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Quantification of total beta-glucan, ganoderic acid A and antioxidant activity of *Ganoderma lucidum* (Lingzhi) powder

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Abstract

This study aimed to investigate the polysaccharide, triterpene and antioxidant properties of *Ganoderma lucidum* powder obtained through commercial extraction and spray drying from various species of *G. lucidum*. The different species of *G. lucidum*, MG2 and G2, were used in these studies. The dried mushroom was ground and stored in an airtight black polythene bag at room temperature until use. Commercial water extraction (100°C in 76 min) and spray drying (inlet temperature 140°C and outlet temperature 80°C) were used to produce lingzhi powder. *Ganoderma lucidum* MG2 and G2 were extracted and then spray-dried with and without maltodextrin to make lingzhi powder. It was found that the species and presence of maltodextrin influence the antioxidant activity of *G. lucidum* (lingzhi powder). From the analysis of *G. lucidum* MG2 and G2 powder after spray drying, it was found that *G. lucidum* MG2 had the highest total amount of antioxidants by the ferric-reducing antioxidant power (FRAP) and trolox equivalent antioxidant capacity (TEAC) methods, with *G. lucidum* powder from MG2 found to be greater than G2. The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) revealed that *G. lucidum* G2 had better antioxidation properties. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method showed that extracts from *G. lucidum* G2, followed by *G. lucidum* MG2 offered antioxidant activity. A high-performance liquid chromatographic (HPLC) analysis of β-glucan from *Ganoderma* to differentiate the various species of the genus *Ganoderma*. Results show that the highest β-glucan content ranged from 9.9149 to 38.8291 mg/100 mg. It was found of ganoderic acid A varying from 0.8502 to 0.9241 mg/100 mg was found to contribute to local medicinal mushroom habitats and significantly contributed to the accumulation of bioactive compounds or nutraceutical products, such as β-glucan content.

1. Introduction

Ganoderma lucidum is one of the most famous medicinal mushroom species and has been used in traditional medicine in the Far East for more than 2,000 years (Wachtel-Galor *et al.*, 2011; Wasser *et al.,* 2005). The fruiting body of *G. lucidum*, called *lingzhi* in China and *reishi* in Japan, has been used since ancient times for the treatment of a series of diseases including hepatitis, arthritis, nephritis, bronchitis, asthma, arteriosclerosis, hypertension, cancers, immune regulation and gastric ulcers (Russell *et al.,* 2006). Mushrooms are increasingly being explored as a valuable source of nutrients and highly bioactive secondary metabolites. *Ganoderma lucidum* (commonly called Lingzhi in China) is used as an elixir of life to promote health and longevity in East Asian countries (Bao *et al.,*2002; Chan *et al.,* 2003). It is commercially available in the forms of raw mushrooms, tea, powder and capsule extract and contains two major groups of bioactive triterpene, polysaccharide, protein, peptides and amino acid components (Wasser*,* 2002). The polypore fungus *G. lucidum* provides bioactive compounds containing several triterpenoids and polysaccharides that are reported to possess anti-cancerous and immunomodulatory properties (Benzie and Strain., 1999). Hot water analysis to determine the recovery efficiency of polysaccharides and triterpenoids. Extraction is an important step for the recovery of bioactive compounds from the plant material. Extraction technology must be safe for the operating personnel and

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the consumers and inexpensive to use, at the same time it must be environmentally friendly. The free radical scavenging activity of *G. lucidum* was encouraged by the presence of total phenol content (Chen *et al.,* 2008). Polysaccharides, triterpenoids and other several components of *G. lucidum* illustrated antioxidant activity (Chen *et al.,* 2008). *Ganoderma lucidum* is presently popular and used in the production of functional foods. From several previous studies, it is known that *G. lucidum* comprises potential phytoconstituents which are concomitant with the advancement of good health as well as used for alternative medicine. Up to now, the HPLC method has been widely used in the analysis of triterpenes and related compounds in *G. lucidum*. Cai *et al.* 2001 reported the effective use of RP-HPLC (YWG-C_{18} column) for quantifying ganoderic acid contents, specifically ganoderic acid A, C, D, and E, in both cultured and wild *G. lucidum*. This method was asserted to be exceptionally appropriate for the quality control of herbal formulations containing Lingzhi. A binary gradient HPLC approach for the concurrent quantitative study of six triterpenoids ganoderic acids C2, B, AM1, K, H, and D in *G. lucidum* and its allied species has recently been published by Wang et al. (2006).

Meanwhile, Gao *et al.* (2003) observed that high quality dried *G. lucidum* is essential either for direct consumption or further processing into pharmaceutical or healthcare products. Its bioactive ingredients are extracted from the fruiting body and spray-dried into powder form, followed by processing the powder into capsules or incorporating it into drugs and beverages. Spray drying is a method of producing a dry powder from a liquid or slurry by rapidly drying with heat. Spray drying is the preferred method of drying for many thermally sensitive materials such as foods and pharmaceuticals. It is the most frequently used method in the food sector due to its low cost and simplicity of operation. It offers a very short drying contact time (a few seconds) for the core materials in the drying chamber. The objectives of this study were to determine β-glucan, ganoderic acid A and antioxidant activity in the *G. lucidum* powder to determine the differences between species MG2 and G2.

2. Materials and methods

2.1 Materials

Two separate *G. lucidum* species sources were used in this study: *G. lucidum* MG2 obtained from a local market in Chiang Mai, Thailand and *G. lucidum* G2 imported from China Figure 1. The dried mushroom was ground to 4 mm particles using a cutting mill and an ultra centrifugal mill, then stored at room temperature in an airtight black polythene bag until used.

Figure 1. The sample of Lingzhi cultivars *G. lucidum* G2 imported from China (A) and *G. lucidum* MG2 from Chiang Mai (B).

2.2 Ganoderma lucidum powder preparation

Using ground *G. lucidum* at a ratio of 20 kg per 1000 kg of water in commercial water extraction (high-speed extractor), temperatures were maintained at 100°C for 76 mins. The extracted solution was filtered via a falling film evaporator model (VPF-200L) at 60°C until it was completely dry. *Ganoderma lucidum* MG2 and G2 were extracted and evaporated, followed by spray drying using spray drier model SDG-100 NF. The inlet temperature was 140°C and the outlet temperature was 80°C and the process was conducted with and without maltodextrin to produce *G. lucidum* powder. The extracted solution was filtered through analytical filter paper and the filtrate was evaporated to dryness using a rotary evaporator. A quantity of 100 mg of dry extract was dissolved in 25 mL of HPLC water using temperatures from 150°C for 15 mins and filtered through a 0.45 μm membrane filter unit. Then, each sample solution was analyzed by HPLC.

2.3 High-performance liquid chromatography analysis

2.3.1 Analysis of beta-glucan

The samples and standard B-glucan were analyzed by HPLC separation with a Luna C18 column (250 × 4.6 mm) with a 5 μm internal diameter. The mobile phase was acetonitrile (ACN) 100% with flow rate of 0.5 ml/min. The injection volume for the sample and standard solution was 10 μL. The detection occurred at UV light at 305 nm wavelength.

2.3.2 Analysis of ganoderic acid A

The samples and standard ganoderic acid A were analyzed by HPLC separation with a Luna C18 column (150 × 2.00 mm) with a 5 μm internal diameter. The mobile phase A consisted of 0.1% Trifluoroacetic acid in Mili-Q water and the mobile phase B was acetonitrile 5 % for 5 mins, then increasing to 95 % Acetonitrile over 20 mins, before holding at 95% for 5 mins with a flow rate of 0.4 mL/min. The injection volume for the sample and standard solution was 2.0-10 μL. The detection occurred at UV light at 250±4.0 nm wavelength.

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2.4 Antioxidant assay

2.4.1 Ferric-reducing antioxidant power assay

Following the modified method of Benzie and Strain (1999), the ferric-reducing antioxidant power (FRAP) assay of the hot water extraction (HWE) of *G. lucidum* was performed. The FRAP assay was a modified version of the method according to Benzie and Strain (1999) as previously described by Thetsrimuang *et al*. 2011). FRAP reagent was prepared by mixing 1 unit of $FeCl_3{\bullet}6H_2O$ solution (20 mM), 1 unit of TPTZ (2,4,6-tripyridyl-s-triazine) solution and 10 units of acetate buffer (0.5 M, pH 3.6). The reagent was warmed to 37° C. The sample of 20 µL was added to 980 µL of FRAP reagent. The ability to reduce power was measured at an absorbance of 593 nm after initial mixing and after 90 min of reaction.

2.4.2 1,1-Diphenyl-2-picrylhydrazyl assay

The ability of the hot water extraction (HWE) of *G. lucidum* to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was determined according to the method of Brand-Williams *et al*. (1995) with minimal modification. The DPPH assay was performed as described previously following the transformation of the DPPH radical to its reduced, neutral form. A total of 10 µL of each extract or standard compound was added to 60 µL of DPPH reagent and mixed with 180 µL of methanol. Absorbance was measured spectrophotometrically at 515 nm after 30 mins of reaction. The antioxidant activity was expressed as a concentration of the sample that inhibited the DPPH radical formation by 50% (EC_{50}) and the experiment was performed in triplicate.

2.4.3 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid assay

The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay (Arnao *et al*., 2001) was used to measure the radical scavenging activity of the extracts and standard compound, propyl gallate (PG). A total of 10 mL of each extract or standard compound (Trolox, vitamin E analogue) (except in a control probe when only the solvents were used) was added to 290 µL of ABTS or distilled water. Absorbance was measured spectrophotometrically at 515 nm after 30 mins of reaction. The same procedure was repeated with PG as positive control. The absorbance was measured at 734 nm after 5 mins of reaction. All the samples were made in triplicate and mean values of ABTS scavenging activity were expressed as milligram Trolox equivalents (TE) per gram of dry weight (mg TE/g dw), calculated according to the standard curve.

For the statistical analysis, SPSS software was used to analyze data, and results were expressed as mean ± standard error mean (SEM). One-way analysis of variance (ANOVA) and least significant difference (LSD) tests were utilized to decide the distinctions among the methods. An estimation of $p \leq 0.05$ was thought to be statistically significant.

3. Results and discussion

3.1 High-performance liquid chromatography instrumentation and chromatographic conditions

3.1.1 Beta-glucan content

Beta-glucan extraction from the dried mycelia of two separate *G. lucidum* species sources was used in this study: *G. lucidum* MG2 obtained from a local market in Chiang Mai, Thailand and *G. lucidum* G2 imported from China was examined via HPLC. HPLC chromatogram of β-glucan a shown in Figure 2. The amounts of β-glucan content in the two separate *G. lucidum* species are shown in Table 1. The results showed that the values of β-glucan content exhibited significant differences ($p \leq 0.05$) The difference between *G. lucidum* MG2 and G2 with maltodextrin added during the spray drying process was significant ($p \leq 0.05$), while the β-glucan content ranged from 9.9149 to 38.8291 mg/100 mg. It has been reported that *Ganoderma* is number one among medicinal mushrooms, and it has been considered the "king of medicinal mushrooms". *Ganoderma lucidum* belongs to a large group of polypore fungi and has been used as a traditional therapeutic mushroom for over 4,000 years (Zhang *et al*., 2016). Some of the more efficacious compounds of *Ganoderma* are 1,6-branched and contain 1,3-β-glucan and have been reported to inhibit tumor growth by stimulating the immune system via the activation of macrophages. It is widely known that the cell walls of mushrooms contain a high number of polysaccharides, including β-glucan. Most of the bioactive β-glucan in mushrooms has been found to be complex with peptides. Hot water is the most popular β-glucan extraction technique since it fractionates only water-soluble β-glucan into the aqueous extract. The

Table 1. β-glucan content and ganoderic acid A of *Ganoderma lucidum* (Lingzhi) powder (mg/100 mg).

Medicinal mushrooms	β-glucan	Ganoderic acid A ^{ns}
G. lucidum MG2	27.4867±4.08^{b}	0.9241±0.15
G. lucidum G2	16.1796±0.48^{bc}	0.8502±0.01
G. lucidum MG2 maltodextrin	38.8291±4.43^{a}	0.8650±0.01
G. lucidum G2 maltodextrin	9.9149±1.29^{c}	0.8720±0.00

Values are presented as mean±SEM. Values with different superscripts within the same column are statistically significantly different at 95% with posthoc LSD test.
^{ns} no significant difference at 95%.

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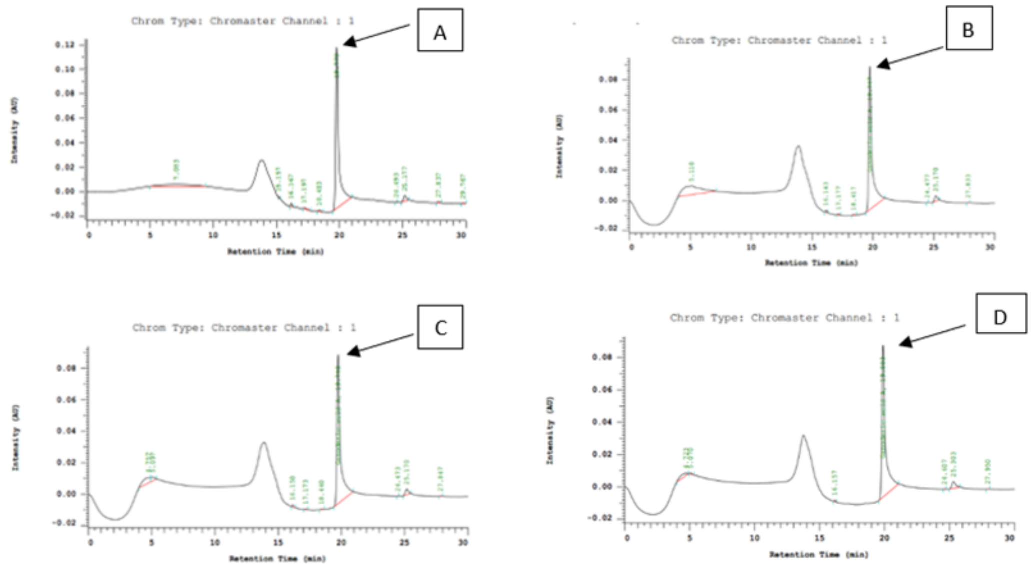

Figure 2. HPLC chromatograms for the Ganoderic acids A. *G. lucidum* MG2 (A), *G. lucidum* G2 (B), *G. lucidum* MG2 maltodextrin (C) and *G. lucidum* G2 maltodextrin (D).

water-insoluble β-glucan has therefore been isolated by other extraction techniques. Previous authors (Toledo, 2013) found that the concentration of β-glucan varied from 27.0 to 89.0 mg/g in dehydrated mushrooms. These values are higher than those obtained in the present study, in which the lower β-glucan concentrations. Different strains may have different amounts of β-glucan. Moreover, environmental factors such as vegetation, soil and process for extraction and production of lingzhi powder may also affect composition. Shao *et al.* (2019) reported a spray-drying microencapsulation of *G. lucidum* polysaccharide (GLP) employing maltodextrin-protein as wall materials for enhanced stability and bioaccessibility using maltodextrin as wall materials, retaining about 50% of β-glucan after 10 weeks of storage. Microcapsules showed a good chance for active ingredients of edible fungi to be used in the food industry.

3.1.2 Ganoderic acid A content

HPLC chromatogram of *ganoderic acid A* was shown in Figure 3. The validated analytical method was applied to the dried mycelia of two separate *G. lucidum* species sources which were used in this study via HPLC. Table 1 shows details of the extracts of *G. lucidum*. It was found that the concentration of ganoderic acid A varied from 0.8502 to 0.9241 mg/100 mg in dehydrated mushrooms. Fruiting bodies of *G. lucidum* were the best sources of the five ganoderic acids. For the *G. lucidum* species sources used in this study, *G. lucidum* MG2 obtained from a local market in Chiang Mai, Thailand, and *G. lucidum* G2 imported from China, the amount of each ganoderic acid as shown in Table 1. For example, Wang *et al*. (2006) reported that *G. sinense* contained low levels of ganoderic acids, which were estimated mostly by extrapolation of standard curves below LOQ. No data about the amounts of ganoderic acids in *G. applanatum* were presented in the literature.

3.2 Antioxidant activity

3.2.1 2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid

Free radical quantitative analysis was conducted with samples from *G. lucidum* MG2 and G2 with maltodextrin added to lingzhi via spray drying. The findings are shown in Table 2. ABTS scavenging activity was tested by ABTS (2,2'-Azino-bis-(3-ethybenzothia zoline-6-sulfonic acid). ABTS sought to determine the EC_{50} value of *G. lucidum* extract powder to assess the differences between the MG2 and G2 species, both with and without maltodextrin. It was found that *G. lucidum* G2 value of 0.10 g sample/L had the best antioxidation activity, followed by *G. lucidum* MG2 value of 0.13 g sample/L, compared to the curve. The standard of Trolox from the ABTS method is shown in Table 2. Wang *et al.* (2019) reported that a low EC_{50} value indicates strong antioxidant activity in a tested sample. Lingzhine E and lingzhine F showed equivalent ABTS+ scavenging activities, with EC_{50} values of closely the positive control (trolox), which had an EC_{50} value.

3.2.2 1,1-Diphenyl-2-picrylhydrazyl

Antioxidant activity is one of the functions provided by various bioactive components of *G. lucidum* MG2 and G2. The activity assay assesses the ability of the spray-dried powder to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals and considers the effects of different species and the use of maltodextrin. From Table 2, the study of antioxidant activity through *in vitro* free radical binding capacity by DPPH method showed that extracts from *G. lucidum* G2 had the greater antioxidant activity, followed by *G. lucidum* MG2. The

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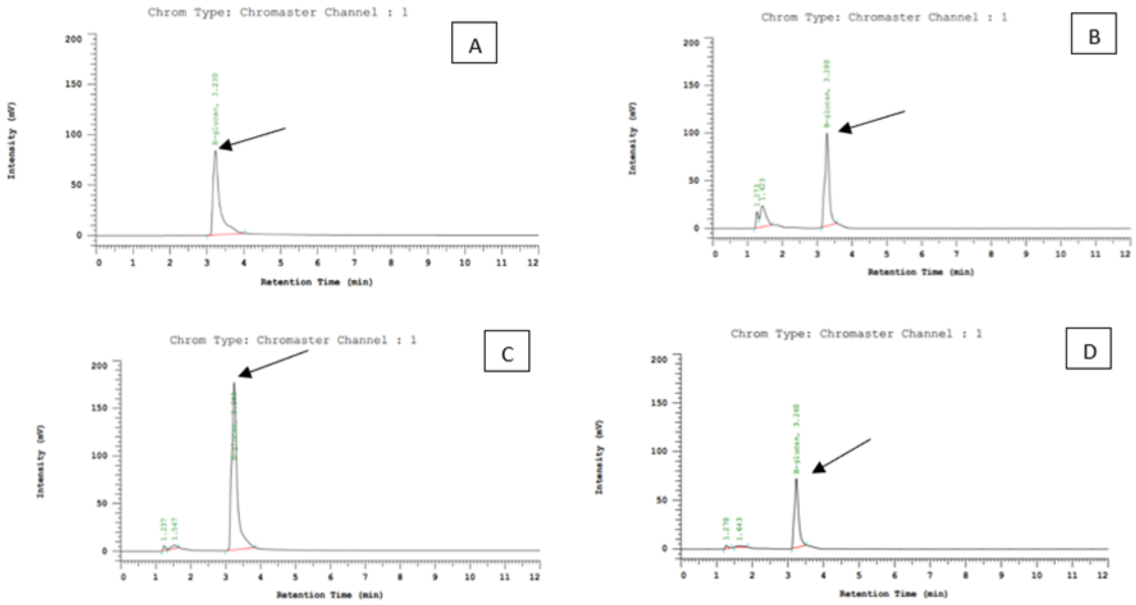

Figure 3. HPLC chromatograms for the β-glucan for *G. lucidum* MG2(A), *G. lucidum* G2 (B), *G. lucidum* MG2 maltodextrin (C) and *G. lucidum* G2 maltodextrin (D).

concentration of the substance was able to reduce the concentration of DPPH (1,1-diphenyl-2-picryl-hydrazyl) by 50%, giving the value (EC_{50}) of 0.57 to 0.58, respectively. *G. lucidum* G2 with maltodextrin and *G. lucidum* MG2 with maltodextrin were found to be antioxidative but could not be quantified with this method, because when the reaction occurs, the solution is cloudy. Furthermore, when testing the antioxidant activity of DPPH, it was found that *G. lucidum* MG2 had the highest free radical scavenging ability, followed by *G. lucidum* G2. When testing DPPH of the extracts compared with the standard alpha-tocopherol, this was shown in the form of Trolox equivalent antioxidant capacity (TEAC)/g sample extract. It was also found that DPPH scavenging was the highest with TEAC values of 12.65 and 12.64 mg TE/g dw, respectively, which may be caused by various factors, such as cable characteristics. Varieties, temperature, geography, and minerals used for cultivation also contribute to the differences. Moreover, the extraction of *G. lucidum* along with maltodextrin may lead to the DPPH antioxidant activity assay solution becoming cloudy. At the time of analysis, *G. lucidum* MG2 was a mature mushroom-yielding strain, with high spores and content. Klaus *et al*. (2011) reported that reducing power and chelating effect on ferrous ions were normalized and expressed as EC_{50} values which represented the effective concentration of each mushroom extract required to show 50% of antioxidant properties. A lower EC_{50} value corresponds to the higher antioxidant activity of the mushroom extract. *Ganoderma lucidum* showed very good antioxidant activity, which was confirmed by a low EC_{50} value.

3.2.3 Ferric-reducing antioxidant power assay

Reducing efficiency of *G. lucidum* extracts was observed using the FRAP method, which is a method for analyzing the electron transport/electron capacity of *G. lucidum* coarse union samples as electron carriers of the samples by the extract. Of the two species of *G. lucidum*, MG2 acts as an electron donor to the [Fe^{3+}-TPTZ] complex, causing the iron atoms to be reduced and converted to the hexagonal compound of [Fe^{2+}-TPTZ]. The reducing efficiency values of crude extracts from the two species of *G. lucidum* and extraction methods with the addition of maltodextrin are shown as follows in Table 2. It was found that *G. lucidum* MG2 had the highest total amount of antioxidants by the FRAP and TEAC methods, and the total amount of phenolic compounds was 13.49 mg TE/g dw, while for *G. lucidum* G2 the value was 12.11 mg TE/g dw. With maltodextrin, the value for *G. lucidum* G2 was 5.11 mg TE/g dw. while for *G. lucidum* MG2 with maltodextrin, the value was 0.36 mg TE/g dw. These differences may be attributed to various factors including the species and the environment. Veljović *et al.* (2017) found that ethanol extracts of *G. lucidum* exhibited significantly higher antioxidant activity, with values ranging from 4.32 to 6.65 FRAP units and 2.52 to 6.00 mmol Trolox Equivalent. In contrast, a special brandy infused with the same amount of *G. lucidum* (40 g/L) showed much lower antioxidant activity, measuring at 0.432 FRAP units and 1.043 mmol Trolox Equivalent (Pecić *et al*., 2016). The extraction process concentrates on the bioactive compounds, leading to a significant increase in antioxidant capacity in the ethanol extracts compared to the brandy.

4. Conclusion

Table 2. Antioxidant activity of *Ganoderma lucidum* (Lingzhi) powder.

Antioxidant assay	Samples	EC_{50} (g sample/L)	TEAC (mg TE/g dw)
ABTS	*G. lucidum* MG2	0.13±0.01	22.38±0.61
	G. lucidum MG2 maltodextrin	1.03±0.02	3.02±0.06
	G. lucidum G2	0.10±0.02	30.77±2.32
	G. lucidum G2 maltodextrin	0.63±0.03	5.01±0.20
DPPH	*G. lucidum* MG2	0.57±0.01	12.65±0.29
	G. lucidum MG2 maltodextrin	a	a
	G. lucidum G2	0.58±0.05	12.64±0.97
	G. lucidum G2 maltodextrin	a	a
FRAP	*G. lucidum* MG2	ND	13.49±2.02
	G. lucidum MG2 maltodextrin	ND	0.36±0.43
	G. lucidum G2	ND	12.11±0.82
	G. lucidum G2 maltodextrin	ND	5.11±0.12

Values are presented as mean±SEM. Values with different superscripts within the same column are statistically significantly different at 95% with posthoc LSD test. EC_{50}: Effective concentration at 50%, TEAC: Trolox equivalent antioxidant capacity, a: ability to resist oxidation but cannot be quantified, ND: not detected.

HPLC analysis of the triterpenoid contents of *Ganoderma* is a simple and easy method to differentiate the species of *Ganoderma*. This should prove beneficial to the research fields of classification, biological activity, and structural determination. In the present study, the profiles of two separate *G. lucidum* species sources were used: *G. lucidum* MG2 obtained from a local market in Chiang Mai, Thailand, and *G. lucidum* G2 imported from China differed significantly in all analyzed samples. This present work suggested an accurate and sufficient method for quantitative evaluation, which is suitable for quality evaluation of *Ganoderma* products. It can be also concluded that the geographical distributions, growth conditions, and substrates might be the key to differences in producing chemical compositions.

Conflict of interest

The authors declare no conflict of interest.

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