Study on Nutritional Composition of Fungus Ganoderma Lucidum

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Abstract

Ganoderma lucidum (G. lucidum) (also known as Reishi or LingZhi) is a medicinal mushroom known since ancient times and widely used in Traditional Asian Medicine. Even in modern times, it is recommended as a functional food and alternative treatment for various diseases of the body. Compounds with biological activity are numerous, but there are three classes of compounds that have been intensively studied and their therapeutic activity has been proven by both in vitro and in vivo tests. Polysaccharides, terpenes and phenolic compounds are the most abundant biocompounds. They mainly exhibit antioxidant, antitumour, anti-inflammatory, anti-aging, cardioprotective, hapatoprotective activities and are involved in metabolic regulatory processes. In the present study, we aimed to determine the nutritional values in the alcoholic extract of the fruiting body of the mushroom G. lucidum, purchased from a mushroom farm. The total content of polysaccharides and polyphenols was determined spectrophotometrically. The antioxidant activity was determined by DPPH and FRAP methods. The results obtained correlate with the reference studies and we conclude that the analysed mushroom, G. lucidum, is a mushroom with nutritional and therapeutic potential for the human organism.

Keywords: polysaccharides, polyphenols, antioxidant, antitumour, anti-inflammatory, anti-aging

Introduction

Ganoderma lucidum (G. lucidum) is a fungus that has been known under various names in traditional Asian medicine since ancient times. It is most commonly found under the names Reishi and Lingzhi. It has proven to be a "source of longevity" as its

long medicinal use (approximately 2000 years), which is common among the Asian population, has led to the promotion of natural remedies and alternative treatments [1]. Its medicinal benefits cover a wide range of acute and chronic diseases such as hypertension, diabetes, asthma, liver and kidney diseases, neurological disorders, inflammatory diseases, cancer, and it acts on oxidative stress, thus having an anti-aging effect and exhibit antimicrobial activity [1-6] In terms of chemical composition, the mushroom species G. lucidum contains more than 400 compounds with biological activity, but according to the scientific literature, polysaccharides and triterpenes are found in the most significant amount. Lipids, proteins, peptides, vitamins and minerals are part of the chemical composition of fungal species, with justified biological activities that each of them possess [7,8].

The most studied bioactive compounds in the mushroom composition are carbohydrates, especially polysaccharides (glucose, galactose, xylose, fucose, rhamnose), which have been shown to have pharmacological benefits in terms of antioxidant, antitumour and antimicrobial activities. The second class of bioactive compounds that have attracted the interest of researchers are terpenes, but triterpenes have been found in significant amounts, such as: ganoderic acid, ganoderol, lucidenic acid [9]. The beneficial activities reported for triterpenes are antioxidant, antitumour, anti-inflammatory, antimicrobial and antiviral [10]. The phenolic class is also one of the most studied classes in mushroom composition, but the polyphenolic acids exert most of the biological activity, these are: gallic acid, p-hydroxybenzoic acid, p-coumaric acid and protocatechuic acid. Polyphenolic acids have been shown to contribute significantly to antioxidant, anti-inflammatory and antimicrobial activities [11].

Due to numerous studies on bioactive compounds and their diversity in the mushroom species G. lucidum, we wanted to determine the nutritional profile to obtain clear evidence on the content of the mushroom of interest, polysaccharides and polyphenols to confirm the presence of antioxidant activity.

Materials, Equipments and Methods

G. lucidum characterisation

The Ganoderma lucidum mushroom sample was purchased from a mushroom farm in Mehedinti County, Romania. The mushroom was cultivated under optimal conditions, in bags with sawdust substrate, wood trunks and grains, at a controlled temperature of 20-24°C for about 90 days [12]. Figure 1 shows the specimen we took at work, G. lucidum (Reishi) mushroom, characterised by a reddish-brown cap, with a shiny surface, woody texture with a bulky body, weighing 0.25g, width 8-9 cm and length 9-10 cm.

We brought the mushroom to the powder stage by cleaning it with a brush, then dried the specimen at a temperature of 24-25°C for 15 days, cut it into small pieces and then passed it through a grinder to become powder. In order to obtain the finest powder

(Figure 2), the powder obtained was passed through a sieve with a mesh size of 0.87 mm [13].

Figure 2. G. lucidum mushroom fruiting body (personal archive)

Figure 3. G. lucidum mushroom in powdered form obtained after grinding

Equipments

All laboratory equipment used in this study has been metrologically verified to ensure calibration and reproducibility of experiments with accurate measurements.

G. lucidum ethanolic extract

In order to obtain the ethanolic extract, Soxhlet apparatus was prepared for extraction. Thus, 3 g of the obtained G. lucidum powder was subjected to 5 refluxes for 2.5 h (30 min refluxing) using 300 mL of 96% ethyl alcohol. The alcohol filtrate and dried ethanolic extract were obtained by low pressure filtration using Whatmann No. 1 filter paper [14].

The extraction yield (%) was calculated using the equation (1):

$$\text{Extraction Yield } \% = \left(\frac{\text{practical mass obtained}}{\text{theoretical mass}}\right) \text{X } 100 \quad (1)$$

Nutritional composition of G. lucidum fruiting bodies

Moisture content

Moisture content was determined according to the standards of the Association of Official Analytical Chemistry (AOAC) [15]. 3 g of dried mushroom was placed in an oven at 105°C for 2.5-3 h, during which time the mushroom had a constant weight. After drying, the sample was placed in a desiccator for cooling. The results were obtained using equation (2):

$$\text{Moisture } \% = \frac{(W_1 - W_2) \text{ x } 100}{W_1} \quad (2)$$

where, W_1 is mass (g) of sample before drying; W_2 is mass (g) of sample after drying.

Ash content

Ash determination is an important step in mineral content analysis. We determined the ash content by the conventional ashing method at high temperature, 600°C for 2 hours. The resulting ash is placed in a desiccator to keep it away from atmospheric moisture until it cools down [15]. The result is expressed as a percentage and calculated using the following equation (3):

$$\text{Ash \% (dry weight)} = \frac{M_{ASH}}{M_{DRY}} \text{x } 100 \quad (3)$$

where, W_{ASH} is ash content obtained; W_{DRY} is dry sample before incineration.

Crude protein content

The crude protein content of the analysed mushroom was determined by the total nitrogen method (N x 6.25). The total nitrogen content was determined by the Kjeldahl method. A UdK DK6 digester equipped with a 127 distillation unit was used for the analysis. The sample was first mineralised with sulphuric acid in the presence of mercury (Hg) and selenium (Se) catalysts. After alkalinisation of the sample, ammonia is released by alkaline steam distillation and captured in boric acid solution followed by titration with hydrochloric acid. The amount of protein is calculated by multiplying % N by 6.25. The result is then expressed as a percentage relative to the amount of mushroom powder used in the analysis [15, 16].

Crude fat content

Crude fats were determined following the method described by Roy et al. 2018. Thus, they were extracted with pethroleum ether in a Soxhlet apparatus for 4.5-5 hours. After evaporation of the ether, the fatty residue obtained was determined gravimetrically. The results are expressed in percentages, referred to the amount of powder taken in work [15, 17].

Dietary fibre content

A simple and inexpensive gravimetric method was used for the determination of crude fibre. Fat and non-fibrous matter were removed from the sample using an acid-alkaline mixture. The insoluble fibre residue obtained was washed, filtered and dried. The result was weighed and the fibre content was estimated as a percentage by weight of the sample analysed [18].

Total carbohydrates

Carbohydrate was calculated by difference using the equation (4) described by Food and Agriculture Organization (FAO) 2003, adapted by Singh et al. 2020 [19, 20]:

$$\text{Total carbohydrates (\% dry weight)} = 100 - (\text{\% moisture} + \text{\% ash} + \text{\% protein} + \text{\% fat}) \quad (4)$$

Determination of total polysaccharide content

For total carbohydrate analysis, the aqueous extract of the fruiting body of G. lucidum was prepared. 25 g of the powder was extracted with 250 mL of bidistilled water at a temperature of 24-25°C for 24 hours on a mechanical shaker. At the end of the time, the solution was filtered and the precipitate was allowed to dry. The extraction yield was calculated using the above equation (1). The total polysaccharide content of the aqueous solution was determined by the phenol-sulphuric acid method. 0,1 mg of the aqueous extract was mixed with 0,5 ml of 4 % phenol (prepared immediately before use) to which 2,5 ml of concentrated sulphuric acid was added. The mixture was homogenised. Polysaccharide content was determined using a Turner 690 spectrophotometer (Figure 3) at a wavelength of 490 nm [16, 21]. The absorbance of the samples was compared with the glucose calibration curve according to the following equation (5):

$$y = 0.0136x + 0.0735 \quad (5)$$

where, y - sample absorbance measured at 490 nm; x - carbohydrate concentration, $R^2 = 0.9958$ (correlation coefficient).

Figure 4. Spectrophotometer Turner 690

Determination of antioxidant activity by different assays

Total phenolic content (TPC)

The total phenolic content of the 96% alcoholic extract was determined by the method described by Salamah et al. 2018, adapted to the present working conditions. Folin-Ciocalteu reagent (1:10 v/v) was used, and as a standard we used gallic acid solution, prepared before use, in different concentrations 50-450 mg/mL. We mixed 0.5 mL of each concentration of gallic acid obtained with 2.5 mL of Folin-Ciocalteu reagent, to which we added 1.2 mL of 7.5% Na_2CO_3. The absorbance is read at 765 nm on a Turner 690 spectrophotometer. Take 0.5 mL of the ethanolic extract of G. lucidum and mix with 2.5 mL of Folin-Ciocalteu reagent, followed by 1.2 mL of 7.5% Na_2CO_3. The mixture is left in the dark for 30-40 minutes and the absorbance is read

at a wavelength of 765 nm. The results are expressed in milligrams gallic acid equivalent (GAE) per gram of dry weight (dw) [22]. The absorbance was compared with the gallic acid calibration curve using the following equation (6):

$$y=0.0078x+0.1861 \quad (6)$$

where, y - sample absorbance measured at 765 nm; x - total phenolic content; R^2 = 0.9959.

DPPH assay

The antioxidant activity of the extract obtained from the fungus G. lucidum was measured by free radical scavenging activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay. 2 mg powder of the fruiting body of G. lucidum was taken and dissolved in methanol, over which a DPPH solution with a concentration of 20 mg/L was added. The resulting mixture was left in the dark for 30 minutes. Ascorbic acid was used as a positive control. Absorbance was measured using a Turner 690 spectrophotometer at a wavelength of 517 nm [16, 23]. The DPPH activity was determined according to equation (7):

$$\% \text{ Inhibition} = A_{control}\left(\frac{A_{sample} - A_{blank}}{A_{control}}\right) x\ 100 \quad (7)$$

where, A_{sample} - abosorbance of the sample read at 517 nm; $A_{control}$ - DPPH absorbance without powder mixture; A_{blank} - Ascorbic acid absorbance.

Ferric Reducing Antioxidant Power assay

The method is based on the ability of the sample to reduce Fe^{3+} ions to Fe^{2+}. For the determination, 2.9 mL of FRAP reagent was mixed with 0,1 mL of the ethanolic extract obtained at different concentrations. After mixing, the sample was left in the dark for 30 minutes. Absorbance was measured using a Turner 690 spectrophotometer at a wavelength of 593 nm against the blank solution [16, 24]. The standard solution was ascorbic acid solution and the results are expressed in µg ascorbic acid equivalent (AAE) per mL of the extract.

Statistical analysis

All determined tests were performed in triplicate. Data are expressed as means ± standard deviation (SD). The results were studied using SPSS 16.0 parametric tests. One-way ANOVA was utilized in order to correlate the chemical composition data of the mushroom species. When differences were found, the Duncan multiple comparison test was used, and differences were considered significant at $p < 0.05$.

Results and Discussion

Nutritional properties and chemical composition of the fungus

The results obtained for the nutritional profile and chemical composition of the mushroom of interest are presented in Table 1, obtained by adapting to the available working conditions. The yield of the aqueous extraction was 52.92%.

Table 2. Nutritional profile of Ganoderma lucidum

No.	Nutritional compound (g/100g)	Sample 1	Sample 2	Sample 3	Value (Mean±SD)
1	Moisture	38.70	33.33	41.93	37.98 ± 4.34
2	Ash	9.26	8.51	9.46	9.07 ± 0.50
3	Crude protein	5.25	5.10	6.14	5.49 ± 0.56
4	Crude fats	1.75	1.70	2.04	1.83 ± 0.18
5	Dietary fibres	2.89	2.91	2.90	2.9 ± 0.01
6	Total carbohydrates	33.47	39.49	28.44	33.80 ± 5.53

In the determinations carried out, it can be observed that the calculated moisture content has a value relatively close to that of El Sheikha 2022 [8]. The total carbohydrate content has the highest value in the nutrient profile, corresponding to the estimated reports of Gharib et al. 2022 [12] and Rahman et al. 2020 [24]. The ash, fibre and crude fat contents have values close to those estimated by other authors such as Gharib et al. 2022, Rahman et al. 2020 and Stojkovic et al. 2014 [12,24,25]. In our case, the crude protein content was low compared to the literature reviewed [12,24].

Based on the results obtained and the reports of other authors observed in different studies, we concluded that the climatic conditions and the soil origin of the reference mushrooms have a great importance for the nutritional profile. Thus, we observed that in regions with a warmer climate, favourable for mushroom development, the contents in the chemical composition may vary in significant percentages compared to the nutritional profile obtained from the fruiting body of the mushroom we analysed [8, 24].

The amount of bioactive compounds is closely correlated with physicochemical factors and soil enzyme activity. Studies investigating the effect of soil quality on Ganoderma lucidum metabolites have reported that differences in soil depth and soil enzyme activity play a significant role in metabolite concentration [26, 27]. However, a detailed knowledge of the composition of bioactive compounds and an in-depth substrate-based analysis of different soils in different geographical regions have not been investigated and should be further investigated [28].

Total polysaccharide content of G. lucidum aqous extract

Polysaccharides are known as macromolecules with antioxidant activity involved in various biological processes. They are considered as one of the most important metabolites of the fungus Ganoderma lucidum, representing a significant amount in its nutritional composition.

The carbohydrate content determined from the aqueous extract of G. lucidum was evaluated at 36.72 ± 0.27 mg/g DW of the extract, calculated according to the equation (5) described above using the glucose calibration curve, as shown in Figure 4.

To determine the total polysaccharide content, the calibration curve was established at different glucose concentrations. The calibration curve was obtained by reading three concentrations of glucose solutions. A calibration curve point was established.

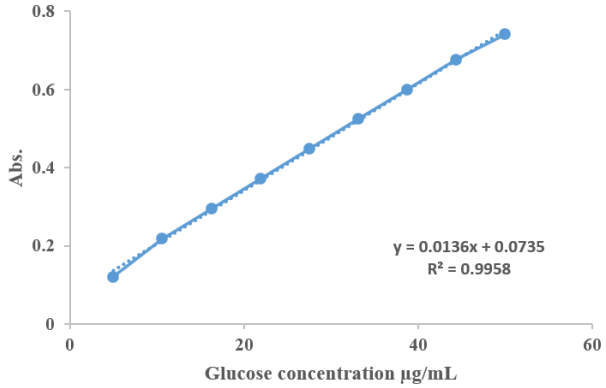

Figure 5. Calibration curve of glucose at different concentrations for the determination of total polysaccharides.

The results obtained for total polysaccharide content are presented in **Table 2**. The reported value is the mean of three experimental readings with standard deviation (SD). Also, values reported by other authors for total polysaccharide content are presented.

Tabel 3. Results on polysaccharide content in G. lucidum compared with other literature data

	Data from the present study (Mean ± SD)	Data from the literature	Reference
Polysaccharide content	36.72 ± 0.27 mg /g DW	655.0 mg/g DW 112.82 mg/g DW 24.06 mg/g DW	[21] [29] [29]

Following studies estimating the total polysaccharide content we observe that the values vary considerably, thus Kozarski et al. 2014, determined a content of 655.0 mg/g DW. Skalicka-Wozniak et al. 2012, determined the total polysaccharide content for Ganoderma lucidum on different wood type substrates and the content ranged from 112.82 to 24.06 mg/g DW [21, 29].

From studies estimating the total polysaccharide content, it can be seen that the values vary considerably, for example, Kozarski et al. 2014, determined a content of 655.0 mg/g DW on fresh wild fruiting bodies. Skalicka-Wozniak et al. 2012, determined the total polysaccharide content for Ganoderma lucidum on different woody substrates and the content ranged from 112.82 on maple substrates to 24.06 mg/g DW on birch substrates [21, 29]. Thus, the medium in which the mushrooms are grown has a strong influence on the amount of polysaccharides, as observed in the literature. The mushroom of interest on which the polysaccharide content was determined was grown on a sawdust substrate and the polysaccharide content was 36.72 mg/g DW.

Antioxidant axtivity

The presence of an excess of reactive oxygen species or a decrease in the level of antioxidant components in the body leads to damage to body tissues by various factors (physical, chemical and physiological) and can cause a variety of irreversible/reversible diseases such as ageing, infertility, development of tumours, infections of the genito-urinary and digestive systems. Antioxidant activity is noted through reactions and mechanisms such as free radical scavenging, free radical reducing capacity and prevention of ionic catalyst chain formation of transition metal catalysts [30]. In the present study, antioxidant activity was determined by determination of total phenolic content, DPPH radical scavenging assay and FRAP method.

Some studies have shown that triterpenoid compounds exert antioxidant activity by reducing free radicals in cancer cells, also have an effect on intracellular levels of reactive oxygen species and endogenous antioxidant enzymes in spleen lymphocytes reducing radiation-induced oxidative damage [31, 32]. Polysaccharide compounds contribute beneficially in reducing oxidative damage induced by reactive oxygen species. Some studies have shown benefits on reducing some forms of cervical carcinoma post γ-radiation [33].

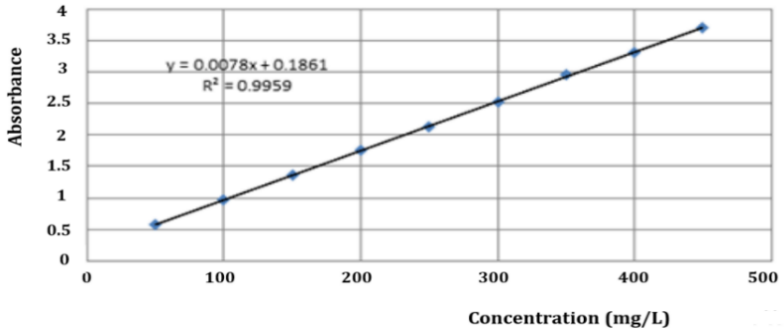

Figure 6. Callibration curve of gallic acid standard [34]

For the determination of antioxidant activity, alcoholic extract was prepared using 96% ethanol. The extraction yield was 65.67%. For the determination of TPC, the calibration curve was generated and the results were obtained using the above equation (6) (Figure 5).

The calibration curve was obtained by absorbance readings, in triplicate, of nine different concentrations of the standard gallic acid solution.

The results are averages of three determinations with standard deviation (SD), obtained for total polyphenol content (TPC) and are shown in Table 3.

Table 4. Results obtained for total phenolic content (TPC)

	Data from the present study	Data from the literature	Reference
TPC	25.66 ± 0. 15 mg GAE/g DW	28.11 mg GAE/g DW 30.91 mg GAE/g DW 39.30 mg GAE/g DW	[35] [36] [37]

The total phenolic content of the fungal species studied, Ganoderma lucidum, was 25.66 ± 00.15 mg GAE/g DW. It was determined using the GAE calibration curve [34]. Consulting the literature, the value obtained is close to the value of 28.11 mg GAE/g d.w. obtained by Gasecka et al. 2016 [35]. Other authors determined TPC and the results were 30.91 mg GAE/g and 39.30 mg GAE/g d.w. reported by Sheikh et al. 2015 and Wu et al. 2015 [36, 37].

The antioxidant capacity tests carried out on Ganoderma lucidum powder by means of the DPPH assay and the FRAP assay gave the results that are presented in Table 4.

Table 5. Results obtained for antioxidant capacity by DPPH and FRAP assays

	Data from the present study	Data from the literature	Reference
DPPH	23.92 ± 0.1 %	24.04 ± 0.33 % 51.3 ± 1.04 %	[24] [38]
FRAP	119.080 ± 50.66 µg/100g	614.83 ± 0.05 µg/100g 49.87 ± 1.58 µMol TE/g	[24] [38]

For DPPH and FRAP assay determinations the results were 23.92 ± 0.1 % respectively 119.080 ± 50.66 µg/100g. Literature indicates approximate values on DPPH test and relative values on FRAP test. Thus Rahman et al. in 2020 reported values for DPPH assay of 24.04 ± 0.33 % and for FRAP assay the value of 614.83 ± 0.05 µg/100g [24]. Kolniak-Ostek et al. in 2022 reported for DPPH assay 51.3 ± 1.04 and for FRAP assay 49.87 ± 1.58 µMol TE/g [38]. The antioxidant properties of compounds from G. lucidum have been intensively studied over the years through numerous studies and thus their antioxidant capacity has been demonstrated [39].

Conclusions

Ganoderma lucidum (Reishi or LingZhi) is a natural source of nutritional interest due to its rich composition. It is well known that G. lucidum has been traditionally used in Asian medicine since ancient times for its proven medical benefits such as anti-inflammatory, antioxidant, antitumour, antimicrobial and antiviral activity. A comprehensive review of the literature shows that Ganoderma lucidum is a mushroom of current interest, and the interest of researchers is mainly focused on the nutritional composition and the quantitative and qualitative determination of compounds with biological activity. In this study, the nutritional composition and antioxidant activity of the G. lucidum mushroom have been highlighted, and the nutritional content observed following the determination of moisture, ash, crude protein, crude fat, dietary fibre and total carbohydrates has been correlated with the results observed in the scientific literature, allowing us to conclude that this mushroom has nutritional properties beneficial to human health.

The total content of polysaccharides was determined using the aqueous extract and the results indicated a relative content compared to studies that determined carbohydrates on the same species of mushroom but with different geographical origins. The differences in the results are due to the geographical location and the growth conditions of the mushroom. It has been observed that factors such as growth substrate, warmer climate and tropical geographical location have a positive influence on the nutritional content and the content of bioactive compounds such as polysaccharides and triterpenes. However, more in-depth studies on the correlation between the concentration of bioactive compounds in the G. lucidum mushroom and the substrate of the ridge soil should be targeted, as this information is currently

insufficient and a detailed analysis would improve the understanding of these differences and the quality of the final product.

In the current study, the antioxidant activity was demonstrated by determining the total phenolic content, DPPH and FRAP assays. The mushroom species is known for its antioxidant activity, especially for its antitumour effect and its activity on oxidative stress reduction processes. The ethanolic extract was used to determine the antioxidant activity for DPPH and FRAP assays and to determine the total phenolic content. The results showed that the mushroom has antioxidant activity, and the results were correlated with several studies in the literature.

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