

Importance of Bioactive Compounds of *Ganoderma lucidum* Extract in Medical Field

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Abstract

Ganoderma lucidum is a well-known medicinal mushroom with a long history, used as an ancient remedy for promoting health and increasing longevity. This species of *Ganoderma* genus is important for our study because it has an interesting chemical composition and pharmacological benefits such as immune suppressor, hypocholesterolaemia, hypotensive, antitumoral, antioxidant, anti-inflammatory agents, and more others. *G. lucidum* chemical composition has many compounds such as vitamins (mainly Vitamin E), polysaccharides, triterpenes and each one of them is offering a distinctive pharmacological effect. This species of *Ganoderma* medicinal mushroom is used extensively for its internal effect on improving immune system, hepatoprotective benefit and additionally, a large variety of people consume *Ganoderma lucidum* as tea infusion and coffee because its antioxidant and reducing stress effects.

Keywords: *Ganoderma lucidum*, antitumoral, polysaccharides, anti-inflammatory, antioxidant.

Introduction

Ganoderma lucidum (*G. lucidum*) is the most known medicinal fungus due to its impressive natural ingredients. Its pharmacological application dates back nearly two thousand years ago [1] and it is known as Lingzhi from Chinese population and as Reishi in Japan [2].

G. lucidum is known as basidiomycete and according to Li, has been called as the “Mushroom of Immortality” [1]. This mushroom has been used in China for healing purpose and has been predominantly used by Asian population for an alternative of

chemotherapy treatment to inhibit cancer or to handle side-effects from chemotherapy medicine [3]. Nowadays, usage of this fungus increased because its potential of reducing obesity, cholesterol, for gout health, decreasing cardiovascular disorders, controlling the diabetes and other metabolic diseases, stimulation of probiotics [4].

William Curtis was a researcher who discovered *Ganoderma lucidum* in 1781 based on grown material in England [2]. According to Moncalvo 1995, in that time confusions were wide between *Ganoderma tsugae* and *Ganoderma lucidum* because of chlamydospore production regarding the distinction of both species. European *G. lucidum* does not produce ovoid chlamydospore like *G. tsugae*, but an interesting fact is American *G. lucidum* produces ovoid chlamydospore. Many other species have been researched and beside these, Adaskaveg 1989 reported that North American *G. lucidum* and European *G. resinaceum* belong to the same species, but Moncalvo 1995 hasn't reported any evidence of these [5].

Work by Sun (2014) clarify difficulties associated with the separation and identification of polysaccharides from *Ganoderma* spp. and settle a high-performance liquid chromatography (HPLC) based methodology to characterise species from *Ganoderma* genus using polysaccharide fingerprint profiling [6].

Such as *Ganoderma* polysaccharides, many other papers were published to value triterpenes, especially Ganoderic Acids, to differentiate between *Ganoderma* species. A study released by Chen 1999 shows a method of analysis of triterpenoids to differentiate between *G. tsugae* and *G. lucidum* [7].

Even though it has a variety of components, polysaccharides and triterpenoids are the most important compounds from this fungus and some authors consider it being responsible for the most of its pharmacological activity [8].

Over the years, *G. lucidum* defines its potential due to its largely acceptance of the population and usage of it for many human conditions. Nowadays, it is known that this fungus has numerous pharmacological effects such as: immunomodulating, anti-atherosclerotic, anti-inflammatory, analgesic, chemo preventive, anti-tumor, radio-protective, sleep-promoting, antibacterial, antiviral, hepatoprotective, anti-diabetic, anti-aging, antioxidative, hypoglycaemic [9].

A study published by Gerenutti et al. 2021, shows an *in vitro* activity of *G. lucidum*, used for evaluation of antitumor, immunomodulatory, antioxidant, neuronal cell protection, antifungal, and antimicrobial activity. Based on author's research in literature, all authors reported *in vitro* antitumoral activity, positive effect of *G. lucidum* extract on immunomodulation, antioxidant activity of the extract, benefits as adjuvant in neurodegenerative diseases, antimutagenic activity of the extract of the body and the mycelia of *G. lucidum* [10].

It is known that the common way to administrate this supplement is oral, but dosage and posology varies from person to person. Searching in literature, *in vivo* tests aren't

so common. So, based on Gerenutti et al. 2021 work, are related some interesting in vivo activity for *Ganoderma lucidum* extract. And these are: evidence-based antitumoral activity against breast cancer, lung cancer, leukemia; testosterone inhibition activity with the result of reducing the malignancy of prostate cancer; protection against idiosyncrasies side effects induced by antitumoral agents and, protection against radiotherapy; hepatoprotective effect was also reported after drug induced hepatotoxicity [10].

Materials and Methods

G. lucidum is found all over the world, growing on multiple hosts. In Europe, it grows on living trees or stumps of oaks or chestnuts, rarely on coniferous trees [11]. The study area is Sub Carpati Region of the Romanian montain chain. We choose this surface in order to contibuite at the development of wild products in the region of Romania and their use in the medical field.

Botanical description

This species belongs to kingdom Fungi, phyla Basidiomycota, Class Agaricomycetes, Order Polyporales, Family Ganodermataceae, Genus Ganoderma and Species *Ganoderma lucidum* [10]. Fungus of this Genus have a shiny surface that is associated with the presence of thickwalled pilocystida embedded in an extracellular melanin matrix [12]. *Ganoderma* is characterised due to its shape and exceptional colors (red, black, blue, white, yellow, purple) of the fruiting body. These characteristics differ between species due to their geographical origins and cultivation environments [13].



Figure 1. *Ganoderma lucidum*

General methods for obtaining composition data

The standard AOAC International (Association of Official Analytical Chemists) (2016) procedures were applied in order to determinate carbohydrates, proteins, lipids and ash. The Kjeldahl method of protein estimation was applied based on the calculation

of total N (Azote). Total lipid content was made by Soxhlet extracting method with petroleum ether. The ash content was determined gravimetrically after incineration at $600 \pm 15^\circ\text{C}$. The carbohydrate content was determined by calculation. The NaCl content was determined by the Mohr method with the modifications made by Taofiq et al. (2017) [14].

***G. lucidum* ethanolic extract**

A simple method of extraction using ethanol is described by Taofiq [14]. The dried powder of *G. lucidum* (3g) was extracted in Soxhlet apparatus for 5-6h (35 ± 5 min/cycle) using ethanol. In the end of this procedure, the final solvent was evaporated under reduced pressure to obtain the dried ethanolic extracts. In addition, to characterise phenolic and triterpenoid compounds, the obtained extracts were dissolved in ethanol (5.5 mg/mL), followed by a filtration through $0.22 \mu\text{m}$ nylon syringe filter and analysed by high performance liquid chromatography equipped with photodiode array detection-mass (HPLC-DAD-ESI/MSn). Alongside with characterising these two bioactive compounds, antioxidant, antityrosinase, anti-inflammatory, antimicrobial activity, and cytotoxicity in human tumour cell or in non-tumour cells lines can start being analysed with this ethanolic extract method, according to Taofiq [14].

Extraction of polysaccharides

A method that determinate the total polysaccharides from *G. lucidum* sample is described by Taofiq, with some modifications of Vazirian et al. 2014, method. Shortly, method is based on the dry sample (1.5 g) extracted by maceration with boiling water and then filtered under vacuum. In addition, this step was repeated twice. Next, filtered extract was lyophilized and fraction of polysaccharide is dissolved in water at a concentration of 5mg/mL. To the obtained solution (1mL), phenol (4%, 25 μL) is added, followed by sulphuric acid (1mL). The final solution is stirred for 30 seconds and then measured using a UV-VIS spectrophotometer, absorbance is read at 548 nm against a blank solution (distilled water). This method uses Starch (5-50 $\mu\text{g}/\text{mL}$) as a standard and results are expressed in mg of starch equivalents per g of dw (dry weight) [14].

Extraction of triterpenoids

Nowadays, even the technology arises, for terpenoids, remain these two classical methods of extraction. Colorimetric method using vanillin-perchloric acid spectroscopic method, which can be estimated the total triterpenoids content, and the other one with determining individual triterpenoids using high-performance liquid chromatography (HPLC) or thin layer chromatography (TLC). For both methods, many authors reported severe conditions of high temperature and strong acid and these methods are considered destructive methods. According to Chen 2012, near infrared (NIR) spectroscopy offer some advantages such as high efficiency, low cost, easy operation, less or no sample preparation and quick data analysis [15].

Results and Discussions

Ganoderma lucidum is a type of medicinal mushroom full of bioactive compounds. In addition to the mainly compounds, there are other biological components of pharmaceutical interest, such as: fat, proteins, ash, carbohydrates, and sodium chloride that are presented in Table 1.

Table 1. Chemical composition of *Ganoderma lucidum*

Component	Mean \pm SD (g/100 g)
Lipids	2.85 \pm 0.03
Proteins	7.85 \pm 0.05
Ash	2.1 \pm 0.15
Total carbohydrates	86.64 \pm 0.2
NaCl	0.56 \pm 0.04

Ganoderma lucidum ethanolic extract reveals tyrosinase activity through the compound Ganodermanondiol which suppresses the expression of tyrosinase-related activity (TRP-1, TRP-2) and microphthalmia-associated transcription factor (MITF) thereby inhibiting the synthesis of melanin. In this case, tyrosinase inhibitors contribute to skin lightening and with the help of another existent compound in the fungus, phenolic acids, reduce the severity of hyperpigmentation and decrease melanin biosynthesis. The extract has also showed anti-inflammatory activity by inhibiting NO production and at this activity phenolic acids and triterpenic acids such as, Ganoderic Acid C1 and Lanostane are contributing to its anti-inflammatory activity. Antitumor activity is present, and also high antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MSSA) and methicillin-sensitive *Staphylococcus aureus* (MRSA) [14].

Polysaccharides from *G. lucidum*

For so long, a lot of scientific attention has been focused on polysaccharides from the *Ganoderma* spp. In fact, polysaccharides represent biological macromolecules with a complex structure of elements and a wide range of physicochemical properties [14]. The content in polysaccharides was 15.4 \pm 0.2 mg starch/g, reported by Taofiq [14].

The most important beneficial function of oligosaccharides in human body are: doesn't stimulate or increase the level of blood glucose, decrease the absorption of released glucose; provide small amount of energy, improve the intestinal environment and maintain intestinal tract's health as probiotic.

Polysaccharides from *Ganoderma* spp. have a good antioxidant effect due to its potential of reducing damage, caused by the free radicals formed in the oxidation

reaction. Polysaccharides act as anti-diabetic agents with the result of lowering blood glucose. Beside these functions, this bioactive compound possesses inhibitive effect on the tumour growth and enhance proliferation of macrophage cells in the immune system as a result of antitumoral agent against breast cancer [17].

Triterpenoids from *G. lucidum*

Studies have shown that over 150 triterpenoids were found in *Ganoderma* spp. and these are: ganoderic, lucidenic, ganodermic, ganoderenic, ganolucidic and applanoxidic acids, lucidones, ganoderals and ganoderols. The content of Triterpenoids reported by Taofiq was 27.2 ± 0.7 mg ursolic acid/g and in Terpenoids was 27.2 ± 0.7 mg linalool/g [14].

Naturally, triterpenoids are a subtype of Terpene composed of one or more isoprene units. Triterpenes isolated from *G. lucidum* spores have shown significantly pharmaceutical activity against many human disorders, including cancer. Studies have shown that Ganoderic acid T, D, F provide strong cytotoxic effects at low concentrations in various human cancer cell lines. Anti-inflammatory and antioxidant properties possess remarkable pharmacological activities [15].

Regarding the benefits of bioactive components, it is known that the antitumoral effect is mostly given by β -glucans from polysaccharides. But its also worth to mention the other important bioactive component, triterpenoid which provides antitumor effect. Beside it's widely discussed and studied effect, this compound also provides antioxidant and immounomodulatory effects, proved by in vitro and in vivo studies.

Table 2. Therapeutic effects and bioactive compounds of *Ganoderma lucidum* reported in literature

Pharmacological effect	Bioactive compound	References
Immunomodulating effect	Ganoderic acids	[18]
Antitumoral, Chemo preventive, Radio preventive effects	β -D-glucans, Heteropolysaccharides, Glycoproteins.	[18][19]
Anti-HIV effect	Triterpenoids	[20]
Anti-diabetic effect	Glycans	[21]
Hepatoprotective effect	Ganoderic acids	[22]
Antiinflammatory effect	Ganoderic acids	[23]
Anti-allergic effect	Ganoderic acids	[23]

These days, *Ganoderma lucidum* and not just this species, are consumed by population almost everyday. Its usage as nutraceutical and pharmaceutical formulation increased its popularity among researchers and of course, pharmaceutical companies. Based on this popularization, it become very popular between Americans and Europeans. The “Mushroom of Immortality” [1] has been utilized as herbal extract like tea infusion and as tonic, known from Traditional Chinese Medicine (TCM), and also as an alternative treatment of neurasthenia, hypertension, diabetes, liver diseases, arthritis, bronchitis, asthma, fatigue, coughing, insomnia, and many other. Is widely used for promoting human vitality and longevity based on TCM medicine.

Nowadays, *G. lucidum* has been recognized as an alternative treatment in the prevention and treatment of leukimia, carcinoma, hearth disease, immune system enhancer, detoxify effect [9][24]. Now, there are more then a hundred brands of different products based on *G. lucidum* that can be found in the market all over the world [5]. Commercially products available are: hydroalcohol extract, dry extract capsules (different dosages), dried extract tablets (different dosages), spore powder (capsules of 100mg). However, there are still no concret reports about its safety in pregnancy, lactation, or administration in children. In addition to the commercially available forms, various extracts of *Ganoderma lucidum* are also found in the form of coffee, powder, tea, spore products, drinks, syrup, toothpaste, soaps, and lotions [10].

Conclusions

G. lucidum is considered a very potential medicinal fungus due to the large pharmacologically bioactive compounds obtained from the fruiting body, spores, and mycelium. Our study highlights a wide variety of biochemically active compounds. The high content of terpenes, polysaccharides and triterpenoids is noteworthy. The second part of the study highlights the pharmacological effects of this fungus that have been used in Traditional Chinese Medicine, but it is also use in recent modern studies.

References

- [1] Li, J., Zhang, J., Chen, H., Chen, X.Q., Lan, L., Liu, C. Complet mitochondrial genome of the medicinal mushroom *Ganoderma lucidum*. PLoS ONE 8:1-11, 2013;
- [2] Wang, X.-C., Xi, R.-J., Li, Y., Wang, D.-M., Yao, Y.-L. The species identity of the widely cultivated Ganoderma, *G. lucidum* (*Ling-zhi*), PLoS ONE 7:1-12, 2012b;
- [3] Gordan, J.-D., Chay, W.-Y., Kelley, R.K., Ko, A.H., Choo, S.P., Venook, A.-P. And what other medications are you talking? *J. Clin. Oncol.* 29:288-291, 2011;
- [4] Jin, X., Ruiz, J., Sze, D.M., Chan, G.C. *Ganoderma lucidum* (Reishi mushroom) for cancer treatment. *Cochrane Db. Syst. Rev.* 6, 2012;
- [5] Bishop, S.K., Kao, C., H., J., Xu, Y., Glucina, P., M., Paterson, M., R., R., Ferguson L., R. From 2000 years of *Ganoderma lucidum* to recent developments in nutraceuticals. *Phytochemistry.* 114:56-65, 2015;

- [6] Sun, X., Wang, H., Han, X., Chen, S., Zhu, S., Dai, J. Fingerprint analysis of polysaccharides from different *Ganoderma* by HPLC combined with chemometrics methods. *Carbohydr. Polym.* 114:432-439, 2014;
- [7] Chen, D.-H., Shiou, W.-Y., Wang, K.-C., Huang, S.-Y., Shie, Y.-T., Tsai, C.-M., Shie, J.-F., Chen, K.-D. Chemotaxonomy of triterpenoid pattern of HPLC of *Ganoderma lucidum* and *Ganoderma tsugae*. *J. Chin. Chem. Soc.* 46:47-51, 1999;
- [8] Cheng, P-G., Phan, C-W., Sabaratnam, V., Abdullah, N., Abdulla, M. A., Kuppusamy, U. R. Polysaccharides-Rich Extract of *Ganoderma lucidum* (M.A. Curtis:Fr) P. Karst Accelerates Wound Healing in Streptozotocin-Induced Diabetic Rats. *Evidence-Based Complementary and Alternative Medicine*, 2013;
- [9] Sanodiya, B., S., Thakur, G., S., Baghel, R., K., Prasad, G., B., Bisen, P., S. *Ganoderma lucidum*: a potent pharmacological macrofungus. *Curr. Pharm. Biotechnol.* 10:717-742, 2009;
- [10] Gerenutti, M., Viroel, F., Jozala, A., Grotto, D., Viana, S. Therapeutic applications of *Ganoderma lucidum*: Progress and Limitations. *Produtos Naturais e Suas Aplicações: da comunidade para o laboratório.* 16:249-267, 2021;
- [11] Copoț, O., Tănase, C., Maxent modelling of the potential distribution of *Ganoderma lucidum* in North-Eastern Region of Romania. *J. Plant Development* 43:133-143, 2017;
- [12] Moncalvo, J.-M., Wang, H.-F., Hseu, R.-S. Gene phylogeny of the *Ganoderma lucidum* complex based on ribosomal DNA sequences. Comparison with traditional taxonomic characters. *Mycol. Res.* 99:1489-1499, 1995;
- [13] Zhao, J., D., Zhang, X., Q. Importance, distribution, and taxonomy of Ganodermataceae in China. Proceedings of Contributed Symposium, 5th International Mycological Congress, Vancouver. August 14-21, 1994;
- [14] Taofiq, O., Heleno, S. A., Calhelha, R. C., Alves, M. J., Barros, L., Gonzales-Paramas, A. M., Berreiro, M. F., Ferreira, I. C. F. R. The potential of *Ganoderma Lucidum* extract as bioactive ingredients in topical formulations, beyond its nutritional benefits. *Food and Chemical Toxicology* 108:139-147, 2017;
- [15] Chen, Y., Xie, M.-Y., Zhang, H., Wang, Y., Nie, S., Li, C. Quantification of total polysaccharides and triterpenoids in *Ganoderma lucidum* and *Ganoderma atrum* by near infrared spectroscopy and chemometrics. *Food Chemistry* 135:268-275, 2012;
- [16] Boh, B., Berovic, M., Zhang, J., Zin-Bin, L. *Ganoderma luicdum* and its pharmaceutically active compounds. *Biotechnol Annu. Rev.* 13:265-301, 2007;
- [17] Alzorqi, I., Singh, A., Manickam, S., Al-Qrimli, H. Optimization of ultrasound assisted extraction (UAE) of β -D-glucan polysaccharides from *Ganoderma lucidum* for prospective scale-up. *Resource-Efficient Technologies* 3:46-54, 2017;
- [18] Zhang, L., X., Mong, H., Zhou, X., B. Effect of Japanese *Ganoderma lucidum* on production of interleukin-2 from murine splenocytes. *Chung-Kuo Chung Hsiu Chien Ho Tsa Chih* 13:613-615, 1993;

- [19] Li, M., C., Lei, L., S., Liang, D., S., X., Z., M., Yuan, J., H., Yang, S., Q., Sun, L., S. Effect of *Ganoderma lucidum* polysaccharides on oxygen free radicals in murine peritoneal macrophages. *Chin. J. Pharmacol. Toxicol.* 14:65-68, 2000;
- [20] Gao, Y., Zhou, S., H., Huang, M., Xu, A. Antibacterial and antiviral value of the genus *Ganoderma* P. Karst species (Aphyllophoromycetidae): a review. *Int. J. Med. Mushrooms* 5:235-246, 2003;
- [21] Gao, Y., Lan, J., Dai, X., Ye, J., Zhou, S., H. A phase I/II study of Lingzhi mushroom *Ganoderma lucidum* (W. Curt.:Fr) Lloyd (Aphyllophoromycetidae) extract in patients with type II diabetes mellitus. *Int. J. Med. Mushrooms* 6(1):33-39, 2004;
- [22] Chen, R., Y., Yu, D., Q. Studies on the triterpenoid constituents of the spores from *Ganoderma lucidum* Karst. *J. Chin. Pharm. Sci.* 2:91-96, 1993;
- [23] Li, J., M., Lin, C., C., Chiu, H., F., Yang, J., J., Lee, S., G. Evaluation of the anti-inflammatory and liver-protective effects of *Anoectochilus formosanus*, *Ganoderma lucidum* and *Gynostemma pentaphyllum* in rats. *Am. J. Chin. Med.* 21:59-69, 1993;
- [24] Zhou, X., W., Su, K., Q., Zhang, Y., M. Applied modern biotechnology for cultivation of *Ganoderma* and development of their products. *Appl. Microbiol. Biotechnol* 93:941-963, 2012;
- [25] Adaskveg, J., E., Gilbertson, R., L. Cultural studies, and genetics of sexuality of *Ganoderma lucidum* and *G. tsugae* in relation to the taxonomy of *G. lucidum* complex. *Mycologia* 78:694-705, 1986;
- [26] Gupta, A., Kirar, V., Keshir, G. K., Gola, S., Yadav, A., Negi, P. S., Misra, K. Wound Healing Activity of an Aqueous Extract of the Lingzhi or Reishi Medicinal Mushroom *Ganoderma Lucidum* (Higher Basidiomycetes). *International Journal of Medicinal Mushroom*, 16(4):345-354, 2014;
- [27] Paterson, M., Russell, B. *Ganoderma* – A therapeutic fungal biofactory. *Phytochemistry* 67:1985-2001, 2006;
- [28] Wachtel-Galor, S., Yuen, J., Buswell, J. A., Benzie, F. F. I. *Ganoderma Lucidum* (Lingzhi or Reishi): A Medicinal Mushroom. *Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition*, 9, 2011.