical plant originally grown in South America and cultivated in dry regions as an important food source (Majdoub, Roudesli, & Deratani, 2001). This genus is endemic to America and up to now 377 species has been recognized. 104 have been wild in Mexico, and 60 of which are endemic to this country (Pérez-Cacho, Galán-Soldevilla, García, & Montes, 2006). In some countries, some species of cactaceae family are used for human consumption due to their highly nutritive value. The components obtained from the cladodes contain a large amount of active ingredients, particularly antioxidant constituents including Vitamin C, Vitamin E, carotenoids, glutathione, flavonoids and phenolic acids etc. (Panico et al., 2005). *O. ficus indica* was introduced to China from Milpa Alta of Mexico in 1998, and mainly cultivated in Hainan province of China.

Water-soluble polysaccharides, long-chain polymers with high molecular weight, are generally present in cell walls of plants with a variety of structures. The compositions of polysaccharide structure are quite different due to different cultivating methods and regions, even though polysaccharides are obtained from the same species (Gonzaga, Ricardo, Heatley, & Soares, 2005). There are many studies on the analysis of chemical components of *O. ficus indica*, some of which involved its polysaccharide composition (Habibi, Heyraud, Mahrouz, & Vignon, 2004; Majdoub, Roudesli, & Deratani, 2001; Matsuhiro, Lillo, Sáenz, Urzúa, & Zárate, 2006; McGarvie & Parolis 1981; Panico et al., 2005). From these studies, we can find that different authors reported very different polysaccharide composition of *O. ficus indica*. It is supposed that the different growing regions, or different sampling fractions cause this difference.

It is known that hydroxyl radical is a powerful oxidant that can react with all biological molecules such as proteins, lipids, and carbohydrates and that oxidative stress can mediate a wide variety of degenerative processes and diseases (Dhalla, Temsah, & Netticadam, 2000; Sayre, Smith, & Perry, 2001; Kovacic & Jacintho, 2001). Common antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene have been suspected of being responsible for liver damage and carcinogenesis (Grice, 1988; Qi et al., 2005). Therefore, research on antioxidants, especially exploration of potent natural compounds with low cytotoxicity from plants, has become an important branch of biomedicine. The water-soluble sulphated polysaccharides extracted from some medicinal plants are shown to have antioxidant potential to scavenge free radicals (Kardošová & Machová, 2006; Kofuji, Isobe, & Murata, 2009).

Although *O. ficus indica* is widely consumed, its chemical analysis and antioxidant activities have received little attention. In view of different geographical conditions and planting methods, in this study, we tried to isolate and purify the polysaccharides of *O. ficus indica* cultivated in China. Physiochemical properties of purified polysaccharides obtained were analyzed, and their associated antioxidant capabilities were further tested.

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2. Materials and methods

2.1. Materials

The dry *O. ficus indica* peeled fruit that cultivated in Hainan province (China) was collected. The standard monosaccharides (rhamnose, D-arabinose, D-glucose, sucrose, and maltose) purchased from Fluka Co. (America). Dextrans of different molecular weights were from Sigma Chemical Co. (St. Louis, MO, USA). Sephadex G-200 column was from Pharmacia Co. (Sweden), and Sephacryl S-400 HR column was from Amersham Co. (USA). All chemicals used were of analytical grade. An ultraviolet-visible spectrophotometer (Unico-UV21202PC) was used (UNICOR, Japan). Total carbohydrate content was determined by the phenol–sulphuric acid method as D-glucose equivalents. GPC was performed with a HPLC system (Waters), using Waters 2410 differential refraction detector and evaporative light-scattering detector (ELSD 2000).

2.2. Isolation and purification of polysaccharide

2.2.1. Isolation of crude polysaccharide

The powder of *O. ficus indica* was ground into fine particles (500 g) and defatted with 95% ethanol at room temperature for 48 h, then extracted with 2 l of distilled water for 2 h at 100 °C and filtered. The residue was further extracted with 750 ml of water for 1 h. The combined aqueous extracts were concentrated in a rotary evaporator under reduced pressure at 50 °C and filtered. Then the filtrate was precipitated by adding ethanol (4 times the volume of aqueous extract) at 4 °C, followed by centrifugation at 4000 rpm for 10 min.

The precipitate was dissolved in 300 ml of water and deproteinized more than 6× with 60 ml of 4:1 $CHCl_3$-n-BuOH as described by Sevag method. The content of residual proteins was measured by Kjeldahl determination. The resulting aqueous fraction was extensively dialyzed against distilled water for 2 days and precipitated again by adding fourfold volume of ethanol. After centrifugation, the precipitate was washed with anhydrous EtOH and then dissolved in water and lyophilized to yield the crude polysaccharide (3.47 g) corresponding to polysaccharide of *O. ficus indica* in the subsequent description.

2.2.2. Purification of crude polysaccharide

Crude *O. ficus indica* polysaccharide (10 mg) was dissolved in distilled water, obtaining the liquid supernatant (1 ml) after centrifugation, applied to a Sephacryl S-400 HR column (1.6 cm × 30 cm). The column was eluted with 0.2 M NaCl. Each fraction of 1 ml was collected at a flow rate of 0.2 ml/min and monitored by the phenol–sulphuric acid method at 490 nm. At the same time, the absorbance value (280 nm) of proteins was determined. To test the homogeneity of the purified polysaccharide, HPLC with Gel-permeation chromatography was used.

2.3. Chemical analysis

2.3.1. Physicochemical property

Different solvents that include distilled water, dilute acids, dilute bases, dilute salts, hot water and organic reagents (ethanol, acetone, ethyl acetate, chloroform) were used to estimate the solubility of *O. ficus indica* polysaccharide. And its specific rotatory power was also evaluated. 25 mg purified *O. ficus indica* polysaccharide was dissolved in 25 ml distilled water, a 100 mm observation pipe was used to determine its optical rotation with sodium light. Specific rotatory power $[\alpha]_D^{20}$ was calculated $[\alpha]_D^{20} = \alpha/LC$; α-optical rotation, L—1 dm, C—0.001 g/ml).

2.3.2. Gel-permeation chromatography (GPC) analysis

The homogeneity and molecular weight of *O. ficus indica* polysaccharide was determined on a Waters HPLC system (717Plus sample injector, 1525 pump; Waters) equipped with Waters TSK G-5000 PW × L TSK and G-3000 PW × L gel column coupled with a Waters 410 differential refractometer. A sample solution (20 μL of 1.0 mg/ml polysaccharide) was injected in each run, with 0.02 M KH_2PO_4 (pH 6.0) as the mobile phase at 0.6 ml/min.

The molecular weight of the purified polysaccharide was determined by a gel chromatography technique. Standard dextrans (six different molecular weights: 3300, 18,300, 100,000, 164,000, 236,000, 333,000 Da) were passed through the column with same analysis condition as *O. ficus indica* polysaccharide. Then, the elution volumes were plotted against the logarithm of their respective molecular weights. The calibration curve of Log MW (molecular weight) of standard dextrans on GPC against their elution volume (EV) was obtained (EV = −2.8085 Log MW + 27.7460). The elution volume of the purified polysaccharide was plotted in the same calibration curve, and the molecular weight was determined.

2.3.3. Polysaccharide component analysis

40 mg *O. ficus indica* polysaccharide was added to 2 M H_2SO_4 (2 ml) for 10 h at 100 °C in a sealed glass tube, the excess acid was completely removed by $BaCO_3$ (50 °C water bath), after centrifugation, liquid supernatant was obtained for the following study. In order to get the equation of calibration curve, different standards with concentration (0.5, 1.0, 1.5, 2.0, 2.5 mg/ml) were injected with 5 μl respectively and their chromatograms were recorded. Authentic standards (rhamnose, arabinose, glucose, sucrose, and maltose) were prepared and subjected to HPLC–ELSD analysis separately. The following chromatographic conditions were used: C_{18} column (4.6 mm × 250 mm, 40 °C), mobile phase (CH_3CN:H_2O = 7:3; rate 1.2 ml/min), ELSD detector, injection volume (5 μL), high-purity helium was used as the carrier gas at a flow rate of 2.4 l/h. Mixed standards and *O. ficus indica* polysaccharide supernatant were also injected under the same way.

2.4. Antioxidant activity assays

2.4.1. Inhibition of pyrogallol autoxidation

Purified *O. ficus indica* polysaccharide was dissolved in distilled water at 200, 400, 600, 800 and 1000 μg/ml. While ascorbic acid (60, 120, 180, 240 and 300 μg/ml) and crude *O. ficus indica* polysaccharide (200, 400, 600, 800 and 1000 μg/ml) were compared. The sample solution (1 ml) was mixed with 2 ml of 0.05 M Tris–HCl buffer (pH 8.2) and incubated at 25 °C in a water bath for 20 min. Then 1,2,3-phentriol (0.4 ml, 5 mM) was added, and the mixture was shaken rapidly at room temperature. The absorbance of the mixture was measured at 325 nm per 20 s against a blank. The scavenging ability for inhibition of pyrogallol autoxidation was calculated using the equation:

$$E\% = \frac{S_{control} - S_{sample}}{S_{control}} \times 100$$

where S_{sample} represents the slope of sample group, $S_{control}$ is the slope of blank control group, where the decrease of S_{sample} indicated an increase in the restraining power. The effect of superoxide anion radical scavenging activity was expressed as EC_{50} (SAR): the amount of the sample necessary to inhibit pyrogallol autoxidation by 50%.

2.4.2. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity was measured according to Fenton method described before (Diez, Livertoux, Stark, Wellman-Rousseau, & Leroy, 2001), with slightly modification. Samples with different concentration (purified *O. ficus indica* polysaccharide: 200,

400, 600, 800 and 1000 μg/ml; crude *O. ficus indica* polysaccharide: 200, 400, 600, 800 and 1000 μg/ml; ascorbic acid: 300, 400, 500, 600, and 700 μg/ml) were prepared, then incubated with 9.0 mM $FeSO_4$ (1.0 ml), 0.3% H_2O_2 (1.0 ml) in 0.5 ml salicylic acid–ethanol solution (9.0 mM) for 30 min at 37 °C. Hydroxyl radical was detected by monitoring absorbance at 510 nm. The total volume of the mixture in each tube was made up to 3 ml by adding the required amount of distilled water. The hydroxyl radical scavenging effect was calculated as follows:

$$E\% = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ and A_{sample} represent the absorbance of blank control group and sample group under 510 nm. The effect of hydroxyl radical scavenging activity was expressed as EC_{50} (HR): the amount of the sample needed to inhibit hydroxyl radical concentration by 50%.

2.4.3. Liver lipid peroxidation inhibiting assay

Malondialdehyde (MDA) is the final product of the lipid peroxidation, MDA levels in liver homogenate of animals were estimated as thiobarbituric acid (TBA) reactive substances (Prasad, 1997). MDA is reacted with TBA under heat (100 °C) and acidic condition to produce red substance which has strong absorption (532 nm) in the aim of measuring of lipid peroxidation. Different livers were respectively removed from *Mus musculus* (mouse). 5 g of liver was washed in cold normal saline, cut into small pieces and grinded. After centrifugation, supernatant liquid was obtained.

Samples with different concentration, liver homogenate (1 ml), Fenton reagents (9.0 mM $FeSO_4$, 0.2 ml; 60 mM H_2O_2, 0.1 ml) were in water bath (37 °C) for 1 h. The total volume of the mixture in each tube was made up to 2.3 ml by adding the required amount of distilled water. Then, the reactions were suspended by adding trichloroacetic acid. 0.7% TBA was added for color reaction (15 min) in boiling water, the absorption (532 nm) was determined after refrigeration and centrifugation. The MDA inhibiting effect was calculated as same as hydroxyl radical scavenging assay. The MDA inhibiting effect was expressed as EC_{50} (MDA): the amount of the sample needed to inhibit MDA concentration by 50%.

3. Results and discussion

3.1. Isolation and purification of polysaccharide

The yield of crude polysaccharides from *O. ficus indica* was 0.694%, which is lower than that of precious literature (Cai, Gu, & Tang, 2008). Crude polysaccharide was sequentially purified through a Sephacryl S-400 HR column, giving two big overlapping elution peaks (Fig. 1a). Fractions (peak 1, time: from 50 to 100 min) were collected and combined because polysaccharide was enriched in these fractions. In addition, it had the weakest absorption which represented little impurity existed (Chen, Xie, Nie, Li, & Wang, 2008). The other fractions containing a large number of low molecular weight polymer were not investigated here. After concentration, the collected fractions were dialyzed and lyophilized for subsequent research.

O. ficus indica polysaccharide was further eluted as a symmetrically single peak and a solvent peak (small) from GPC with HPLC (Fig. 1b). The GPC chromatogram indicated that the purity of *O. ficus indica* polysaccharide obtained was high enough for further analysis.

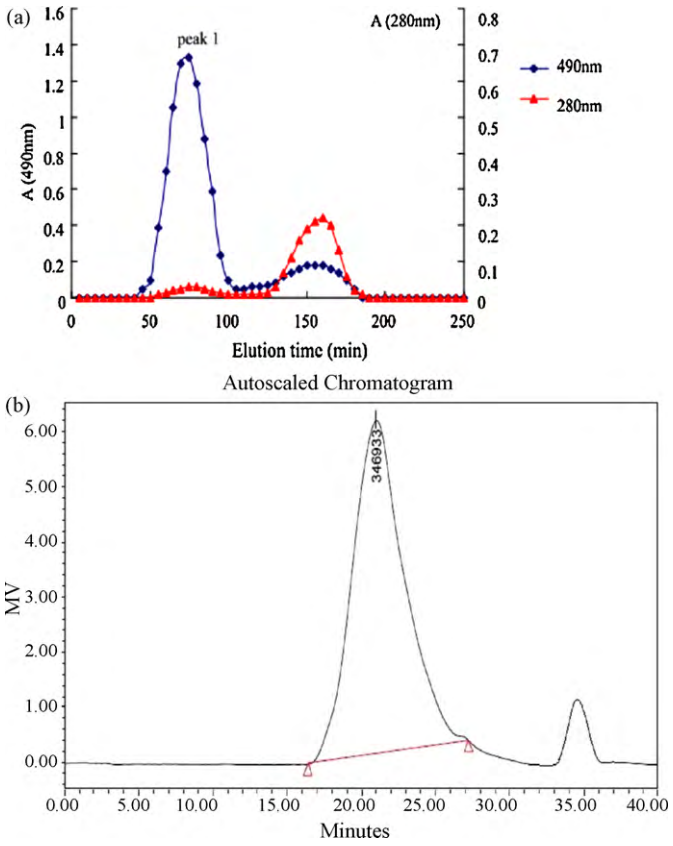

Fig. 1. (a) Sephacryl S-400 HR column elution profile of crude *O. ficus indica* polysaccharide; fluent: 0.2 M NaCl; flow rate: 0.2 ml/min, 1 ml/tube. (b) GPC chromatogram of *O. ficus indica* polysaccharide.

3.2. Chemical analysis of O. ficus indica polysaccharide

3.2.1. Physicochemical properties

O. ficus indica polysaccharide was primrose yellow powder, which is soluble in a majority of inorganic reagents (acids, bases and salts). Specific rotatory power $[\alpha]_D^{20}$ of it is +72.5°. Phenol–H_2SO_4(c) and anthrone–H_2SO_4(c) chromogenic reactions were conspicuous, indicating that the main composition was polysaccharide. I_2–KI reaction had no obvious phenomenon, demonstrating that there is no starch in *O. ficus indica* polysaccharide; α-naphthol–H_2SO_4 was masculine as well, indicating that reducing sugar was present in it; ninhydrin reaction was not obvious and there was little change in color, that was a symbol of little protein.

3.2.2. Molecular weight by GPC

O. ficus indica polysaccharide was further eluted as a single and symmetrically sharp peak from gel-permeation chromatography on TSK G-5000 PW × L and TSK G-3000 PW × L gel column with HPLC. Information can be acquired from the chromatogram coupled with GPC numerical analysis, the retention time was 20.967 min and elution volume was 12.580 ml. Based on the calibration with standard dextrans, the peak with elution volume of 12.580 ml corresponded to number-average molecular weight of 172,591 Da (Ovodov, 2009); GPC chromatogram of *O. ficus indica* polysaccharide is showed in Fig. 1b, its molecular weight of peak position at 346,933 Da.

3.2.3. Polysaccharide composition

The polysaccharide composition was determined by HPLC chromatograms (Ovodov, 2009), and mixed standards chromatogram

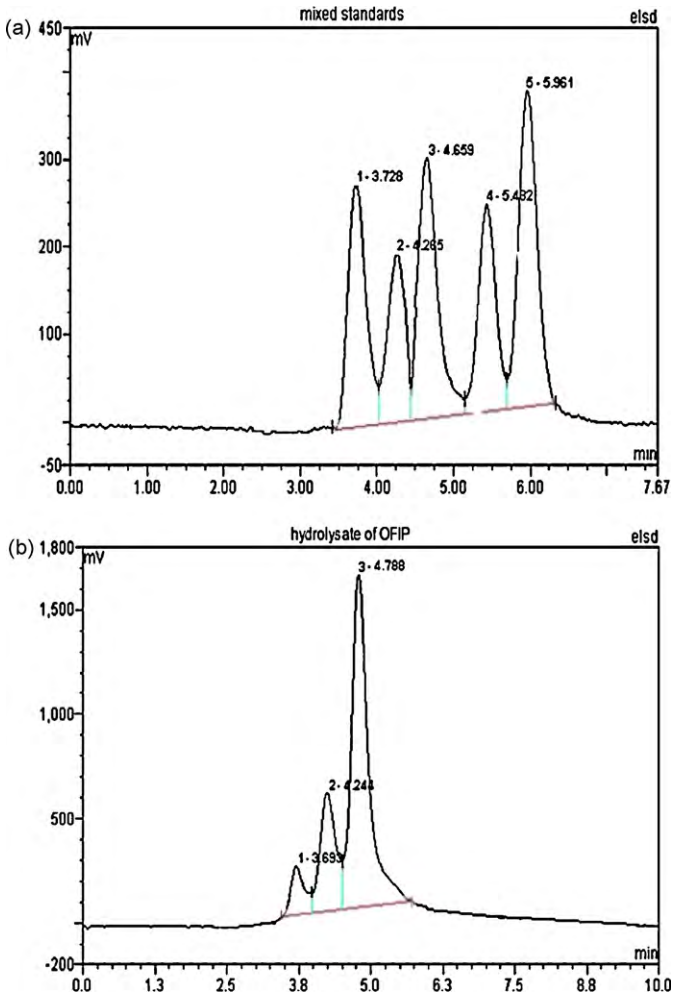

Fig. 2. (a) ELSD chromatogram of mixed standards. According to five preceding chromatograms of saccharide standards, peak 1 represent for rhamnose, 2 for arabinose, 3 for glucose, 4 for sucrose, 5 for maltose. (b) ELSD chromatogram of hydrolysate of *O. ficus indica* polysaccharide.

was shown in Fig. 2a. Five equations of calibration curve and accurate retention time of each saccharide were obtained according to the HPLC chromatograms (Table 1).

The HPLC chromatogram of hydrolysate of *O. ficus indica* polysaccharide indicated that the *O. ficus indica* polysaccharide mainly consisted of glucose (Fig. 2b). After hydrolysis by H_2SO_4, rhamnose, arabinose, glucose were detected by HPLC system. Usually, HPLC chromatograms could give relatively accurate peak area and retention time, combined with the equation of calibration curve and molecular weight, these saccharides (rhamnose, arabinose, and glucose) were found to be present in a molar ratio of 1.00:2.98:2.57. The result is different from those reported previously (Habibi et al., 2004; Majdoub, Roudesli, & Picton, 2001; Matsuhiro et al., 2006; McGarvie & Parolis, 1981; Panico et al., 2007), which is perhaps due to different climates and soil conditions.

Comparing the findings by different authors, we find that the polysaccharide compositions of *O. ficus indica* have a relatively large difference. This difference may be related to different cultivation regions, analysis methods, and samplings (Table 2).

McGarvie and Parolis (1981) found five components in *O. ficus indica*. However, Habibi et al. (2004) reported seven sugar components in the polysaccharides of the peel of *O. ficus indica*, but the seven sugar components were different from other authors' research (Majdoub, Roudesli, & Picton, 2001). In addition, Matsuhiro et al. (2006) found five components in peeled fruits of *O. ficus indica*.

Almost all the researchers focused on its skin and peeled fruits, the results showed that the compositions of polysaccharides were diverse. Results are distinguishing even with the same sampling (e.g. skin). Different analysis method can also cause different results (Matsuhiro et al., 2006). The polysaccharides constituents are easily oxidized with chemical methods, and chromatographic method can get results that are more complete.

3.3. Antioxidant activity analysis

3.3.1. Inhibition of pyrogallol autoxidation

Superoxide anion radical can be generated by pyrogallol autoxidation and produce a colored compound (Chen, Zhang, & Xie, 2005). Resulting from a color change from purple to yellow, the absorbance at 325 nm increased when the superoxide anion was scavenged by an antioxidant, which can represent the content of superoxide radicals and indicate the antioxidant activity of the sample. Superoxide anion is one of the precursors of hydroxyl radicals, it indirectly initiates lipid peroxidation. Apart from that, the presence of superoxide anion can magnify the cellular damage because it produces other kinds of free radicals and oxidizing agents.

The scavenging activity of the three solutions increased with the increase of concentration, ascorbic acid displayed the highest scavenging ability (Fig. 3a). EC_{50} (HR) of ascorbic acid, crude *O. ficus indica* polysaccharide and *O. ficus indica* polysaccharide was 189.1, 628.5 and 665.7 μg/ml, respectively. Although the ability of crude and purified *O. ficus indica* polysaccharide was weaker compared with ascorbic acid, they can scavenge superoxide anion well in higher concentration. The effect of crude polysaccharide was better than purified polysaccharide, which maybe attributed to the purification process. After sophisticated purification, many high anti-oxidation components such as glycopeptide and conjugated proteins were cleaned out. Overall, the results indicated that crude and purified *O. ficus indica* polysaccharide had a strong scavenging power for inhibition of pyrogallol autoxidation at low addition quantity.

3.3.2. Activity of scavenging hydroxyl radical

Hydroxyl radicals were the most harmful free radical and were mainly responsible for the oxidative injury of bimolecular generated by reaction of Fe(II) complex with H_2O_2 in the presence of acid. Salicylic acid has the ability to absorb •OH to bring coloring material. Added hydroxyl radical scavengers compete with salicylic acid, which makes the content of coloring material down. The method

Table 1
Retention time and equation of calibration curve of saccharide standards.

Standards	Retention time (min)	Calibration curve (*C*, mg/ml)	r^2
Rhamnose	3.737	Log area = 1.29837 + 1.05405 Log *C*	0.9963
Arabinose	4.024	Log Area = 0.63432 + 1.84639 Log *C*	0.9879
Glucose	4.617	Log Area = 1.63977 + 1.20426 Log *C*	0.9986
Sucrose	5.364	Log Area = 1.73281 + 0.95423 Log *C*	0.9959
Maltose	5.955	Log Area = 1.77283 + 0.85650 Log *C*	0.9856

Table 2
Analysis results of polysaccharide extracted from O. ficus indica by different researchers.

Regions/fractions	Methods	Arabinose	Galactose	Galacturonic acid	Glucose	Glucuronic acid	Xylose	Rhamnose	Uronic acid	References
South Africa/no data	Methylation analysis and periodate oxidation	+	+	+			+	+		McGarvie and Parolis (1981)
Monastir Tunisian Sahel, May 1998/peeled nopals	Methanolyse and silylation followed by gas chromatography	+	+	+	+	+	+	+		Majdoub et al. (2001)
Semi-arid lands of Morocco/skin	Methylation and reduction–methylation analysis	+	+		+		+	+	+	Habibi et al. (2004)
Tiltil, November/fruits	Gel-permeation chromatography (water-insoluble polysaccharides)	+	+				+	+		Matsuhiro et al. (2006)
	Gel-permeation chromatography (water-soluble polysaccharides)		+				+	+		
Catania, sicily, January 2005/cladodes	Methylation	+	+	+			+	+		Panico et al. (2007)
Hainan province, China/peeled fruit	Gel-permeation chromatography (water-soluble polysaccharides)	+			+			+		Present

was used to evaluate the hydroxyl radicals scavenging ability of natural compounds (Boligon et al., 2009).

The results of hydroxyl radical scavenging activities of ascorbic acid, crude and purified *O. ficus indica* polysaccharide were given in Fig. 3b. All the samples exhibited obvious scavenging activity on hydroxyl radical in a concentration-dependent manner. EC_{50} (HR) of the three (ascorbic acid, crude and purified *O. ficus indica* polysaccharide) were found to be 456.4, 566.0, 631.8 μg/ml respectively.Effect of *O. ficus indica* polysaccharide on •OH-induced lipid peroxidationEffects of various amounts of ascorbic acid, crude and purified *O. ficus indica* polysaccharide solution on the •OH-induced production of malondialdehyde (MDA) in mouse liver and chicken

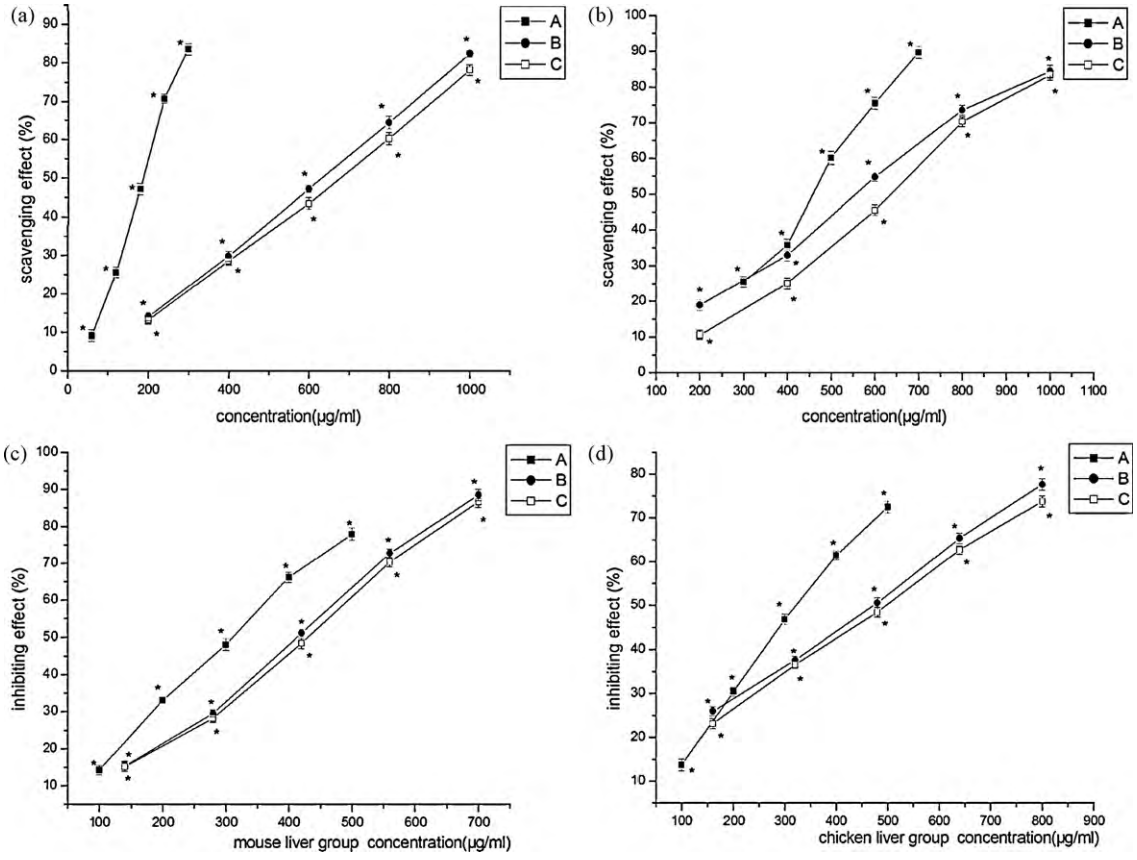

Fig. 3. The effect of superoxide anion radical (a), hydroxyl radical (b) scavenging activity and the inhibiting effect of MDA in mouse liver (c) and chicken liver (d) on different concentrations. Both ascorbic acid (A) and crude *O. ficus indica* polysaccharide (B) were compared with purified *O. ficus indica* polysaccharide. Values are represented as mean ± standard deviation ($n = 3$) of them. * represents that the statistics are significantly different ($P < 0.01$) compared with blank control group.

liver homogenate were investigated to determine if they could prevent or reduce •OH-induced lipid peroxidation. The results of mouse liver homogenate were summarized in Fig. 3c, EC_{50} (MDA) of ascorbic acid, crude and purified polysaccharide were 313.3, 409.5, 422.1 μg/ml respectively. When it comes to chicken liver, from Fig. 3d, EC_{50} (MDA) of them were 333.8, 462.5, and 493.7 μg/ml. All of them produced a concentration-dependent decrease in the •OH-induced formation of MDA. The results suggested that *O. ficus indica* polysaccharide reduced •OH-induced lipid peroxidation by scavenging •OH.

O. ficus indica polysaccharide produced a concentration dependent reduction in the formation of superoxide anion radical and hydroxyl radical. It produced a marked decrease in the production of MDA in both mouse and chicken liver homogenates as expected. The reduction in lipid peroxidation in liver homogenate by *O. ficus indica* polysaccharide is probably due to its scavenging of hydroxyl radical. Since the peroxidation process in homogenate takes place mainly in the lipid phase of the cell membrane (Prasad, Laxdal, Yu, & Raney, 1996), *O. ficus indica* polysaccharide, which is a fairly polar molecule, is less effective here than ascorbic acid in the aqueous phase. On the other hand, in most cases the ability of scavenging free radical of crude *O. ficus indica* polysaccharide is stronger than purified *O. ficus indica* polysaccharide. Presumably, some plants that have high antioxidation are due to the synergy effect of many nutrient substances (Trombetta et al., 2006).

4. Conclusion

O. ficus indica polysaccharides were isolated and purified polysaccharide was confirmed to be of high purity. The water-extracted polysaccharide was composed of rhamnose, arabinose, and glucose with the molar ratio of 1.00:2.98:2.57. *O. ficus indica* polysaccharide can effectively inhibit the hydroxyl radical generated by Fenton system, and shows a good performance of the hydroxyl radical scavenger. *O. ficus indica* polysaccharide can also effectively inhibit the generation of malondialdehyde in microsomes of mice liver and chicken liver. The future challenge is to define the 3D structure of *O. ficus indica* polysaccharides and the structure–function relationship.

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