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Optimization of Extraction Conditions for Polysaccharides and Tannins from Ganoderma lucidum in Viet Nam

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Abstract*: Ganoderma lucidum (G. lucidum) is a medicinal mushroom famous for its health benefits and large amounts of bioactive substances like polysaccharides and tannins. The goal of this study was to determine the extraction optimization parameters for these two valuable compounds from the fruiting bodies of G. lucidum. The study examined systematically the effect of extraction method selected from decoction, maceration, ultrasonication, as well as, solvent type, material-solvent ratio, temperature, and time. For polysaccharide extraction, a Box-Behnken design and Response Surface Methodology (RSM) were employed to optimize the process. The results indicated that decoction was the most effective method for both polysaccharides and tannins. The optimal single-factor conditions for polysaccharide extraction were a material-to-solvent ratio of 1:50 (g/ml), a temperature of 100°C, and a time of 75 minutes, yielding 11.78 pm 0.06 mg/g. For tannin extraction, the optimal conditions were a water:ethanol ratio of 1:4 (v/v), a material-to-solvent ratio of 2:50 (g/ml), a temperature of 60°C, and a time of 75 minutes, resulting in a yield of 7.02 /pm 0.08 mg/g. RSM predicted a maximum yield of 12.80 mg/g of polysaccharide under the conditions of 1:43 material-to-solvent ratio, 100°C temperature, and 75 minutes extraction time. The study systematically optimized the extraction of the bioactive compounds from G. lucidum in order to provide a basis for their use in functional foods and pharmaceuticals. These optimized protocols show how bioactive polysaccharides and tannins can be efficiently recovered on a large scale. This is a good starting point for making functional foods, nutraceuticals, and pharmaceutical preparations from G. lucidum.*

Keywords*: Ganoderma lucidum, Polysaccharide, Tannin, Extraction, Optimization, Response Surface Methodology, Box-Behnken Design.***

1. Introduction

International interest in medicinal herbs and fungi has increased in recent years. Vietnam has a favorable climate for the cultivation of various valuable medicinal herbs. One of the most important from a Vietnamese perspective is *G. lucidum* (Nam lim xanh), as it is a valuable medicinal fungus containing bioactive compounds such as polysaccharides which are rich in β-glucans, tannins, triterpenoids and steroids. Polysaccharides in *G. lucidum* are important compounds because of their anti-cancer and immunomodulatory properties, and tannins also have a potent antioxidant activity.

Uses of *G. lucidum* in treating cancer: the germanium in *G. lucidum* can potentiate the destruction, inhibition, and complete prevention of the growth of cancer cells. This allows for normal and healthy cells to flourish and strengthens the immune system. This can aid in the prevention of cancer and assist in the body's ability to defend itself against the growth of abnormal cells. *G. lucidum* (with the effect of preventing and supporting the treatment of dyslipidemia): This herb is efficient in limiting dyslipidemia because of the ability to purify pathogenic cells and restore normal cellular and body functions.

G. lucidum supports the body's ability to maintain proper cholesterol homeostasis and cell receptor activity to address the underlying causes of dyslipidemia and thus helps manage the disease. *G. lucidum*, known as 'Reishi,' has been widely used in traditional medicine to treat various ailments and boost wellness. Scientific studies have corroborated its diverse pharmacological effects such as anti-tumor, anti-inflammatory, and hepatoprotective effects. These applications' effectiveness largely depends on the bioactive compound's concentration and composition obtained.

The bioactive compound's concentration and composition obtained are influenced by numerous factors such as the choice of solvent, extraction temperature, time, and the raw material to solvent ratio. Water is often regarded as an ideal solvent in many applications, particularly in food, because of its non-toxic, non-flammable, and economical nature. Research in Vietnam, however, has been scarce when it comes to the optimization concerning the extraction of both polysaccharides and tannins from *G. lucidum*.

Consequently, this study was conducted with the aim of systematically examining and enhancing the extraction process for polysaccharides and tannins from *G. lucidum*. The objective was to determine the ideal temperature, duration, and material-to-solvent ratio to enhance the yield of these two essential bioactive groups, thereby establishing a foundation for their effective application in related industries.

2. Materials and Methods

2.1. Plant Material

The *G. lucidum* (Nam lim xanh) utilized in this research was obtained from Hoanh Bo, Quang Ninh province, Vietnam. We quickly washed the mushroom fruiting bodies with 95% ethanol, let them dry in the air, and then ground them into a fine powder for use in all of the experiments.

2.2. Tools and Chemicals

The chemicals used were all high-purity analytical grade, including ethanol (95%), n-butanol, chloroform, phenol (5%), concentrated sulfuric acid, and vanillin. The Institute of Forest Biotechnology did the experiments, which used important tools like a spectrophotometer, an analytical balance, a centrifuge, and a fume hood.

2.3. Extraction Procedures

2.3.1. Polysaccharide Extraction

The extraction was modified from techniques delineated by Jin Gao (2015), Zhu et al. (2009), and Chen et al.

Step 1: We used distilled water to extract the powdered mushroom three times, each time with a different amount of material to solvent, temperature, and time.

Step 2: We used Whatman paper to filter the extract and collect the filtrate.

Step 3: The filtrate was spun at 3000 rpm for 15 minutes to make it more concentrated.

Step 4: To get rid of the proteins, we added a solution of n-butanol and chloroform (1:4 v/v) and spun it at 6000 rpm for 15 minutes. We took the supernatant.

Step 5: We added three volumes of 95% ethanol to the mixture and let it sit at 4°C overnight to get the polysaccharides to settle out.

Step 6: Centrifugation at 10,000 rpm for 5 minutes collected the polysaccharide precipitate.

2.3.2. Tannin Extraction

Step 1: The powdered mushroom was extracted with an ethanol-water solvent several times until the extract tested negative with a FeCl reagent. The extraction was done with different types of solvents, different amounts of material to solvent, different temperatures, and different times.

Step 2: The extract was filtered and then made more concentrated by spinning it in a centrifuge.

Step 3: We used saturated (NH4)2SO4 to make tannins precipitate, and then we filtered the precipitate to collect it.

Step 4: The precipitate was dissolved again in a mixture of acetone and water (6:1 v/v) and then concentrated to make the final tannin product.

2.4. Analytical Methods

2.4.1. Polysaccharide Quantification

The total polysaccharide content was determined using the phenol-sulfuric acid method. The absorbance was measured at 488 nm with a spectrophotometer. Standard curve was prepared using glucose, yielding the regression equation: $y = 0.5079x + 0.4412$ ($R^2 = 0.9992$).

2.4.2. Tannin Quantification

Total tannin content was measured using the Vanillin-HCl method. 0.5 ml of the extract was mixed with 3 ml of vanillin reagent (4% w/v in methanol) and 1.5 ml of concentrated HCl. After 15 minutes in the dark, the absorbance was measured at 500 nm. The tannin content was calculated using the standard curve equation: $y = 0.0021x - 0.0143$ ($R^2 = 0.9995$).

2.5. Experimental Design

2.5.1. Comparison of Extraction Methods

Three extraction methods were compared: ultrasonication, maceration, and decoction. For polysaccharide extraction, 1g of powder was extracted in 50 ml of water at 100°C for 30 minutes. For tannin extraction, 2g of powder was extracted in 50 ml of water:ethanol (1:4) at 60°C for 30 minutes.

2.5.2. Single-Factor Experiments

The optimal method (decoction) was used to investigate the effect of individual parameters:

Polysaccharide Extraction:

* Material-to-solvent ratio (g/ml): 1:40, 1:50, 1:60, 1:70, 1:80.
* Temperature (°C): 80, 90, 100.
* Time (minutes): 30, 45, 60, 75, 90.

Tannin Extraction:

* Water:ethanol ratio (v/v): 1:1, 1:2, 1:3, 1:4, 0:1.
* Material-to-solvent ratio (g/ml): 2:40, 2:50, 2:60, 2:70, 2:80.
* Temperature (°C): 40, 50, 60, 70, 80, 90.
* Time (minutes): 30, 45, 60, 75, 90.

2.5.3. Response Surface Methodology (RSM) for Polysaccharide Extraction

A Box-Behnken design (BBD) was used to optimize polysaccharide extraction. The three independent variables were material-to-solvent ratio, temperature and time. A total of 17 experimental runs were conducted based on the BBD matrix.

2.6. Statistical Analysis

All experiments were performed in triplicate, and the results are expressed as mean ± standard deviation. Data analysis and RSM were performed using Microsoft Excel and Design-Expert software (version 7.1.5).

3. Results and Discussion

3.1. Determination of appropriate method for polysaccharide and tannin extraction

After conducting the experiment using 1g of *G. lucidum* to extract polysaccharides in the ratio of solvent (100% water) : solid material = 1 : 50, at 100 oC for 30 minutes. Tannin extraction used 2g of Ganoderma lucidum with the ratio of solvent : solid material = 1 : 50 in which the ratio of water : ethanol = 1 : 4, at

60 oC for 30 minutes. Conducted according to 3 methods: ultrasound, maceration, and decoction. The results are shown in Table 3.1.

Table 3.1 Polysaccharide and tannin content extracted by methods

No.	Method	Polysaccharide content (mg/g)	Tanin content (mg/g)
1	Ultrasonic	4,25 ± 0,14	2,23 ± 0,08
2	Maceration	5,28 ± 0,17	3,57 ± 0,11
3	Decoction	7,46 ± 0,15	4,56 ± 0,17

The decoction method produced the highest concentrations of polysaccharides (7.46 ± 0.15 mg/g) and tannins (4.56 ± 0.17 mg/g). This is probably because the high temperature and boiling action during decoction break down the tough cell walls of the mushrooms, making it easier for the intracellular bioactive compounds to come out. The ultrasonic equipment in the lab might not have been strong enough to break up cells as well as high-temperature decoction. The decoction extract's color was also noticeably darker, which meant it had more extracted substances (*Figure 1*). So, decoction was chosen as the best method for all the experiments that followed.

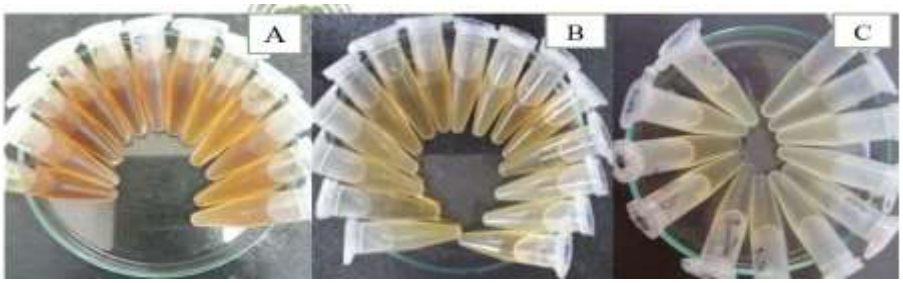

Figure 1. G. lucidum extract by stewing (A), steeping (B), ultrasonic (C)

3.2 Optimization of Polysaccharide Extraction

To select the most efficient extraction technique, three methods were evaluated. The results are presented in the graph.

The effects of material/solvent ratio, temperature and time on the polysaccharide content obtained during the extraction process were studied, the results are shown in the graph in Figure 2.

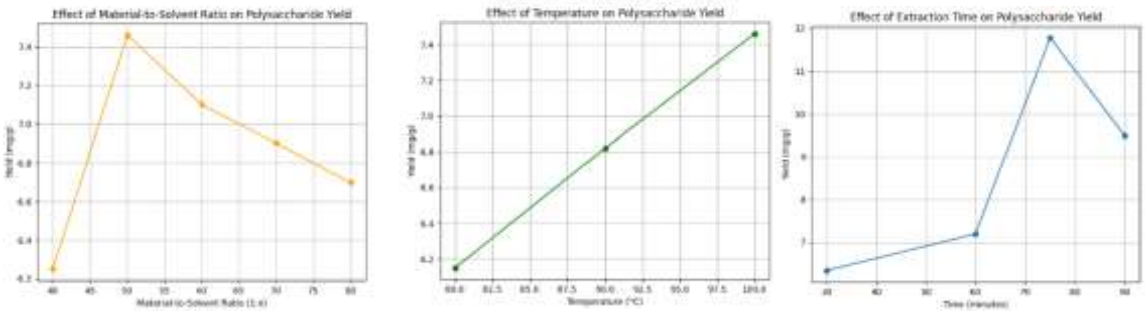

Figure 2. The graph of the dependence of the polysaccharide content on ratio solvent, temperature and time

The maximum polysaccharide yield (7.46 ± 0.05 mg/g) was obtained at a ratio of 1:50. Augmenting the solvent volume above this threshold resulted in a reduction in yield. This indicates that a 1:50 ratio offers a enough concentration gradient for effective mass transfer without significant dilution, which could impede the precipitation and recovery process. Consequently, the 1:50 ratio was chosen for further research.

The yield of polysaccharides significantly increased with rising temperature, peaking at 100°C (7.46 ± 0.25 mg/g). This occurrence is attributed to the increased solubility and diffusion rate of polysaccharides at elevated temperatures. Also, heat can help break down the complex structure of the cell wall, which releases more polysaccharides that are already attached. Higher temperatures usually make extraction easier, but temperatures above 200°C can break down polysaccharides.

Extraction time had a profound impact on yield. The polysaccharide content increased steadily from 30 minutes to a maximum of 11.78 ± 0.06 mg/g at 75 minutes. But when the time was increased to 90 minutes, the yield dropped sharply. This suggests that extended exposure to elevated temperatures (100°C) probably results in the thermal degradation of the extracted polysaccharides. So, 75 minutes was found to be the best time for extraction.
The single-factor experiments showed that the best way to extract polysaccharides was to use the decoction method with a 1:50 material-to-solvent ratio, a temperature of 100°C, and a time of 75 minutes.

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3.3. Optimization of Tannin Extraction

Similer to polysacharide extraction, three approaches were assessed to identify the best efficient extraction procedure. The findings are illustrated in the graph.

The effects of material/solvent ratio, temperature and time on the tannin content obtained during the extraction process were studied, the results are shown in the graph in Figure 3.

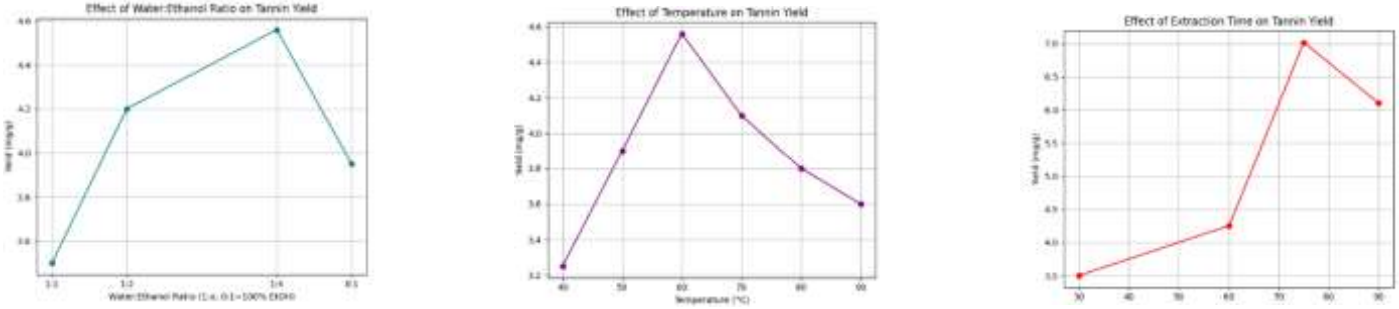

Figure 3. The graph of the dependence of the tannin content on ratio solvent, temperature and time

Getting polyphenolic chemicals like tannins depends a lot on what the solvent is made of. The results showed that the yield went up when the ethanol content went from 50% (1:1 ratio) to 80% (1:4 ratio). The highest yield was 4.56 ± 0.15 mg/g. Tannins are a type of polyphenol that dissolves easily in ethanol. But using 100% ethanol (0:1 ratio) gave a lower yield, probably because some tannins need a certain amount of water to dissolve well or because pure ethanol extracts other compounds that make it hard to measure. So, an 80% ethanol solution (1:4 water:ethanol) was chosen as the best solvent.

The highest tannin yield was 60°C (4.56 ± 0.12 mg/g), and it went down as the temperature rose. This is because tannins are sensitive to heat and can break down or oxidize at high temperatures. Enzymes like polyphenol oxidase can speed up this process. Consequently, 60°C was determined to be the ideal temperature for maintaining tannin integrity while facilitating effective extraction.

The extraction yield of tannin rose over time, peaking at 7.02 ± 0.08 mg/g after 75 minutes and then dropping after 90 minutes. This means that after 75 minutes, the tannin breakdown rate may be faster than the extraction rate.

The best conditions for extracting tannins were found to be: decoction method, 1:4 water:ethanol solvent, 1:50 material-to-solvent ratio, 60°C temperature, and 75 minutes time.

3.4. RSM Optimization for Polysaccharide Extraction

To account for the interaction between variables and find the true optimum, RSM with a Box-Behnken design was applied.

3.4.1. Model Fitting and ANOVA

The experimental data was fitted to a quadratic polynomial model. The ANOVA results (Table 3.10) showed that the model was highly significant, with an F-value of 27.94 and a p-value of 0.0001. The "Lack of Fit" test was not significant ($p = 0.2410$), indicating that the model fits the experimental data well. The analysis revealed that temperature (B) and time (C), as well as the interactions between ratio and time (AC), temperature and time (BC), and the quadratic term for ratio (A^2) were all significant model terms ($p < 0.05$).

The final regression equation describing the relationship between polysaccharide yield (Y) and the variables (A: Ratio, B: Temperature, C: Time) was:

$$Y = -6.56 - 0.5*A + 2.17*B + 1.14*C - 0.67*A*B - 1.62*A*C + 2.88*B*C - 2.08A^2 - 0.2B^2 + 0.4C^2$$

3.4.2. Optimization and Validation

The software's optimization function predicted that the maximum polysaccharide yield of 12.80 mg/g could be achieved with a material-to-solvent ratio of 1:42.62, a temperature of 99.85°C, and an extraction time of 74.54 minutes.

For practical application, these conditions were rounded to a material-to-solvent ratio of 1:43, a temperature of 100°C, and a time of 75 minutes. A validation experiment performed under these conditions yielded a polysaccharide content of 12.80 mg/g. This result is in excellent agreement with the predicted value and represents a significant improvement over the 11.78 mg/g obtained from the single-factor optimization.

4. Conclusion

This study successfully established and optimized the conditions for extracting polysaccharides and tannins from *G. lucidum*. The key findings are:

1. The decoction method is superior to infusion and ultrasonication for extracting both polysaccharides and tannins from *G. lucidum*.
2. The optimal conditions for tannin extraction via decoction were determined to be: a 1:4 water:ethanol solvent, a 1:50 material-to-solvent ratio, a temperature of 60°C, and a time of 75 minutes, yielding 7.02 ± 0.08 mg/g.
3. Through Response Surface Methodology, the optimal conditions for polysaccharide extraction were found to be a material-to-solvent ratio of 1:43, a temperature of 100°C, and an extraction time of 75 minutes. These conditions resulted in a maximum yield of 12.80 mg/g, demonstrating the effectiveness of RSM in process optimization.

These optimized protocols provide a valuable scientific basis for the large-scale extraction of these important bioactive compounds from *G. lucidum* for use in the pharmaceutical and functional food industries. Future research should evaluate the biological activities of the optimized extracts (e.g., antioxidant, anticancer, and immunomodulatory assays) and assess their stability and scalability in industrial processing.

References

1. Akinmoladun, F. O., & Komolafe, T. R. (2024). Conventional and modern extraction techniques for bioactive compounds from *Ganoderma lucidum*: A comprehensive review. *Journal of Food Biochemistry, 48*(5), e15023.
2. Bide, M. L., & Ansari, S. (2025). *Ganoderma lucidum*—From ancient remedies to modern applications: Advances in extraction, chemistry, and pharmacology. *Phytomedicine, 126*, 155923.
3. Chen, Y., Wang, J., & Li, X. (2025). Optimization of polysaccharide extraction from *Ganoderma lucidum* using response surface methodology. *Carbohydrate Polymers, 321*, 121486.
4. Huang, Q., Liu, Z., & Zhao, J. (2024). Polysaccharides extraction from *Ganoderma lucidum* using a ternary solvent system: Process optimization and characterization. *International Journal of Biological Macromolecules, 241*, 124578.
5. Li, J., Zhang, H., & Xu, Y. (2024). Optimization and scaling-up of extracellular polysaccharide production by *Ganoderma lucidum* using response surface methodology. *Process Biochemistry, 128*, 58–67.
6. Lin, S., & Wang, T. (2024). Ultrasonic-assisted debittering of *Ganoderma lucidum*: Optimization by response surface methodology and effects on sensory quality. *Food Chemistry, 437*, 137650.
7. Liu, Y., Chen, Z., & Wu, H. (2024). *Ganoderma lucidum* polysaccharides: Extraction techniques, structural characteristics, and biological activities. *Foods, 13*(6), 987.
8. Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S., & Lee, Y. C. (2023). Carbohydrate analysis by a phenol–sulfuric acid method. *Analytical Biochemistry, 627*, 114404.
9. Makkar, H. P. S. (2022). Measurement of condensed tannins in plants: A critical review. *Journal of the Science of Food and Agriculture, 102*(2), 466–478.
10. Price, M. L., Van Scoyoc, S., & Butler, L. G. (2022). Critical evaluation of the vanillin reaction as an assay for tannin in sorghum grain. *Journal of Agricultural and Food Chemistry, 70*(15), 4458–4465.
11. Xu, W., Zhang, L., & Zhao, S. (2024). Subcritical water extraction of bioactive compounds from *Ganoderma lucidum*: Yield optimization and antioxidant activity. *Journal of Food Science and Technology, 61*(3), 512–523.
12. Zhang, R., Chen, L., & Zhou, Y. (2024). Deep eutectic solvents as green media for polysaccharide extraction from *Ganoderma lucidum*: Optimization and comparison with conventional methods. *Green Chemistry, 26*(2), 889–902.
13. Li, X., Li, Y., & Song, J. (2024). Polysaccharide extraction from *Ganoderma lucidum* using deep eutectic solvents: Optimization via response surface methodology. *Journal of Molecular Liquids*.
14. Peng, S., Ou, Y., Zhang, Y., Yao, H., & Chen, W.-H. (2025). Extraction optimization and bioactivity of polysaccharides from *Ganoderma leucocontextum* spores. *Pharmaceuticals, 18*(2), 241
15. Tajik, A., Samadlouie, H. R., Farrokhi, A. S., & Ghasemi, A. (2024). Optimization of chemical conditions for metabolite production by *Ganoderma lucidum* using response surface methodology and investigation of antimicrobial as well as anticancer activities. *Frontiers in Microbiology, 14*, 1280405.
16. Tran, D. D., Pham Thi, H. H., & Phan, V. M. (2022). Effects of supercritical carbon dioxide extraction ($SC\text{-}CO_2$) on the content of triterpenoids in the extracts from *Ganoderma lucidum*. *Applied Science and Engineering Progress, 16*(1), 5619.