

Separation of Biomolecules from *Ganoderma lucidum* with Special Reference to Polysaccharides

R. Sasidhara¹, B. Vinuthna¹ and T. Thirunalasundari^{2*}

¹Department of Biotechnology, Dhanalakshmi Srinivasan College of Arts & Science for Women, Perambalur - 621 212, India.

²Department of Biotechnology, Bharathidasan University, Tiruchirappalli - 620 024, India.

(Received: 27 December 2011; accepted: 21 February 2012)

Mushrooms have been valued as high tasty nutritional and nutraceutical foods throughout the world. At present 270 species of mushrooms are known to have various nutritional and therapeutic properties. Numerous species of wild mushrooms are consumed as a delicacy all over the world. Credible evaluation of their nutritional value has so far been limited due to fragmentary knowledge of their composition and mainly due to the very limited information on the availability of their constituents. The biomolecules like polysaccharides, proteins produced by *Ganoderma lucidum* gain importance for their antimicrobial and antioxidant properties. Polysaccharides of mushrooms are used for their pharmacological properties as immunostimulators. The mushrooms screened in this study are good producers of exopolysaccharides (EPS) and intracellular polysaccharides (IPS). *Ganoderma lucidum* was the best producer of polysaccharide among all the strains. The significance of EPS and IPS was well established in medicine (1-3). They are well known due to their antidiabetic, antitumor and hepatoprotective activities, and so are likely to be used in the preparation of novel drugs. It is suggested that a thorough study on the optimization and characterization of these biomolecules is essential to exploit this mushroom on an industrial scale.

Keywords: Mushrooms, nutraceutical, immunostimulators, biomolecules, *Ganoderma lucidum*, polysaccharides, medicinal properties.

Mushroom is a macrofungus with a distinctive fruiting body which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand⁴. Many bioactive compounds have been found in mushrooms, some of which inhibit the growth of cancer cells *in-vitro*, have antiviral activity *in-vitro* or have other health benefits *in-vivo*. These substances may be useful as starting materials for the development of chemotherapeutic agents in cancer treatment and for other ailments⁵.

There are many biologically active metabolites present in the mycelium of mushrooms grown in submerged culture⁶. Researchers investigating *Ganoderma* for bioactivity have found active compounds in the liquid cultivated mycelium⁷⁻⁹ and in the culture medium^{10, 11}. The spores have also been found to be a valuable source of bioactive compounds^{12, 13}.

Ganoderma is a member of the polypores, a group of fungi characterized by the presence of pores instead of gills on the lower side of the fruiting body. *Ganoderma lucidum* is considered to be one of the most beautiful shelf fungi. It is distinguished by its varnished, red surface. It is a saprophytic fungus that tends to grow more prolifically in warm climates on decaying hardwood logs and stumps. Under commercial cultivation

* To whom all correspondence should be addressed.
Tel.: +914312407086; Fax: +914312407045
E-mail: drttns@bdu.ac.in, drttns@gmail.com

conditions, *Ganoderma lucidum* is normally grown on artificial saw dust or logs.

The major bioactive polysaccharides isolated from *Ganoderma* species are glucans, particularly β -1-3 and β -1-6 D-glucan. The basic structure is β -1-3 D-glucopyranan with 1 to 15 units of β -1-6 monoglucosyl sidechains¹⁴. Other polysaccharides of *Ganoderma lucidum* are heteropolysaccharide glycoprotein (polysaccharides conjugated to proteins) or a group of polysaccharides known as ganoderans A, B and C¹⁵.

Attention has recently been focused on the development of immunotherapeutic compounds that can identify and eliminate cancer cells as foreign matter (non-self), or be able to act on substances such as immunopotentiators, immunoinitiators and biological response modifiers (BRM)¹⁶.

Garibova *et al.*,¹⁷ believed that therapeutic properties of *Ganoderma lucidum* are determined by its unique chemical composition. It contains proteins, lipids, polysaccharides, phenolic compounds, steroids, terpenoids, and microelements, including germanium, which ensures intensive oxygen consumption by cells. Phenolic compounds have significant biological and pharmacological properties, and some have demonstrated remarkable ability to alter sulfate conjugation. The bioactivity of phenolics may be related to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals¹⁸. *Ganoderma lucidum* has been in many clinical studies with animals and humans, reporting the beneficial results. The high-molecular-weight polysaccharides from the cell walls of *G. lucidum* are physiologically active. They are used against various diseases like diabetes, Alzheimer's disease, dyspnea, leucopenia and insomnia. They also show hepatoprotective effect in animal and human studies. Hence the present study was undertaken with the objective of screening the potential mushrooms for useful polysaccharides and determining the content of biomolecules in mushrooms.

MATERIALS AND METHODS

Isolation and maintenance of macrofungi

The fruiting bodies of basidiomycetous

fungi were collected in rainy season from the surroundings of Warangal district, Andhra Pradesh from decaying wood, living trees, soil and decaying leaf litter. A total of 10 fungal species were isolated, which were mostly belonging to Polyporales, Aphyllophorales or Agaricales. The characteristics of the fruit bodies were corticoid, effused, steroid, effuoro reflexed, coralloid, dimidiate, cyphelloid, polyporoid, agaricoid or boletoid. The fruit bodies were photographed before collection for identification and then were preserved by sealing in polythene bags. A small piece of fruiting body was dipped in 0.01% mercuric chloride to remove the surface contamination and washed several times with distilled water to remove the traces of mercuric chloride and transferred aseptically on 3% malt agar slants. Slants were incubated for 5 to 7 days and were observed. The mycelium collected from the growing edge was transferred into fresh malt agar slant and incubated further for 5 to 7 days. This was repeated 2 to 3 times to get pure isolates and was stored at 4°C. Approximately 2 mm² of mycelial mat was removed from slants and was allowed to grow on malt agar slants for 7 days and was used for further studies.

Screening for exopolysaccharide and endopolysaccharide

Exopolysaccharide

A liquid culture medium (g/L) with peptone 1.0; yeast extract 2.0; K₂HPO₄ 1.0; MgSO₄·7H₂O 0.2; (NH₄)₂SO₄ 5.0; glucose 20.0 (pH 6.0) was selected in preliminary studies as it was ideal for exopolysaccharide production by basidiomycetes (19). Erlenmeyer flasks containing 100ml of sterilized culture media were inoculated with the suspension of fungal mycelium grown on malt extract agar slants and were incubated at 27°C for both still and shake flask cultures. Shake flask cultures were kept on rotary shaker at 150g for 14 days.

Endopolysaccharide

The mushrooms were grown in liquid culture media for 7 and 14 days and the lower part of mycelial mat was separated from the media. Mycelial mat was washed with distilled water, lyophilized and weighed for their dry weight. The dried mycelium was washed with 80% ethanol, submerged in distilled water, autoclaved at 120°C (1.5 atm) for 1 hour and centrifuged. The filtrate was mixed with equal volume of isopropanol,

stirred vigorously and then left overnight at 4°C for precipitation. The precipitated intracellular polysaccharide was centrifuged at 3,000 g for 20 min, redissolved with distilled water and centrifuged again. The IPS was weighed after lyophilization of the supernatant.

Screening for polysaccharide

7 and 14 days culture was filtered to separate fungal biomass which was washed twice with distilled water and quantified as dry weight (105°C to constant weight). Isopropanol was added to the culture filtrate (1:1 v/v) and after 24hrs at 4°C, the precipitated biopolymer was separated by centrifugation (4,000 g for 15 min) and also quantified as dry weight.

Preparation of sample

1 gm of dry mat was macerated in 10 ml of Tris-HCl (pH 6.6) and centrifuged at 300 g for 15 min. 5 ml of TCA was added to the supernatant. The precipitate formed was used for protein estimation and the supernatant was taken to quantify carbohydrate. 10 ml of 80% ethanol was added to 1 gm of dry mat, macerated and then centrifuged at 300 g for 30 min and the supernatant was taken to quantify amino acids.

Quantification of biomolecules of *Ganoderma lucidum*

The polysaccharides in the mat of the *Ganoderma lucidum* were analyzed by anthrone method²⁰. The proteins in the mat of the *Ganoderma lucidum* were analyzed according to Lowry *et al.*,²¹. Amino acid content was quantified by ninhydrin method²⁰. Total phenolic constituent was determined by employing the methods given in the literature²² involving Folin–Ciocalteu reagent and gallic acid as standard.

RESULTS AND DISCUSSION

Based on the fruit body characters, the fungi were identified by Friesian classification system proposed by Swedish Mycologist Elias Fries²³. All the species produced white coloured mycelium and grow by spreading over the medium two days after incubation. The collected fungal strains were screened for the production of EPS. All the strains produced exopolysaccharide in different quantities in still culture (Table 1). The best yield was shown by *Ganoderma lucidum* which produced a biomass of 6.8 g.dr.wt/l after

Table 1. Screening of polysaccharides from Basidiomycetes Species

Basidiomycetes Species	Incubation Period in days	Biomass g.dr.wt/l	IPS (mg/g)	EPS (g/l)
<i>Ganoderma lucidum</i>	7	6.8	551.70	6.8
	14	6.2	482.70	6.2
<i>Schizophyllum commune</i>	7	1.2	325.00	5.2
	14	3.0	309.52	5.8
<i>Daedaleopsis confragosa</i>	7	4.2	500.00	5.0
	14	4.4	200.00	3.6
<i>Pleurotus ostreatus</i>	7	4.6	450.00	5.8
	14	5.0	277.00	3.4
<i>Abortiporus biennis</i>	7	3.2	318.00	5.8
	14	3.6	311.00	5.4
<i>Trichaptum biforme</i>	7	1.6	352.94	5.2
	14	5.0	300.00	3.4
<i>Tephrocybe ambusta</i>	7	1.8	470.00	5.0
	14	1.8	333.00	4.8
<i>Pycnoporus coccineus</i>	7	4.0	142.00	4.6
	14	4.8	360.00	4.6
<i>Calvatia sculpta</i>	7	3.2	181.18	4.6
	14	3.6	142.85	4.0
<i>Antrodiella semisupiforme</i>	7	1.0	500.00	6.6
	14	1.8	200.00	4.6

Table 2. Biomolecules of *Ganoderma lucidum* 10 and 20 days after incubation

Biomolecules	Quantity of Biomolecules in mg/g Duration of Incubation	
	10 th Day	20 th Day
Carbohydrates	300	160
Proteins	260	180
Amino Acids	520	310
Phenols	22	35

7 days of incubation and 6.2 g.dr.wt/l after 14 days of incubation. The least biomass was produced by *Antrodiella semisupiforme* after 7 and 14 days of incubation i.e., 1.0 g.dr.wt/l and 1.8 g.dr.wt/l respectively. There is no relation between biomass and EPS production and in some cases a considerable decrease of biopolymer was observed after 14 days of incubation. Rosana *et al.*,²⁴ reported the screening of basidiomycetes for the production of EPS and biomass in submerged culture. According to them, different strains of *Schizophyllum commune*, *Pycnoporus sanguinus* and *Trametes villosa* showed different biomass and polymer production. In this study, *Ganoderma lucidum* was found to be the best producer of IPS with a yield of 551 and 482 mg/g after 7 and 14 days of incubation respectively. Also, *Ganoderma lucidum* produced the highest content of EPS when compared to other strains with a yield of 6.8 g/l and 6.2 g/l after 7 and 14 days of incubation.

Biomolecules like Proteins, Carbohydrates, Amino Acids, and Phenols were estimated in *Ganoderma* on 10th day and 20th day of incubation (Table 2). Among the different biomolecules looked for, amino acids were found to be maximum, which ranged between 310–520 mg/g. Kim *et al.*,²⁵ studied the amino acid components of *Ganoderma lucidum* and a mixture of glutamine and glutamic acid, indicated glx, was the greatest component at 1.01 mg/g. The presence of appreciable amount of amino acids in *Ganoderma* sp. makes it a potential valuable product for future research in the food industry and medical drugs development.

The content of carbohydrates was 300 mg/g after 10 days of incubation and reduced after 20 days of incubation (160 mg/g). The reduction was around half with increase in number of days

of incubation time. As the main carbon and energy source for most fungi, carbohydrate may play an important role in cell growth and polysaccharide production. Total content of soluble sugars or polyols were determined in other species of mushrooms like, *Agaricus blazei* and *Boletus edulis* to be 150-225 mg/g²⁶. They noticed an amount of 6.96-20.8 mg/g in soluble sugars in the *P. eryngi* contributed a sweet taste. The antitumor polysaccharide differs greatly in their sugar composition and consequently in chemical structure but one common feature is their relatively high molecular weight²⁵. It has been reported that polyglucans with a higher molecular weight (10^{4-6} daltons) tend to have greater water solubility and therefore, have more effective antitumor activity⁵. Water insoluble polysaccharides in *Ganoderma* species known as diet fibres have displayed antitumor activity. Antitumor activity *in-vitro* has also been linked to the frequency of polysaccharide branching, which changes with each stage of mycelial growth²⁷.

Ten days culture of *Ganoderma lucidum* showed more protein content (260 mg/g) than 20th day (180 mg/g). Vetter and Rimoczi²⁸ reported the highest crude protein content together with the highest digestibility of 92% in cultivated *Pleurotus ostreatus* at a cap diameter of 5-8 cm. The composition of the mushroom proteins seems to be of a higher nutritional value than that of most plant proteins²⁹.

The total phenolic content of *G. lucidum* was estimated by the Folin cioclteau method as 35.0 mg GAE/gm of dry extract. The total phenolic content per 1 g of dry extract was higher than that reported for garlic extract (0.98 mg GAE per gram of extract)³⁰, but much lower than reported for content of total phenols per gram of asparagus extract (46.95 mg GAE/g extract)³¹. The phenolic content of mushrooms could make a significant contribution to the antioxidant properties. Human diet containing medicinal mushrooms possessing antioxidative properties would be potentially useful to help human body to reduce oxidative damage Medicinal mushrooms, including *Ganoderma lucidum* containing phenolic compounds have shown limitless potential in functional foods and medicinal industries³². The broad-spectrum medicinal property of *G. lucidum* might be due its significant antioxidative activity. Earlier reports

also confirm this conclusion³³.

Rich flora of *Ganoderma* is available in South India, therefore, exploitation of the polysaccharides of this mushroom would be highly beneficial for healthcare. Overall, our report from the present analysis should be ground data for undertaking further study, and the information will be useful for investigating new mushroom materials for food additives and human health.

ACKNOWLEDGMENTS

The authors extend profuse gratitude to the Management, Dhanalakshmi Srinivasan College of Arts & Science for Women, Perambalur, Tamil Nadu, India for their moral support. Also the authors thank Dr. M.A. Singara Charya, Professor, Department of Microbiology, Kakatiya University, Warangal, Andhra Pradesh, India for his valuable suggestions and help.

REFERENCES

- Reshetnikov SV, Tan K-K. Higher Basidiomycota as a Source of Antitumor and Immunostimulating Polysaccharides (Review). *International Journal of Medicinal Mushrooms*. 2001;**3**(4):34.
- Bao X, Liu C, Fang J, Li X. Structural and immunological studies of a major polysaccharide from spores of *Ganoderma lucidum* (Fr.) Karst. *Carbohydrate Research*. 2001;**332**(1):67-74.
- Berovic M, Habijanac, J., Zore, I., Wraber, B., Hodzar, D., Boh, B., Pohleven, F. Submerged cultivation of *Ganoderma lucidum* biomass and immunostimulatory effects of fungal polysaccharides. *Journal of biotechnology*. 2003;**103**(1): 77-86.
- Chang ST. Mushroom biology-a new discipline. *The Mycologist*. 1992; **6**: 64-5.
- Mizuno T. Bioactive molecules of mushrooms: food function and medicinal effect of mushroom fungi. *Food Rev Int*. 1995; **11**: 7-21.
- Lorenzen K, Anke, T. Basidiomycetes as a source for new bioactive natural products. *Current Organic Chemistry*. 1998; **2**(4):329.
- Sone Y, Okuda R, Wada N, Kishida E, Misaki A. Structures and antitumor activities of the polysaccharides isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. *Agricultural and Biological Chemistry*. 1985;**49**(9):2641-53.
- Lin LJ, Shiao, M.S., Yeh, S.F. Seven new triterpenes from *Ganoderma lucidum*. *Journal of natural products*. 1988; **51**(5): 918-24.
- Gao Y, Zhou, Shufeng, Wen, Jianbo, Huang, Min, Xu, Anlong. Mechanism of the antiulcerogenic effect of *Ganoderma lucidum* polysaccharides on indomethacin-induced lesions in the rat. *Life sciences*. 2002; **72**(6):731-45.
- Tasaka K, Akagi, M., Miyoshi, K., Mio, M., Makino, T. Anti-allergic constituents in the culture medium of *Ganoderma lucidum*. (I) Inhibitory effect of oleic acid on histamine release. *Inflammation Research*. 1988; **23**(3):153-6.
- Song CH, Byung, K.Y., Kyung, S.O.O.R.A., Shon, D.H., Park, E.J., Geon, I.L.G.O., Kim, Y.H. Hepatoprotective effect of extracellular polymer produced by submerged culture of *Ganoderma lucidum* WK-003. *Journal of Microbiology and Biotechnology*. 1998; **8**(3): 277-9.
- Zhu HS, Yang XL, Wang LB, Zhao DX, Chen L. Effects of extracts from sporoderm-broken spores of *Ganoderma lucidum* on HeLa cells. *Cell Biology and Toxicology*. 2000;**16**(3):201-6.
- Min BS, Gao, J.J., Hattori, M., Lee, H.K., Kim, Y.H. Anticomplement activity of terpenoids from the spores of *Ganoderma lucidum*. *Planta medica*. 2001;**67**(9):811-4.
- Mizuno T. Antitumour polysaccharides of polyporaceae fungi, *Ganoderma applanatum* and *Fomitopsis pinicola*. In 3rd Symposium on *Ganoderma lucidum*; Seoul, Korea1991.
- Hikino H, Konno, C., Mirin, Y., Hayashi, T. Isolation and hypoglycemic activity of ganoderans A and B, glycans of *Ganoderma lucidum* fruit bodies. *Plant Med*. 1985; **4**: 339-40.
- Gao Y, Zhou, Shufeng. The Immunomodulating Effects of *Ganoderma lucidum*(Curt.: Fr.) P. Karst. (Ling Zhi, Reishi Mushroom) (Aphyllphoromycetidae). *International Journal of Medicinal Mushrooms*. 2002; **4**(1):11.
- Garibova L, Antimonova, AV, Zav'yalova, LA, Krasnopol'skaya, LM. Growth and Morphological Characteristics of the Reishi mushroom *Ganoderma lucidum* as Functions of Cultivation Conditions. *Mikol Fitopatol*. 2003; **37**(3): 14-9.
- Decker EA. Phenolics: prooxidants or antioxidants. *Nutr Rev*, 1997;**55**: 396-407.
- Kim HM, Park, M.K., Yun, J.W. Culture pH affects exopolysaccharide production in submerged mycelial culture of *Ganoderma lucidum*. *Applied Biochemistry and Biotechnology*. 2006; **134**(3): 249-62.
- Jayaraman J. Laboratory Manual in Biochemistry. New Delhi, India: New Age International (P) Limited; 1981. 180 p.

21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951; **193**(1):265-75.
22. Kahkonen MP, Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S., . Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry.* 1999; **47**: 3954-62.
23. Alexopoulos CJ. Introductory Mycology. II ed. New York: John Wiley and Sons; 1962.
24. Rosana M, Valeria, C., Ramos, B.V.L. Screening of basidiomycetes for the production of exopolysaccharide and biomass in submerged culture. *Revista de Microbiologia.* 1999; **30**: 77-84.
25. Kim S, Kim, ES, Kim, YS. Studies on the polysaccharide extracted from *Ganoderma lucidum*. *Journal of The Korean Society of Food and Nutrition.* 1995; **24**: 147-53.
26. Tsai S-Y, Tsai, H.-L., and Mau, J.-L. . Antioxidant properties of *Agaricus blazei*, *Agrocybe cylindrace* and *Boletus edulis*. *Food Science and Technology.* 2007; **40**:1392-402.
27. Wang G, Zhang, J., Mizuno, T., Zhuang, C., Ito, H., Mayuzumi, H., Okamoto, H., Li, J. Antitumor active polysaccharides from the Chinese mushroom Songshan lingzhi, the fruiting body of *Ganoderma tsugae*. *Bioscience, biotechnology, and biochemistry.* 1993; **57**(6): 894-900.
28. Vetter J, and Rimoczi, I. . Crude, digestible and indigestible protein in fruiting bodies of *Pleurotus ostreatus*. *Zeitschrift für Lebensmittel Untersuchung and Forschung.* 1993; **197**:427-8.
29. Belitz HD GW, Schieberle. Food Chemistry. 3rd Revised ed. New York: Springer Berlin, Heidelberg; 2004.
30. Queiroz YS, Ishimoto EY, Bastos DHM, Sampaio GR, Torres EAFS. Garlic (*Allium sativum* L.) and ready-to-eat garlic products: in vitro antioxidant activity. *Food Chemistry.* 2009; **115**(1): 371-4.
31. Z. Zekovic IM, Z., Lepojevic, S. Vidovic, M. Radojkovic, S. Milosevic, S. Jokic. In 47th Meeting of the Serbian Chemical Society. 2009; Serbia.
32. Kim M-Y, Seguin, Philippe, Ahn, Joung-Kuk, Kim, Jong-Jin, Chun, Se-Chul, Kim, Eun-Hye, Seo, Su-Hyun, Kang, Eun-Young, Kim, Sun-Lim, Park, Yool-Jin, Ro, Hee-Myong, Chung, Ill-Min. Phenolic Compound Concentration and Antioxidant Activities of Edible and Medicinal Mushrooms from Korea. *Journal of agricultural and food chemistry.* 2008; **56**(16): 7265-70.
33. Sheena N, Ajith T, Janardhanan K. Anti-inflammatory and anti-nociceptive activities of *Ganoderma lucidum* occurring in South India. *Pharmaceutical Biology.* 2003; **41**(4):301-4.