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Research Article

Proximate analysis, phtochemical screening and antioxidant activity of different strains of ganoderma lucidum (Reishi Mushroom)

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Abstract

In this study, proximate analysis, phytochemical screening and antioxidant activity of two strains of medicinal mushroom *Ganoderma lucidum* (arbitrarily named strain 5 and 7) and their mix, cultivated in Bangladesh National Mushroom Development Institute, have been determined. The mix was used to determine whether it contains higher nutritive value than strain 5 and 7 alone. Protein content per 100 gm of strain 5, 7 and mix had been found to be 278.85mg, 298.69mg and 286.19mg, respectively. Lipid content estimated were 2.43gm, 1.96 gm and 2.4gm, respectively, while that of ash were 4.42 gm, 6.11gm and 3.93gm, respectively. *Ganoderma lucidum* strain 7 contained highest amounts of total phenol, total flavonoid, ascorbic acid and reducing sugar than the others. Among the three strains evaluated in the present study, *G. lucidum* 7 contained highest nutritional and medicinal components. Thus, *G. lucidum* 7 might be an ideal food supplement to the consumers.

Introduction

Ganoderma lucidum, a medicinal macrofungi, has been hailed in the orient since ancient times [1]. Content of numerous bio-components has allured this mushroom globally [2]. The last decade has witnessed *G. lucidum* – based pharmaceuticals, cosmetics and toiletries. These are produced from different parts of the mushroom, including mycelia, spores, and fruit body. The specific applications and attributed health benefits of *G. lucidum* include control of blood glucose levels, modulation of the immune system, hepatoprotection, bacteriostasis, and more. Though beliefs regarding the health benefits of are based largely on anecdotal evidence, traditional use and cultural mores, recent reports provide scientific support to some of the ancient claims of the health benefits of *G. lucidum* [3]. More extensive study is called for elucidating the health benefits provided by this mushroom especially for the ever increasing food and nutrition oriented therapeutic purposes. In this regard, proximate analysis and phytochemical screening of *G. lucidum* spur high.

Oxidative stress (OS) stands at the root of multiple diseases. Thus, search for potent antioxidant has got momentum. Ganoderma lucidum has been reported possessing antioxidant prowess of different sorts. Its antioxidant capacity varies from strain to strain even from parts to parts of the same strain. There is hardly any study reporting the proximate analysis, phytochemical screening and antioxidant studies of different strains and mix of different strains of this mushroom. Thus, the present study has been aimed at elucidating these unraveled issues of two strains named arbitrarily as 5 and 7 of G. lucidum and their mix.

Materials and methods

Mushroom collection and preparation

Fruiting bodies of *G. lucidum* were collected from Bangladesh

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national mushroom development institute and cut into small pieces. Small pieces were dried under the sun followed by hot air oven at the temperature 55°C until proper drying. After drying, the dried chips were ground into coarse powders using blender having high capacity grinding power. Then the powder were stored in air tight container with necessary markings for identification and kept in cool, dark and dry place for further investigation. Hot water extract (HWE) of *G. lucidum* was prepared following the method of Rahman, et al. (2016) [4].

Proximate analysis

Following the procedure established by the Association of Official Analytical Chemists (AOAC), the analyses were performed [5]. Analyses included the determination of crude protein, crude fat, ash, crude fiber, moisture and carbohydrate. The percentage of all the fractions (crude protein, crude fat and ash) were added together and subtracted from 100 to obtain the total carbohydrate percentage.

Antioxidant studies

I. Qualitative screening for antioxidant activity

Determination of phenols (Ferric chloride test)

Following the method of Soloway and Wilen (1952) [6], ferric chloride test was performed to assess the phenolics in the HWE of *G. lucidum*.

Determination of flavonoids (Alkaline reagent test)

Following the method of Rahman, et al. (2017) [7], Alkaline reagent test was performed to assess the flavonoids in the HWE of *G. lucidum*.

Determination of ascorbic acid

Following the method of Schmall and Pifer (1953) [8], HWE of *G. lucidum* was assessed for the presence of ascorbic acid.

II. Quantitative estimation of phytochemical constituents

Total phenolics content (TPC) assay

Following the modified method of Singleton, et al. (1999) [9], content of total phenolics in the HWE of *G. lucidum* was performed.

Total flavonoid content (TFC) assay

Following the modified method of Chang, et al. (2002) [10], content of total flavonoids in the HWE of *G. lucidum* was performed.

Ascorbic acid content assay

Following the modified method of Omaye, et al. (1979) [11], content of ascorbic acid in the HWE of *G. lucidum* was performed.

Total protein content assay

Following the modified method of Lowry, et al. (1951) [12], content of total protein in the HWE of *G. lucidum* was performed.

Reducing sugar content assay

Following the modified method of Nelson-Somogyi (1944) [13], content of reducing sugar in the HWE of *G. lucidum* was performed.

Antioxidant assay

Ferric reducing antioxidant power (FRAP) assay

Following the modified method of Benzie and Strain (1996) [14], ferric reducing antioxidant power (FRAP) assay of the HWE of *G. lucidum* was performed.

DPPH(1,1-diphenyl-2-picryl-hydrazyl) Free radical scavenging activity assay

The ability of the HWE of *G. lucidum* to scavenge 2,2diphenyl-1-picrylhydrazyl (DPPH) free radicals was determined according to the method Brand-williams (1995) [15] with little modification.

Statistical analyses

All the experiments were performed in triplicate and the data presented as mean ± SEM. Statistical package SPSS version 20 was used. Analyses were carried out using one-way analysis of variance (ANOVA) and the differences among means were further analyzed by least significance difference (LSD) at 95% level ($P \leq 0.05$).

Results

Proximate composition

Carbohydrate content per 100 gm of strain 5, 7 and mix had been found to be 37.18 gm, 51.65 gm and 44.91 gm, respectively (Table 1). Protein content per 100 gm of strain 5, 7 and mix had been found to be 278.85mg, 298.69mg and 286.19mg, respectively (Table 1). Lipid content per 100 gm of strain 5, 7 and mix had been found to be 2.43mg, 1.96 mg and 2.4 mg, respectively (Table 1). Fiber content per 100 gm of strain 5, 7 and mix had been found to be 12.48mg, 12.23 mg and 14.67 mg, respectively (Table 1). Ash content per 100 gm of strain 5, 7 and mix had been found to be 4.42 mg, 6.11 mg and 3.93 mg, respectively (Table 1). Moisture content per 100 gm of strain 5, 7 and mix had been found to be 10.27 mg, 12.61 mg and 12.19 mg, respectively (Table 1).

Phytochemical and antioxidant content

As shown in Table 2, content of ascorbic acid, total polyphenol, total flavonoid, reducing sugar was higher in *G. lucidum* 7 than those of *G. lucidum* 5 and mix. Similar trend was observed for FRAP and DPPH free radical scavenging potentiality. Thus, among the three strains studied in this experiment, *G. lucdium* 7 contained the highest phytochemical and antioxidant capacity.

Discussion

Strains of *G. lucidum* studied in the present experiment (strain 5, 7 and their mix), vary from each other with respect

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Table 1: Proximate analysis of *Ganoderma lucidum* strains (%dry weight).

Proximate composition	Ganoderma lucidum-*5*	Ganoderma lucidum-*7*	Ganoderma lucidum-*mix*
Carbohydrate	37.18±0.15[a]	51.65±0.1337[b]	44.91±0.17[c]
Crude protein	278.85±0.124[a]	298.69±0.188[b]	286.19±0.294[c]
Crude lipid	2.43±0.124[a]	1.96±0.188[a]	2.4±0.294[a]
Fiber	12.48±0.106[a]	12.23±0.260[a]	14.67±0.241[a]
Ash	4.42±0.0.121[a]	6.11±0.249[b]	3.93±0.187[a]
Moisture	10.27±0.43[a]	12.61±0.38[b]	12.19±0.33[a]

Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level (P ≤ 0.05) with post hoc least significance difference (LSD) test.

Table 2: Phytochemical and antioxidant content of *Ganoderma lucidum* strains.

Phytochemical and antioxidant content	Ganoderma lucidum-*5*	Ganoderma lucidum-*7*	Ganoderma lucidum-*mix*
Ascorbic acid (mg/100g)	30.58±1.13[a]	32.2±1.16[a]	31.3±1.18[a]
Total polyphenol (mg/100g)	33.30±1.98[a]	43.49±1.67[b]	34.87±1.55[c]
Total flavonoid (mg/100g)	34.09±1.66[a]	38.08±1.47[b]	36.93±1.58[ac]
Reducing sugar (g/100g)	0.922±0.02[a]	1.22±0.03[a]	1.24±0.035[a]
FRAP (µg/100g)	175.66±0.03[a]	723.33±0.04[b]	614.83±0.05[c]
DPPH scavenging (%)±	24.27±0.43[a]	23.66±0.38[a]	24.04±0.33[a]

Results are expressed as mean±SEM. Results are expressed as mean±SEM. Mean values with different lower case superscripts (a–c) represent statistically significant difference at 95% level (P ≤ 0.05) with post hoc least significance difference (LSD) test.

to nutritional and antioxidant content. Protein content of *G. lucidum* 7 is higher than that of the 5. Our findings show close proximity with those of Breene (1990) [16]. However, compared with those of Chang and Buswell (1996) [17], fibre and ash content were found higher and carbohydrate and lipid content were found lower in in this study.This variation may be due to the different growth of the strains. Also, growth substrate, the method of cultivation, stage of harvesting and time interval between harvest and measurement methods might contribute to variation in the nutritional status of the mushroom strains. Content of ascorbic acid was also higher in *G. lucidum* 7 than that of the 5. Ascorbic acid is a strong antioxidant that directly interacts with a broad spectrum of harmful reactive oxygen species, terminates the chain reaction initiated by free radicals via electron transfer, and is involved in the regeneration of other antioxidants, to their functional state.

Total phenolics content (TPC) and total flavonoids content were also higher in the *G. lucidum* 7 than that of the 5. Phenolic acid is the main phenolic compounds in mushrooms. Ganoderma-based other phenolics include gallic acid, tannic acid, protocatechuic acid and gentisic acid [4]. Flavonoids are the plant pigments responsible for plant colors and exert their health-promoting activities through their high pharmacological potentials as radical scavengers [4]. Flavonoids are the antioxidants that can prevent or delay the oxidation of substrates even when it is present in low concentrations, so as to prevent oxidation by the pro-oxidants (ROS and RNS). These non-enzymatic antioxidants (phenolic and flavonoids) react with the pro-oxidants leading to inactivation. In the redox reaction, the antioxidants act as reluctant and serve as the first-line defense to suppress the formation of free radicals. The flavonoids have strong inherent ability to modify the body's reaction to allergens, viruses and carcinogens. Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. They have anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activity [4]. Thus, the reducing ability of *G. lucidum* 7 was found to be the highest among the three samples.

Conclusion

Three strains of *G. lucidum* 5, 7 and their mix were analyzed for proximate and phytochemical composition as well as for antioxidant potentiality. The mix was used to evaluate whether it contains higher nutritive and medicinal components than strain 5 and 7 alone. Among them, strain 7 had been found the best in terms of nutritional and antioxidant capacities. Thus, this strain of *G. lucidum* could be cultivated in large scale and studied for further therapeutic usage.

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