



Antioxidant properties and antioxidant components of extracts from mushroom *Ganoderma sinensis*

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Abstract

Ganoderma sinensis (Zi Ling zhi) is an endemic mushroom in China, which has been used as medicine or food for centuries, particularly in Asian countries. *G. sinensis* is available in form of log-cultivated and sawdust-cultivated fruit bodies, solid-fermented products (SFP) and liquid-fermentation mycelia. Based on these four forms derived by *Ganoderma sinensis*, methanolic and hot water extracts were prepared and then their antioxidant properties were investigated. Moreover, antioxidant components in both two extracts, including ascorbic acid, tocopherols, β -carotene and total phenols, were analyzed. Both two extracts from the four forms of *G. sinensis* showed high antioxidant activities of 69.69-99% at 20 mg/ml (mg extract/ml) and low EC₅₀ values of 0.95-10.00 mg/ml (mg extract/ml). EC₅₀ values in reducing power of both two extracts from the four forms of *G. sinensis* were in the range of 0.16-9.23 mg/ml. At 20 mg/ml, both two extracts from the four forms scavenged 2,2-diphenyl-1-picrylhydrazyl (DPPH) by 93.83%, except for hot water extracts from SFP being scavenging ability of 71.98%. Both two extracts could scavenge hydroxyl radicals, while the scavenging activity of hot water extracts from mycelia increased to 97.94% at 20 mg/ml. Total phenols in both two extracts from four forms were the major naturally occurring antioxidant components at the range of 19.76-60.36 mg/g. Due to low EC₅₀ values of the *G. sinensis*, it could be developed as a new dietary supplement and functional food.

Key words: *Ganoderma sinensis*, antioxidant activity, reducing power, scavenging ability, antioxidant components.

Introduction

Reactive oxygen/nitrogen species generated in the human body can cause oxidative damages associated with many degenerative diseases such as atherosclerosis, coronary heart diseases, aging and cancer¹. It has been recognized that there is an inverse association between consumption of some mushroom and mortality from degenerative diseases, which could be partly attributed to their antioxidants². The health promoting effect of antioxidants from mushrooms is thought to arise from their potential effects on the reactive oxygen/nitrogen species³. In addition, antioxidants have been widely used in food industry to prolong the shelf life. However, there is widespread agreement that some synthetic antioxidants such as butylhydroxyanisole and butylhydroxytoluene need to be replaced with natural antioxidants due to their potential health risks and toxicity⁴. Therefore, it is very important to find out new sources of safe and inexpensive antioxidants of natural origin.

Medicinal mushroom such as *G. sinensis* have been used to treat human diseases in the East for centuries. People are increasingly interested in medicinal mushrooms because of their good therapeutic performance and low toxicity. Since traditional Chinese medicines and food are believed to share a common origin in Chinese tradition, it is not easy to distinguish traditional Chinese medicines from food. In fact, many traditional Chinese medicines

have been used as flavors, pigments and foods. In recent years, studies on antioxidant properties of Chinese medicinal mushrooms have increased remarkably due to more interest in their potential of being used as a rich and natural source of antioxidant compounds⁵⁻¹¹.

Ganoderma sinensis (Zi Ling zhi) is a medicinal mushroom in China, which has been used as medicine and food for centuries, particularly in Asian countries. *G. sinensis* is available in form of log-cultivated and sawdust-cultivated fruit bodies, solid-fermented products (SFP) and liquid-fermentation mycelia. Normally, log-cultivated *G. sinensis* is harvested from plastic bags filled with log of sawtooth oak in 4-6 weeks later after fruiting, while sawdust-cultivated *G. sinensis* is harvested from plastic bags filled with sawdust in 2-4 weeks later after fruiting. In order to shorten cultivation period, two different fermentation methods were also used for *G. sinensis* production, including the liquid and solid fermentation. Liquid-fermentation mycelia and solid-fermented products (SFP) are alternative or substitute products of log-cultivated and sawdust-cultivated fruit bodies. The nutritional values of the four forms of *G. sinensis* have been studied. However, the antioxidant properties of the four forms of *G. sinensis* are not available in the previous studies.

Accordingly, our objective was to evaluate and compare the

antioxidant properties of methanolic and hot water extracts from the log- and sawdust-cultivated fruit bodies, solid-fermented products and liquid-fermentation mycelia derived from *G. sinensis*. Antioxidant activity, reducing power and scavenging abilities on 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydroxyl radicals were investigated. The contents of antioxidant components of both two extracts were also discussed.

Materials and Methods

Mushroom fruit bodies, SFP and mycelia: The pure culture of *Ganoderma sinensis* was originally obtained from the Department of the Strain, Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. Log- and sawdust-cultivated fruit bodies (4 weeks old) were harvested from the mushroom room of Sichuan Academy of Agricultural Sciences and air-dried in an oven at 40°C for 2-3 days before sample preparation. SFP and mycelia were obtained from the Cellular and Molecular Lab of Chinese Materia and Medica, Sichuan.

Solid-fermented products were solid-state fermented in polypropylene bag filled with culture medium. The medium consisted of 76% corncobs, 24% sawdust and pH 6.5. The culture condition was 27°C in growth chamber. After 40 days of incubation, SFP were harvested.

Mycelia were grown in a 50-litre fermentor with a 30-litre working volume. The medium consisted of 2% cane-sugar, 0.5% yeast extract, 0.5% peptone, 0.3% ammonium sulfate, 0.3% magnesium sulfate heptahydrate, 0.3% potassium dihydrogen phosphate, pH 6.5. The working conditions were established at 27°C; aeration rate at 0.5 vvm; agitation speed at 60 rpm and the inoculum rate at 10 ml/l. After 7 days of incubation, the mycelia were harvested at the sugar concentration decreased to 0.1 g/l. The mycelia were obtained by centrifugation at 4°C, 8000g for 15 min and the precipitates were washed with deionised water. Finally, the mycelia were freeze-dried into powders. For each of log- and sawdust-cultivated fruit bodies, SFP and mycelia, three dried samples were randomly selected for analysis.

Preparation of methanolic extracts and water extracts: After fine powders (20 meshes) were obtained using a mill, a subsample (10 g) was extracted by stirring with 100 ml of methanol at 25°C at 20 g for 24 h and filtered with Whatman No.4 filter paper. The residues were then extracted again as the previous description with two additional 100 ml. The combined methanolic extracts were then rotary-evaporated at 40°C to dryness. In addition, another subsample (10 g) was heated with 200 ml deionized water at reflux for 3 h. The mixture was cooled to room temperature and filtered with Whatman No. 4 filter paper. The residues were then refluxed with two additional 100 ml of deionized water as described above. The combined hot water extracts were freeze-dried with Modulyod freeze-dryer (Thermo). Both the dried extracts obtained by two methods were redissolved in corresponding extraction solvent to a concentration of 20 mg/ml and stored at 4°C for future uses.

Determination of antioxidant activity: The antioxidant activity was determined by the conjugated diene method¹². Each extract (0.1-20 mg/ml, 100 µl), was mixed with 2 ml of 10 mM linoleic acid emulsion (Sigma Chemical Co., St. Louis, MO) in 0.2 M sodium

phosphate buffer (pH 6.5) in test tubes and placed in darkness at 37°C to accelerate oxidation. After incubation for 15 h, 6 ml of 60% methanol in deionised water was added into each tube, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer. The antioxidant activity (AOA) was calculated based on the formulas: $AOA (\%) = [(\Delta A_{234} \text{ of control} - \Delta A_{234} \text{ of sample}) / \Delta A_{234} \text{ of control}] \times 100$. The blank is the deionised water only and the control consisted of water and the reagent solution without the extract. The AOA value of 100% indicates the strongest antioxidant activity. The EC₅₀ value (mg extract/ml), the effective concentration at which the antioxidant activity is 50%, was determined by interpolation from linear regression analysis. Ascorbic acid and α -tocopherol (all from Sigma) were used for comparison.

Determination of reducing power: The reducing power was determined by the method of Oyaizu¹³. Each extract (0.1-20 mg/ml, 2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide (Sigma), and the mixtures were incubated at 50°C for 20 min. The 2.5 ml of 10% trichloroacetic acid (w/v, Wako) were added and the mixtures were centrifuged at 200 g for 10 min. The upper layers (5 ml) were mixed with 5 ml of deionised water and 1 ml of 0.1% ferric chloride (Wako). The absorbance was measured at 700 nm against the blank. The higher absorbance indicates a higher reducing power. The EC₅₀ value (mg extract/ml) is the effective concentration at which the absorbance was 0.5 for the reducing power. Ascorbic acid and α -tocopherol were used as reference.

Determination of 1,1-diphenyl-2-picrylhydrazyl radical scavenging ability: Each extract (0.1-20 mg/ml, 4 ml) in corresponding extraction solvent was mixed with 1 ml of methanolic solution containing DPPH (Sigma) radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously and still stood for 30 min in the dark, and the absorbance was then measured at 517 nm against with that of a blank¹⁴. The scavenging ability was calculated with the formula: $\text{scavenging ability} (\%) = [(\Delta A_{517} \text{ of control} - \Delta A_{517} \text{ of sample}) / \Delta A_{517} \text{ of control}] \times 100$. The EC₅₀ value (mg extract/ml) is the effective concentration at which the DPPH radicals were scavenged by 50%. Ascorbic acid and α -tocopherol were used for comparison.

Determination of hydroxyl radical scavenging ability: The hydroxyl radical reacted with the nitron spin trap 5,5-dimethyl pyrroline-N-oxide (DMPO, Sigma) and the resultant DMPO-OH adducts were detected with an electron paramagnetic resonance (EPR) spectrometer. The EPR spectrum was recorded 2.5 min after mixing 200 µl of each extract (0.1-20 mg/ml, 200 µl), with corresponding extraction solvent, with 10 mM hydrogen peroxide (Sigma), 200 µl of 10 mM ferrous sulfate (Sigma) and 200 µl of 10 mM DMPO using a BrukerEMX-10 EPR spectrometer by the following settings: 3480-G magnetic field, 1.0 G modulation amplitude, 0.5 s time constant and 200 s scan period¹⁵. The scavenging ability was calculated by subtracting the relative EPR signal intensity from 100. The relative EPR signal intensity was calculated as follows: $\text{The relative EPR signal intensity} (\%) = [h\Delta H^2 (\text{sample}) / h\Delta H^2 (\text{control})] \times 100$; wherein h is the width of the peak and ΔH is the length of the peak. The EC₅₀ value (mg

extract/ml) is the effective concentration at which the hydroxyl radicals are scavenged by 50%. Ascorbic acid and α -tocopherol were used for comparison.

Determination of antioxidant components: Ascorbic acid was determined according to the method of Klein and Perry¹⁶. Each methanolic extract (20 mg) was extracted with 10 ml of 1% metaphosphoric acid (Union) for 45 min at room temperature and filtered through Whatman No. 4 filter paper. The filtrate (1 ml) was mixed with 9 ml of 2,6-dichloroindophenol (Sigma) and the absorbance was measured within 15 s at 515 nm against that of a blank. Content of ascorbic acid was calculated based on the calibration curve of authentic L-ascorbic acid.

Tocopherols were extracted and analyzed according to the method of Carpenter¹⁷. Each extract (50 mg) was suspended in 6 ml of pyrogallol (6% in 95% ethanol) and 4 ml of 60% aqueous potassium hydroxide solution, and the resulting mixture was saponified at 70°C for 20 min. Deionised water (15 ml) was added and the mixture was extracted with 15 ml of *n*-hexane. The organic layer was washed with deionised water to neutral, dried over anhydrous sodium sulphate and rotary-evaporated to dryness. The residue was redissolved in 5 ml of *n*-hexane and filtered prior to HPLC injection onto a high-performance liquid chromatograph (HPLC).

The HPLC system consisted of a Waters 1525 Binary HPLC pump, a 20 μ l sample loop, and a 2487 Dual λ Absorbance detector. The mobile phase was acetonitrile/methanol, 85:15 (v/v), at a flow rate of 1.0 ml min⁻¹ and UV detection was at 295 nm. Content of each tocopherol was calculated on the basis of the calibration curve of each authentic tocopherol (Sigma).

β -Carotene was extracted and analysed as described by Rundhaug *et al.*¹⁸. The mobile phase was acetone/methanol/acetonitrile (1:2:2) at a flow rate of 0.7 ml/min and UV detection was at 470 nm. Content of β -carotene was calculated on the basis of the calibration curve of authentic β -carotene (Sigma). The HPLC system was the same as for the tocopherols assay.

Total phenols were determined according to the method of Taga *et al.*¹⁹. Each methanolic extract (20 mg) was dissolved in a solution of 5 ml of 1.3% HCl in methanol/deionised water (60:40, v/v) and the resulting mixture (100 μ l) was added to 2 ml of 2% aqueous sodium carbonate solution. After 3 min, 100 μ l of 50% Folin-Ciocalteu's phenol reagent (Sigma) were added to the mixture. After 30 min standing, the absorbance was measured at 750 nm against that of a blank. The content of total phenols was calculated on the basis of the calibration curve of gallic acid (Sigma).

Statistical analysis: For each extract from log-cultivated and sawdust-cultivated fruit bodies, SFP and mycelia, three samples were prepared for assays of every antioxidant attribute and component. The experimental data were subjected to an analysis of variance for a completely random design as described by Steel *et al.*²⁰ to determine the least significant difference at the level of 0.05.

Results and Discussion

Extraction yield: The yield ratios of both two extracts in the four forms of *G. sinensis* were obtained: mycelia > sawdust-cultivated fruit bodies ~ log-cultivated fruit bodies ~ SFP (Table 1). The yield of mycelia was highest mainly due to the most components

Table 1. Yields of methanolic and hot water extracts from *Ganoderma sinensis* (log- and sawdust-cultivated fruit bodies (LCFB and SCFB), solid-fermented products (SFP) and mycelia).

	Methanolic extracts		Hot water extracts	
	Yield ^a (g)	Extraction %	Yield (g)	Extraction %
LCFB	0.99±0.04	9.95B ^b	0.82±0.03	8.17B
SCFB	0.77±0.06	7.72B	0.74±0.04	7.36B
SFP	0.79±0.01	7.88B	0.73±0.01	7.27B
Mycelia	4.29±0.45	42.91A	3.57±0.23	35.71A

^a Extracted from dried materials (10.00 g). Each value expressed as mean \pm standard deviation (n = 3).

^b Means with different letters within a column are significantly different (P<0.05).

contained in the mycelia being small and water-soluble^{21, 22}. However, there was not significant difference between the yields of the hot water and methanolic extracts in the four forms. The use of hot water to extract soluble components in the four forms seemed to be the procedure of Chinese medicine preparation or the herbal tea cooking. Therefore, the study of two distinct extraction methods is better to understand that hot water extracts would be more valuable for these products used in human diets.

Antioxidant activity: By the conjugated diene method, the methanolic extracts from log- and sawdust-cultivated fruit bodies and SFP showed high antioxidant activities of 80.2, 61.96 and 60.03%, respectively, at 2.5 mg/ml. At the 20 mg/ml, the antioxidant activities of the methanolic extracts from log- and sawdust-cultivated fruit bodies and SFP were 89.04-98.05%, which was similar to that of α -tocopherol. On the other hand, the hot water extracts showed moderate antioxidant activities of 48.82-50.52% at 2.5 mg/ml, except for that of hot water extract from mycelia being 31.27% (Fig. 1). The antioxidant activities of ascorbic acid were 45.11% at 2.5 mg/ml (Fig. 1). It was obvious that the hot water extracts from log- and sawdust-cultivated fruit bodies and SFP were as effective as ascorbic acid in inhibiting the peroxidation of linoleic acid.

Similarly, the methanolic extract from fruit bodies of *G. tsugae* showed high antioxidant activities of 96.8% at 20 mg/ml. On the contrary, the hot water extracts showed low antioxidant activities of 58.5%^{7, 8}. With regard to antioxidant activity, the hot water extracts from *G. sinensis* as well as *G. tsugae* were less effective than the methanolic extracts. The difference of antioxidant activities between hot water and methanolic extracts might be attributed to the contents of antioxidant components contained in the extracts.

Mau *et al.*⁶ found that the methanolic extracts from fruit bodies of *G. lucidum* showed moderate antioxidant activity of 46.4% at 0.5 mg/ml and high antioxidant activity of 93.6% at 20 mg/ml. The methanolic extract from mycelia of *G. lucidum* showed a low antioxidant activity of 10.4-19.3% at 0.5-20 mg/ml. In addition, methanolic extracts from fruit bodies of *G. tsugae* showed moderate antioxidant activities of 46.4-49.3% at 2.5 mg/ml and high antioxidant activities of 93.6-96.8% at 20 mg/ml, whereas the methanolic extract from mycelia showed a low antioxidant activity of 10.4-19.3% at 0.5-20 mg/ml⁸. Compared with *G. lucidum* and *G. tsugae*, the log-cultivated fruit bodies and mycelia of *G. sinensis* contained more components effective in inhibiting the oxidation of linoleic acid. Besides, the methanolic extracts from fruit bodies were more effective in antioxidant activities than the methanolic extracts of mycelia from *Ganoderma* species mentioned above.

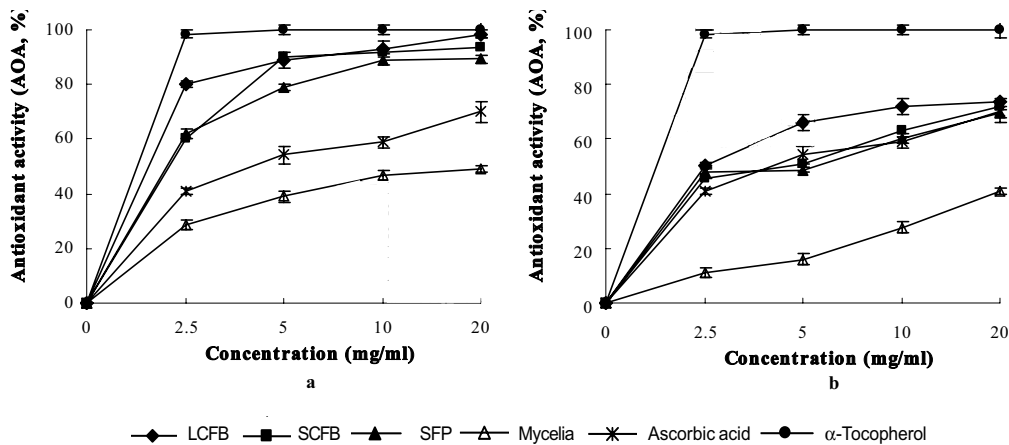


Figure 1. Antioxidant activity of methanolic extracts (a) and hot water extracts (b) from *Ganoderma sinensis*. Each value expressed as mean \pm standard deviation (n = 3).

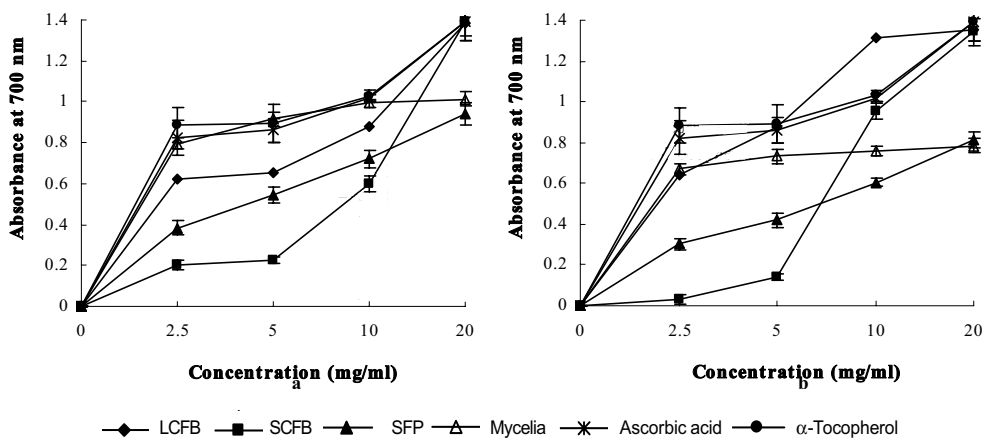


Figure 2. Reducing power of methanolic extracts (a) and hot water extracts (b) from *Ganoderma sinensis*. Each value expressed as mean \pm standard deviation (n = 3).

Reducing power: At 20 mg/ml, the methanolic extracts from log- and sawdust-cultivated fruit bodies, SFP and mycelia were capable of reducing powers at 1.38, 1.39, 0.94 and 1.01, respectively while those of the hot water extracts from corresponding forms were 1.35, 1.34, 0.81 and 0.79, respectively (Fig. 2). However, reducing powers of ascorbic acid and α -tocopherol were 1.39 and 1.38 at 20 mg/ml, respectively.

Similarly, at 20 mg/ml, reducing powers of the methanolic extracts from fruit bodies and mycelia of *G. tsugae* were 1.05 and 1.00, respectively, while those of the hot water extracts were 1.08 and 0.95, respectively⁷. Similar results were also found in fruit bodies and mycelia of *G. lucidum*, *A. cylindracea* and *A. camphorate*^{6, 23}. It revealed that the reducing power of the hot water and methanolic extracts were almost same, for medicinal mushrooms mentioned above. However, the methanolic extracts from fruit bodies of other *Ganoderma* species, including *G. tsugae*, *G. lucidum* and *G. lucidum antler*, showed strong reducing powers of 1.05, 1.03 and 0.81 at 1.5 mg/ml, respectively⁶. The difference in reducing power among the methanolic extracts from the same species of *Ganoderma* might be due to the difference in the strains studied.

1,1-Diphenyl-2-picrylhydrazyl radical scavenging ability: The methanolic extracts from log- and sawdust-cultivated fruit bodies,

SFP and mycelia possessed high DPPH• radical scavenging abilities of 60.04-91.96% at 2.5 mg/ml. The hot water extracts from the four forms of *G. sinensis* showed moderate DPPH• radical scavenging abilities of 49.39-66.37% at 2.5 mg/ml (Fig. 3). The methanolic extracts were more effective in scavenging DPPH• than the hot water extracts from the four forms. At 2.5 mg/ml, α -tocopherol revealed excellent DPPH• scavenging abilities of 93.38%. However, at 2.5-20 mg/ml, ascorbic acid showed considerable low scavenging ability of 37.12-47.78%.

Similarly, DPPH radical scavenging abilities of the methanolic extracts from fruit bodies of *G. tsugae* was 88.4% at 5 mg/ml but that of the hot water extracts was only 79.3% at 20 mg/ml⁷. DPPH radical scavenging abilities (89.92% at 5 mg/ml) of the methanolic extracts from fruit bodies of *G. sinensis* were similar to that of fruit bodies from *G. tsugae*. However, the hot water extracts from fruit bodies of *G. sinensis* were higher in DPPH radical scavenging abilities (96% at 20 mg/ml) than that of *G. tsugae*. In addition, scavenging abilities of methanolic extracts from other commercial mushrooms (silver ears, jin ears and tree oyster mushrooms) were 42.9-69.9% at 10 mg/ml²⁴. At 1-20 mg/ml, the scavenging abilities of hot water extracts from fruit bodies and mycelia from *Agrocybe cylindracea* were in the range of 58.3-66.2% and 47.7-76.1%, respectively, and hot water extracts from fruit bodies and mycelia

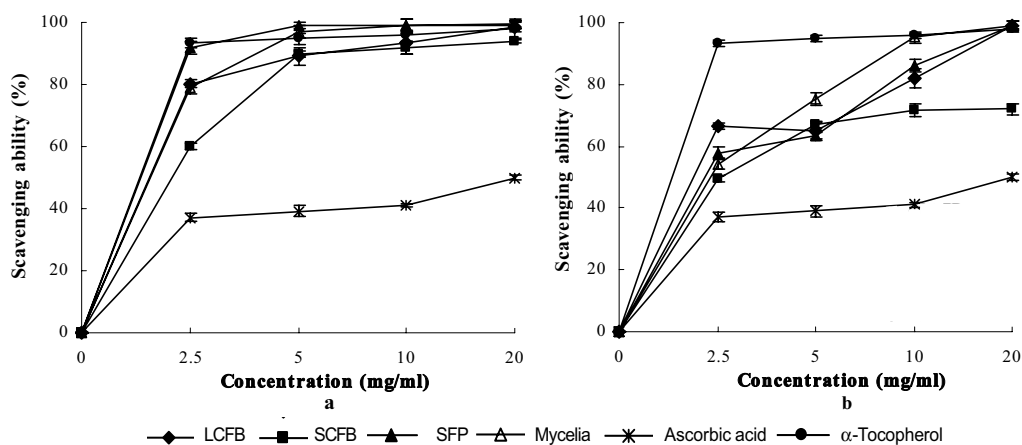


Figure 3. Scavenging ability of methanolic extracts (a) and hot water extracts (b) from *Ganoderma sinensis* on 1,1-diphenyl-2-picrylhydrazyl radicals. Each value expressed as mean \pm standard deviation (n = 3).

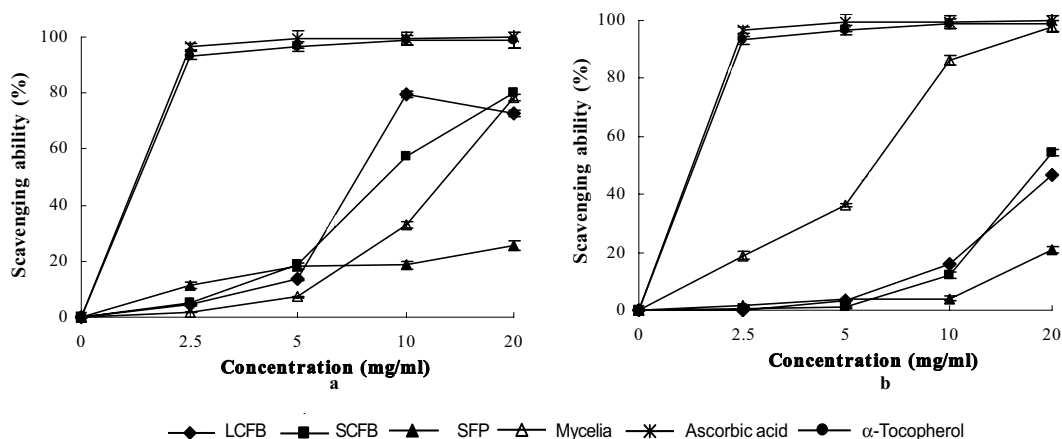


Figure 4. Scavenging ability of methanolic extracts (a) and hot water extracts (b) from *Ganoderma sinensis* on hydroxyl radicals. Each value expressed as mean \pm standard deviation (n = 3).

of *Pleurotus citrinopileatus* were 52.3% and 48.3%, respectively^{23, 25}. It seemed that the methanolic extracts from fruit bodies of *G. sinensis* as well as *G. tsugae*, were more effective in DPPH radical scavenging than those from the fruit bodies of silver ears, jin ears and tree oyster. On the other hand, hot water extracts from fruit bodies and mycelia of *G. sinensis* were more effective in DPPH• scavenging than those of the corresponding forms from *A. cylindracea* and *P. citrinopileatus*.

Hydroxyl radical scavenging ability: At 20 mg/ml, the hydroxyl scavenging abilities of the methanolic extracts from log- and sawdust-cultivated fruit bodies and SFP were 72.55, 79.9 and 25.51%, respectively, whereas the scavenging ability of the hot water extracts from corresponding forms were 46.86, 54.28 and 21.09%, respectively (Fig.4). Obviously, the OH radical scavenging abilities of the methanolic extracts from fruit bodies and SFP were higher than those of the hot water extracts. At 2.5 mg/ml, the OH radical scavenging ability of ascorbic acid was 93.28%.

Mau *et al.*⁸ found that at 5-20 mg/ml, the OH radical scavenging abilities of the methanolic extracts from mature and baby fruit bodies of *G. tsugae* were in the range of 1.49-9.66% and

2.69-6.9%, respectively, and less effective than those from the hot water extracts (in the range of 23.23-72.37% and 19.55-73.69%, respectively). In addition, at 40 mg/ml, the methanolic extracts from speciality mushrooms scavenged OH radicals by 39.6-75.0%, whereas those from commercial mushrooms showed scavenging abilities of 29.2-36.6%^{6, 24}. Compared with the different mushroom species mentioned above, both two extracts from fruit bodies and mycelia of *G. sinensis* showed high effect on scavenging OH radicals. It indicated that the fruit bodies and mycelia from *G. sinensis* were good scavengers to hydroxyl radicals.

EC₅₀ values in antioxidant properties: The antioxidant properties of both two extracts from log- and sawdust-cultivated fruit bodies, SFP and mycelia were summarized in Table 2 and the results are presented as EC₅₀ values. Effect on antioxidant properties was negatively correlated with EC₅₀ value. Both two extracts from four forms of *G. sinensis* showed the EC₅₀ values in antioxidant activities and reducing powers were in the range of 0.95-10.00 mg/ml and 0.16-9.23 mg/ml, respectively. EC₅₀ values in DPPH scavenging ability of the methanolic extracts from the four forms of *G. sinensis* were less than 1 mg/ml, while those of the only hot water extracts

Table 2. EC₅₀ values of methanolic and hot water extracts from *Ganoderma sinensis* in antioxidant properties.

	EC ₅₀ value ^a (mg extract/ml)			
	LCFB	SCFB	SFP	Mycelia
Methanolic extracts				
Antioxidant activity	0.95±0.15C ^b	1.09±0.43B	1.12±0.28B	5.33±1.01A ^c
Reducing power	1.21±0.20C	9.23±0.10A	4.05±0.04B	1.63±0.30C
Scavenging ability on DPPH radicals	0.95±0.14A	0.93±0.35A	0.19±0.04C	0.46±0.03B
Scavenging ability on OH radicals	9.28±0.19A	6.04±0.06B	>20	8.44±0.19AB
Water extracts				
Antioxidant activity	1.73±0.31C	3.88±0.55B	3.88±0.89B	10.00±0.02A
Reducing power	2.21±0.11B	5.78±0.15A	5.95±0.07A	0.16±0.01C
Scavenging ability on DPPH radicals	>20	>20	2.23±0.13A	0.24±0.06B
Scavenging ability on OH radicals	18.95±0.19A	>20	>20	5.33±0.78B

^aEC₅₀ value: the effective concentration at which the antioxidant activity was 50%; the absorbance was 0.5 for reducing power; and 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]) or hydroxyl (•OH) radicals were scavenged by 50%, respectively. EC₅₀ value was obtained by interpolation from linear regression analysis. ^bEach value is expressed as mean ± standard deviation (n = 3). Means with different letters within a row at a specific EC₅₀ are significantly different (P<0.05). ^cObtained by extrapolation from linear regression analysis.

from fruit bodies were higher than 20 mg/ml. The methanolic extracts showed low EC₅₀ values (<10 mg/ml) in scavenging OH radicals, except for that of the methanolic extracts from SFP increasing to 20 mg/ml. EC₅₀ values in OH[•] scavenging abilities of the hot water extracts from log-cultivated fruit bodies and mycelia were 18.95 and 5.33, respectively.

The potent antioxidant activity, reducing power and free radical scavenging ability of both two extracts in the four forms of *G. sinensis* were well shown by those low EC₅₀ values, which were similar to the previous paper²⁵. Several diseases were associated with oxidative damage, including cardiovascular disorders, neurasthenia and cancer¹. One possible way to help fight against these diseases is to increase antioxidant defences by intaking of external antioxidant^{26,27}. Mushrooms such as *G. sinensis*, which is a natural source rich in antioxidant, not only demonstrated good antioxidant properties *in vitro* but also are able to increase antioxidant defences *in vivo*²⁸. In addition, antioxidant properties of nature antioxidant *in vitro* could be correlated with those *in vivo*²⁶. Therefore, it is not surprising that intrinsic antioxidant properties of *G. sinensis in vitro* were demonstrated and then it could function *in vivo*.

Although α-tocopherol and BHT have good antioxidant properties and thus are extensively applied in food industry, it has been criticized for a long time mainly due to possible toxic side effect of these antioxidants²⁹. *Ganoderma* were deeply

demonstrated that they possessed significant antimutagenic and antigenotoxic activities without toxic effect^{30,31}. *G. sinensis*, which belongs to the *Ganoderma* family, might be non-toxic additive and functional food. Furthermore, in this investigation, it was found that both two extracts from the four forms of *G. sinensis* possessed good antioxidant properties. Therefore, in addition to their therapeutic effects, *G. sinensis* in human diets might serve as possible protective agent to help humans reduce oxidative damage. The extracts could be added in emulsion with antioxidation prevention or be developed for the sucrose of nature antioxidant³². The fruit bodies could be formulated into bread as a health-promoting functional food.

Antioxidant components: Total antioxidant components varied among the extracts by hot water and methanol: log-cultivated fruit bodies > sawdust-cultivated fruit bodies > SFP > mycelia. The antioxidant components, including ascorbic acid, tocopherols and total phenols, were included in the extracts from log- and sawdust-cultivated fruit bodies, SFP and mycelia (Table 3). Total phenols were one of the major naturally occurring antioxidant components found in both extracts at the range of 19.76-60.36 mg /g (Table 3). However, the contents of ascorbic acid were relatively less in the hot water extracts from the four forms of *G. sinensis* and were not detected in the methanolic extracts. The tocopherols were not found in the hot water extracts from sawdust-cultivated fruit bodies, SFP and mycelia. β-Carotene was not detected in both two extracts in this study.

Comparing with antioxidant components from the extracts of *G. sinensis*, total phenols were one of the major naturally occurring antioxidant components. Indeed, phenolic compounds are heavily investigated for their potential good effect on human health. Components such as BHT or gallate are known as effective antioxidants and the main antioxidative compounds rich in the fruits and vegetables^{27,33}. Moreover, the antioxidant capabilities of the extracts from grape fruits and *Chamaecyparis obtusa var. formosana* correlated with their content of total phenols^{34,35}. Therefore, total phenols might be responsible for the antioxidant properties. Besides, antioxidant properties of phenolic compounds are correlated with pharmacological

Table 3. Contents of ascorbic acid, tocopherol and total phenols of methanolic and hot water extracts from *Ganoderma sinensis*.

Compound	Content (mg/g)			
	LCFB	SCFB	SFP	Mycelia
Methanolic extracts				
Ascorbic acid	n.d ^b	n.d	n.d	n.d
Tocopherol	0.78±0.11A ^a	0.56±0.20B	n.d	0.09±0.01C
Total phenols	52.96±0.58A	39.86±0.04B	24.0±0.15C	19.76±0.23D
Water extracts				
Ascorbic acid	3.02±0.05A	1.47±0.09B	0.11±0.03C	0.13±0.05C
Tocopherol	0.28±0.11	n.d	n.d	n.d
Total phenols	60.36±0.56A	47.25±0.20B	35.3±0.15C	25.6±0.34D

^aEach value is expressed as mean ± standard deviation (n = 3). Means with different letters within a row are significantly different (P<0.05). ^bNot detected.

effect³¹. It indicated that these antioxidant properties could contribute partly to the therapeutic benefits of the certain claims of *G. sinensis*.

Conclusions

The present study demonstrated that *G. sinensis* in four forms possessed potent antioxidant and free radical scavenging abilities and reducing powers, of which could be mainly derived from antioxidative compounds such as phenols. These antioxidant properties could contribute partly to the therapeutic benefits of certain traditional claims of *G. sinensis*. In addition, on the basis of the results obtained, the antioxidant properties of *G. sinensis* might be beneficial to the antioxidant protection system of the human body.

G. sinensis is available in the form of log- or sawdust-cultivated fruit bodies, SFP and liquid-fermentation mycelia. Traditionally, it was believed that the biological and pharmacological activity of the log-cultivated fruit bodies was higher than that of sawdust-cultivated. However, in our recent investigation, the antioxidant properties of sawdust-cultivated fruit bodies were similar to those from the log-cultivated one. The log cultivation might cause ecological risks due to sawtooth oak woods utilized excessively. The sawdust cultivation is friendly to environment and efficient to the agro-industrial residues utilization based on sawdust and corncob being mainly utilized as cultivation substrates. In light of environment protection and agro-industrial residues efficient utilization, sawdust-cultivated fruit bodies are more beneficial to antioxidant and functional food. Among the four forms of *G. sinensis*, the mycelia showed the largest extraction ratio. However, it costs too much compared with SFP by solid fermentation methods. The solid fermentation to produce natural antioxidants, therefore, is one of appropriate methods in the additives-industry production. In light of the economic and environmental issues, sawdust-cultivated fruit bodies could be used as antioxidant and functional food, while SFP could be developed into antioxidant additives.

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