Journal of Applied Pharmaceutical Science Vol. 9(04), pp 001-007, April, 2019
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2019.90401
ISSN 2231-3354

Antimutagenic and antioxidative effects of polysaccharides isolated from the water extract of *Ganoderma lucidum*

Hyeong-Ryeol Lee, Heung-Bin Lim*
College of Agriculture, Life and Environment Sciences, Chungbuk National University, Cheongju, Korea.

ARTICLE INFO

Received on: 19/11/2018
Accepted on: 16/03/2019
Available online: 18/04/2019

Key words:
Ganoderma lucidum, *β*-glucan, total polyphenolic content, antimutagenic activity, antioxidant activity.

ABSTRACT

This study was conducted to investigate the antimutagenic and antioxidative effects of *Ganoderma lucidum*. *Ganoderma lucidum* was extracted with hot-water (GLW), and then partially purified with crude glycoside extract (GLG) and crude polysaccharide extract (GLP). The yield from GLW, GLG, and GLP was 7.63%, 1.38%, and 1.04%, respectively. *β*-glucan content from GLW, GLG, and GLP was 15.2%, 29.3%, and 48.0%, respectively. The total polyphenolic content from GLW, GLG, and GLP was 33.1, 16.5, and 5.8 mg gallic acid equivalent/g, respectively. Regardless of the bacterial strain, the three extracts did not induce mutations up to a maximum concentration of 5,000 μg/plate. All three extracts showed antimutagenic effects. GLP, which had the highest content of *β*-glucan, was the most effective in inhibiting mutations. Alternatively, the antioxidant activity, measured by 2,2-diphenyl-1-picrylhydrazyl and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging assays, was relatively high in GLW, which had the highest total polyphenolic content. Overall, *G. lucidum* extracts were genetically safe, and *β*-glucan was confirmed to be the major factor influencing the antimutagenic activity, whereas polyphenol was the major factor influencing the antioxidant activity of *G. lucidum*.

INTRODUCTION

A mutation is a change in genetic information due to DNA sequence changes. Mutations can occur naturally during the DNA repair process in humans and can also be caused by environmental factors, such as chemicals, metals, radiation, stress, diet, and tobacco. Changes in the DNA sequence due to mutations affect the structure or function of the encoded protein, leading to changes in genetic traits. Mutations are one of the leading causes of cancer, and more than 90% of cancers are reported to be caused by mutations due to the environmental factors (Anand *et al*., 2008). Reactive oxygen species are also generated by the same environmental factors that cause mutations; the generation of excess reactive oxygen species causes oxidative stress. Oxidative stress refers to a state in which the balance of the body's antioxidant system is disrupted. Reactive oxygen species also react with DNA and proteins in the cell to induce or promote mutations, thereby causing cancer (Pisoschi and Pop, 2015).

Antimutagenic activity that inhibits mutagenesis, includes preventing mutation-causing events, inactivating mutagens through chemical interactions before they affect the genes, and stopping the mutation process of genes damaged by mutagens (Mitscher *et al*., 1996). Several studies have been actively conducted on natural products to identify antimutagens because they exhibit various physiological activities while being less toxic. Recently, the antimutagenic activity of various natural materials, such as cat's claw and guava, have been reported (Almeida *et al*., 2017; da Costa *et al*., 2015; Di Sotto *et al*., 2015; Gontijo *et al*., 2018; Nag *et al*., 2015; Todorova *et al*., 2015; Zahin *et al*., 2017).

Ganoderma lucidum, common name Lingzhi, is a medicinal mushroom from the family Ganodermataceae distributed in China, Korea, and Tropics. In traditional Chinese medicine, *G. lucidum* has been used to improve health and prolong life (Zhang *et al*., 2003). *Ganoderma lucidum* has also been reported to have various physiological properties, such as anti-cancer, anti-inflammation, anti-diabetes, antioxidant, antibacterial,

*Corresponding Author
Heung-Bin Lim, College of Agriculture, Life and Environment Sciences, Chungbuk National University, Cheongju, Korea.
Email: heungbin @ chungbuk.ac.kr

anti-aging, and immunity enhancement effects (Bhardwaj *et al*., 2014; Boh, 2013; Cai *et al*., 2016; Ferreira *et al*., 2015; Ma et al., 2015; Socala *et al*., 2015; Wang *et al*., 2014). It has been reported that *G. lucidum* contains more than 400 physiologically active ingredients, including triterpenoids, polysaccharides, nucleotides, sterols, steroids, fatty acids, and proteins. Among them, the major physiologically active compound is known to be a polysaccharide-type *β*-glucan (Boh *et al*., 2007; Wong *et al*., 2014).

Meanwhile, Lakshmi et al. (2003; 2006) reported that when the Ames test was carried out with *G. lucidum* collected from the Thrissur region in southern India, antimutagenic activity was found against benzo[*a*]pyrene-induced mutations in the *Salmonella typhimurium* TA98 and TA100 strains. However, these studies were conducted with *G. lucidum* collected in the tropics and from in ethanol and methanol extracts; additionally, there have been no other reports on the antimutagenic activity of *G. lucidum*.

In the present study, *G. lucidum* native to Korea was used. The polysaccharide-type *β*-glucan, which is known as the physiologically active component of *G. lucidum*, was partially purified and used as a sample and not as an extract. The mutagenic, antimutagenic, and antioxidant effects of the *β*-glucan from *G. lucidum* were investigated.

MATERIALS AND METHODS

Materials and extraction

In this study, dried fruiting bodies of Ganoderma lucidum were used (Fig. 1). Fruiting bodies of *G. lucidum* were collected from Mt. Songni, located in Boun-gun, Chungcheongbuk-do, Korea. The Fruiting bodies powder (100 g) was added to distilled water (20 l) and subjected to hot-water extraction at 100°C for 3 hours. This process was carried out three times, individually. Each hot-water extracted mixture was filtered and vacuum-filtered (8 mm Advantec) in a rotary vacuum evaporator, and fully concentrated.

Figure 1. Dried fruiting body of *G. lucidum* and its powder.

Partial purification

Three concentrates obtained by hot-water extraction of *G. lucidum* were partially purified as the *G. lucidum* water extract (GLW), *G. lucidum* glycoside extract (GLG), and *G. lucidum* polysaccharide extract (GLP) (Fig. 2).

GLW was prepared by lyophilizing the *G. lucidum* concentrate and cooling to −70°C.

GLG was prepared by adding 99% ethanol to the *G. lucidum* concentrate four times and allowing the mixture to stand at 4°C for 24 hours to precipitate the carbohydrates (Zhao *et al*., 2010). The precipitate was then recovered and lyophilized. The final result was used as GLG.

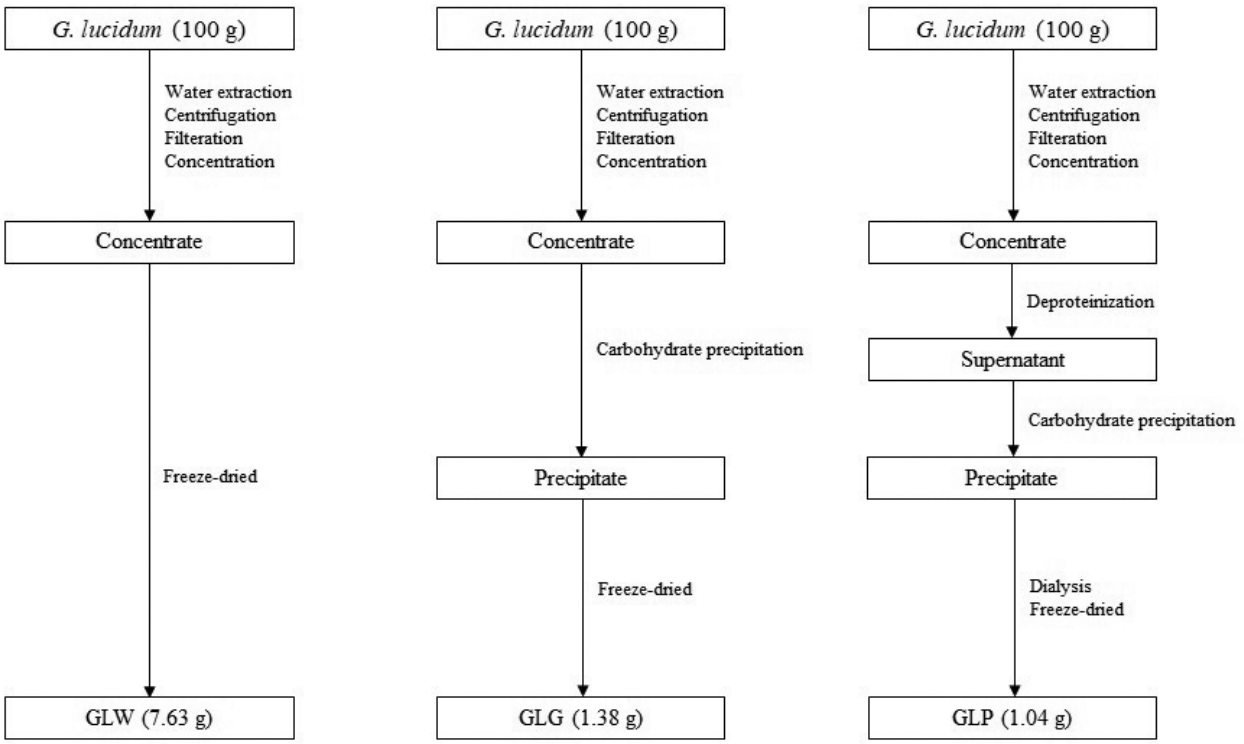

Figure 2. Partial purification scheme of polysaccharides from *G. lucidum*.

The purification process of GLP is as follows: the *G. lucidum* concentrate was adjusted to pH 3 by adding a 20% trichloroacetic acid solution and allowed to stand at 4°C for 12 hours. Next, centrifugation (1,700 × g, 10 minutes) was performed, and only the supernatant (deproteinization solution) was collected. This procedure was repeated twice (Huang *et al*., 2010). The carbohydrates from the supernatant were precipitated in the same manner as in the GLG purification procedure. The precipitate was collected and dialyzed (to remove low-molecular-weight carbohydrates) and then lyophilized. GLP was obtained as the final product. The yield (%) of each extract was determined as follows: weight (g) of each extract obtained per 100 g of the dry *G. lucidum*.

Determination of carbohydrate and β-glucan content

The total carbohydrate content was determined by using the phenol-sulfuric acid method (Dubois *et al*., 1956) with glucose as the standard. To each sample (250 µl), 5% phenol (250 µl), and H_2SO_4 (750 µl) were added and incubated at 22°C for 30 minutes. Then, the absorbance was measured at 470 nm with a spectrophotometer.

β-glucan content was measured using a Mushroom and Yeast *β*-glucan Assay kit (Megazyme, Wicklow, Ireland). Glucose was used as the standard, and *β*-glucan content was expressed as total glucan content minus *α*-glucan.

The total carbohydrate and *β*-glucan content were calculated as milligrams (mg) of glucose equivalent (GE) per gram (g) of extract using the calibration curve equation of glucose.

Mutagenicity test

Mutagenicity was assessed in *Salmonella typhimurium* strains according to the methods described by Maron and Ames (1983) and in the Organization for Economic Cooperation and Development (OECD) (1997) Guideline (Test no. 471). *Salmonella typhimurium* TA98 (frameshift mutation) and TA100 (base pair mutation) strains, which are used for the Ames test, were purchased from Korea Biotechnology Research Institute. Prior to the mutagenic and antimutagenic tests, the strains were routinely subjected to genetic analysis with histidine and biotin-dependence, biotin-dependence, histidine-dependence, ultraviolet radiation (*uvr*) B mutation, *rfa* mutation, R-factor, and spontaneous return tests. 2-aminoanthracene (2-AA) (1.0 µg/plate), an indirect mutagen (in the presence of the liver metabolizing system, S-9), was used as the positive control. DMSO was added to the agar plate as a negative control. The concentration of the *G. lucidum* extracts was 500–5,000 µg/plate.

Agar (2 ml) was placed in a sterile tube at 45°C. Then, the S-9 mixture (500 µl), sample (100 µl) dissolved in DMSO, and *S. typhimurium* (100 µl, 1–2 × 10^9 CFU/ml) were added sequentially. The tube was then vortexed for 2–3 seconds and plated on a glucose agar plate. After incubation for 48 hours in a 37°C incubator, the number of revertant colonies on each plate was counted using an automatic colony counter.

Antimutagenicity test

An antimutagenic activity test was performed according to the method described by Maron and Ames (1983). Agar (2 ml) was placed in a sterile tube at 45°C. Then, the S–9 mixture (500 µl), sample (50 µl), *S. typhimurium* strain (100 µl, 1–2 × 10^9 CFU/ml), and indirect-mutagen, 2-AA (50 µl), were added sequentially. The tube was then vortexed for 2–3 seconds and plated on a glucose agar plate. After incubation for 48 hours in a 37°C incubator, the number of revertant colonies on each plate was counted using an automatic colony counter. At the same time, positive and negative controls were used for all tests, and all tests were performed in triplicate at each test concentration.

The inhibition activity was calculated as follows:

$$\text{Inhibition (\%)} = (M - B)/(M - A) \times 100$$

where M = number of revertant colonies by the mutagen, A = number of spontaneous revertant colonies, and B = number of revertant colonies when the sample and mutagen were both added to the test strains.

Radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay was performed by modifying the method described by Blois (1958). A sample (200 µl) was added to a DPPH solution (0.4 mM, 800 µl), diluted in methanol, and allowed to react at the room temperature for 30 minutes. The absorbance was measured at 520 nm using a spectrophotometer.

The 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay for total antioxidant capacity was carried out by modifying the method of Re *et al*. (1999). When potassium persulfate (2.6 mM) was added to the ABTS solution (7.4 mM), the ABTS cation was formed after 12–18 hours. One hundred microliter of the sample was added to the activated ABTS solution (1 ml), and the absorbance was measured at 735 nm after 10 minutes.

The radical scavenging activity was calculated as the rate of decrease in sample absorbance relative to the blank absorbance (100%). The percentage of scavenging activity of radicals was calculated using the following equation:

$$\text{Radical scavenging activity (\%)} = (A\,\text{blank} - A\,\text{sample}/A\,\text{blank}) \times 100$$

where A blank and A sample are the absorbances of the control and extract, respectively. All samples were analyzed in triplicate.

For both DPPH and ABTS assays, L-ascorbic acid was used as a standard. The RC_{50} (concentration required for a 50% reduction of radicals) was calculated using the graph of radical scavenging activity versus extract concentration.

Total polyphenolic content determination

The total polyphenolic content was determined by the method described by Dewanto *et al*. (2002). The sample (100 µl, 1.0 mg/ml) was added to a 2% Na_2CO_3 solution (2 ml) and reacted with the 50% Folin–Ciocalteu reagent (100 µl) for 30 minutes at the room temperature.

Gallic acid was used as a standard and the absorbance at 750 nm was measured using a spectrophotometer. The total polyphenolic content was calculated as milligrams (mg) of gallic acid equivalent (GAE) per gram (g) of extract using the calibration curve equation of gallic acid.

Table 1. Yield, total carbohydrate, and *β*-glucan content of *G. lucidum* extracts.

Extract	Yield (%)	Total carbohydrate content (GE g/100 g)[1]	Glucan content (GE g/100 g)[2]		
			Total	*α*-glucan	*β*-glucan
GLW	7.63	32 ± 7[c]	16.4 ± 1.9	1.2 ± 0.6	15.2 ± 2.2[c]
GLG	1.38	53 ± 4[b]	30.2 ± 1.6	1.0 ± 0.7	29.3 ± 2.3[b]
GLP	1.04	80 ± 9[a]	48.9 ± 0.5	0.9 ± 0.1	48.0 ± 0.5[a]

[1,2]Total carbohydrate content and glucan content analyzed as glucose equivalent (GE) g/100 g of extract.
Data were expressed as mean ± SD of three repeated experiments ($n = 3$). Different letters (a–c) in each sample are significantly different at $p < 0.05$ by Tukey's multiple comparison test.

Table 2. Evaluation of mutagenic activity of *G. lucidum* extracts in *S. typhimurium* TA 98 and TA 100 with S9 enzyme system.

Strain	Treatment (μg/plate)	GLW	GLG	GLP
S. typhimurium TA98	Negative control[1]	25 ± 3	25 ± 3	25 ± 3
	Positive control[2]	531 ± 31	531 ± 31	531 ± 31
	500	26 ± 5	26 ± 3	30 ± 5
	1,000	26 ± 2	22 ± 5	25 ± 7
	2,500	29 ± 3	21 ± 1	26 ± 5
	5,000	32 ± 2	19 ± 1	29 ± 6
S. typhimurium TA100	Negative control	118 ± 16	118 ± 16	118 ± 16
	Positive control	657 ± 15	657 ± 15	657 ± 15
	500	149 ± 12	108 ± 3	122 ± 2
	1,000	139 ± 4	113 ± 4	127 ± 7
	2,500	147 ± 5	133 ± 11	134 ± 11
	5,000	136 ± 1	142 ± 7	131 ± 6

[1]The number of spontaneous revertant colonies (means ± SD) determined without the addition of the samples, only with the vehicle, DMSO.
[2]Means the number of revertant induced with 2-AA (0.5 μg/plate, with metabolic activation).

Statistical analysis

Data obtained from the experiments are presented as means ± standard deviations (SD) of three replicates. IBM Statistical Package for the Social Sciences 22 (SPSS Inc., Chicago, IL) was used as a statistical program. A one-way analysis of variance was conducted to compare the results obtained from testing each type of *G. lucidum* extracts. Tukey's multiple comparison test was used to determine the significance of the differences between the groups. Differences at $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Ganoderma lucidum has long been used in traditional Chinese medicine for health promotion and life extension. *Ganoderma lucidum* has many bioactive ingredients, but polysaccharide-type *β*-glucan is known to be the main active ingredient (Boh *et al*., 2007). The purpose of this study was to partially purify polysaccharide, the major physiologically active ingredient of *G. lucidum*, and to examine its antimutagenic and antioxidant activities. Previous studies have reported various physiological activities for polysaccharides, but the results were diverse due to various factors such as differences in the extraction method of polysaccharides and differences in the content of active ingredients depending on the country of origin. In this study, polysaccharides were purified using hot water extraction and the antimutagenic activity of the polysaccharides was first reported.

The yield, carbohydrate, and β-glucan content

Table 1 shows the yield, total carbohydrate content, and *β*-glucan content of GLW, and the partially purified GLG and GLP from wild *G. lucidum*. The yields of GLW, GLG, and GLP were 7.63%, 1.38%, and 1.04%, respectively. The total carbohydrate content of GLW, GLG, and GLP was 32 ± 7, 53 ± 4, and 80 ± 9 g GE/100 g, respectively, and the ratio increased significantly as the partial purification proceeded. Moreover, the percentage of *β*-glucan in the total carbohydrate content was 15.2% ± 2.2% for GLW, 29.3% ± 2.3% for GLG, and 48.0% ± 0.5% for GLP. The percentage increased greatly as the partial purification progressed. When the yield of *β*-glucan according to the solvent was compared, the percentage of *β*-glucan in the water extract obtained by Kozarski et al. (2012), the ethanol extract obtained by Veljović et al. (2017), and the GLP obtained in this study was 41.4% ± 1.4%, 15.64% ± 0.01%, and 48.0% ± 0.5%, respectively. Among them, the GLP we obtained in this study had the highest amount of *β*-glucan.

Mutagenicity test

The Ames test was performed to investigate whether the metabolites of the *G. lucidum* extracts cause mutations. According to the OECD guidelines, mutagenicity is considered to be present if the number of revertant colonies treated with an unknown sample is more than twice the number of spontaneous revertant colonies. Table 2 shows the number of revertant colonies of *S. typhimurium* TA98 and TA100 mutants when treated with *G.*

Table 3. Evaluation of antimutagenic activity of *G. lucidum* extracts against 2-AA induced mutation in *S. typhimurium* TA 98 and TA 100 with S9 enzyme system.

Strain	Treatment (μg/plate)	GLW		GLG		GLP	
		Revertants/plate	Inhibition rate (%)	Revertants/plate	Inhibition rate (%)	Revertants/plate	Inhibition rate (%)
S. typhimurium TA98	Negative control[1]	25 ± 3		25 ± 3		25 ± 3	
	Positive control[2]	583 ± 14^{a}		583 ± 14^{a}		583 ± 14^{a}	
	50	531 ± 6^{b}	9.2	540 ± 10^{b}	7.5	550 ± 15^{ab}	5.9
	100	498 ± 12^{c}	15.2	485 ± 8^{c}	17.6	530 ± 13^{b}	9.5
	250	486 ± 12^{cd}	17.3	454 ± 12^{d}	23.1	480 ± 11^{c}	18.5
	500	468 ± 5^{e}	20.6^{A}	400 ± 12^{e}	32.8^{B}	368 ± 8^{d}	38.5^{C}
S. typhimurium TA100	Negative control	122 ± 6		122 ± 6		122 ± 6	
	Positive control	679 ± 19^{a}		679 ± 19^{a}		679 ± 19^{a}	
	50	627 ± 15^{b}	9.3	631 ± 17^{ab}	8.6	620 ± 14^{b}	10.6
	100	596 ± 17^{bc}	14.9	587 ± 20^{bc}	16.5	591 ± 11^{b}	15.8
	250	575 ± 11^{c}	18.7	568 ± 15^{c}	19.9	544 ± 14^{c}	24.2
	500	525 ± 21^{d}	27.6^{A}	495 ± 22^{d}	33.0^{A}	398 ± 10^{d}	50.4^{B}

[1]The number of spontaneous revertant colonies determined without the addition of the samples, only with the vehicle, DMSO.
[2]Means the number of revertant induced with 2-AA (0.5 μg/plate, with metabolic activation).
[a–c]Values (means ± standard derivation of triplicate experiments) in the same column followed by the different letter are significantly different at $p < 0.05$ by Tukey's multiple comparison test.
[A–C]When groups compared with GLW at 500 concentration, values [Inhibition rate (%)] in the each samples followed by the different letter are significantly different at $p < 0.05$ by Tukey's multiple comparison test.

lucidum extracts (500–5,000 μg/plate) in the presence of the liver metabolizing enzyme (S-9 fraction), which digests the extract into its metabolites. The number of spontaneous revertant colonies was 25 ± 3 in TA98 and 118 ± 16 in TA100. When treated with 2-AA (1.0 μg/plate), which is a representative mutagenic compound, the number of revertant colonies was significantly higher, 531 ± 31 in TA98 and 657 ± 15 in TA100. On the other hand, when the TA98 culture was treated with *G. lucidum* extracts, regardless of the increased concentration of the extract, the number of revertant colonies did not deviate from the OECD guideline standard and showed no statistically significant increase. In addition, the TA100 strain culture showed no significant difference in the number of revertant colonies, even when the concentration of the *G. lucidum* extracts was increased. Thus, we concluded that the metabolites of *G. lucidum* extracts, produced by the S-9 fraction, did not induce mutagenesis.

Antimutagenicity test

After confirming that *G. lucidum* extracts did not induce mutations, the Ames test was performed to investigate the antimutagenic activity of GLW against 2-AA-induced mutations in *S. typhimurium* TA98 and TA100 strains. The antimutagenic effect of *G. lucidum* extracts is shown in Table 3. In the presence of the S-9 fraction, the number of spontaneous reversion mutations was 25 ± 3 in the TA98 strain and 122 ± 6 in the TA100 strain. On the other hand, the number of revertant mutations after 2-AA treatment was 583 ± 14 in TA98 and 679 ± 19 in TA100, which was significantly higher than that of spontaneous revertant mutations. *Ganoderma lucidum* extracts (50–500 μg/plate) showed antimutagenic activity in both TA98 and TA100 strains in a dose-dependent manner for mutations induced by 2-AA ($p < 0.05$). At the highest treatment concentration (500 μg/plate), the mutagenic-inhibition percentage of GLW, GLG, and GLP was 20.6%, 32.8%, and 38.5%, respectively, in the TA98 strain, and 27.6%, 33.0%, and 50.4%, respectively, in the TA100 strain. GLP showed a statistically significant difference from GLW and GLG at the concentration (500 μg/plate) at which the antimutagenic activity was the highest ($p < 0.05$).

In a study on the antimutagenic effect of *G. lucidum*, Lakshmi et al. (2003) reported that the *G. lucidum* ethanol extract (5,000 μg/plate) inhibited mutations induced by benzo[*a*]pyrene by 65.0% in the TA98 strain and 70.3% in the TA100 strain. Lakshmi et al. (2006) also reported that the methanol extract of *G. lucidum* (3,000 μg/plate) inhibited mutations induced by benzo[*a*] pyrene by 60.7% in the TA98 strain and 59.6% in the TA100 strain. 2-AA and benzo[*a*]pyrene are both carcinogenic precursors, which are metabolized by the cytochrome (CY)P450 enzyme in the liver. Their metabolites (N-hydroxyaryl amine, benzo[*a*] pyrene diol epoxide) covalently link to DNA to form adducts, leading to mutations (Murata and Kawanishi, 2011). Therefore, it is suggested that *G. lucidum* inhibited the (CY)P450 enzyme involved in metabolizing 2-AA and benzo[*a*]pyrene producing the antimutagenic activity. In addition, antimutagenic and anti-cancer effects of certain *β*-glucans (Mantovani et al., 2008), and antimutagenic effects of specific *β*-glucan derivatives due to their radical scavenging ability (Krizková et al., 2003) have been reported. Therefore, it is suggested that *β*-glucan, in the form of the *G. lucidum* polysaccharide used in this study, can directly or indirectly contribute to the antimutagenic activity. In this study, all three extracts of *G. lucidum* showed antimutagenic activity in a dose-dependent manner, and GLP, which had the highest *β*-glucan content, showed the highest antimutagenic activity. Based on the results of this and previous studies, it was concluded that *β*-glucan of the GLW extract acted as a major contributor to its antimutagenic activity. Therefore, we suggest that studies to identify the *β*-glucan components of *G. lucidum*, and studies on the inhibitory effect of *β*-glucan on the CYP450 enzymatic activity required for bioactivity of 2-AA should be carried out in the future.

Table 4. DPPH and ABTS radical scavenging ability and total polyphenolic content of *G. lucidum* extracts.

Sample	DPPH	ABTS	Total polyphenolic content (mg GAE/g)[2]
	RC_{50} (μg/ml)[1]		
Ascorbic acid	490.39 ± 19.21[a]	470.92 ± 35.62[a]	
GLW	2,067.47 ± 51.18[b]	1,017.98 ± 35.62[b]	33.1 ± 1.9[a]
GLG	4,432.81 ± 168.06[c]	1,570.17 ± 70.86[c]	16.5 ± 1.0[b]
GLP	N.D[3]	2,549.61 ± 153.36[d]	5.8 ± 0.6[c]

[1]RC_{50} value is the effective concentration at which the DPPH and ABTS radicals were reduced by 50%.
[2]Total polyphenolic content analyzed as GAE mg/ g of extract.
[3]Not detected.
Data were expressed as mean ± SD of three repeated experiments (n = 3). Different letters (a–d) in each sample are significantly different at $p < 0.05$ by Tukey's multiple comparison test.

Antioxidant activity of G. lucidum extracts

Reactive oxygen species are important as indirect and direct initiators of mutagenesis. Some studies suggest that antioxidant properties may have an inhibitory effect on DNA mutagenesis induced by oxidative stress (Jena, 2012). Given that 2-AA mediated genetic toxicity contributes to release of reactive oxygen species, the free radical scavenging activity of the *G. lucidum* extracts may play an important role in antimutagenic activity (Hasnat *et al*., 2013).

Ganoderma lucidum is reported to have various antioxidative effects. These effects include the inherent antioxidative effect of triterpenoids, antioxidative effect from an increase of specific enzyme activity, antioxidative effect from the formation of a *β*-glucan-protein complex, and antioxidant effect from an increase in *β*-glucan activity (Hasnat *et al*., 2013; Jia *et al*., 2009; Smina *et al*., 2011; XiaoPing *et al*., 2009; Zhu *et al*., 1999). However, there have been no reports on the ability of *β*-glucan to have its own radical scavenging activity. Therefore, DPPH and ABTS assay were carried out to investigate the antioxidant effect of *β*-glucan in *G. Lucidum* extracts.

The results are shown in Table 4. The results of the radical scavenging ability measurement are shown in Table 4. At the concentration of 1,000 μg/ml, DPPH radical scavenging activity (RC_{50} = 2,067.47 ± 51.18 μg/ml) and ABTS radical scavenging activity (RC_{50} = 1,017.98 ± 35.62 μg/ml) were the highest in GLW. Thus, the results in this study suggested that the *β*-glucan is not directly related to radical scavenging activity.

Total polyphenolic content

Polyphenolic compounds exhibit anti-cancer and antioxidant properties (Balasundram *et al*., 2006). The evidence accumulated by the *in vitro* tests showed that the antioxidant capacity of the plant extract was positively correlated with the total polyphenolic content (Canadanovic-Brunet *et al*., 2005; Oboh *et al*., 2008). The total polyphenolic content of *G. lucidum* extracts is shown in Table 4. Total polyphenolic content was highest in GLW (33.1 ± 1.9 mg GAE/g), while GLP (5.8 ± 0.6 mg GAE/g) showed the lowest polyphenolic content. The reason for the total polyphenolic content of the GLP low is thought to be due to loss of valuable compounds, such as polyphenols in the purification process of polysaccharide. As a result of radical scavenging ability and total polyphenolic content of *G. lucidum* extracts, it was concluded that the antioxidant effect of *G. lucidum* was due to the polyphenolic compound rather than the polysaccharide-type *β*-glucan.

CONCLUSION

Overall, we analyzed the antimutagenic and antioxidant activities of *G. lucidum* by partially purifying polysaccharide-type *β*-glucan from *G. lucidum* and confirming its contributing factors. *Ganoderma lucidum* contains many polysaccharides with anti-cancer activity. In this study, purified polysaccharide-type *β*-glucan showed antimutagenic activity. Therefore, it can be suggested that G. lucidum is excellent for not only preventing cancer but also in preventing mutations. Further studies on the toxicity, isolation, and characterization of *β*-glucan in *G. lucidum* should be performed. We suggest that purified *β*-glucan may be used as a raw material for future herbal medicines and health supplements.

FINANCIAL SUPPORT AND SPONSORSHIP

None.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

REFERENCES

Almeida I, Soares L, Lucio F, Cantagalli L, Reusing A, Vicentini V. Chemotherapeutical effects of the herbal medicine *Uncaria tomentosa* (Willd.) DC. Genet Mol Res, 2017; 16(3):gmr16039782.

Anand P, Kunnumakara AB, Sundaram C, Harikumar KB, Tharakan ST, Lai OS, Sung B, Aggarwal BB. Cancer is a preventable disease that requires major lifestyle changes. Pharm Res, 2008; 25(9):2097−116.

Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence, and potential uses. Food Chem, 2006; 99(1):191–203.

Bhardwaj N, Katyal P, Sharma AK. Suppression of inflammatory and allergic responses by pharmacologically potent fungus *Ganoderma lucidum*. Recent Pat Inflamm Allergy Drug Discov, 2014; 8(2):104–17.

Blois MS. Antioxidant determinations by the use of a stable free radical. Nature, 1958; 181(4617):1199–200.

Boh B. *Ganoderma lucidum*: a potential for biotechnological production of anti-cancer and immunomodulatory drugs. Recent Pat Anticancer Drug Discov, 2013; 8(3):255–87.

Boh B, Berovic M, Zhang J, Zhi-Bin L. *Ganoderma lucidum* and its pharmaceutically active compounds. Biotechnol Annu Rev, 2007; 13:265–301.

Cai Z, Wong CK, Dong J, Jiao D, Chu M, Leung PC, Lau CBS, Lau CP, Tam LS, Lam CWK. Anti-inflammatory activities of *Ganoderma lucidum* (Lingzhi) and San-Miao-San supplements in MRL/lpr mice for the treatment of systemic lupus erythematosus. Chin Med, 2016; 11(1):23.

Canadanovic-Brunet JM, Djilas SM, Cetkovic GS, Tumbas VT. Free-radical scavenging activity of wormwood (*Artemisia absinthium* L) extracts. J Sci Food Agric, 2005; 85(2):265–72.

da Costa MC, Regina M, Cilião Filho M, Linde GA, do Valle JS, Paccola-Meirelles LD, Colauto NB. Photoprotective and antimutagenic activity of *Agaricus subrufescens* basidiocarp extracts. Curr Microbiol, 2015; 71(4):476–82.

Dewanto V, Wu X, Liu RH. Processed sweet corn has higher antioxidant activity. J Agric Food Chem, 2002; 50(17):4959–64.

Di Sotto A, Mazzanti G, Savickiene N, Staršelskytė R, Baksenskaite V, Di Giacomo S, Vitalone A. Antimutagenic and antioxidant activity of a protein fraction from aerial parts of *Urtica dioica*. Pharm Biol, 2015; 53(6):935–8.

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem, 1956; 28(3):350–6.

Ferreira IC, Heleno SA, Reis FS, Stojkovic D, Queiroz MJ, Vasconcelos MH, Sokovic M. Chemical features of *Ganoderma* polysaccharides with antioxidant, antitumor and antimicrobial activities. Phytochemistry, 2015; 114:38–55.

Gontijo DC, Diaz MAN, Brandão GC, Gontijo PC, Oliveira AB, Fietto LG, Leite JPV. Phytochemical characterization and antioxidant, antibacterial and antimutagenic activities of aqueous extract from leaves of *Alchornea glandulosa*. J Toxicol Environ Health A, 2018; 81(16):805–18.

Hasnat A, Pervin M, Lim BO. Acetylcholinesterase inhibition and in vitro and in vivo antioxidant activities of *Ganoderma lucidum* grown on germinated brown rice. Molecules, 2013; 18(6):6663–78.

Huang GL, Yang Q, Wang ZB. Extraction and deproteinization of mannan oligosaccharides. Z Naturforsch C, 2010; 65(5–6):387–90.

Jena NR. DNA damage by reactive species: mechanisms, mutation and repair. J Biosci, 2012; 37(3):503–17.

Jia J, Zhang X, Hu YS, Wu Y, Wang QZ, Li NN, Guo QC, Dong XC. Evaluation of in vivo antioxidant activities of *Ganoderma lucidum* polysaccharides in STZ-diabetic rats. Food Chem, 2009; 115(1):32–6.

Kozarski M, Klaus A, Nikšić M, Vrvić MM, Todorović N, Jakovljević D, Van Griensven LJLD. Antioxidative activities and chemical characterization of polysaccharide extracts from the widely used mushrooms *Ganoderma applanatum, Ganoderma lucidum, Lentinus edodes* and *Trametes versicolor*. J Food Compost Anal, 2012; 26(1):144–53.

Krizková L, Duracková Z, Sandula J, Slamenová D, Sasinková V, Sivonová M, Krajcovic J. Fungal beta-(1-3)-D-glucan derivatives exhibit high antioxidative and antimutagenic activity in vitro. Anticancer Res, 2003; 23(3B):2751–6.

Lakshmi B, Ajith TA, Jose N, Janardhanan KK. Antimutagenic activity of methanolic extract of *Ganoderma lucidum* and its effect on hepatic damage caused by benzo [*a*] pyrene. J Ethnopharmacol, 2006; 107(2):297–303.

Lakshmi B, Ajith TA, Sheena N, Gunapalan N, Janardhanan KK. Antiperoxidative, anti-inflammatory, and antimutagenic activities of ethanol extract of the mycelium of *Ganoderma lucidum* occurring in South India. Teratog Carcinog Mutagen, 2003; 23(S1):85–97.

Ma HT, Hsieh JF, Chen ST. Anti-diabetic effects of *Ganoderma lucidum*. Phytochemistry, 2015; 114:109–13.

Mantovani MS, Bellini MF, Angeli JP, Oliveira RJ, Silva AF, Ribeiro LR. beta-Glucans in promoting health: Prevention against mutation and cancer. Mutat Res, 2008; 658(3):154–61.

Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity test. Mutat Res, 1983; 113(3–4):173–215.

Mitscher LA, Telikepalli H, McGhee E, Shankel DM. Natural antimutagenic agents. Mutat Res, 1996; 350(1):143–52.

Murata M, Kawanishi S. Mechanisms of oxidative DNA damage induced by carcinogenic arylamines. Front Biosci (Landmark Ed), 2011; 16(3):1132–43.

Nag D, Ghosh M, Mukherjee A. Antimutagenic and genoprotective effects of *Saraca asoca* bark extract. Toxicol Ind Health, 2015; 31(8):696–703.

Oboh G, Raddatz H, Henle T. Antioxidant properties of polar and non-polar extracts of some tropical green leafy vegetables. J Sci Food Agric, 2008; 88(14):2486–92.

OECD. Test No. 471: Bacterial reverse mutation test. Organization for Economic Co-operation and Development, Paris, France, 1997.

Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. Eur J Med Chem, 2015; 97:55–74.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med, 1999; 26(9–10):1231–7.

Smina TP, De S, Devasagayam TP, Adhikari S, Janardhanan KK. *Ganoderma lucidum* total triterpenes prevent radiation-induced DNA damage and apoptosis in splenic lymphocytes in vitro. Mutat Res, 2011; 726(2):188–94.

Socala K, Nieoczym D, Grzywnowicz K, Stefaniuk D, Wlaz P. Evaluation of anticonvulsant, antidepressant-, and anxiolytic-like effects of an aqueous extract from cultured mycelia of the Lingzhi or Reishi medicinal mushroom *Ganoderma lucidum* (Higher Basidiomycetes) in mice. Int J Med Mushrooms, 2015; 17(3):209–18.

Todorova T, Pesheva M, Gregan F, Chankova S. Antioxidant, antimutagenic, and anticarcinogenic effects of *Papaver rhoeas* L. extract on *Saccharomyces cerevisiae*. J Med Food, 2015; 18(4):460–7.

Veljović S, Veljović M, Nikićević N, Despotović S, Radulović S, Nikšić M, Filipović L. Chemical composition, antiproliferative and antioxidant activity of differently processed *Ganoderma lucidum* ethanol extracts. J Food Sci Technol, 2017; 54(5):1312–20.

Wang J, Yuan Y, Yue T. Immunostimulatory activities of *β*-D-glucan from *Ganoderma lucidum*. Carbohydr Polym, 2014; 102(1):47–54.

Wong KL, Wong RN, Zhang L, Liu WK, Ng TB, Shaw PC, Kwok PC, Lai YM, Zhang ZJ, Zhang Y, Tong Y, Cheung HP, Lu J, Sze SC. Bioactive proteins and peptides isolated from Chinese medicines with pharmaceutical potential. Chin Med, 2014; 9(1):19.

XiaoPing C, Yan C, ShuiBing L, YouGuo C, JianYun L, LanPing L. Free radical scavenging of *Ganoderma lucidum* polysaccharides and its effect on antioxidant enzymes and immunity activities in cervical carcinoma rats. Carbohydr Polym, 2009; 77(2):389–93.

Zahin M, Ahmad I, Aqil F. Antioxidant and antimutagenic potential of *Psidium guajava* leaf extracts. Drug Chem Toxicol, 2017; 40(2):146–53.

Zhang HN, He JH, Yuan L, Lin ZB. In vitro and in vivo protective effect of *Ganoderma lucidum* polysaccharides on alloxan-induced pancreatic islets damage. Life Sci, 2003; 73(18):2307–19.

Zhao L, Dong Y, Chen G, Hu Q. Extraction, purification, characterization and antitumor activity of polysaccharides from *Ganoderma lucidum*. Carbohydr Polym, 2010; 80(3):783–9.

Zhu M, Chang Q, Wong LK, Chong FS, Li RC. Triterpene antioxidants from *Ganoderma lucidum*. Phytother Res, 1999; 13(6):529–31.

How to cite this article:
Lee H-R, Lim H-B. Antimutagenic and antioxidative effects of polysaccharides isolated from the water extract of *Ganoderma lucidum*. J Appl Pharm Sci, 2019; 9(04):001–007.