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Full Length Research Paper

In vitro antioxidant activity of Polysaccharide from *Sedum aizoon* L. extracts

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Crude polysaccharide was isolated from the stem of *Sedum aizoon* L. Antioxidant activity of crude polysaccharide was evaluated with 2, 20-diphenyl-l-picrylhydrazyl (DPPH), 2, 20-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), superoxide radical scavenging, hydroxyl radical scavenging and reducibility. It exhibited a powerful ABTS radical and hydroxyl radical scavenging character when compared with the standard Vitamin C. However, the antioxidant test indicated that crude polysaccharide had no significant effect on DPPH.

Key words: *Sedum aizoon* L., antioxidant activity, polysaccharide.

INTRODUCTION

Sedum aizoon L. is a well known medicinal, edible and ornamental plant in oriental countries. Leaves and young stems are raw, ornamental and cooked (Li, 1996; Tian et al., 2000; Che et al., 2004). The whole grass has been used to cure palpitation and insomnia in Chinese countries for a long time (Wan, 2007). It is considered to have the property of activating blood genesis, maintaining hemostasis, detoxification and pain relief (Wang, 1990; The Committee of Chinese Herbals, 1999; Xu and Wan, 2002). The previous research has also proved that it can be used as a medicine in cardiovascular disease, hyperlipidemia and other illnesses (Che et al., 2004). Chemical constituents have been identified, such as carbohydrates, alkaloids, flavonoids etc (Chen and Xu, 2009; Guo et al., 2007; Lin et al., 2008). However, limited studies have reported the antioxidant activity of polysaccharides from *S. aizoon*. Antioxidant supplementation constitutes important defenses against a variety of diseases and environmental stress. Therefore, in this study, we reported for the first time the antioxidant activities *in vitro* of polysaccharide from *S. aizoon*.

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MATERIALS AND METHODS

Plant materials

The stem of *S. aizoon* was collected from Fujian Province, P. R. China, in July 2009 and identified by Prof. Zhang Yong-Tian. A voucher specimen (*No.*090701) was deposited with the College of Chemical Engineering, Huaqiao University.

Chemicals

Vitamin C was purchased from Sigma Chemical Co., ABTS radical was purchased from Merck, and DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical was purchased from Sigma-Aldrich. However, methanol, petroleum ether, ethanol, acetic anhydride and all other chemicals and reagents were of grade AR.

Extracting crude polysaccharide

The dried stems (100 g) were crushed, and then the powder was extracted with petroleum ether at 65℃ for 1.5 h. The residue was further extracted with 80% ethanol at 90℃ for 1.5 h, filtered and dried, and then extracted with double-distilled water at 100℃ for 2 h thrice. All extracts were combined, filtrated and concentrated using a rotary evaporator at 55℃ under reduced pressure. The proteins in the product of condensation were removed using the Sevag reagent (Navarini et al., 1999) several times. After removal of the Sevag reagent, the extract was precipitated with four volumes of ethanol and the mixture was kept overnight at 4℃.

The precipitate was collected by centrifugation at 4000 rpm for 20 min, washed successively with petroleum ether, acetone and ethanol, dissolved in water, and then the procedure of precipitation was performed with iteration. Finally, the extract was dialyzed against deionized water for 72 h and recovered by freeze-drying, which produced a crude polysaccharide (DNP).

Scavenging ability of DPPH radical

Measurement is based on methods developed by Shimada et al. (1992), with some modifications. Vitamin C was used as the reference material. Briefly, 0.1 mmol/L solution of DPPH in methanol was prepared and 1.0 ml of this solution was added with 3.0 ml of the purification polysaccharides of various concentrations (10-2000 µg/ml) in water. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm (A_{517}). The DPPH scavenging effect was calculated using the following equation:

DPPH scavenging effect (%) = 〔$(A_0-(A-A_b))/ A_0$〕×100

Where A_0 is A_{517} of DPPH without sample, A is A_{517} of sample with DPPH, and A_b is A_{517} of the sample without DPPH.

ABTS assay

It was determined by Re et al. (1999) with some modifications. The ABTS cation radical was produced by the reaction of 7 mmol/L ABTS solution and 2.45 mmol/L potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 h. Before use, this solution was diluted with ethanol to get an absorbance of 0.70±0.02 at 734 nm(A_{734}) in a final volume of 0.2 ml, with various concentrations (10 to 2000 µg/ml) added to 2.0 ml of ABTS solution and mixed vigorously. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. The inhibition percentage of ABTS radical was calculated using the following formula:

ABTS scavenging effect (%) = 〔$(A_0-(A-A_b))/ A_0$〕× 100

Where A_0 is A_{734} of ABTS without sample, A is A_{734} of sample with ABTS, and A_b is A_{734} of sample without ABTS.

Hydroxyl radical scavenging assay

It was operated according to the method of Smironoff and Cumbes, (1989) and Wang et al. (2008), with some modifications. Different concentrations (10 to 2000 µg/ml) of the samples were incubated with 2.0 mmol/L EDTA-Fe (0.5 ml), 3%H_2O_2 (1.0 ml) and 360 µg/ml crocus in 4.5 ml sodium phosphate buffer (150.0 mmol/L, pH 7.4) for 30 min at 37℃, and hydroxyl radical was detecte d by monitoring the absorbance at 520 nm(A_{520}). The hydroxyl radical scavenging effect was calculated using the following equation:

hydroxyl radical scavenging effect (%) = 〔$(A_C-A_S)/ A_C$〕×100

Where A_S is A_{520} of sample, and A_C is A_{520} of the control. In the control, sample was substituted by distilled water, and sodium phosphate buffer was replaced by H_2O_2.

Superoxide radical scavenging assay

It was determined by Zhao et al. (2003). The percentage of inhibition effect was calculated according to the following formula:

O_2 scavenging assay effect (%) = 〔$(A_0-A_b)/ A_0$〕×100

Where A_0 is the absorbance of the extract blank, and A_b is the absorbance of the extract addition.

Determination of reducibility

It was measured by Yen and Chen (1995).

Data handing

Results were expressed as means ± standard deviations of three replicated determinations. SPSS 8.0 software (SPSS, Chicago, IL, USA) was used for data analysis.

RESULTS AND DISCUSSION

The polysaccharide was obtained from the stem of *S. aizoon* by water-extraction and ethanol-precipitation. The total yield of crude polysaccharides was 5.21%.

Figure 1 shows the scavenging capacity of polysaccharide on DPPH (Vitamin C as the positive control). Among the levels used in the experiment, 2 mg/ml was the strongest one with a scavenging rate of 28.38%, and the standard rate of Vitamin C was 99.07% at the same concentration. It was obvious that the DPPH radical scavenging activity of Vitamin C was stronger than that of DNP at every concentration. The low scavenging capacity of DNP on DPPH was possibly due to the phenolic compounds which could not act as hydrogen donor antioxidants.

The effect of scavenging ABTS radicals is useful for evaluating the total antioxidant power of complex mixtures of various plants (Huang et al., 2008). It is obvious that ABTS scavenging activity of polysaccharide is strong in Figure 2. For the positive control, Vitamin C showed an excellent scavenging activity on ABTS at the half inhibition concentration (EC_{50}) = 0.005 mg/ml. Polysaccharide was also observed to have a strong scavenging activity, with an EC_{50} value of 0.4 mg/ml. Particularly, in a higher dose (1.0 mg/ml), polysaccharide exhibited very high radical scavenging activity (87.94), and Vitamin C as standard was measured for 96.45% at the same concentration.

The results of hydroxyl radical scavenging activities of polysaccharide and Vitamin C are given in Figure 3. The Vitamin C showed high radical scavenging activity (90%) at about 0.2 mg/ml. Polysaccharide exhibited weaker scavenging effect than Vitamin C at every concentration, but at the concentration of 1 mg/ml, the scavenging activity was close to Vitamin C. Therefore, polysaccharide has an appreciable scavenging power on hydroxyl radicals. The result showed that polysaccharide had no significant effect on O_2 radical scavenging (Figure 4). It was observed that the inhibition rate was above 80% at

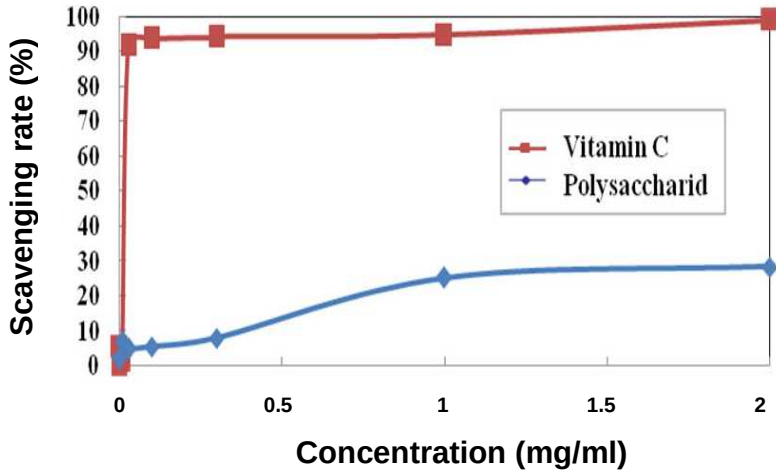

Figure 1. DPPH radical scavenging activity of polysaccharide.

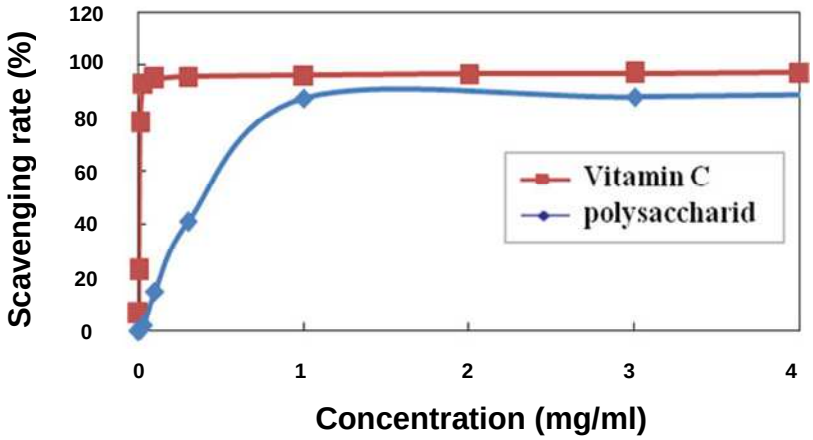

Figure 2. The ABTS radical scavenging activity of polysaccharide.

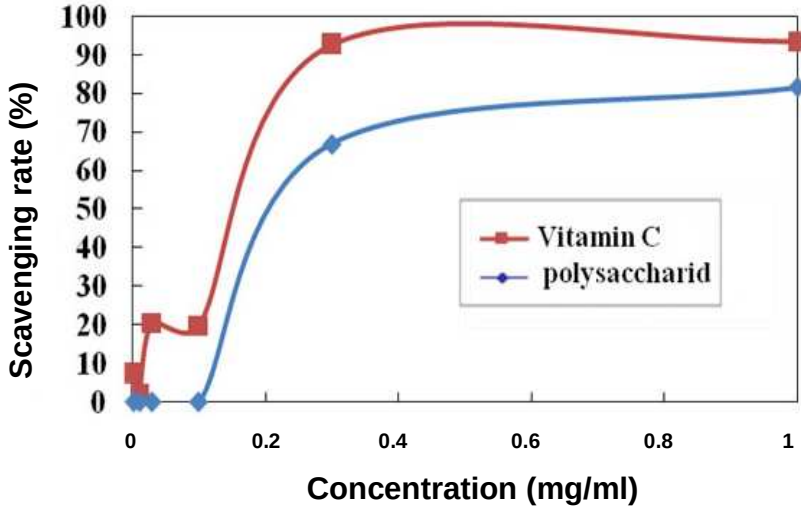

Figure 3. The Hydroxyl radical scavenging activity of polysaccharide.

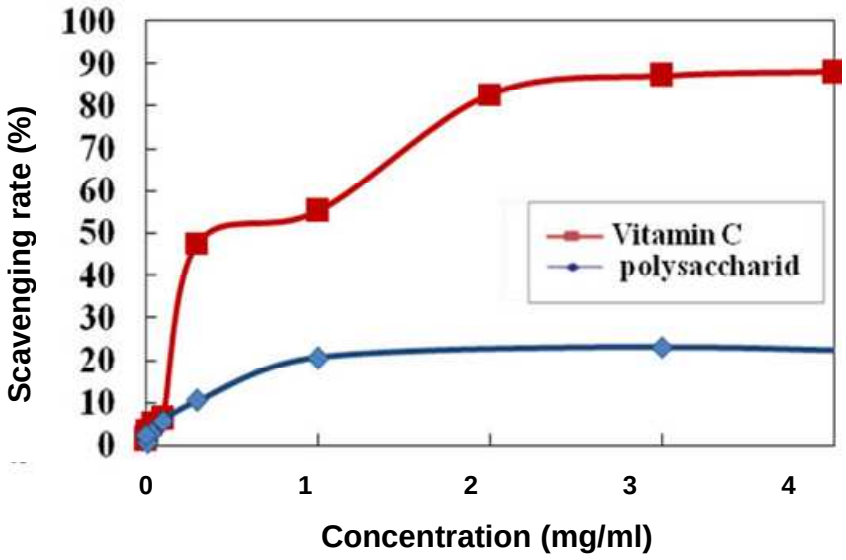

Figure 4. The O_2 radical scavenging activity of polysaccharide.

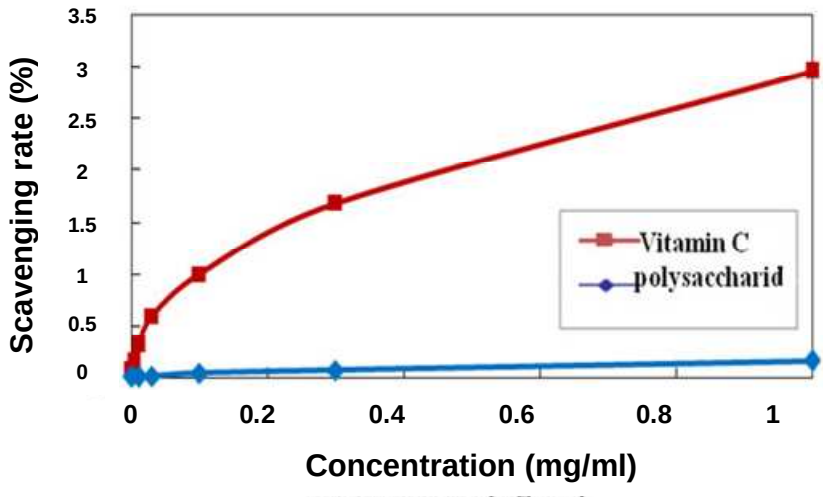

Figure 5. The reducibility radical scavenging activity of polysaccharide.

2 mg/ml in the positive control, while the scavenging O_2 activity of polysaccharide was below 25% for different concentrations.

The result indicated that polysaccharide exhibited very low effects of reducibility (Figure 5). The inhibition rate was above 0.16% at 0.003 mg/ml in positive control, while scavenging reducibility activity of polysaccharide was below 0.16% for different concentrations, and was far lower than that of Vitamin C with the same concentration.

Conclusion

In our studies, polysaccharide from S. *aizoon* was found to exhibit excellent antioxidant activities in the scavenging ABTS radicals and hydroxyl radical *in vitro.* As a result, S. *aizoon* L. seems to be a good source of natural antioxidant.

However, further investigation of *S. aizoon* L. will be carried out in our subsequent work.

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