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Polysaccharide Fractions from *Sarcodia ceylonensis* and their Antioxidant Properties *In vitro* and *In vivo*

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ABSTRACT

Background: The oxidative stress induced cell damage triggers both the physiological process of aging and many pathological progressions that eventually lead to serious health problems. Lots of researches exhibited that some polysaccharides have been demonstrated to play an important role as free radical scavenger for the prevention of oxidative damage in living organisms. So the purpose of the present investigation was to elucidate the isolation of the polysaccharide from Sarcodia ceylonensis and evaluate its antioxidant activities. **Materials and Methods:** Three polysaccharide fractions (SCP-40, SCP-60 and SCP-80) from *Sarcodia ceylonensis* were obtained by using the method of water-extraction and ethanol-precipitation. In order to evaluate the antioxidant activity in vitro of these polysaccharides, the radicals scavenging activity against ABTS, DPPH and hydroxyl radicals was determined. **Results:** The results showed SCP-40 exhibited strong scavenging effects on these radicals. For in vivo assays, the polysaccharide SCP-40 was found to increase the levels of antioxidant enzymes superoxide dismutase and to decrease the malondiadehyde content in blood serum. **Conclusion:** The polysaccharide SCP-40 from *Sarcodia ceylonensis* should be explored as a novel potential antioxidant.

Key words: *Sarcodia ceylonensis*, polysaccharide, antioxidant activity

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INTRODUCTION

The polysaccharides not only are energy resources but play key biological roles in many life processes as well. The structure and mechanisms of pharmaceutical effects of bioactive polysaccharides on diseases have been extensively studied. Lots of researches exhibited that some polysaccharides have been demonstrated to play an important role as free radical scavenger for the prevention of oxidative damage in living organisms (Wu *et al*., 2007; Tsiapali *et al*., 2001). Seaweeds are the most abundant source of polysaccharides, as alginates, agar and agarose as well as carrageenans polysaccharides are widespread in marine algae. Some polysaccharides from marine algae exhibited much strong biological activities, such as polysaccharide from Gracilaria birdiae can prevents naproxen-induced gastrointestinal damage in rats (Silva *et al*., 2012). Sulfated polysaccharides isolated from sphaerococcus coronopifolius (Rhodophytha, Gigartinales) and boergeseniella thuyoides (Rhodophyta, Ceramiales) have antiviral activities (Bouhlal *et al*., 2011). Polysaccharides in marine sponges have anti-HIV activity (Esteves *et al*., 2011). Sulfated polysaccharides from *Sargassum plagiophyllum* showed higher anticancer and antioxidant activity (Suresh *et al*., 2013). And also, some polysaccharides from marine algae have significant antioxidant properties, such as *Sargassum graminifolium* (Zhang *et al*., 2012), *Fucus vesiculosus* (Ruparez *et al*., 2002; De Souza *et al*., 2007).

Sarcodia ceylonensis is a red algae and commonly consumed as seafood and as medical resource for its hypolipidemic and immune enhancement effect (Xia, 1999). There are abundant polysaccharides in *Sarcodia ceylonensis*. But, up to now, the purification and biological activity of the polysaccharide from *Sarcodia ceylonensis* has not been reported. Therefore, the purpose of the present investigation was to elucidate the isolation and purification of water-soluble polysaccharide from *Sarcodia ceylonensis*, as well as to evaluate its antioxidant activities *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials and chemicals: Malondiadehyde (MDA) assay kit and superoxide dismutase (SOD) assay were purchased from the Institute of Biological Engineering of Nanjing Jianchen (Nanjing, China). 2, 2-azinobis-6-(3-ethylbenzothiazoline sulfonic acid (ABTS) radical was purchased from Merck (Darmstadt, Germany). 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and vitamin C were purchased from Sigma (St.Louis, MO, Petroleum ether,

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ethanol, ethyl acetate, N-butanol and all other chemicals and reagents were analytical grade.

Extraction the polysaccharide from *Sarcodia ceylonensis*: The *Sarcodia ceylonensis* were thoroughly washed with water, dried at 60°C and then powdered with a pulverizer. The powder was extracted in 6 times the volume (v/m) 0.4% HCl for 8 h. The solution was separated from *Sarcodia ceylonensis* residues by successive filtration through gauze and the residues were extracted with 0.4% HCl (6 times the volume) for three times. All extracts were combined, neutralized with NaOH, filtered. Then all extracts were concentrated using a rotary evaporator at 55°C. The extract was deproteinized four times using the Sevag reagent (Navarini *et al*., 1999). After removal of the Sevag reagent, the extract was precipitated by adding ethanol (two-thirds times the volume of aqueous extract) and the mixture was kept overnight at 4°C to yield the SCP-40. After filtration and centrifugation, the solution was successively precipitated by adding ethanol until the concentration of ethanol got to 60 and 80%, which to yield the SCP-60 and SCP-80, respectively. After washed successively with ethyl acetate and acetone, three polysaccharides were dissolved in double-distilled water and subsequently dialyzed against deionized water for 72 h and lyophilized.

ABTS radicals scavenging assay: The radicals scavenging activity of the four samples against $ABTS^+$ radicals were measured using the methods of Re *et al*. (1999) and Luo (2008) with some modifications. $ABTS^+$ was produced by reacting 7 mmol L^{-1} of $ABTS^+$ solution with 2.45 mmol L^{-1} of potassium persulphate and the mixture would be kept in the dark at room temperature for 16 h. In the moment of use, the $ABTS^+$ solution was diluted with ethanol to an absorbance of 0.70±0.02 at 734 nm. Each sample (0.2 mL) with various concentrations (62.5-4000 μg mL^{-1}) were added to 2 mL of $ABTS^+$ solution and mixed vigorously. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured. The $ABTS^+$ scavenging effect was calculated by the following Eq:

$$\text{ABTS scavenging effect (\%)} = \frac{\text{Ao-(A-Ab)}}{\text{Ao}} \times 100$$

where, Ao is A734 of ABTS without sample, A is A734 of sample and ABTS and Ab is A734 of sample without ABTS.

DPPH radicals scavenging assay: In the present test, DPPH scavenging activities of the different fractions were measured according to the method of Fan *et al*. (2009), with some modifications. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added with 3.0 mL of the samples of various concentrations (62.5-4000 μg mL^{-1}). The solution was kept at room temperature for 30 min and the absorbance at 517 nm (A517) was measured. The DPPH scavenging effect was calculated as follows:

$$\text{DPPH scavenging effect (\%)} = \frac{\text{Ao-(A-Ab)}}{\text{Ao}} \times 100$$

where, Ao is A517 of DPPH without sample, A is A517 of sample and DPPH and Ab is A517 of sample without DPPH.

Hydroxyl radicals scavenging assay: The antioxidant activity was determined by hydroxyl radicals as described by Luo *et al*. (2010), with some modifications. Briefly, different concentrations (0.001-4.0 mg mL^{-1}) samples were incubated with 2 mM EDTA-Fe (0.5 mL), 3% H_2O_2 (1.0 mL) and 0.36 mg mL^{-1} crocus in 4.5 mL sodium phosphate buffer (150 mM, pH 7.4) for 30 min at 37°C and hydroxyl radical was detected by monitoring absorbance at 520 nm. The hydroxyl radical scavenging effect was calculated as follows:

$$\text{Scavenging effect (\%)} = \frac{\text{Ao-As}}{\text{Ao}} \times 100$$

where, A_0 is the A_{520} of control and As is the A_{520} of sample.

Antioxidant activity *in vivo*: Kunming mice (provided by Sichuan Academy of Medical Science, China), weighing in the range of 18-22 g, were kept in separated cages at a temperature of 21±1°C and 50-60% of relative humidity. They underwent 12 h light and dark cycles with free access to food and water. All of the mice were evenly and randomly divided into five groups of ten mice each. Group I was given D-galactose and normal laboratory diet; Group II was given D-galactose, Vc (100 mg kg^{-1} day^{-1}) and normal laboratory diet; Group III was given D-galactose, the sample at a dose of 200 mg kg^{-1} day^{-1} and normal laboratory diet; Group IV was given D-galactose, the test sample at a dose of 100 mg kg^{-1} day^{-1} and normal laboratory diet; Group V was given D-galactose, the sample at a dose of 50 mg kg^{-1} day^{-1} and normal laboratory diet. The dose of D-galactose of each group was 100 mg kg^{-1} day^{-1} b.wt. (Lv *et al*., 2007). Twenty-four hours after the last drug administration, blood samples were obtained from the eyepit of the mice and processed for serum. The concentrations of MDA in blood serum from the mice were determined with an MDA Assay Kit. The SOD activities were determined with an SOD Assay Kit.

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Statistical analysis: The data were presented as Mean±Standard Deviation. Statistical analysis was conducted with the SPSS 16.0 software package.

RESULTS

Scavenging effects of SCP-40, SCP-60 and SCP-80 on ABTS: ABTS assay is often used in evaluating antioxidant power of natural products. In the experiment, the scavenging abilities of three polysaccharides (SCP-40, SCP-60 and SCP-80) on ABTS free radicals were shown in Fig. 1. As seen from the figure, the scavenging power of all samples correlated well with increasing concentrations. Moreover, vitamin C showed valuable high radical scavenging activity (89.9-91.4%) in the higher doses (from 2.0 to 4.0 mg mL^{-1}). At the same time, SCP-40 and SCP-80 exhibited strong scavenging activity (70.5-79.1% for SCP-40, 50.8-77.1% for SCP-80) in the higher doses (from 2.0 to 4.0 mg mL^{-1}), which were close to that of vitamin C. But the scavenging activity of SCP-60 was far lower than that of the other samples. Therefore, it was obvious that the polysaccharide of SCP-40 has strong antioxidant activity in the higher doses, followed by SCP-80. SCP-60 has no significant effect on ABTS radical scavenging.

Effect of scavenging DPPH radicals: In this experiment, the scavenging ability of the purified polysaccharides and Vc on DPPH free radical were examined in the concentration range of 62.5-4000 $\mu g\ mL^{-1}$ using the DPPH colorimetric assay (shown in Fig. 2). The results manifested Vc and SCP-40 in all concentrations dose-dependent DPPH radical scavenging activities. Furthermore, the scavenging activity of Vc increased very significantly with increasing concentrations, at 500 $\mu g\ mL^{-1}$, the scavenging effects has got to 90.1%. At the same time, SCP-40 exhibited good scavenging activity especially in the high dose. However, the polysaccharides SCP-60 and SCP-80 had very low scavenging effects on DPPH at every dose. Therefore, the results exhibited that SCP-40 have a certain degree of effects on DPPH radical scavenging. SCP-80 and SCP-60 has no significant scavenging activities on DPPH radical.

Scavenging effects of polysaccharides on hydroxyl radicals: The results of hydroxyl radical scavenging activities of SCP-40, SCP-60 and SCP-80 compared to those of vitamin C are shown in Fig. 3. All samples exhibited obvious hydroxyl radical scavenging activities in a concentration-dependent manner. These three polysaccharides were found to have the ability to scavenge hydroxyl radicals at concentrations between 1000 and 4000 $\mu g\ mL^{-1}$. The ability of SCP-40 was the strongest, followed by SCP-80 and SCP-60. At high dose, SCP-40 showed an excellent hydroxyl radical scavenging

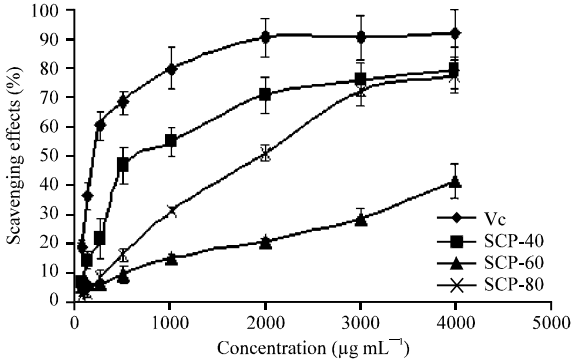

Fig. 1: The scavenging effects of polysaccharides on ABTS radicals. Results are presented as Means±Standard deviations. Different polysaccharide fractions correlated well with increasing concentrations. In the high doses, Vc, SCP-40 and SCP-80 exhibited strong scavenging activity. Especially as SCP-40, at 4.0 mg mL^{-1}, the scavenging effect on ABTS radical close to that of vitamin C

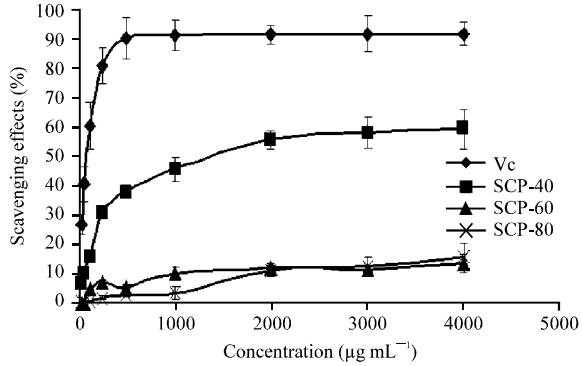

Fig. 2: The scavenging effects of polysaccharides on DPPH radicals. Results are presented as Means±Standard Deviations. Vc and all polysaccharides in all concentrations dose-dependent. The scavenging effects of Vc was the strongest, followed by SCP-40. However, the polysaccharides SCP-60 and SCP-80 had no significant scavenging effects on DPPH

activity (92.5% at 1000 $\mu g\ mL^{-1}$ and 92.8% at 4000 $\mu g\ mL^{-1}$) among all the polysaccharide samples, also close to Vitamin C (94.6%). The hydroxyl radical scavenging ability decreased in the order of SCP-40>SCP-80>SCP-60. Therefore, these results clearly showed that SCP-40 has potential antioxidant ability of scavenging hydroxyl radical.

Antioxidant activity *in vivo*: According to the results above, the effects of antioxidant activities *in vitro* of

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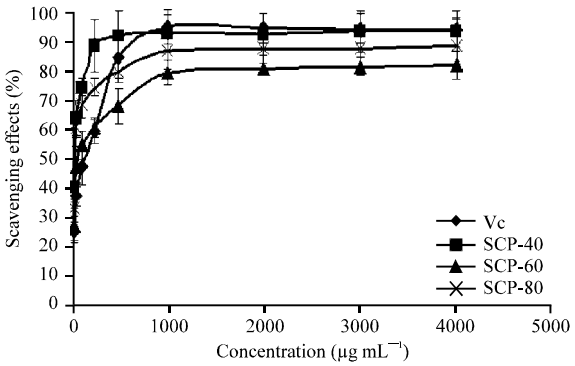

Fig. 3: The scavenging effects of polysaccharides on hydroxyl radicals. Results are presented as Means±Standard Deviations. All polysaccharides and Vc exhibited obvious hydroxyl radical scavenging activities in a concentration-dependent manner. In the high doses (1000 and 4000 μg mL^{-1}), all samples exhibited significant scavenging effects on hydroxyl radicals. The ability of SCP-40 was the strongest, followed by SCP-80 and SCP-60

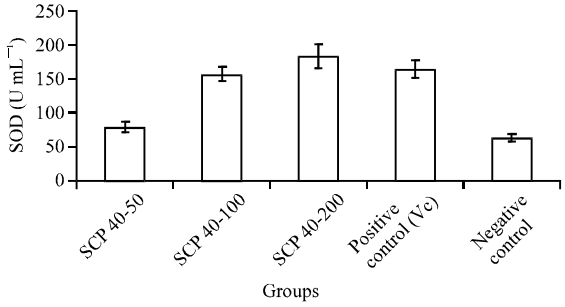

Fig. 4: SOD activity analysis in mice. Results are presented as Means±Standard Deviations. The positive control is Vitamin C at the concentration of 100 mg kg^{-1}. SOD activities of different doses of SCP-40 exhibited dose-dependent behavior. At the high dose of 200 mg kg^{-1}, SOD activity of u mL^{-1} was 182.9 u mL^{-1}, which was higher than that of vitamin C ($p<0.05$). So, SCP-40 could enhance the SOD activity in the mice

SCP-40 exhibited strongest free radical scavenging effects among the three polysaccharides, therefore, in order to investigate in-depth the antioxidant activity of the polysaccharide SCP-40, the antioxidant activity in vivo was tested. The results are shown in Fig. 4. SOD activities of different doses of SCP-40 exhibited dose-dependent behavior. At 100 mg kg^{-1}, SCP-40 exhibited high SOD activity and the SOD activity value of SCP-40 was 156.6 U mL^{-1}, which was close to that of

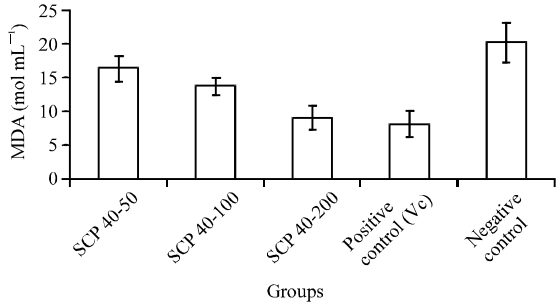

Fig. 5: Determination of MDA contents in blood serum from the mice. Results are presented as Means±Standard Deviations. The figure exhibits a significant pattern of a decreasing MDA concentration in blood serum with increasing SCP-40 concentration. At high dose, the concentration of MDA was close to that of the positive control. This can be interpreted as a significant effect of SCP-40 at high concentrations on MDA scavenging in the mice

the positive control (163.7 U mL^{-1}). At the high dose of 200 mg kg^{-1}, particularly, SOD activity of U mL^{-1} was 182.9 U mL^{-1}, which was higher than that of vitamin C ($p<0.05$). However, the SOD activity at low concentrations was much less evident, which is similar to that of the negative control. The results were therefore an indication of enhancement SOD activity of SCP-40 for high concentrations.

The concentrations of MDA in blood serum from the mice were determined with a MDA Assay Kit. Briefly, the samples added with TBA were heated in an acidic environment, then, the absorbance of the resulting solution was measured at 532 nm. The results in Fig. 5 exhibit a significant pattern of a decreasing MDA concentration in blood serum with increasing SCP-40 concentration. At 200 mg kg^{-1}, the concentration of MDA was 8. 97 nmol mL^{-1}, close to that of the positive control (8.09 nmol mL^{-1}) ($p<0.05$). This can be interpreted as a significant effect of SCP-40 at high concentrations on MDA scavenging *in vivo*.

DISCUSSION

The generation of Reactive Oxygen Species (ROS) is an unavoidable consequence of life in an aerobic environment. However, the uncontrolled production of ROS is involved in onset of many human diseases such as cancer, atherosclerosis, reperfusion injury and hepatic injury, as well as in degenerative processes associated with aging (Halliwell and Gutteridge, 1989; Ke *et al*., 2009; Zou *et al*., 2008) through lipid peroxidation, DNA damage (Halliwell and Aruoma, 1991) and inhibition of protein synthesis (Martin and Dean, 1991) and so on.

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Antioxidants can delay or prevent oxidation of cellular oxidative substrates. Synthetic antioxidants are widely used since they are effective and cheaper than natural ones. However, the safety and toxicity of synthetic antioxidants have brought great concerns (Imaida *et al*., 1983). It is essential to develop and utilize effective and natural antioxidant to protect the body from ROS damage. Therefore, enhancement of body antioxidant defenses through natural and safe antioxidants would seem to provide a reasonable and practical approach to reduce the oxidative stress to human body. Previous studies indicated that polysaccharides antioxidant activity might be closely related to chemical properties and structural characteristics of polysaccharides (Chen *et al*., 2008). Therefore, discovery and evaluation of polysaccharides extracted from plants and fungus as new safe compounds for functional foods or medicine has become a hot research spot.

On the basis of the above results, the water-extracted crude polysaccharide from *Sarcodia ceylonensis* were obtained by using the methods of water-extraction and ethanol-precipitation. Antioxidant in vitro indicated that SCP-40 exhibited a powerful scavenging effect on hydroxyl radicals and ABTS radical, which may be comparable to Vitamin C. For *in vivo* assays, the polysaccharide SCP-40 was found to increase the levels of antioxidant enzymes (SOD) and to decrease the MDA content in blood serum. It was confirmed that SCP-40 can protect tissues against oxidative damages. Enhanced SOD activity in mice blood serum also can be related to the *in vivo* antioxidant activity of SCP-40. With such strong antioxidant ability, SCP-40 was identified as a potential antioxidant.

Sure, in order to corroborate the antioxidant ability of SCP-40 and research the new drug, further investigation of the mechanism of antioxidant activities will be carried out in our later work.

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