International Journal of Medicinal Mushrooms, 17(12): 1171–1177 (2015)

Antioxidant Potential of Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (Higher Basidiomycetes) Cultivated on *Artocarpus heterophyllus* Sawdust Substrate in India

P. Rani,* Merlin Rajesh Lal, Uma Maheshwari, & Sreeram Krishnan

Department of Biotechnology, PSG College of Technology, Coimbatore, Tamil Nadu, India

*Address all correspondence to: P. Rani, Department of Biotechnology, PSG College of Technology, Peelamedu, Coimbatore 641 004, Tamil Nadu, India; Tel.: +91-422-2572177; Fax: +91-422-2573833; rani@bio.psgtech.ac.in

ABSTRACT: The artificial cultivation of *Ganoderma lucidum (*MTCC1039) using *Artocarpus heterophyllus* as sawdust substrate was optimized and free radical scavenging activities of the generated fruiting bodies were investigated**.** The choice of *A. heterophyllus* as substrate was due to its easy availability in South India. Sawdust supplemented with dextrose medium yielded better spawn hyphae and early fruiting body initiation (15 days). The biological yield obtained was 42.06 ± 2.14 g/packet and the biological efficiency was 8.41 ± 0.48%. Both aqueous and methanolic extracts of fruiting body were analyzed for radical scavenging activity. Methanolic extract showed maximum scavenging activity for 1,1-diphenyl-2-picrylhydrazyl (IC_{50} = 290 µg/ml) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (IC_{50} = 580 µg/ml), whereas aqueous extract had better scavenging for ferric reducing antioxidant power (IC_{50} = 5 µg/ml). Total phenolic content and total antioxidant capacity were significantly higher in methanolic extract ($p < 0.01$). A positive correlation existed between the phenolic content and antioxidant activity. Our results indicated that fruiting bodies of *G. lucidum* cultivated in sawdust medium possess antioxidant property, which can be exploited for therapeutic application.

KEY WORDS: medicinal mushrooms, *Ganoderma lucidum*, cultivation, dextrose, sawdust, free radical scavenging, DPPH, ABTS, FRAP

ABBREVIATIONS: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; **BHA,** butylated hydroxyanisole; **BHT,** butylated hydroxyl toluene; **DPPH,** 1,1-diphenyl-2-picrylhydrazyl; **FRAP,** ferric reducing antioxidant power assay; **RSA,** radical scavenging assay; **TPTZ,** tripyridyltriazine

I. INTRODUCTION

Lingzhi or reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst. (Ganodermataceae, higher Basidiomycetes), is an utmost important polypore mushroom renowned for its medicinal properties. This oriental fungus is widely used as a remedy for promotion of health and longevity in China and other Asian countries.[1] Both fruiting bodies and cultured mycelia of *G. lucidum* are effective in the treatment of hepatopathy, hypertension, hyperglycemia, and neoplasia.[2] The fruiting bodies, culture mycelium, and culture broths derived from a list of 650 species and seven intraspecific taxa from 182 genera of higher hetero- and homobasidiomycetes are found to contain pharmacologically active polysaccharides.[3] Compared to culture broth, fruiting body extracts contain higher polysaccharide content. It is also reported that the concentration of polysaccharides in medicinal mushrooms varies with different stages of the fruiting body and storage conditions.[4,5]

Biologically active antitumor and antioxidant polysaccharides are reported in higher Basidiomycetes mushrooms.[6] Phenolics, triterpens, and polysaccharides in *G. lucidum* are reported to play a major role in curtailing oxidative stress. The ability of different mushroom species to utilize various substrates depends on both fungal and substrate association. There are many artificial fungal cultivation methods in use, among which the sawdust method possesses

1521-9437/15/$35.00

a distinct advantage. The sawdust of tree species like *Mangifera indica, Artocarpus heterophyllus*, *Dalbergia sissoo*, *Eucalyptus camaldulensis*, *Albizia procera*, *Borasus flabellifer*, and *Albizia richardiana* have been widely used as substrates for *G. lucidum* cultivation.[6] *Artocarpus heterophyllus* (jackfruit) can be utilized as sawdust substrate due to its wide cultivation in tropical Asia.

Mushrooms readily accumulate many bioactive metabolites.[7] The phenolic compounds contribute directly to the antioxidative action,[8] and it was also reported that in some common edible mushrooms, the total phenolic compounds were directly associated with antioxidant activity and also participated in stabilizing lipid peroxides.[9,10]

The objective of the present study was to optimize the growth conditions for generation of *G. lucidum* fruiting bodies using *A. heterophyllus* sawdust substrate and to validate its antioxidant properties using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), and ferric reducing antioxidant power (FRAP) assays.

II. MATERIALS AND METHODS

A. Mushroom Material

G. lucidum (MTCC 1039) obtained from the Microbial Type Culture Collection & Gene Bank (MTCC; Chandigarh, India) was used in this study. The vegetative phase of *G. lucidum* was grown using malt extract medium consisting of 1 g peptone, 20 g malt extract, and 20 g nutrient broth (Himedia, Mumbai, India), pH 5.0. The stock culture was also maintained on the same 1 liter of the medium. To study the effect of carbon source such as dextrose, fructose, maltose, sucrose, and lactose on the mycelial growth, an equal quantity (20 g/l) was provided independently in the medium. The fungal biomass was collected after 14 days and dried at 60°C for 6 h and the dry weight was determined.

B. Spawn Preparation

The substrate for spawn production was prepared by using corn seeds. Fresh corn (1 kg) was cooked and spread in clean filter tissue, dried, and then mixed with different combinations of calcium carbonate, dextrose, and sawdust as shown in Table 1. The mixtures were then packed in bags, sterilized, and inoculated with mycelium of *G. lucidum* grown in malt extract agar. The bags were incubated vertically at 27°C for 40 days in complete darkness. The quality of developed spawn was analyzed visually for mycelial growth.

TABLE 1: Media Composition (in Grams) for Spawn Production (1 kg) in Six Groups*

Group	Dextrose	$CaCO_3$	Sawdust
1	15	–	10
2	15	–	–
3	15	15	10
4	15	15	–
5	–	15	10
6	–	15	–

*Composition is for per bag cultivation.

C. Production of *G. lucidum* Fruiting Bodies

For generation of fruiting bodies, the bags were filled with sawdust medium containing 1 kg sawdust obtained from hardwood of *A. heterophyllus*, 15 g rice bran, and 20 g dextrose. The moisture content was maintained at 60% and the bags were sterilized and cooled. The bags were then spawned at 25–30°C with the grain spawn developed on calcium carbonate, dextrose, and sawdust containing medium. After primordial formation, a cut of size 1 × 1 cm^2 was made in the middle of the bags and it was incubated in more than 800 lux light at 27°C with 85% humidity. Proper aeration was maintained and water was sprayed four to five times a day. The fruiting bodies were harvested, dried, and the biological yield (in grams/packet) was calculated. The biological efficiency was calculated using the formula:

Biological efficiency (%) = [Total biological yield (g)/Total dry substrate used (g)] × 100.

D. Extraction of Dried *G. lucidum*

G. lucidum fruiting bodies were dried at room temperature and 100 g was taken and chopped well. Extraction of antioxidant metabolites from sample was done with 100% (v/v) methanol and water as solvents independently for 10 h in a soxhlet apparatus. The extracts were dried using a vacuum flash evaporator and stored in an amber bottle for further use. The obtained crude extract was resuspended in respective solvents to obtain a final concentration of 0.2–1.0 mg/ml for DPPH, 1.0 mg/ml for ABTS assays, and 5–30 µg/ml for FRAP assay.

E. Measurement of Total Phenolic Content

The fungal phenolic content was determined using the Folin-Ciocalteu method.[11] One milliliter of the extract was added to 10 ml of the deionized water and 0.5 ml of Folin's reagent (2.0 N) and 3.0 ml of NA_2CO_3 (200 mg/ml), and mixed well. The mixture was vortexed and allowed to stand at room temperature for 15 min. The absorbance of the sample was measured at 725 nm using a UV-visible spectrophotometer. Gallic acid was used as a reference standard and the phenolic content was calculated as equivalents of gallic acid/g dry weight.

F. Measurement of Total Antioxidant Property

The total antioxidant capacity of the extracts was estimated by the phosphomolybdate method. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate-Mo (V) complex at acidic pH 5.0. An aliquot of 1.0 ml extract was combined with 1.0 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the solution was measured at 695 nm against solvent blank in a UV-visible spectrophotometer. The antioxidant capacity of the extract was expressed as equivalents of butylated hydroxyl toluene (BHT)/g dry weight.

G. Radical Scavenging Assays

1. DPPH

The DPPH scavenging activity of mushroom was estimated according to the procedure described by Shirwaikar et al.[12] First, an aliquot of 1.0 ml of the sample extracted at different concentration was added to the test tubes with 0.5 ml of 0.1 mM DPPH radical in 1.5 ml of methanol. The mixture was shaken vigorously and left to stand for 20 min in the dark at room temperature. The absorbance of the reaction mixture was determined at 517 nm in a UV-visible spectrophotometer. BHT (0.2–1.0 mg/ml) was used as reference standard.

2. ABTS

The ABTS radical scavenging assay was estimated according to the method of Shirwaikar et al.[12] ABTS cationic solution was prepared by mixing 1 ml of 100 mM potassium dichromate and 25 ml of 10 mM ABTS and was kept overnight in the dark at room temperature. Five-hundred microliters of ABTS radical cationic solution was added to 2.0 ml of the crude extract of varying concentration. The mixtures were left undisturbed for 15 min and the absorbance was recorded at 734 nm. BHT (0.2–1.0 mg/ml) was used as a reference standard.

3. FRAP

The ferric reducing ability of the extracts was determined by measuring the blue colored Fe (II)-triazine compound formed from oxidized Fe (III) by the action of electron donating antioxidants.[13] The FRAP reagent was prepared with 2.5 ml of 10 mM TPTZ, 2.5 ml of 20 mM $FeCl_3$ in 25 ml of 0.3 M acetate buffer; 1.0 ml of suitably diluted extracts were mixed with 500 µl of FRAP reagent and incubated at 37°C for 30 min. The absorbance of the mixtures was measured at 595 nm. BHT (5–30 µg/ml) was used as a reference standard.

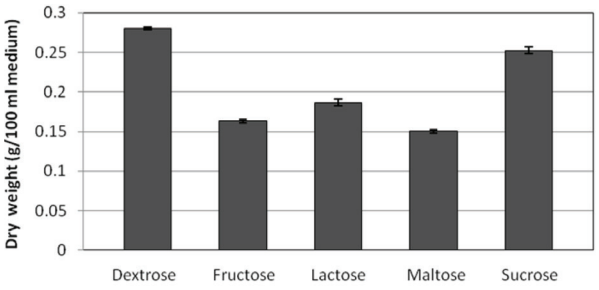

FIG. 1: Effect of carbon sources on mycelial biomass yield. Medium was supplemented with different carbon sources at the concentration of 20 g/l.

H. Statistical Analysis

All the experiments were performed in triplicate and the results were expressed as means ± standard deviations. The results were analyzed using the Student's *t*-test and correlation analysis was done using SPSS 10.0. software.[14]

III. RESULTS AND DISCUSSION

A. Effect of Carbon Source on Mycelia Growth

In the present study, to evaluate the effect of different carbon sources, *G. lucidum* was cultured on malt extract medium with different carbon sources. The fungal biomass was collected after 14 days and the dry weight was determined. The mycelial growth was maximum in dextrose-containing medium, compared to media containing fructose and maltose. Dextrose produced maximum amount of biomass followed by sucrose, lactose, fructose, and maltose, as shown in Fig. 1. Dextrose has been widely used as a carbon source for the cultivation of mushrooms and there are reports that cellulose and glucose are good carbon sources for the biomass production of *G. lucidum*.[15]

B. Quality of Spawn

Spawn grains are reported to be very effective in introducing pure fungal cultures into different growth substrates as well as to increase mushroom yield.[16] In the present study, corn was used for spawn production with various combinations of additives, namely, $CaCO_3$, dextrose, and sawdust, as shown in Table 1. After 40 days of incubation, the quality of spawn was analyzed based on density of the mycelial growth. Among the different combinations of medium used in the study, spawn developed in the medium containing calcium carbonate, dextrose, and sawdust was found to possess better quality than the other combinations. The comparative quality of spawn in different medium is as follows: calcium carbonate + dextrose + sawdust > calcium carbonate + dextrose = dextrose + sawdust > calcium carbonate + dextrose > calcium carbonate. Reports also show that this formulation was successful even in scale-up operations by mushroom growers in the United States and Canada.[17] Wheat and corn spawns were reported to be similar in characteristics and both induced the longest stipe lengths and heaviest carpophore net weights for *Psathyerella atrumbonata*.[18]

C. Generation of Fruiting Bodies

G. lucidum spawn was inoculated on the sawdust medium supplemented with rice bran and dextrose. At the end of the vegetative spawn run, whitish mature mycelia started to form dense growth and gradually developed into primordia and rose above the surface of the medium as whitish rounded mounds.

The formation of fruiting bodies was initiated on the 11th day and subsequently matured caps were harvested. After the first harvest, the bags were incubated again and the fruiting bodies produced were harvested. For the *A. heterophyllus* sawdust-supplemented medium, the biological yield obtained was 42.06 ± 2.14 g/packet and the biological efficiency was 8.41 ± 0.48%. The biological efficiency varied widely depending on the kinds of sawdust used, bran, and their combinations.[19] Biological efficiencies of *G. lucidum* grown on the sawdust of different tree species was reported as follows: *M. indica* (10.25%), *Tectona grandis* (7.00%), *Albiza procera* (9.63%), and mixed sawdust (11.00%).[20] The results of the present study indicated that sawdust of *A. heterophyllus* could also be used as an effective substrate for the growth of *G. lucidum* based on

biological efficiency when compared with sawdust obtained from other tree species.

D. Free Radical Scavenging Potential of Fruiting Bodies Extracts

The fruiting body of *G. lucidum* was extracted with methanol and aqueous solvents independently. The solubles present in the methanolic and aqueous extracts were 3.375 and 6.825 g per 100 g dry weight, respectively. The extracts were assessed for their antioxidant potential using DPPH, ABTS, and FRAP assays.

1. DPPH and ABTS Assays

In the present study, the methanolic extract of *G. lucidum* showed better DPPH scavenging activity (IC_{50} = 290 µg/ml) compared to the aqueous extract (IC_{50} = 2389 µg/ml) (Fig. 2A). This could be due to the presence of an increased amount of phenolic and triterpenoic compounds in the methanolic extract. Similar results have also been reported elsewhere.[21–23]

In ABTS assay, direct generation of ABTS radical monocation ($ABTS^{*+}$) without the involvement of any intermediary radical was analyzed. The radical cation was formed prior to addition of the antioxidant test system, rather than the continual generation of the radical in the presence of antioxidant. Radical scavenging activity of extract was evaluated by monitoring decolorization. This method is commonly used to screen both lipophilic and hydrophilic antioxidants. In the present study, the ABTS scavenging activity (Fig. 2B) of methanolic extract (IC_{50} = 580 µg/ml) was higher when compared to aqueous extract (IC_{50} = 1419 µg/ml). The reduced scavenging activity of aqueous extracts observed in the present study could be due to changes in the phenolic and triterpenoic antioxidants induced by the hydrogen bonding nature of polar solvents as reported by Pedrielle et al.[24]

2. FRAP Assay

The ferric reducing activity (Fig. 2C) of mushroom extracts was also monitored. Aqueous extracts

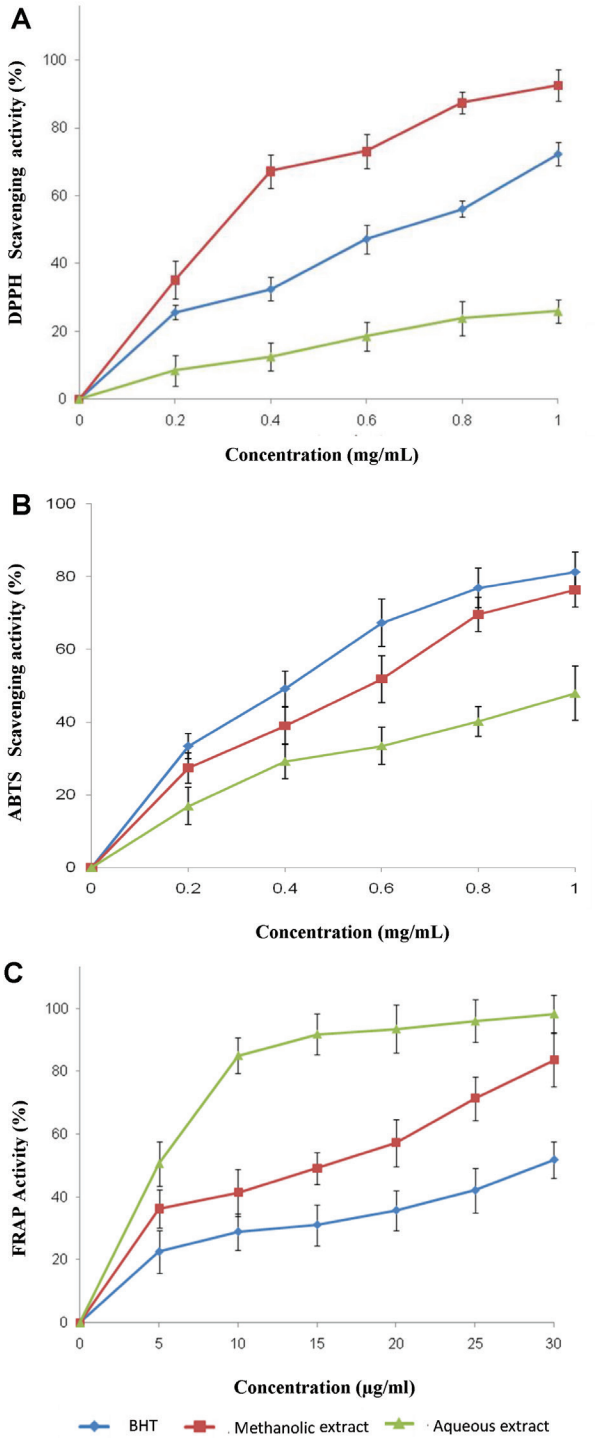

FIG. 2: Antioxidant capacity assays of aqueous and methanolic extracts of *Ganoderma lucidum* fruiting bodies: (A) DPPH scavenging activity. (B) ABTS radical scavenging activity. (C) ferric reducing antioxidant power assay (FRAP).

TABLE 2: Phenolic Content and Total Antioxidant Capacity of *Ganoderma lucidum*

Extract	Phenolic Compounds (mg gallic acid equivalent/g dry weight)	Antioxidants (mg BHT equivalent/g dry weight)
Methanolic extract (n = 3)	39.05 ± 0.65	95.89 ± 0.14
Aqueous extract (n = 3)	32.62 ± 0.61 [a]	85.90 ± 0.21 [a]

Values are expressed as mean ± standard deviation.
[a] Significant at $p < 0.01$ (t-test)

showed better scavenging effect (IC_{50} = 5 μg/ml) than the methanolic extracts (IC_{50} = 16.8 μg/ml). FRAP scavenging activity of aqueous extract probably could be contributed by the polysaccharides present in the extract. Isolated polysaccharides from *G. lucidum* were also shown to augment the superoxide dismutase activity in mice contributing to the prevention of radiation damage.[25]

3. *Phenolic Content and Antioxidant Capacity*

Phenolics are known to contribute largely to antioxidant potential. In the present study, the concentration of phenolic compounds (Table 2) was significantly greater in the methanolic extract ($p < 0.01$) when compared to aqueous extracts of *G. lucidum*. The total antioxidant content was also higher in the methanolic extract ($p < 0.01$) compared to the aqueous extract, suggesting that phenolics could be the major antioxidants in *G. lucidum*. Our results are in accordance with Rawat et al.,[26] who also reported that the phenolic content in *G. lucidum* was higher in the methanolic extracts. Polyphenols present in the methanolic extracts could contribute to the increased antioxidant capacity. Goli et al.[27] also reported that the concentration of phenolics in the extract was dependent on the type of solvent used. Positive correlation was also observed between phenolic content and antioxidant capacity in methanolic extract ($r = 0.98$, $p < 0.01$) and aqueous extract ($r = 0.97$, $p < 0.01$). Apart from phenolics, other phytochemicals can also contribute to the antioxidant property.[23] Studies have found that triterpenes and polysaccharides are the other major physiological constituents contributing to the antioxidant capacity.[1,28]

The results of the present study also emphasize that both methanolic and aqueous extracts of *G. lucidum* fruiting body cultivated on sawdust medium have potent antioxidant activity as evidenced from the DPPH, ABTS, and FRAP assays. This study provides new insights about the cultivation methods that could enhance the yield of fruiting bodies, which are a rich source of antioxidants.

IV. CONCLUSIONS

The results of the present study revealed that dextrose could be used as a good carbon source for the mycelial growth of *G. lucidum* on *A. heterophyllus* sawdust medium. Dextrose supplementation produced good quality spawn and better generation of fruiting bodies with biological efficiency of 8.41 ± 0.4%. The aqueous and methanolic methanolic extracts of fruiting bodies exhibited a potent free radical scavenging activity as shown by ABTS and FRAP assays. This could be contributed by both phenolic compounds and polysaccharides present in the extracts. Positive correlation was observed between the phenolic content and antioxidant capacity. Thus, *G. lucidum* could be cultivated at a large scale using jack tree sawdust as substrate and the generated fruiting bodies could be exploited for therapeutic application due to their high free radical scavenging activity.

REFERENCES

1. Shiao MS, Lee KR, Lin LJ, Wang CT. Natural-products and biological activities of the Chinese medicinal fungus Ganoderma lucidum. Food Phytochem Cancer Prevent. 1994;547:342–54.
2. Hsu SC, Ou CC, Li JW, Chuang TC, Kuo HP, Liu JY, Chen CS, Lin SC, Su CH, Kao MC. Ganoderma tsugae extracts

inhibit colorectal cancer cell growth via G(2)/M cell cycle arrest. J Ethnopharmacol. 2008;120:394–401.
3. Wasser SP. Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med Mushrooms. 2010;12(1):1–16.
4. Minato K, Mizuno M, Ashida H, Hashimoto T, Terai H, Tsuchida H. Influence of storage conditions on immunomodulating activity of Lentinus edodes. Int J Med Mushrooms. 1999;1:243–50.
5. Minato K, Mizuno M, Kawakami S, Tatsuoka S, Denpo Y, Tokimato K Tsuchida H. Changes in immunomodulating activities and content of antitumour polysaccharides during growth of two mushrooms, Lentinus edodes (Berk.) Sing and Grifola frondosa (Dicks:Fr.) S.F. Int J Med Mushrooms. 2001;3:1–7.
6. Wasser SP. Medicinal mushroom science: current perspectives, advances, evidences and challenges Biomed J. 2014;37(6):345–56.
7. Halliwell B. Antioxidants in human health and disease. Annu Rev Nutr. 1996;16:33–50.
8. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of harnjyur (Chyrsanthemum morifolium Ramat). Lebensmittel-Wissenschaft and Technologie. 1999;32:269–77.
9. Barros L, Ferreira MJ, Queirσs B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem. 2007;103:413–9.
10. Yen GC, Duh PD, Tsai CL .Relationship between antioxidant activity and maturity of peanut hulls. J Agric Food Chem. 1993;41:67–70.
11. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Technol Metallurgy. 2005;40:255–60.
12. Shirwaikar A, Shirwaikar A, Rajendran K, Punithaa ISR. In vitro antioxidant studies on the benzyl tetra isoquinoline alkaloid berberine. Biol Pharm Bull. 2006;29:1906.
13. Benzie IEF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal Biochem.1996;23:70–6.
14. SPSS. SPSS Inc., 10th ed. Chicago: SPSS; 1999.
15. Babitskaya NG, Bisko NA, Scherba VV, Mitropolskaya NY. Some biologically active substances from medicinal mushroom Ganoderma lucidum (W. Curt.:Fr.) P. Karst. Aphyllophoromycetidae). Int J Med Mushrooms. 2003;5:301–6.
16. Kadiri M. Production of grain mother and planting spawns of Lentinus subnudus Berk. Biosci Res Commun. 1999;11:307–14.
17. Nwanzem PI, Khanm A, Amehm JB, Umoh VJ. The effect of spawn grains, culture media, oil types and rates on carpophore production of Lentinus squarrosulus (Mont.) Singer. Afr J Biotechnol. 2005;4:1285–9.
18. Chen AW. Growing Ganoderma mushrooms. Mushroom grower's handbook 1: Oyster mushroom cultivation [Internet] Mushword; 2004 [cited 2015 Jan 11]. Available from Netlibrary: http://mushroomtime.org/wp-content/uploads/2014/06/02-Mushroom-Growers-Handbook-1-Oyster-Mushroom-Cultivation-MUSHWORLD.pdf.
19. Erkel E I. The effect of different substrate mediums on yield of Ganoderma lucidum (Fr.) Karst. J Food Agric Environ. 2009;7:841–4.
20. Hossain K, Sarker NC, Kakon AJ, Khan AS, Ahmed S. Cultivation of reishi mushroom (Ganoderma lucidum) on sawdust of different tree species. Bangladesh. J Mushrooms. 2009;3:1–5.
21. Ajith TA, Janardhanan KK. Antioxidant and antihepatotoxic activities of Phellinus rimosus (Berk.) Pilat. J Ethnopharmacol. 2002;81:387–91.
22. Gezer K, Duru M E, Kivrak I, Turkoglu A, Mercan N, Turkoglu H, Gulcan S. Free radical scavenging capacity and antimicrobial activities of wild edible mushroom from Turkey. Afr J Biotechnol. 2006;5:1924–8.
23. Saltarelli R, Ceccaroli P, Lotti M, Zambonelli A, Buffalini M, Casadei L, Vallorani L, Stocchi V. Biochemical characterisation and antioxidnat activity of mycelium of Ganoderma lucidum from central Italy. Food Chem. 2009;116:143–51.
24. Pedrielle P, Pedulli GF, Skibsted LH. Antioxidant mechanism of flavonoids. Solvent effect on rate constant for chain breaking reaction of quercetin and epicatechin in autooxidation of methyl linolenate. J Agric Food Chem.2001;4:3034–40.
25. Pillai GT, Nair CKK, Janardhanan KK. Polysaccharides isolated from Ganoderma lucidum occurring in Southern parts of India, protects radiation induced damages both in vitro and in vivo. Environ Toxicol Pharmcol. 2008;26:80–5.
26. Rawat A, Mohsin M, Negi PS, Sah AN, Singh S. Evaluation of polyphenolic contents and antioxidant activity of wildly collected Ganoderma lucidum from central Himalayan hills of India. Asian J Plant Sci Res. 2013;3:85–90.
27. Goli AH, Barzegar M, Sahari MA. Antioxidant activity and total phenolic compounds of pistachio (Pistachia vera) hull extracts. Food Chem. 2005;92:521–5.
28. Tseng YH, Yang JH, Mau JL. Antioxidant properties of polysaccharides from Ganoderma tsugae. Food Chem. 2008;107:732–8.