

Antioxidant Property of Fruiting Bodies of *Ganoderma Lucidum*

R. Radhika^{1*}, S. K. Sundar¹ and R. Anuradha²

¹Department of Microbiology, M. R. Government Arts College, Mannargudi

²Department of Biochemistry, Sengamala Thayar Educational Trust Women's College
(Autonomous), Mannargudi

ABSTRACT

Ganoderma lucidum is one of the mushroom fungi, its fruiting body showed nutritious as well as medicinal value. Oxidants are generated during metabolism, which leads to multiple metabolic disorders. Medicinal mushroom contains a specific chemical which maintains equilibrium between oxidants or free radicals and antioxidants. In this study five different methods were adopted to assess antioxidant power of the *Ganoderma lucidum* aqueous and ethanol extracts. Results revealed that best antioxidant efficiency was noted with DPPH method with 319.0 µg/ml IC₅₀ for ethanol extract and 347.3 µg/ml IC₅₀ for aqueous extract. All the methods proved the efficiency of *Ganoderma lucidum* extracts as an antioxidant. Superoxides, nitric oxides could be responsible for defense mechanism of any tissue, which is responsible for survival of any organisms. *Ganoderma lucidum* could be a therapeutic agent responsible in preventing metabolic disorders. Chemicals of this fungi could be responsible for these biological activities.

Key word: Antioxidant, free radical scavenger, *Ganoderma lucidum*, fruiting body.

INTRODUCTION

Mushrooms are the source of food and medicine. They possess biologically active chemicals, which are responsible for antiviral, antibacterial, antifungal and antiparasitic activities (Romi Singh, 2017). It also revealed the presence of low fat content, which make mushroom as a low calorie food and food of choice for those who suffering from hypertension, arthosclerosis, diabetes and obesity (Rakrudee *et al.*, 2017). Energy production is directly proportional to the oxidation of food materials. Oxidation released free radicals as a end product which acts on cells and tissues and brings out oxidative stress, this leads to metabolic diseases like diabetes, coronary heart diseases, hypertension etc., Maintenance of equilibrium between free radical production and antioxidant defence is an essential feature required by any organisms or animals for their better survival. Mushrooms are nutritionally functional food and a source of physiologically beneficial and nontoxic medicines. They have been used in folk medicine throughout the world since ancient times (Wasser and Weis, 1999). Medicinal mushrooms have an established history of use in traditional oriental therapies. Medicinal effects have been demonstrated for many traditionally used mushrooms (Ooi and Liu, 2000), including extracts of *Ganoderma lucidum*.

Antioxidants dealing with an important role in the process of prevention and treatment of a variety of diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves (Sies,1997). The antioxidants in the human diet are of great interest as possible protective agents to help human body to reduce oxidative damage. To prevent lipid oxidation food industries have long using synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as preservatives in food products which are restricted due to their carcinogenic effects and has led to increased interest

in antioxidant substances from natural resources (Naveena et al., 2008). Free radicals or reactive oxygen species scavengers are several species of mushroom which have made mushrooms attractive as nutritionally beneficial foods and as a source for drugs development (Guerra-Dore et al., 2007). Barros *et al.*, (2007) reported that mushroom flavonoids can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system. Free radical scavenging is a generally accepted mechanism for phenolic antioxidants to inhibit lipid oxidation (Bors *et al.*, 1987). Edible mushrooms are widely consumed and have been valued as a medical resource. The antioxidant properties of the mushroom can be undoubtedly attributed to the rich total phenolics, flavonoids and vitamin C (Rajasekaran and Carmel, 2014). Having known the importance of antioxidants and *Ganoderma lucidum*, the present study was under taken to screen antioxidant power of the extracts of *Ganoderma lucidum*.

MATERIALS AND METHODS

Collection of *Ganoderma lucidum*

Ganoderma lucidum was collected as wild from the paddy fields of Thiruvavur (Dt), Tamil Nadu and identified and authenticated in the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore. The selected strains were multiplied on potato dextrose agar (PDA) petriplates and slant culture was also maintained for further analysis.

Processing and Extraction

Ganoderma lucidum is dried completely, powdered using mechanical grinder and extracted using water and ethanol.

In-vitro antioxidant assay

Antioxidant activity of *Ganoderma lucidum* was assessed by making use of the following five methods. They are DPPH radical scavenging activity (Bliss, 1958), Reducing power assay (Yen and Duh, 1994), Nitric oxide scavenging activity (Soler- Evans et al., 1997), Superoxide radical scavenging activity (PMS-NADH System) (Nishimiki et al., 2009) and H₂O₂ scavenging activity (Ali et al., 2009).

Assessment of % inhibition and IC₅₀

Radical scavenging activity of extracts and standard were expressed in terms of % inhibition. It is calculated by using the formula $[(A_{\text{Control}} - A_{\text{Sample}}) / A_{\text{Control}}] \times 100$. Where A_{Control} is the absorbance of the control, and A_{Sample} is the absorbance in the presence of the sample of aqueous and alcoholic extracts. The IC₅₀ value is defined as the concentration (in µg/mL) of extracts that produced 50% antioxidant effect. $IC_{50} = \text{Concentration of extract} / \% \text{ inhibition} \times 50$.

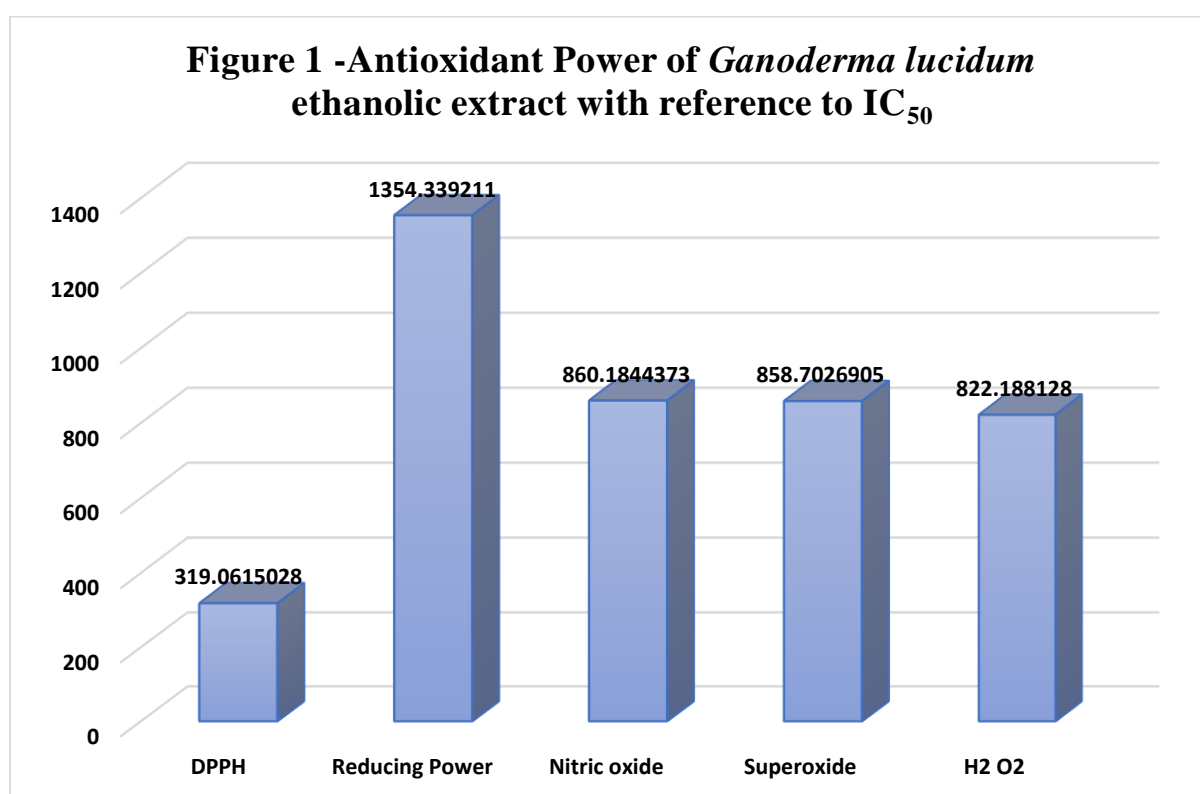
RESULTS

DPPH Assay Spectrophotometric assay of DPPH mediated free radical scavenging activities of alcoholic extracts of *Ganoderma lucidum* and standard were presented in table 1. Result revealed that *Ganoderma lucidum* ethanolic extract at 100 µg/ml concentration showed 54.2±1.1 free radical scavenging power with 319 µg/ml of IC₅₀ (Table 1 and Figure 1). Low percentage of reducing power scavenging power was noted with ethanol extract (25.2±1.3%),

which may due to iron content present in the mushroom. Higher concentrations of *Ganoderma lucidum* ethanol extracts were needed for effective antioxidant efficiency.

Table 1: *In-Vitro* Free Radical scavenging effect of *Ganoderma lucidum* ethanol extract

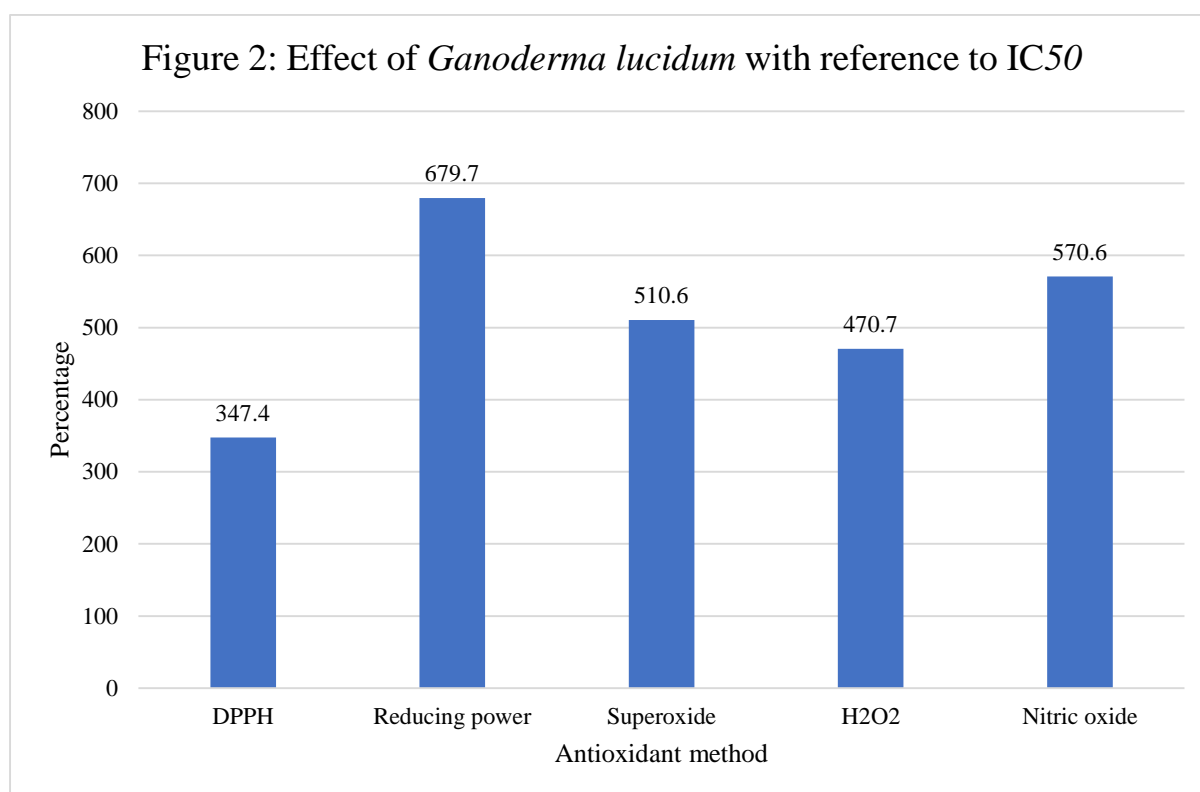
S. No	Concentration of extract	DPPH	Reducing power	Superoxide	H ₂ O ₂	Nitric oxide
1	100 µg/ml	54.2±1.1	7.55±0.6	15.2±0.7	17.2±1.7	22.2±0.8
2	250 µg/ml	60.2±0.9	12.8±0.4	21.7±1.2	22.7±1.2	24.2±0.6
3	500 µg/ml	74.2±0.8	17.5±0.7	26.5±0.8	26.5±0.8	27.1±0.5
4	750 µg/ml	88.5±0.6	21.8±0.8	33.4±0.5	32.4±0.5	33.9±0.6
5	1000 µg/ml	93.5±0.8	25.2±1.3	37.6±0.7	37.0±0.4	37.3±1.1
6	Ascorbic acid (50 µg/ml)	56.3±3.8	42.0±3.0	34.6±1.5	23.6±3.2	37.0±3.61



Aqueous extracts were also used to assess antioxidant activity using all five methods. When compared to ethanolic extract, aqueous extracts were effectively play a vital role as a good antioxidant agent. All methods revealed that aqueous extracts produce morethan 55% free radical scavenging power at 1000 µg/ml with highest in DPPH method and lowest in reducing power scavenging method (Table 2). Similarly, IC₅₀ concentration also lowered in aqueous extract than ethanolic extract. Only 315 µg/ml extract is enough to produce enough free radical scavenging activity (Figure 2). Variable results were illustrated when extracts tested with different methods.

Table 2: *In-Vitro* Free Radical scavenging effect of *Ganoderma lucidum* aqueous extract

S. No	Concentration of extract	DPPH	Reducing power	Superoxide	H ₂ O ₂	Nitric oxide
1	100 µg/ml	46.3±0.4	12.6±1.3	24.6±0.6	32.6±0.3	16.3±0.8
2	250 µg/ml	54.2±1.3	24.7±2.6	37.3±2.3	39.7±2.3	27.8±0.6
3	500 µg/ml	66.8±2.4	33.5±0.7	46.8±0.3	46.9±1.6	39.2±1.5
4	750 µg/ml	80.4±0.6	44.1±1.6	53.9±0.6	57.6±0.3	51.6±1.3
5	1000 µg/ml	89.6±3.4	55.6±1.3	63.7±1.7	71.3±0.3	68.3±0.3
6	Ascorbic acid (50 µg/ml)	56.3±3.8	42.0±1.0	34.6±1.5	23.6±3.2	37.0±3.61



Discussions

Mushrooms are nutritionally functional food and a source of physiologically beneficial and nontoxic medicines. They have been used in folk medicine throughout the world since ancient times (Wasser and Weis, 1999). Medicinal mushrooms have an established history of use in traditional oriental therapies. Mushrooms are usually used as —Biological Response Modifiers – which will modify the host's biological response by a stimulation of the immune system which may result in various therapeutic effects. Mushrooms have anti-cancer, liver protective, analgesic, sedative and anti-radiation properties and have therapeutic effects in gastric and duodenal ulcer, rheumatoid arthritis and navasthenia. Mushrooms are low-calorie, high protein diet with almost no sugars and starch and thus suitable for diabetic people and also for people with obesity, hypertension and heart diseases. Both edible and poisonous mushrooms have medicinal properties and are used in specific diseases. They possess antibacterial, antifungal and antiviral properties.

Oxidation is vital to living organisms for the production of energy to provide fuel in biological process. Oxidative damage caused by free radicals may be related to aging and disease. A free radical is any species which contains one or more unpaired electrons and is capable of independent existence. Free radicals that are produced during natural metabolism of aerobic cells are mostly in the form of reactive oxygen species (ROS). The most reactive oxygen species (ROS) include superoxide anion (O_2^-), hydroxyl radical (OH^\cdot), hydrogen peroxide radical (HO_2^\cdot). The nitrogen derived free radicals are nitric oxide anion (NO^-) and peroxynitrite anion ($ONOO^-$) (Sittichai et al., 2017). Most of the free radicals once produced are neutralized by cellular antioxidant defenses (enzymes and non-enzymatic molecules). Maintenance of equilibrium between free radicals production and antioxidant defense is an essential condition for adequate organism functioning (Valko et al., 2007; Ferreira et al., 2009). In fact, the noncontrolled production of free radicals has been related to more than one hundred diseases including several kinds of cancer, diabetes, etc. Tissues contain several compounds called antioxidants that inhibit free radicals. The reason antioxidants are important to an organism's physical well being comes from the fact that oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as the superoxide anion, hydrogen peroxide, singlet oxygen and the hydroxyl radical. Almost all organisms are well protected from free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols, glutathione and flavonoids. Xanthine oxidase is one of the main enzymatic sources of reactive oxygen species (ROS) in vivo (Sanchez-Moreno, 2002). Xanthine oxidase in normal tissue is a dehydrogenase enzyme that transfers electrons to nicotinamide adenine dinucleotide (NAD^+) as it oxidize xanthine or hypoxanthine to uric acid. Under certain stress conditions, such as oxidative stress, by oxidation of essential thiol groups the dehydrogenase is converted to an oxidase enzyme or by limited proteolysis (Sanchez-Moreno, 2002). Living organisms have evolved a number of mechanisms that are protective against lipid peroxidation or oxidant stress. Some are enzymatic, blocking the formation of reactive compounds, others may scavenge for reactive compounds or act in reducing oxidant stress by unknown mechanisms. Free radicals or reactive oxygen species scavengers are several species of mushroom which have made mushrooms attractive as nutritionally beneficial foods and as a source for drugs development (Guerra-Dore et al., 2007). Barros et al. (2007) reported that mushroom flavonoids can act as free radical scavengers to terminate the radical chain reactions that occur during the oxidation of triglycerides in the food system. Among various naturally occurring substances, mushrooms may prove to be one of the useful candidates in the search for an effective antioxidant with free radical scavenging activity. Free radical scavenging is a generally accepted mechanism for phenolic antioxidants to inhibit lipid oxidation (Bors et al., 1987). Mushrooms contain biologically active polysaccharides in fruit bodies, cultured mycelium, and secrete substances into culture broth. Edible mushrooms are widely consumed and have been valued as an medical resource. Many research studies have found that some species of mushrooms are having therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects. Mushroom polysaccharides prevent oncogenesis, show direct antitumor activity against various allogeneic and tumors, prevent metastasis and have antioxidant properties (Wasser, 2002). They have a wide variety of secondary metabolites, including phenolic compounds, polypeptides, terpenes and steroids. (Rajasekaran and Carmel, 2014).

References

1. Barros, L., Ferriera, M J., Queiros, B., Ferreira, ICFR., and Bapista, P., 2007. Total phenols, ascorbic acid, β -carotene and lycopene in portugese wild edible mushrooms and their antioxidant activities. *Food Chem.*, 103: 413-419.
2. Bors, W., Erben-Russ, and Saran, 1987. Fatty acid peroxy radicals: Their generation and reactivities. *J. Electronana Che.*, 232: 37-49.
3. Chen, Y., Bicker, W., Wu, J., Xie, M.Y., and Lindner, W., 2010. Ganoderma species discrimination by dual- mode chromatographic fingerprinting: A study on stationary phase effects in hydrophilic interaction chromatography and reduction of sample misclassification rate by additional use of reversed- phase chromatography. *J. Chromatography*, 1217(8): 1255-1265.
4. Demir, F., Uzun, F.G., Durak, D., and Kalender, Y., 2011. Subacute chlorpyrifos induced oxidative stress in rat erythrocytes and the protective effects of catechin and quercetin. *Pesticide. Biochem. And Physiol.*, 99: 77-81.
5. Guerra-Dore, CMP. Azevedo, TCG. de Souza, MCR. Rego, LA. de Dantas, JCM. Silva, FRF. Rocha, HAO, Baseia, IG, and Leite, E.L., 2007. Antiinflammatory, antioxidant and cytotoxic actions of glucanrich extract from *Geastrum saccatum* mushroom. *Int. Immunopharma.*, 7(9): 1160-1169.
6. Naveena, B.M., Sen, A.R., Vaithiyanathan, S., Babji, Y., and Kondaiah, N., 2008. Comparative efficacy of pomegranate juice, pomegranate rind powder extract and BHT as antioxidants in cooked chicken patties. *Meat Sci.*, 80(4): 1304-1308.
7. Ooi, V.E.C., and Liu, F., 2000. Immunomodulation and anti-cancer activity of polysaccharideprotein complexes. *Curr. Med. Chem.*, 7: 715-728.
8. Prabin Shrestha, Bishnu Joshi, Jarina Joshi, Rajani Malla, and Lakshmaiah Sreerama, 2016. Isolation and Physicochemical Characterization of Laccase from *Ganoderma lucidum*-CDBT1 Isolated from Its Native Habitat in Nepal. *BioMed Research International*, 10.
9. Rajasekaran, M., and Carmel, P.S., 2014. Free radical scavenging activity of fruiting body extracts of an edible mushroom, *Volvariella volvacea* (Bull. ex Fr.) Singer: an *in vitro* study. *Asian J. of Biomed and Pharma Sci.*, 4(30): 6-11.
10. Rakrudee Sarnthima, Saranyu Khammaung, and Piyawan Sa-ard, 2017. Culture broth of *Ganoderma lucidum* exhibited antioxidant, antibacterial and α -amylase inhibitory activities. *J. Food Sci. Technol.*, 54(11): 3724-3730.
11. Romi Singh. 2017. A Review on Different Benefits of Mushroom. *IOSR Journal of Pharmacy and Biological Sciences* (IOSR-JPBS), 12(1): 107-111.
12. Sanchez-Moreno, S., 2002. Review: Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. Tech Int.*, 8: 121-137.
13. Sies, H. 1997. Oxidative stress: oxidants and antioxidants. *Experimen Physiol.*, 82(2): 291-295.
14. Sittichai Sillapapongwarakorn, Somchai Yanarojana, Darawan Pinthong, Amnuay Thithapandha, Jiraporn Ungwitayatorn, and Porntip Supavilai. 2017. Molecular docking based screening of triterpenoids as potential G-quadruplex stabilizing ligands with anti-cancer activity. *Bioinformation*, 13(9): 284-292.
15. Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T., Mazur, M., and Telser, J., 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem Cell Biol.*, 39: 44-84.

16. Wasser, S.P., and Weis, A.L., 1999. Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspectives (Review). *Int. J. Med. Mushrooms*, 1: 31-62.
17. Soler- Evans CA, Miller NJ, Paganga G. Antioxidant properties of phenolic compounds : *Trends Plant Sci.*, 1997; 2: 152- 159.
18. Blois MS. Antioxidant determinations by the use of a stable free radical: *Nature* 1958 ; 181: 1199- 1200.
19. Yen GC, Duh PD. Scavenging Effect of Methanolic Extracts of Peanut Hulls on Free Radical and Active Oxygen Species: *J Agric Food Chem* 1994; 42: 629- 632.
20. Nishimiki M, Rao NA, Yagi K. Pomegranate juice. A heart - healthy fruit juice: *Nutrition Rev* 2009; 67: 49- 56.
21. Ali EM, Fazel NS, Mohammed NS. Antioxidant activity of leaves and inflorescence of *Eryngium caucasicum* at flowering stage: *Pharmacog Rev* 2009; 1suppl 6: 435- 439.