Antioxidative activities of the polysaccharides extracted from the mushroom *Ganoderma lucidum*

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ABSTRACT

Dried carpophores of *Ganoderma lucidum* were exposed to the hot water extraction and alcohol precipitation to obtain polysaccharides, which were than refined by the dialyses. Dry polysaccharide extract (GL-I) was partially purified and used for the investigation of antioxidant properties. The antioxidant activity was determined by the conjugated diene method, ascorbic acid and α-tocopherol were used as the positive control. DPPH free radical scavenging activity assay was performed with ascorbic acid, BHT and α-tocopherol as the positive control. For determining reducing power ascorbic acid was used as the positive control. Chelating ability on ferrous ions was performed by addition of ferrozine to initialize reaction, while citric acid and EDTA were used for comparison. GL-I showed high antioxidant activity of 85.7 ± 0.7%, at 10 mg/ml. Reducing power reached a plateau of 3.4 ± 0.1 at 20 mg/ml, while GL-I chelated 81.6 ± 3.6 % of ferrous ions at 20 mg/ml. At 10 mg/ml, scavenging ability on DPPH radicals of GL-I increased to 96.8 ± 2.5%. The antioxidative activities of GL-I were concentration dependent and increased with increasing concentration. Regarding the fact that some of synthetic antioxidants, additives may possess mutagenic activity, using wild mushrooms in diet as sources of naturally-derived antioxidants might be beneficial for human health in reducing oxidative demage.

Keywords: Ganoderma lucidum; antioxidant activity; polysaccharides; extract;

INTRODUCTION

Highly reactive free radicals, especially oxygenderived radicals are capable to oxidize biomolecules and can result in cell death and tissue damage. These processes can be responsible for aging which is associated with different degenerative diseases [1]. Antioxidant compounds have important ability to trap free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Naturally occurring antioxidants can be found in whole grains, fruits, vegetables, teas, spices and herbs. Mushrooms have also been reported as organisms with antioxidant activity which is correlated with their phenolic and polysacchride compounds [2, 3, 4]. *Ganoderma lucidum* (Leyss.:Fr.) Karst, commonly known as lacquered mushroom is one of the most often used mushrooms in traditional medicine of Far Eastern people. Because of its bitter taste and wooden build it is not suitable for nutrition, but the bioactive substances extracted from this mushroom possess very important medicinal characteristics [5].
Objective of this investigation was to evaluate antioxidant activity, reducing power, scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals and chelating effect on ferrous ions of GL-I obtained from fruiting bodies of *G. lucidum*.

MATERIALS & METHODS

Preparation of polysaccharides
100 g of fine mushroom powder was washed with 96% ethanol at room temperature for 24 h under stirring, filtered and dried in vacuum (60 min at 42°C). Dried filtercake was extracted with 2 l Milli-Q water (MQ) by autoclaving (45 min at 121°C), the extract was chilled and centrifuged for 20 min at 9000 g. Supernatant was concentrated by heating to 10% of its initial volume and polysaccharides were precipitated by addition of 2 volumes of cold 96% ethanol and left at 4°C overnight. After centrifugation the pellets were washed with 70% ethanol and dried in vacuum. Dry pellets were dialysed against MQ for 24 h at room temperature to remove residual small molecules as polyphenols, peptides and polysaccharides < 8-10 kD. After centrifugation the high molecular weight polysaccharides (GL-I) were ethanol precipitated and vacuum dried.

Antioxidant activity

The antioxidant activity was determined by the conjugated diene method [6] with slight modification. Each polysaccharide powder (0.1 to 10 mg/ml, 100μl) in MQ was mixed with 2 ml of 10 mM linoleic acid emulsion in 0.2 M sodium phosphate buffer. Then 6.5 mM Tween 20 was added to provide a stable emulsion and the mixture was incubated for 15 h in darkness, at 37°C while shaken to accelerate oxidation. Then 0.2 ml of the antioxidant mixture was added to 6 ml absolute methanol. The absorbance of the supernatant mixture was measured at 234 nm against a blank. Ascorbic acid and α-tocopherol were used as the positive control.

DPPH free radical scavenging activity assay

The assay was done according to the modified method of Bilos [7]. In the first series each polysaccharide powder (0.1–10 mg/ml, 2 ml) in MQ was mixed with 1 ml freshly prepared DMSO solution of 0.2 mM DPPH. In the second series each sample was mixed with 1 ml DMSO solution. The reaction mixture was vortexed vigorously for 1 min and kept in dark at 20°C for 40 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm against the blank. Ascorbic acid, BHT and α-tocopherol dissolved in DMSO were used as the positive control [8].

Reducing power

The reducing power was determined according to the method of Oyaizu [9]. Each polysaccharide powder (0.1 to 20 mg/ml, 2.5 ml) in MQ was mixed with 2.5 ml 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was vortexed and incubated at 50°C for 20 min. Then 2.5 ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 2000 g for 10 min. The upper layer (5 ml) was mixed with 5 ml of MQ and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against a blank. Ascorbic acid was used as the positive control.

Chelating ability on ferrous ions

Chelating ability was determined according to Dinis et al. [10]. Each polysaccharide powder (0.1 to 20 mg/ml, 1 ml) in MQ was mixed with 3.7 ml of MQ and 0.1 ml of 2 mM ferrous chloride. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. Citric acid and EDTA were used for comparison.

EC_{50} values in antioxidant properties

The results of antioxidant activity, DPPH free radical scavenging activity, reducing power and chelating effect on ferrous ion were normalized and expressed as EC_{50} (mg/ml) values which are the effective concentration of mushroom extract that are required to show 50% antioxidant properties. A lower EC_{50} value corresponds to higher antioxidant activity of the mushroom's extract.

Statistical analysis

All measurements were done in triplicate and data were expressed as mean ± standard deviation.

RESULTS & DISCUSSION

GL-I showed high antioxidant activity of 85.7 ± 0.7%, at 10 mg/ml and alredy 62.6 ± 0.7% at 1 mg/ml (***Figure 1***). Antioxidant activities of ascorbic acid and α-tocopherol were 64.7 ± 0.1 and 65.0 ± 0.5% at 10 mg/ml. GL-I was more effective than ascorbic acid and α-tocopherol at the highest concentration tested in inhibiting the peroxidation of linoleic acid. EC_{50} value of the antioxidant activity for GL-I was low, 1.2 mg/ml. However, α-tocopherol showed excellent antioxidant activity (EC_{50} < 0.1 mg/ml), while ascorbic acid was also very active as shown by its low EC_{50} value (1.6 ± 0.0 mg/ml).

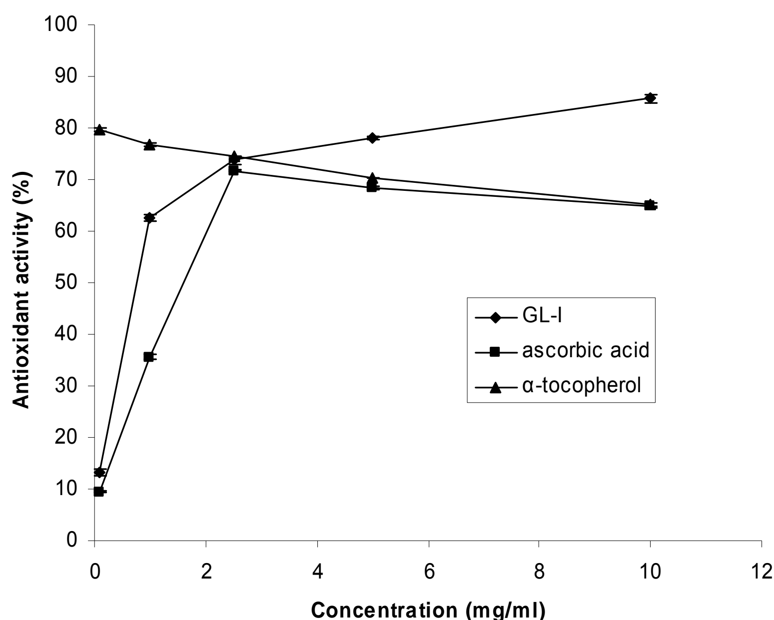

Figure 1. Antioxidant activity of polysaccharide extract (GL-I) from *Ganoderma lucidum*. Each value is expressed as mean ± standard deviation (n = 3).

GL-I scavenged DPPH radicals by 96.8 ± 2.5%, at 10 mg/ml (***Figure 2***). However, ascorbic acid and BHT, scavenged DPPH radicals by 87.6 ± 0.3 and 55.2 ± 0.2% at 10 mg/ml, whereas α-tocopherol scavenged DPPH radicals by 96.1 ± 0.1% at 5 mg/ml. With regard to scavenging ability on DPPH radicals, EC_{50} valueof GL-I was very low, 0.15 mg/ml. Ascorbic acid and α-tocopherol were both excellently scavenging DPPH radicals (EC_{50}<0.1 mg/ml), while BHT was a good DPPH radicals scavenger (EC_{50} = 8.5 ± 0.0 mg/ml).

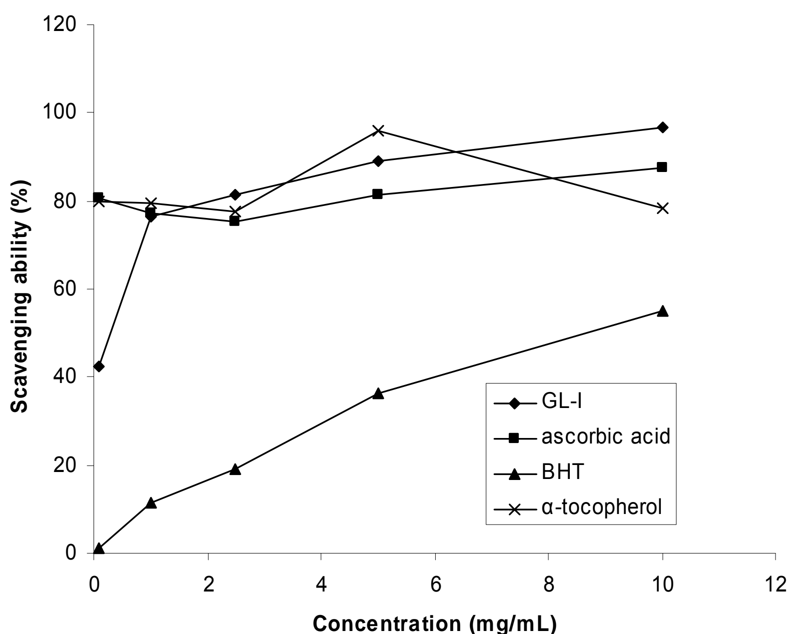

Figure 2. Scavenging ability of polysaccharide extract (GL-I) from *Ganoderma lucidum*. Each value is expressed as mean ± standard deviation (n = 3).

The reducing power of GL-I reached a plateau of 3.4 ± 0.1 at 20 mg/ml (***Figure 3***), same like a positive control, ascorbic acid. EC_{50} value of the reducing power for GL-I was 0.5 mg/ml, whereas ascorbic acid showed excellent reducing activity (EC_{50}<0.1 mg/ml).

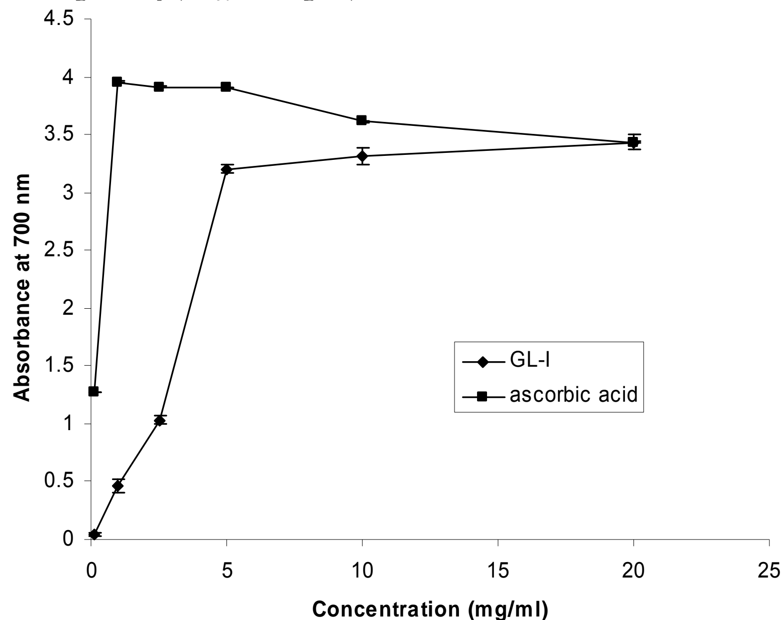

Figure 3. Reducing power of polysaccharide extract (GL-I) from *Ganoderma lucidum*. Each value is expressed as mean ± standard deviation (n = 3).

GL-I chelated 81.6 ± 3.6% of ferrous ions at 20 mg/ml (***Figure 4***). However, the chelating effect of the synthetic metal chelator, EDTA was 100% at 0.1-20 mg/ml, while citric acid was not a strong chelator in this assay, i.e. only 10.3 ± 0.1% at 20 mg/ml. EC_{50} values of the chelating abilities on ferrous ions for GL-I was 3.8 mg/ml and < 0.1 mg/ml for EDTA.

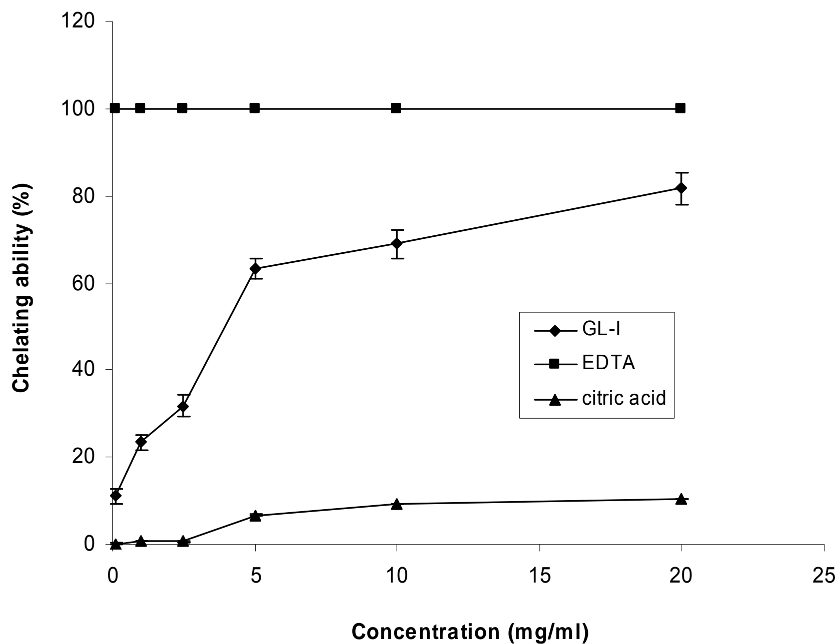

Figure 4. Chelating ability of polysaccharide extract (GL-I) from *Ganoderma lucidum*. Each value is expressed as mean ± standard deviation (n = 3).

CONCLUSION

The antioxidative activities of hot water extracted polysaccharides from *Ganoderma lucidum* in all assays were concentration dependent and increased with increasing concentration. Although EDTA is an excellent metal chelator, whereas ascorbic acid, α-tocopherol and BHT appear good in antioxidant activity, reducing power and scavenging ability on DPPH radicals, these compounds are synthetic antioxidants, respectively additives and may be replaced with natural antioxidants in food and pharmaceuticals. Regarding the fact that some of them, like BHT may possess mutagenic activity [11], using mushrooms in the daily diet as sources of naturally-derived antioxidants might be beneficial for human health in preventing or reducing oxidative demage. To deliver food products with attractive flavors, odors and textures, or a longer shelf life, food processors must apply methods for controlling oxidation, the process by which fats and oils turn rancid. Once this has initiated, the rate of deterioration progresses rapidly. To prevent this process food grade antioxidants could be applied [12]. Mushrooms and their extracts could play a role in that.

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