Spectral Analysis and Phytochemical Screening of *Ganoderma lucidum* Mushroom Extracts

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Abstract

Ganoderma lucidum is a medicinal mushroom known by various names, the most popular being *LingZhi* or *Reishi*. Even today, this mushroom is used for its adjuvant effect in various diseases such as cardiovascular diseases, metabolic diseases, neurodegenerative diseases, diseases of the immune system, and, above all, for its antioxidant effect in the treatment of cancer. It contains numerous bioactive components, but the most abundant are polysaccharides, triterpenes, and phenolic compounds, which confer biological activity against various acute and chronic diseases. In the present study, we aimed to carry out a general analysis of the composition of different crude extracts of *G. lucidum* using a UV-VIS spectrophotometer by reading the absorbance in a fixed range (200-900 nm). The results obtained were correlated with those reported in the literature. At the same time, a pharmacognostic study was carried out on the mushroom extracts, and the results were detailed. According to the results obtained, we can conclude that *G. lucidum* is a mushroom rich in bioactive compounds.

Keywords: antioxidant activity, UV-VIS spectroscopy, phytochemical screening, bioactive compounds, polysaccharides.

Introduction

Ganoderma lucidum, also known as *Reishi*, has been used in traditional Chinese medicine for over two thousand years for its various health benefits due to its diverse bioactive compounds. The species, *Ganoderma lucidum*, contains over 400 identified bioactive compounds, of which polysaccharides and triterpenes are particularly

important [1]. These components are responsible for numerous therapeutic effects, including anti-tumor activity, the ability to stimulate the immune system, inhibit tumor growth, and reduce hypercholesterolemia [2]. The triterpenes in *Ganoderma lucidum* have been shown to reduce cholesterol levels, improve lipid profiles, and have antihypertensive effects as the bioactive components contribute to blood pressure control [3]. Some biocompounds in *Ganoderma lucidum* have anti-aging effects, antioxidant and cell degeneration prevention properties and it helps to maintain skin health and slows down the aging process [4].

Recently, polysaccharide and triterpene compounds have attracted the attention of researchers due to the complex structure of biocompounds and the pharmacological properties they possess. These two classes of compounds have similar effects that are mediated by different mechanisms of action. For example, polysaccharides stimulate the immune system by activating NK (natural killer) cells, T lymphocytes, and macrophage activation, as well as increasing the production of IL-1 cytokines; they stimulate apoptosis of cancer cells and also stimulate cell regeneration in affected cancer tissues [5]. Some polysaccharide compounds, such as fucogalactans and ganoderan, have the ability to protect the liver from fibrotic effects [6]. Among the triterpene compounds, ganoderic acids have remarkable activity, which has been shown to inhibit tumor cell proliferation by blocking the cell cycle, especially in liver, breast, and prostate cancer; they also contribute to the reduction of oxidative stress through their antioxidant properties due to their highly hydroxylated structures [5, 7].

This study aims to analyze and identify the physico-chemical bio-compounds present in *Ganoderma lucidum* through spectrophotometric and pharmacognostic methods. The pharmacognostic analysis qualitatively assessed the presence of various bioactive compounds, including proteins, carbohydrates, lipids, triterpenes, and phenolic compounds. Bioactive components were extracted using conventional hot water extraction (HWE) and Soxhlet extraction techniques. A comprehensive compositional analysis of *Ganoderma lucidum* via spectrophotometric and analytical methods highlights the abundance of specific compounds of interest, enabling subsequent purification and fractionation for more targeted investigations. Spectrophotometric measurements were conducted to detect and quantify biologically active compounds within a fixed wavelength range (200–900 nm), identifying specific absorption peaks characteristic of these compounds.

Materials and Methods

Sample collection

A mushroom farm in Mehedinți County, Romania, was used to purchase the *Ganoderma lucidum* mushroom. The mushroom was cultivated for approximately 90 days at a controlled temperature of 20-24°C in bags containing sawdust, logs, and berries under optimal conditions. In order to get the mushroom to the powder stage, we first cleaned it with a brush, then dried the sample at a controlled temperature of

25°C for 15 days, and then we cut it into pieces and ground it with a rasp. The grated product was passed through a 0.87 mm mesh sieve to obtain the fine powder [8].

Ganoderma lucidum extracts

Two concentrations of ethyl alcohol, 70% and 96%, were used for the alcoholic extraction. Two extraction methods were used, namely alcoholic extraction by maceration and Soxhlet extraction. To obtain the 70% and 96% ethanolic extractions by maceration (cold), 10 g of powder was weighed, and a volume of 100 mL of 70% and 96% ethyl alcohol was added. The mixture was kept in a cool place away from light for 12 days, shaking the containers occasionally. Finally, the extracts were filtered under reduced pressure, and the liquid (extract) was stored in a refrigerator (at 4°C) until analysis [9].

To obtain the 96% ethanolic extract using the Soxhlet apparatus (hot), 10 g of product was weighed into the cartridge and 300 mL of solvent was weighed. The extraction was subjected to 5 refluxes for 2.5 hours (30 minutes per reflux cycle). Finally, after filtration under reduced pressure, the extraction liquid was collected and stored in a refrigerator (at 4°C) until analysis [10, 11]. For aqueous extraction, 0.2 g of powder was weighed into 500 mL of bidistilled water. The mixture was left on a rotary shaker for 24 hours. Finally, the extract was collected by filtration through Whatmann No. 1 filter paper and stored in a refrigerator (at 4°C) until analysis [9].

Equipment

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

Spectral analysis

In the present study, we used the VWR UV-6300 PC double-beam spectrophotometer VWR UV-6300 PC (**Figure 1**) for spectral analysis. The spectral range between 200-900 nm was used to reveal the biocompounds in the crude, unpurified extract of *Ganoderma lucidum*. However, 70% and 96% "cold" ethanolic extract, 96% "hot" ethanolic extract and aqueous extract were analyzed.

Figure 1. VWR UV-6300 PC double-beam spectrophotometer

The aim of using spectral methods was to determine the different biocompounds present in the composition of the fungus, which further will help us to understand the diversity of this species in terms of chemical composition and abundance of active biocompounds. The samples were read against a blank solution of 96% ethyl alcohol.

Qualitative phytochemical screening

Based on the physicochemical methods used in phytochemical studies, we conducted a pharmacognostic study to identify the classes of bioactive compounds that may be present in the fungal species *Ganoderma lucidum*. The importance of these intuitive analyses lies in the possibility of focusing on the secondary metabolites of the fungus. Methods for the determination of the compounds are described and adapted to the actual working conditions [12-15].

Proteins*.* They were determined by the Biuret Test; 2 mL of filtrate is mixed with 1 drop of 2% $CuSO_4$ and 1 mL of 95% ethanol, over which excess 0.1 N KOH is added. The pink coloration appearing in the ethanolic layer represents the presence of proteins.

Reducing sugars. Before analysis, a solution of Benedict's reagent was prepared. 0.5 mL of filtered extract is mixed with 0.5 mL of Benedict reagent. The mixture is heated in a water bath for 4-5 min. The greenish coloration in the test tube confirms the presence of reducing sugars.

Tannins. Tannins were determined by the Braymer test. 1 mL of filtered extract and 3 mL of bidistilled water were mixed with 3 drops of 5% $FeCl_3$ solution. The bluish-green coloration confirms the presence of tannins.

Terpenoids. Triterpene compounds were determined by the Salkowski test. 1 mg powder is treated with 2 mL chloroform and subsequently filtered. 2-3 drops of 96% H_2SO_4 are added to the resulting mixture. The sample is shaken well and allowed to stand. The yellowish coloration in the lower layer indicates the presence of triterpenoids.

Glycosides. Determination was carried out by the Borntrager test. To start, a few drops of concentrated HCl are added over 50 mg of powder. The sample is left in the water bath for 2 hours for the hydrolysis reaction and filtered. 2 mL of the filtrate obtained and 3 mL of chloroform are shaken well. Observe the separation of the chloroform layer over which a few drops of a 10% ammonium solution are added. The pink coloration confirms the presence of glycosides.

Phenolic compounds. The $FeCl_3$ test is used for determination. A small amount (2 mL) of the filtered extract is mixed with 2-3 drops of 5% $FeCl_3$. The very dark bluish-green coloration of the reaction indicates the presence of phenolic compounds.

Phytosterols*.* Determination was carried out by the Libermann-Bouchard test. 50 mg of the filtered extract is dissolved in 2 mL of acetic anhydride to which 2 drops of 96%

H2SO4 are added. A greenish coloration is observed indicating the presence of phytosterols.

Alkaloids. Determination by the Wagner test. A Wagner reagent solution was prepared before analysis. First, 50 mg of powder was mixed with 3 mL of dilute HCl. The mixture was then filtered. 2 mL of the resulting filtrate was mixed with 2 drops of Wagner reagent. Obtaining a dark brown precipitate indicates the presence of alkaloids.

All assays were performed in triplicate.

Results and Discussions

Qualitative phytochemical screening

Phytochemical screening shows the presence or absence of different compounds in the mushroom composition. Thus, in **Table 1** are highlighted both the chemical methods used and the type of extracts studied: aqueous extracts and "cold" alcoholic extracts of 96% and 70% concentration, as well as the results obtained for the compounds identified.

Table 1. Qualitative determinations of extracts obtained from the mushroom species *Ganoderma lucidum*.

Compounds	Reaction	Aquous extract	96% ethanolic extract	70% ethanolic extract
Proteins	Biuret Test	++	+++	+++
Carbohydrates	Benedict Test	+++	+++	++
Lipid compounds	Heating	+	+	+
Saponins	Foaming	++	++	+
Tannins	Braymer Test	++	++	+
Triterpenes	Salkowski Test	++	++	++
Glycosides	Borntrager Test	+++	++	+
Phenolic compounds	$FeCl_3$ Test	++	++	++
Sterols	Libermann-Bourchard	-	+	+
Alkaloids	Wagner Test	-	+	+

+; ++; +++: express weak, moderate and significant result of the presence of different compounds.

The analysis revealed the presence of all studied compounds in the alcoholic extracts. Proteins were significantly abundant in both the 96% and 70% ethanol extracts, followed by carbohydrates. Carbohydrates showed a notable presence in both the aqueous extract and the 96% ethanol extract, while their presence was moderate in the 70% ethanol extract.

Triterpenes and phenolic compounds were moderately present across all three extracts. The aqueous extract exhibited the most diverse range of compounds but had lower concentrations of sterols and alkaloids. In contrast, sterols and alkaloids were detected in the ethanolic extracts, though in lower concentrations compared to other compounds. Lipid compounds were consistently identified in all extracts but at low concentrations. Tannins and saponins were moderately present in the aqueous extract and the 96% ethanol extract, whereas their levels were lower in the 70% ethanol extract. In the aqueous extract, proteins, carbohydrates, and glycosides were observed in significant amounts.

Comparing these results with findings from the literature highlights notable similarities. Islam et al. (2018) conducted a phytochemical screening of *Ganoderma lucidum* aqueous extracts, reporting a significant presence of carbohydrates, saponins, and glycosides [12]. Similarly, Orole et al. (2016) analyzed the mushroom's composition in organic solvents (ethyl acetate and N-hexane) and identified flavonoids, phenolic compounds, sterols, triterpenoids, and glycosides [13]. Thapa et al. (2022) investigated the composition of both aqueous and ethanolic extracts, reporting flavonoids, terpenoids, and saponins in both extracts, along with tannins in the aqueous extract [14].

Spectral analysis

Spectroscopic techniques are essential for both qualitative and quantitative evaluation in pharmaceutical analysis. They provide critical insights into the presence of active components and secondary metabolites, as demonstrated in the analysis of *Ganoderma lucidum* extracts. In this study, UV-VIS spectroscopy was employed to evaluate the UV-visible fingerprint of the extracts.

The analyzed extracts, prepared using different concentrations and methods, were crude and had not undergone fractionation or purification. The primary objective of studying these crude extracts was to corroborate the findings of the pharmacognostic analysis. This global analysis of *Ganoderma lucidum* composition aimed to identify predominant bioactive compounds and secondary metabolites, providing an understanding of its diverse chemical profile.

UV-VIS Spectra Results

Figure 2 presents the overlaid absorption spectra for all four extracts across a wavelength range of 200–900 nm. The 70% ethanolic extract prepared via maceration (red) displayed absorption peaks between 200–425 nm. The 96% ethanolic maceration extract (blue) exhibited peaks between 200–350 nm, while the hot Soxhlet 96% ethanolic extract (pink) showed peaks from 200–325 nm and an additional peak in the range of 390–400 nm. The aqueous extract (purple) revealed two distinct absorption peaks within the 200–300 nm range. Notably, the ethanolic extracts (both hot and cold) showed specific absorbance maxima between 200–400 nm, with the highest absorbance value of 3.5 uab.

These results confirm the presence of numerous bioactive compounds in *Ganoderma lucidum* extracts. However, purification and fractionation of the extracts are essential for future studies to achieve precise quantitative determinations of individual components.

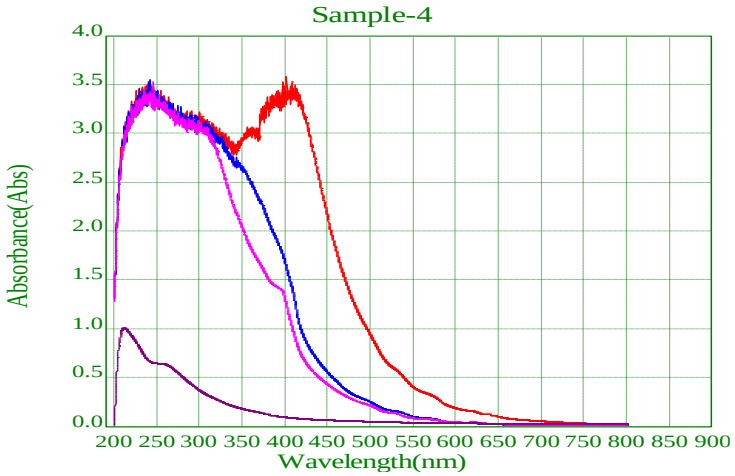

Figure 2. Spectral analysis for extracts: “cold” ethanolic 70% (red), “cold” ethanolic 96% (blue), “hot” ethanolic 96% (pink) and aquous extract (purple)

Figure 3 illustrates the absorption spectra of all four extracts within the 200–400 nm wavelength range, providing further insight into the presence of various components. In the aqueous extracts, components exhibited a maximum absorbance of approximately 1 uab, followed by a gradual decline in absorbance throughout the 250–345 nm range. For the alcoholic extracts, absorbances increased significantly within the 200–240 nm range, reaching a maximum of 3.5 uab. Beyond this, absorbances uniformly decreased between 250–310 nm. In the 310–345 nm range, the decrease varied with different slopes depending on the extract. The 70% ethanolic extract showed a slight increase in absorbance in the 345–365 nm range, peaking at 3.0 uab. A notable but small jump in absorbance was observed at 365 nm, followed by a steady increase from 365–400 nm. These variations in absorbance across the 200–400 nm spectrum highlight the presence of a rich composition of biocompounds, each exhibiting specific absorption patterns at different wavelengths.

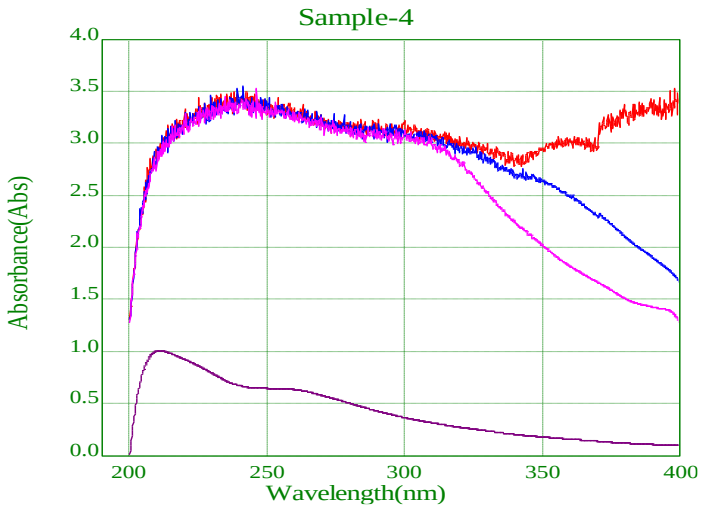

Figure 3. Closer view of spectra for extracts: "cold" ethanolic 70% (red), "cold" ethanolic 96% (blue), "hot" ethanolic 96% (pink) and aquous extract (purple)

Spectral analyses on *Ganoderma lucidum* are confirmed by other studies in the literature. A study conducted by Zhao et al. in 2010 on purified extracts of *Ganoderma lucidum* revealed the presence of polysaccharide compounds using a UV-VIS spectrophotometer in the spectral range 200-400 nm [16].

Figure 4a shows the results from the spectral analysis of the aqueous extract in the 200-800 nm range. From the pharmacognostic study, polysaccharides that were solubilized in water were found in the aqueous extracts. The wavelength range was 200-800 nm, and a significant absorption peak was recorded at 210 nm for an absorbance of 1 uab. **Figure 4b** shows an aqueous extract from *Ganoderma lucidum*, which was fractionated into two fractions, namely, GP-1 and GP-2, by Zhao et al. (2010) [16]. The authors showed that the two fractions had absorption peaks in the spectral range 210-230 nm. [16].

These results, obtained by us and by the team of Zhao et al., allow us to demonstrate the existence of polysaccharides in the aqueous extract of polysaccharides in the composition of the fungus *Ganoderma lucidum*.

Figure 4a and **Figure 4b** show a comparison between the UV-VIS spectral analysis of the aqueous extract of *Ganoderma lucidum* and the spectral analysis of the two purified polysaccharide fractions of the aqueous extract of *Ganoderma lucidum* obtained by Zhao et al. (2010) [16].

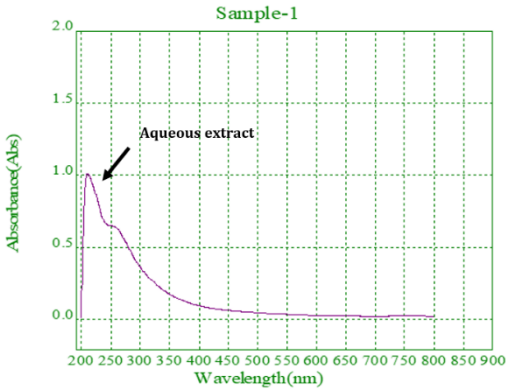

Figure 4a. Own results of spectral analysis of aqueous extracts of *Ganoderma lucidum*

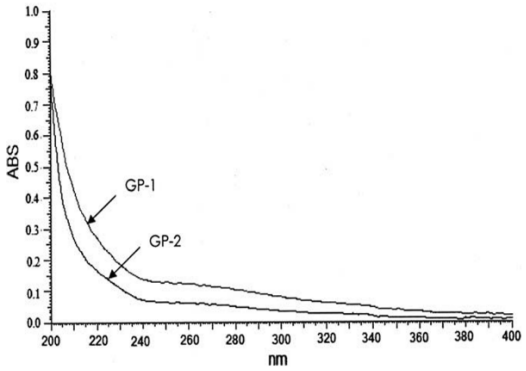

Figura 4b. Results obtained by spectral analysis of aqueous extracts of *Ganoderma lucidum* by Zhao et al. (2010) [16].

For future determinations, in order to obtain clearer values on the abundance of polysaccharide compounds in the composition of the mushroom we have been working with, it is necessary to purify and fractionate the polysaccharides from the extract obtained.

Conclusion

This study aimed to perform a comprehensive compositional analysis of *Ganoderma lucidum* through various pharmacognostic evaluations. Phytochemical screening identified numerous bioactive compounds, including polysaccharides, triterpenes, and phenolic compounds, which are known for their significant roles in treating various acute and chronic diseases. Additionally, proteins, lipids, saponins, tannins, glycosides, sterols, and alkaloids were also detected.

The compositions of four different extracts—one aqueous and three ethanolic extracts prepared at varying concentrations and temperatures—were analyzed using spectral methods. These analyses confirmed the presence of a diverse range of bioactive compounds. A comparative spectral analysis further validated the presence of polysaccharides in the aqueous extracts, consistent with findings reported by other researchers in the literature.

Future research could focus on the purification and detailed characterization of specific active biocompounds. Additionally, in vitro and in vivo studies should be conducted to explore further pharmacological effects, offering deeper insights into the therapeutic potential of *Ganoderma lucidum*.

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