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REVIEW ON GANODERMA LUCIDUM – KING OF HERBS; CULTIVATION METHODS AND ITS THERAPEUTIC APPLICATIONS

Microbiology

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ABSTRACT

Ganoderma lucidum is used as traditional medicine for longevity and vivacity for over two thousand years in Asian countries. It has many pharmacological properties like anti-inflammatory, antitumor, immune modulatory, antibacterial, antiviral, antioxidant, green synthesis of silver nanoparticles and other biological activities. This activity is due to presence of various bioactive compounds namely polysaccharides, triterpenoids, fatty acids, proteins and trace elements in spore, mycelia and fruiting bodies of *Ganoderma lucidum.* Artificial cultivation of this mushroom include saw dust bag method and liquid state fermentation by using cheap carbon sources like hardwood sawdust, rice straw, wheat straw and nitrogen sources namely wheat bran, rice bran along with maintaining proper environmental conditions like temperature, relative humidity, aeration, pH plays a important role in development of spores, mycelia and fruiting bodies. This article will enlarge knowledge to people, and contribute a beneficial reference for research and production on *Ganoderma lucidium.*

KEYWORDS

Bioactive compounds, *Ganoderma lucidium,* Longevity, Liquid state fermentation, Pharmacological properties, Silver nanoparticles

1. INTRODUCTION

Ganoderma lucidum is a fungi belongs to Basidiomycetes division , order polyporales and is a wood decaying organism with many medicinal values. In Japan it is mentioned as Reishi and Lingzi in China [1,2]. Lingzhi shows peculiar character having doubled walled basidiospores which may differ from other polyporales [3]. Fruiting bodies of *Ganoderma* species have bitter taste, hard, not having fleshy texture, hence not considered as edible mushroom [4]. Since 2000 years Lingzhi occupies a major source of medicine through out world wide. Powders, dietary supplements are the products prepared from mycelia, spores and fruiting body of Lingzhi are available commercially [5]. More than four hundred different bioactive compounds, which mainly include polysaccharides, triterpenoids, nucleotides, sterols, fatty acids, proteins and trace elements have been isolated and identified from spores, mycelia and cap of Reishi. These bioactive compounds are used to treat various complications namely headache, hypertension, bronchitis, nephritis, lupus erythematoxis, leucopoenia, cardiovascular problems and cancer [6,7]. Extract of Reishi also used in synthesis of silver nanoparticles [8]. Artificial cultivation of mycelial biomass, spore and fruiting body of *Ganoderma lucidum* has become essential as the demand in international market is in constant increase [9]. Presently saw dust bag and liquid state medium procedures are preferable for production of Reishi mycelia, spores and cap and second method involves the production of fungi mycelia biomass and its metabolic products. For development of whole mushroom may be influenced by media and various process parameters. Cheap carbon sources like rice straw, saw dust, wheat straw and nitrogen sources which include what bran, rice bran along this substrates, physical factors like temperature, aeration, relative humidity and pH are maintained [10].

2. Cultivation Procedure

2.1 Nutritional and environmental factors

The mycelia growth, fruit body yield and different biologically active compounds synthesis in *Ganoderma lucidum* are influenced by substrate and environmental conditions [10]. Substrate namely hard wood sawdust, cotton seed hull, rice straw, wheat straw and other agricultural by products acts as carbon source. Along carbon source, nitrogen is also a major component without nitrogen protein could not be synthesised and mycelia do not grow. The nitrogen rich materials like wheat bran, rice bran, corn powder are added to media [12]. Inorganic elements like potassium, calcium, sodium, magnesium, phosphorous, sulphur are also essential, but these components are available in substrate. Calcium sulphate, potassium dihydrogen phosphate, magnesium sulphate still need to be added to media, especially calcium sulphate reason is that it maintains pH value of substrate, change the void spaces in the medium, proper air flow, fixing nitrogen and improves the quantity of sulphur and calcium elements [5]. For proper mycelia growth and to avoid oxygen deficiency in media, 60 – 65% moisture level should be maintained. Temperature is required for enzymatic reactions during the development process of the mycelia and fruit bodies. Lingzhi mycelia can grow with in a temperature range of 20^0c to 35^0c and optimum temperature is 25^0c - 30^0c. Fruiting body will become yellow and rigid when temperature is below 20^0c and easily die at temperature above 35^0c [13]. 60 – 65% relative humidity is maintained for mycelia growth and for fruiting body development relative humidity should be increased to a level of 85% - 95%. Lingzhi is an aerobic fungi, requires oxygen to support life activities. It is necessary to absorb oxygen and release carbon dioxide during whole growing period, When the concentration of carbon dioxide is higher than 0.1% the fruiting body does not grow normally, only when carbon dioxide is below 0.1% , the fruiting body becomes large, thick with a rounded cap and short stipe. Light shows inhibitory effect on Lingzhi, but weak light promotes primordium differentiation and cap formation. 500 to 1000 lux is required for primordial formation, for fruit body development it is at 3000 to 50,000 lux [9].

2.2 Substitute cultivation - Bag method

Sawdust substrate mixture (65% wet weight and 35% dry weight was prepared), moisture level of the mixture was maintained at 65%. To this substrate mixture 10% rice bran or wheat bran and 3% calcium carbonate are added [14]. This mixture is filled in heat resistant polypropylene bag of size 18x25 cm and tightly packed, for this bag neck is prepared with heat resistant poly vinyl chloride tube. A hole of about 2/3 deep was made at center of bag with sharp end stick to place inoculum. The neck was plugged with cotton and covered with a brown paper and these bags were sterilized at 115^0c for 45 to 60 minutes and substrate is allowed to cool over night [15,16]. One week old pure culture of Lingzhi was inoculated in the hole of center of substrate bag and again plugged with cotton under sterile conditions. The inoculated packets were kept at in almost dark at 25^0c at the time of incubation, and shifted to culture room at 25^0c - 32^0c along with 85 to 95% relative humidity. Proper aeration and spraying of water 3 to 5 times daily is necessary to enhance fruit body development [17]. Formation of fruiting body of Lingzhi requires several months by Bag method, furthermore fruiting bodies and spores are not suitable for direct use as food or pharmaceutical ingredients due to their bitter taste and very hard nature which makes it difficulty to absorb by our body [18]. These problems can be overcome by production of valuable bioactive metabolites with fermentation techniques.

2.3 Fermentation technique – Liquid state fermentation

Formation of mycelia biomass and metabolic products in short time with advantages of less space utilisation with low chance of contamination carried by liquid state fermentation [18]. For culturing of mycelia and production of effective products through fermentation, the level of carbon and nitrogen requirements, pH value, temperature, oxygen, relative humidity are key factors.

In liquid state fermentation the complex carbohydrate source like corn flour, soyabean meal, distillers grains, an organic nitrogen sources are more suitable for production of mycelial biomass and extracellular polysaccharide production compared with inorganic nitrogen sources [19]. Small amount of magnesium sulphate and potassium dihydrogen phosphate are added along with carbon and nitrogen sources to media for better growth of mycelia and production of bioactive compounds. Polysaccharides obtained with in 3 to 5 weeks only through liquid state fermentation [9].

3. Major Bioactive Components

Triterpenoids, polysaccharides, nucleotides, fatty acids, sterols, proteins and trace elements are present in mycelia, fruiting body and spores of Ganoderma *lucidum* [20]. From Lingzhi more than one hundred triterpenes have been isolated, which are of bitter tasting and mainly occurs in form of ganoderic acid, ganosporeric acid [21]. Large amounts of ganodermic acids, triterpene lactones are present in spores than other parts of Reishi mushroom. More than two hundred polysaccharides (Ganopoly) have been isolated from spores, mycelia and fruiting bodies and from broth of liquid culture of Lingzhi [22]. β-D- glucans, heteropolysaccharides with various combinations of glucose, galactose, mannose, xylose, arabinose and fucose have been isolated and characterised with molecular weight ranges from thousands to million Daltons.

Some protein bound sugars also identified [23]. Various fungal immunomodulatory proteins (FIP) namely Lingzhi – 8 (LZ-8), FIP – l29 are present in Reishi mycelia. These proteins are small molecules with similar structure and immunoregulatory activity to immunoglobulin [24]. Adenosine and 5–deoxy–5 methyl sulfinylad–nosine are nucleotides and nucleosides, which are nitrogenous compounds are also isolated from LIngzhi [25]. *Ganoderma lucidum* also contains aminoacids, soluble proteins, sterols, oleic acid, cyclo-octasulfur, an ergosterol peroxide and cerebrosides [26]. Magnesium, zinc, calcium, iron, copper, germanium, manganese are inorganic ions also identified in Reishi fungi. Choline, betaine, tetracosanoic acid, stearic acid, ergosta-7, palmitic acis, behenic acid, tetracosane, hentriacontane and ergosterol are available in spores. Pyrophosphatidic acid is a lipid also isolated from Resihi mushroom [27].

4. Therapeutic Applications

Antiatherosclerotic, immunomodulating, auto-inflmmatory, analgesic, antitumor, chemopreventive, radioprotective, sleep promoting, antiviral (including anti-HIV and Corona virus disease of 2019), antibacterial, hypolipidemic, antifibrotic, hepatoprotective, diabetic, anti oxidative, anti-scavenging, hypoglycrmic, anti-aging and anti-ulcer properties are the pharmacological effects showed by Lingzhi mushroom [28,29,30].

4.1 Antitumor effect

Ganopoly of Resihi exhibit antitumor activity against Sarcoma 180 in mice [27]. Cytotoxic activity in vitro on hepatoma cells showed by triterpenoids like ganodermic acids which are extracted from Lingzhi [31]. Activation of T-helper, natural killer cells, macrophages and other effector cells takes places after binding of polysaccharides on leukocytes surfaces or serum specific proteins, and results in production of cytokines namely tumor necrosis factor (TNF-α), interleukins (IL), interferons (IFN), nitric oxide by the activated effector cells have been increased [32].

4.2 Cholesterol and lipid metabolism

The powdered mycelium of *Ganoderma lucidum* at 5% of the diet of spontaneously hypertensive rats for four weeks, leads to plasma total cholesterol to lowered significantly by 18.6%. Total liver triglyceride and liver cholesterol levels were also decreased in Lingzhi fed group by 46% and 56% [33].

4.3 Hypertension

Systolic blood pressure significantly lowered (approximately 100mg Hg) with out causing difference in heart rate in hypertensive rats when fed with powdered mycelium of Lingzhi at 5% diet for four weeks [34].

4.4 Antibacterial effect

Growth of both gram positive and gram negative bacteria will be inhibited by the antibacterial substances present in Resihi fungi [26]. Gastroduodenal diseases like peptic ulcer, gastric carcinoma and gastritis are associated with *Helicobacter pylori*. Extracts of Lingzhi mushroom has ability to inhibit the growth of *Helicobacter pylori*. Extracts of Resihi mushrom show broad spectrum activity against bacteria due to presence of ganomylin, triterpenoids. Thus, it is important that antibacterial activity of Lingzhi may be useful for those patients with chronic bronchitis and peptic ulcer diseases [35].

4.5 Anti-HIV activity

Different triterpenoids of Lingzhi had showed inhibitory effect against HIV. Lucidenic lactose, lucidenic acid extracted from fruiting body of Resihi inhibits the activity of calf DNA polymerase-α, rat DNA polymerase-β but also shows its action on reverse transcriptase enzyme of HIV-1 [19].Ganoderic acid B and ganoderiol B showed potent inhibitory action on HIV-1 protease [36].

4.6 Silver nanoparticles

Silver nanoparticles were prepared by adding silver nitrate solution with extract of Lingzhi [8]. Low concentration of silver nanoparticles is attractive due to its non-toxicity to the human body, its broad spectrum antibacterial action, and also due to its bactericidal action against multiresistant bacteria like Methicillin-resistant *Staphylococcus aureus* (MRSA}, as well as multi drug resistant *Pseudomonas aeruginosa* [37]. Silver nanoparticles interact with a wide range of metabolic process with in microorganisms, resulting in inhibition of growth, loss of infectivity leading to cell death. Positive charge on the Ag^{+} ion is crucial for its antimicrobial activity through the electrostatic attraction between the negatively charged cell membrane of the microorganisms and the positively charged nanoparticles. Irregular shaped pits formation takes place in the outer membrane that changes membrane permeability, which may lead to release of lipopolysaccharides and membrane proteins due to metal deposition [38].

5. CONCLUSION

Reishi mushroom is named as a herb of longevity and has been used for thousand of years in some countries. A critical need in the field of technology is due for the cultivation of spores, mycelia and fruiting bodies of Lingzhi owing to its demand in the market. More research studies are needed on the therapeutic compounds of Reishi mushroom to assure the efficiency and safety of Lingzhi and to promote the development of commercial functional foods and at same time attention is required on the side effects caused by these products.

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REFERENCES

[1] F.C. Yang and C.B. Liae, "Effect of cultivating conditions on the mycelial growth of *Ganoderma lucidum* in submerged flask cultures," Bioprocess Engineering, 19, 233-236, 1998.
[2] R. Wagner, D.A. Mitchell, G.L. Sassaki, M.A.L.A. Amazonas and M. Berovic, "Current techniques for the cultivation of *Ganoderma lucidum* for the production of biomass, ganoderic acid and polysaccharides," Food Technol Biotechnol, 41, 371-382, 2003.
[3] J.E. Adaskaveg and R.L. Gilbertson, "Basidispores, pilocystidia and other basidiocarp characters in several species of the *Ganoderma lucidum* complex," Mycologia, 80, 493-507, 1988.
[4] S.C. Jong and J.M. Birmingham, "Medicinal benefits of the mushroom *Ganoderma*," Advances in Applied Microbiology, 37, 101-134, 1992.
[5] Subarna Roy, Miskat Ara Akhter Jahan, Kamal Kanta Das, S. Kishore Munshi and Rashed Noor, "Artificial cultivation of *Ganoderma lucidum* (Reishi Medicinal Mushroom) using sawdusts as substrates, " American Journal of Bioscience, 3(5), 178-182, 2015.
[6] Z.X. Ling, A.F. Chen and Z.B. Lin, "*Ganoderma lucidum* polysaccharides enhance the function of immunological effector cells in the immunosuppressed mice," J Ethnopharmacol, 111, 219-226, 1997.
[7] K. Deepalakshmi and S. Mirunalini, "Therapeutic properties and current medicinal usages of medicinal mushroom: *Ganoderma lucidum*," Int J Pharm Pharm Sci, 2, 1922-1929, 2011.
[8] P. Milan, P. Rabin. K.C. Sudip and A. Chandra, "Biosynthesis of Silvernanoparticles using *Ganoderma lucidum* and Assessment of Antioxidant and Antibacterial activity," International Journal of Applied Sciences and Biotechnology. 5(4), 523-531, 2017.
[9] K.K. Hapuarachchi, W.A. Elkhateeb, S.C. Karunarathna, C.R. Cheng, A.R. Bandara and T.C.Wen, "Currents status of global *Ganoderma* cultivation, products, industry and market," Mycosphere, 9(5), 1025-1052, 2018.
[10] S.Baby, A.J. Johnson and B. Govindan, "Secondary metabolites from *Ganoderma*," Phytochemistry, 114, 66-101, 2015.
[11] X.F. Bao, Y. Zhen, L. Ruan and J.N. Fang, "Purification, characterization and modification of T lymphocyte stimulating polysaccharide from spores of *Ganoderma lucidum*," Chemical and Pharmaceutical Bulletin, 50, 623-629, 2002.
[12] W.Z. Xuan, Q.S. Kai and Z. M. Yong, "Applied modern biotechnology for cultivation of *Ganoderma* and development of their products," Appl Microbiol Biotechnol, 93, 941-963, 2012.
[13] A .W. Chen, "Cultivationof the medicinal mushroom *Ganoderma lucidum*,", Int J Med Mushrooms, 1, 263-282, 1999.
[14 M. Azizi, M. Tavana, M, Farsi and F. Oroojalian, "Yield performance of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* using different waste materials as substrates," Int J Med Mushr, 14, 521-527, 2012.
[15] A.J. Kakon, M.B.K. Choudhury and S. Saha, "Mushroom is an ideal food supplement," J Dhaka Med Coll Hos, 18, 58-62, 2012.
[16] M.N. Uddin, S. Yesmin, M.A. Khan, M. Tania, M. Moonmoon and S. Ahmed, "Production of ooyster mushroom in different seasonal conditions of Bangladesh," J Sci Res, 3, 161-167, 2011.
[17] S. Singh, N.S.K. Harsh and P.K. Gupta, "A novel method of economical cultivation of medicinally important mushroom, *Ganoderma lucidum*," Intl J Pharm Sci, 5, 2033-2037, 2014.
[18] R. Wagner, D.A. Mitchell, G.L. Sassaki and M. Berovi, "Current techniques for the cultivation of *Ganoderma lucidum* for the production of biomass, ganoderic acids and polysaccharides," Food Tech Biotechnol, 41, 371-382, 2003.
[19] Q.H. Fang and J.J. Zhong, "Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*," Process Biochemistry, 37, 769-774, 2002.
[20] Y. Gao, S.H. Zhou, G. Chen, X. Dai and J. Ye, "A phase study of a *Ganoderma lucidum*

extract (ganopoly) in patients with advanced cancer," Int J Med Mushrooms, 4(3), 207-214, 2002.
[21] H.W. Kim and B.K. Kim, "Biomedical triterpenoids of *Ganoderma lucidum*," Int J Med Mushrooms, 1(2), 121-138, 1999.
[22] B.S. Min, N. Nakamura, H. Miyashiro, K.W. Bae and M. Hattori, "Triterpens from the spores of *Ganoderma lucidum* and their inhibitory activity against HIV-1 protease," Chem Pharm Bull, 46, 1607-1612, 1998.
[23 Y.Y. Wang, K.H. Khoo and C.H. Lin, Studies on the immunomodulating and antitumor activities of *Ganoderma lucidum* (Rsishi) polysaccharides:functional and proteomic analysis of a fucose containing glycoprotein fraction responsible for the activities, Bioorg Med Chem, 10, 1057-1062, 2002.
[24] F. Li, H.A. Wen, X. Liu, F. Zhou and G. Chen, "Gene cloning and recombinant expression of a novel fungal immunomodulatory protein from *Trametes versicolor*," Protein expression and purification, 82, 339-344, 2012.
[25] T. Mizuno, "Reishi, *Ganoderma lucidum* and *Ganoderma tsugae*: bioactive substances and medicinal effects," Food Rev Int, 11(1), 151-166, 1995.
[26] Y. Gao, S.H. Zhou, M. Huang and A. Xu, "Antibacterial and antiviral value of the genus *Ganoderma*: a review," Int J Med Mushrooms, 5(3), 235-246, 2003.
[27] G.T. Liu, "Recent advances in research of pharmacology and clinical applications of *Ganoderma*," Int J Med Mushrooms, 1(1), 63-68, 1999.
[28] S.C. Jong and J.M. Birmingham, "Medicinal benefits of the mushroom *Ganoderma*," Adv Appl Microbiol, 37, 101-134, 1992.
[29] S.P. Wasser and A.L. Weis, "Medicinal properties of substances occurring in higher Basidiomycetes mushrooms current perspectives (review)," Int J Med Mushrooms, 1(1), 31-62, 1999.
[30] Aly Farag EI Sheikha, "Nutritional Profile and Health Benefits of *Ganoderma lucidum*-Lingzhi, Reishi, or Mannentake as Functional Foods: Current Scenario and Future Perspectives," Foods, 11,1030.https://doi.org/10.3390/foods11071030, 2022
[31] J.O. Toth, B. Luu and G. Ourisson, "Ganoderic acid T and Z cytotoxic triterpenes from *Ganoderma lucidum* (Polyporaceae)," Tetrahedron Lett, 24, 1081-1084, 1983.
[32] J. Battle, T.Z. Ha, W. Browder and D. Williams, "Ligand binding to the (1-3)-beta-D-glucan receptor stimulates NF-Kappa B activities, but not apoptosis in U937 cells, " Biochem Biophys Res Commun, 249, 499-504, 1998.
[33] T.S. Soo, "Effective dosage of the extract of *Ganoderma lucidum* in the treatment of various ailments," In Mushroom Biology and Mushroom Products, Royse, Ed: The Pennsylvania State University, 177-185, 1996.
[34] Y. Kabir, S. Kimura and T. Tamura, "Dietary effect of *Ganoderma lucidum* mushroom on blood pressure and lipid levels in spontaneously hypertensive rats (SHR)," J Nutr Sci Vitaminol, 34, 433-438, 1988.
[35] S.Y. Yoon, S.K. Eo, Y.S. Kim, C.K. Lee and S.S. Han, "Antimicrobial activity of *Ganoderma lucidum* extract alone and in combination with some antibiotics," Arch Pharm Res, 17, 438-442, 1994
[36] D.C.Smith, B.G. Redman, L.E. Flaherty, L. Li, M. Strawderman and K.J. Pienta, "A phase II trial of oral diethylstilbesterol as a second line hormonal agent in advanced prostate cancer," Urology, 52, 257-260, 1998.
[37] H.H. Lara, Ayala-Nunez, N.V. I. Turrent and L.R. Padilla, "Bactericidal effect of silver nanoparticles against multi-resistant bacteria," World Journal of Microbiology and Biotechnology. 26, 615-621, 2010.
[38] B. Srinivas and P. Naga Padma, "Green synthesis of silver nanoparticles using dextran from *Weissella confusa*," International Journal of Science, Environment. 5(2), 827-838, 2016.