NETWORK PHARMACOLOGY-GUIDED DEVELOPMENT OF A *GANODERMA LUCIDUM* SPRAY-DRIED POWDER FOR CANCER THERAPY

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ABSTRACT

Introduction: *Ganoderma lucidum* (GL), a valuable medicinal mushroom in traditional medicine, is recognized for its numerous health benefits, including immune system enhancement, blood pressure regulation, and anti-tumor effects. **Objective**: This study focuses on analyzing the network pharmacology of GL in relation to immuno-oncology, thereby guiding the development of GL spray-dried powder to support cancer treatment. **Materials and methods**: A network pharmacology study was employed to identify target bioactive compounds from GL. The extraction process was optimized using Design Expert 12 with a Box-Behnken design, considering three independent variables: solvent concentration, extraction time, and extraction temperature. The optimal extract was then used to investigate factors influencing the spray-drying process, including excipient ratio, drying temperature, and feed rate. Total triterpenoid and polyphenol contents were quantified using UV-Vis spectrophotometry with ursolic acid and gallic acid as standards. The spray-dried powder was analyzed for ganoderic acid A content using HPLC. **Results**: Molecular pharmacology analysis and literature review identified triterpenoids and polyphenols as key bioactive groups. The optimal extraction conditions were 75% ethanol, 100 °C, 80 minutes, and a material-to-solvent ratio of 1:100 (g/mL). The spray-drying process was most effective with 20% excipient, a drying temperature of 120 °C, and a feed rate of 10%. The average content of ganoderic acid A in the spray-dried powder was 55.76 mg/g. **Conclusion**: The study successfully optimized the extraction and spray-drying processes for GL, resulting in a powder rich in triterpenoids and polyphenols. These findings may provide a foundation for developing safe and effective pharmaceutical products, serving as a reference for future research.

Keywords: *Ganoderma lucidum, network pharmacology, optimization, spray-drying, triterpenoid, polyphenol, ganoderic acid A*

I. INTRODUCTION

Nowadays, despite the remarkable advances made in modern medicine, traditional medicine continues to play a vital role in the prevention and management of chronic diseases, as well as in promoting overall health. Among its therapeutic approaches, the use of medicinal plants has been demonstrated to be effective in modulating the immune system and exerting anti-tumor activities. GL serves as a prominent example highlighting this potential.

GL, commonly known as Lingzhi or Reishi mushroom, belongs to the family Ganodermataceae. It has long been utilized in several Asian countries for its health-promoting properties and its reputed ability to prolong life expectancy. In Vietnam, GL is mainly cultivated in mountainous regions and certain provinces, where its fruiting bodies and spores are harvested, dried, and processed into powders, beverages, or lyophilized (freeze-dried) preparations [1].

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GL contains approximately 140 triterpenes (predominantly lanostane-type triterpenoid saponins), along with phenolic acids and polyphenols (including flavonoids such as quercetin). These bioactive constituents have been associated with regulating blood pressure, reducing cholesterol levels, improving circulatory function, and alleviating respiratory disorders, such as allergic bronchitis and bronchial asthma [8].

Besides, recent advances in network pharmacology analysis have enabled the elucidation of multi-component and multi-target mechanisms of medicinal plants at the molecular level. This approach facilitates the identification of potential bioactive compounds in herbal medicines, guiding extraction processes to achieve higher yields of biologically active substances or compound groups. Additionally, spray-drying technology is widely used in the pharmaceutical and food industries to enhance thermal stability, control particle size, and reduce product moisture content [5].

Based on these considerations, this study conducted a network pharmacology analysis of GL in relation to immunological and cancer-related pathways. The objective was to guide the development of a spray-dried GL powder formulation for potential use as an adjuvant in cancer therapy.

II. MATERIALS AND METHODS

2.1. Materials

GL was cultivated, harvested, and preliminarily processed at Ngoc Anh Lingzhi Mushroom Farm, located in Hamlet 2, An Nhon Tay Commune, Cu Chi District, Ho Chi Minh City, Vietnam, before being packaged in 500 g bags.

2.2. Instruments

In this study, the main instruments employed included: an automatic Soxhlet extractor (model E800, Büchi, Switzerland); an ultrasonic bath (model S100H, Elma, Germany); an infrared moisture analyzer (model MA37-1, Sartorius, Germany); a centrifuge (model Z306, Hermle, Germany); a spray dryer (model B-290, Büchi, Switzerland); a UV-Vis spectrophotometer (model Lambda 365, PerkinElmer, USA); and a high-performance liquid chromatography (HPLC) system equipped with a PDA detector (model Alliance e2695, Waters, USA).

2.3. Chemicals

The chemicals and solvents used in this study included: Folin–Ciocalteu reagent (Merck, Germany); 70% perchloric acid (Alpha, India); vanillin (Shanghai Zhanyun Chemical, China); gallic acid standard (Sigma–Aldrich, USA); ursolic acid standard (Sigma–Aldrich, USA); ganoderic acid A standard (USP, Lot no. F01200, content 0.95 mg/mg); methanol and acetic acid (China); and 96% ethanol (Nguyenlongtech, Vietnam).

2.4. Methods

2.4.1. Network pharmacology analysis

The phytometabolites previously identified in GL were retrieved from the Traditional Chinese Medicine Integrated Database for Immuno-Oncology (TCMIO) [4]. These compounds were then utilized for target protein identification and pathway enrichment analysis to evaluate the anticancer potential of GL.

2.4.2. Investigation and optimization of extraction conditions

2.4.2.1. Investigation of extraction conditions

The extraction parameters examined included ethanol concentration in the extraction solvent (40%, 70%, and 96%), extraction temperature (40, 80, and 120 °C),

and extraction time (50, 100, and 150 minutes).

2.4.2.2. Optimization of the extraction process

A Box–Behnken design was employed to optimize the extraction process, with independent variables comprising ethanol concentration, extraction temperature, and extraction time, while the dependent variables were the total triterpenoid and polyphenol contents. The variable levels (−1, 0, +1) were determined from preliminary experiments. The experimental runs were performed according to the software design, and the results were recorded. The effects of the independent variables were analyzed using ANOVA and regression modeling. Optimal extraction conditions suggested by the software were validated by repeating the experiment six times.

2.4.2.3. Investigation of the raw material-to-solvent ratio

Different raw material-to-solvent ratios were investigated, including 1:50, 1:100, 1:150, and 1:200 (g/mL).

2.4.2.4. Investigation of the spray-drying process

The spray-drying conditions of GL extracts were investigated with respect to carrier agent (maltodextrin) concentration (5, 10, 15, 20, and 25%), inlet temperature (100, 120, 140, 160, and 180 °C), and feed pump rate (5, 10, 15, 20, and 25%).

2.4.3. Determination of total triterpenoid and polyphenol contents

2.4.3.1. Determination of total triterpenoid content in extracts

Five milliliters of the extract were evaporated to dryness and dissolved in methanol. An aliquot of 100 µL was mixed with 150 µL of vanillin–acetic acid (5%) and 500 µL of perchloric acid (70%). The mixture was then heated at 60 °C for 45 minutes, cooled on ice, and supplemented with 2.25 mL of acetic acid. Absorbance was measured at 548 nm, and triterpenoid content was calculated based on a calibration curve of ursolic acid, with methanol as the blank.

2.4.3.2. Determination of total polyphenol content in extracts

Five milliliters of the extract were evaporated to dryness and dissolved in methanol. An aliquot of 500 µL was mixed with 2.5 mL of Folin-Ciocalteu reagent (1:10) and 2 mL of Na_2CO_3 solution (75 g/L), then incubated at 40 °C for 30 minutes. Absorbance was measured at 765 nm, and polyphenol content was calculated based on a calibration curve of gallic acid, with methanol as the blank.

2.4.3.3. Determination of total triterpenoid and polyphenol contents in spray-dried powder

A total of 0.4 g of spray-dried powder was dissolved in 5 mL of methanol, vortexed for 1 minute, sonicated for 20 minutes, and centrifuged at 4500 rpm for 15 minutes. The supernatant was filtered through a 0.45 µm membrane, and 100 µL of the filtrate was subjected to UV-Vis spectrophotometric analysis using the methods described above.

2.4.4. Quantification of ganoderic acid A in spray-dried powder

Sample preparation: Approximately 2 g of the formulation was accurately weighed, mixed with 5 mL of methanol, and vortexed for 30 seconds. The mixture was subjected to ultrasonic extraction for 15 minutes. The supernatant was collected and filtered through a 0.45 µm membrane prior to analysis.

Standard preparation: Approximately 6 mg of ganoderic acid A reference standard was accurately weighed and transferred into a 20 mL volumetric flask. The standard was dissolved in 15 mL of methanol and diluted

to volume with methanol, yielding a stock solution at approximately 300 µg/mL concentration. A series of calibration solutions with concentrations ranging from 10 to 300 µg/mL of ganoderic acid A was prepared by diluting the stock solution.

Blank: Methanol.

Analytical conditions: The analysis was performed under the chromatographic conditions reported in reference [2].

III. RESULTS

3.1. Network pharmacology analysis

A total of 275 compounds of GL were retrieved from the TCMIO database, primarily classified into three groups: triterpenoids, steroids, and other compounds (**Table 1**). Among these, triterpenoids accounted for the highest proportion (66.18%), with their associated biological targets ranking second (44.05%) after the group of other compounds. Furthermore, immuno-oncology network analysis identified central targets including TSHR, MAPK1, and TP53 (Figure 1-A). In addition, the pathway enrichment analysis illustrated in Figure 1-B revealed that the most significantly enriched pathways (with low p-values and high fold enrichment) were predominantly related to cancer, metabolic disorders, and infectious and immune diseases. The pathways with the greatest gene enrichment included bladder cancer, VEGF signaling, and sphingolipid signaling. These were followed by parasitic disease pathways such as *Leishmaniasis* and *Toxoplasmosis*.

Table 1. ***Compound groups of GL retrieved from TCMIO***

Compound group	Quantity	Percentage (%)	Percentage associated with biological targets (%)
Triterpenoids	182	66,18%	44,05%
Steroids	21	7,64%	3,57%
Others	72	26,18%	52,38%

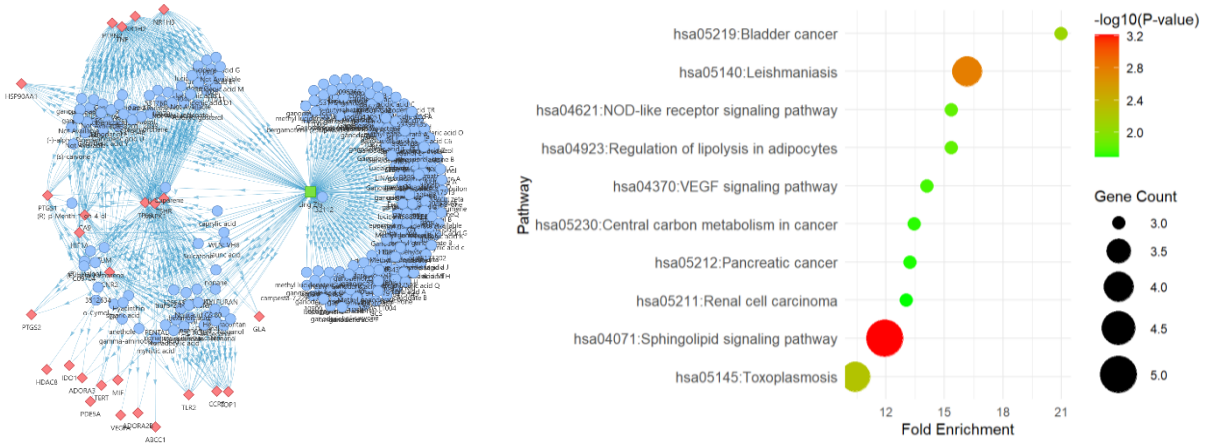

Figure 1. ***Network pharmacology analysis of GL.***

(A) Immuno-oncology network of GL–compound–target interactions (source: http://tcmio.xielab.net/moa);

(B) Top ten statistically significant enriched pathways.

3.2. Investigation and optimization of extraction conditions

3.2.1. Factor level investigation

The effects of ethanol concentration, extraction temperature, and extraction time are illustrated in **Figure 2**. Based on the obtained results, the conditions of 70% ethanol concentration, an extraction temperature of 80 °C, and an extraction time of 100 minutes were selected as the central point (level 0) for the Box–Behnken design in optimizing the extraction process.

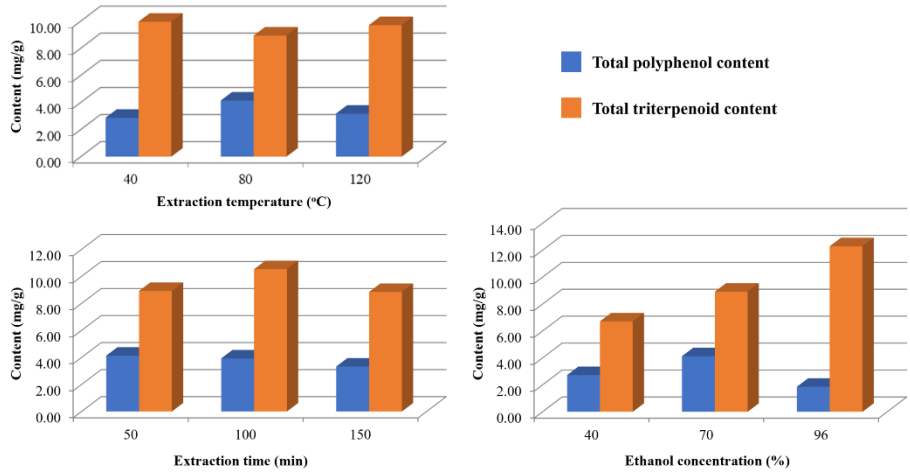

Figure 2. ***Effects of ethanol concentration, extraction temperature, and extraction time on total polyphenol and triterpenoid contents in the extract.***

3.2.2. Extraction process optimization

The experimental data are presented in **Table 2**.

Table 2. ***Experimental results***

No.	Ethanol (%)	Temperature (°C)	Time (min)	Total polyphenol content (mg/g)	Total triterpenoid content (mg/g)
1	40	40	100	2.76	6.33
2	100	40	100	1.84	10.12
3	40	120	100	3.25	5.74
4	100	120	100	1.58	15.28
5	40	80	50	3.18	7.43
6	100	80	50	1.41	16.23
7	40	80	150	2.25	3.53
8	100	80	150	1.06	12.84
9	70	40	50	2.74	10.12
10	70	120	50	3.04	10.31
11	70	40	150	3.25	9.77
12	70	120	150	2.44	11.18
13	70	80	100	3.89	8.45
14	70	80	100	3.85	9.64
15	70	80	100	3.80	8.65
16	70	80	100	3.72	10.15
17	70	80	100	4.18	7.80

The software was used to analyze the experimental data. The effects of the independent variables on the dependent variables were illustrated by 3D response surface plots, as shown in **Figure 3** and **Figure 4**.

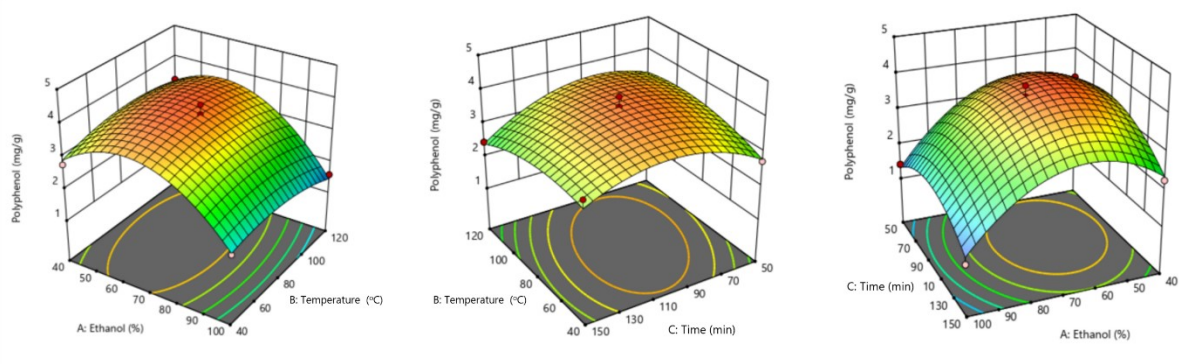

Figure 3. ***3D response surface plots of polyphenol content***

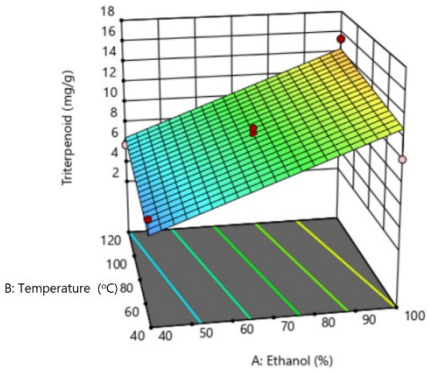

Figure 4. ***3D response surface plots of triterpenoid content***

Based on the experimental data and the constraints between the independent and dependent variables, the software proposed the optimal conditions. The validation of the optimal conditions through experimental trials is presented in **Table 3**.

Table 3. ***Optimal extraction conditions***

Optimal conditions	**Ethanol (%)**	**Temperature (°C)**	**Time (min)**
	75.94%	98.61	80.28
Experimental validation results			
Dependent variable	Lower 95% prediction interval	Mean experimental value	Upper 95% prediction interval
Total polyphenol content (mg/g)	3.27	3.81	3.93
Total triterpenoid content (mg/g)	9.39	16.10	12.80

From the obtained results, the mean value of polyphenol content fell within the confidence interval predicted by the software, whereas the mean value of triterpenoid content did not. Nevertheless, this condition was still selected, as the combined yield of polyphenols and triterpenoids under these extraction conditions was the highest.

3.2.3. Investigation of the raw material-to-solvent ratio

Experiments were conducted under the previously optimized conditions (75% ethanol, extraction temperature of 100 °C, and extraction time of 80 minutes), while varying the raw material-to-solvent ratios at 1:50, 1:100, 1:150, and 1:200 (g/mL). The results are illustrated in Figure 5.

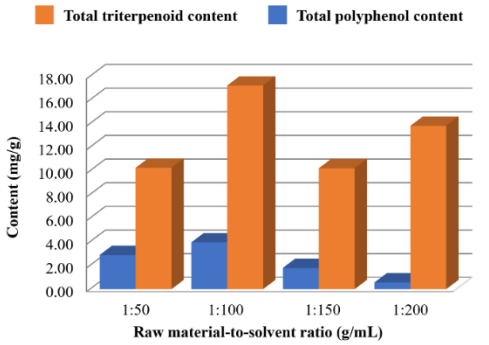

Figure 5. ***Effect of raw material-to-solvent ratio on bioactive compound contents in GL extract***

The results indicate that extraction under the conditions of 75% ethanol, 80 °C, and 100 minutes with a raw material-to-solvent ratio of 1/100 (g/mL) yielded the highest combined content of triterpenoids and polyphenols.

3.2. Investigation of spray-drying conditions

The effects of spray-drying conditions on the content of bioactive compounds are presented in **Figure 6**, **Figure 7**, and **Figure 8**.

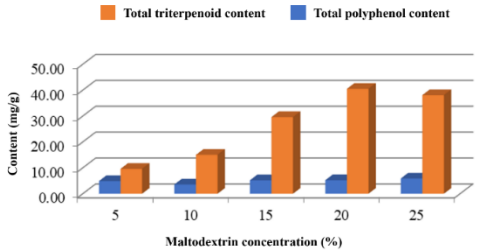

Figure 6. ***Effect of maltodextrin concentration on bioactive compound contents in GL spray-dried powder***

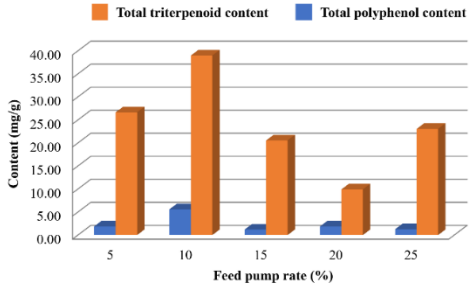

Figure 7. ***Effect of feed pump rate on content of bioactive compounds in the GL spray-dried powder***

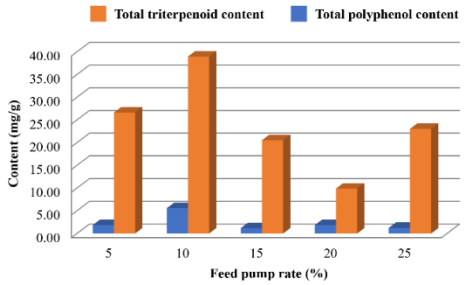

Figure 8. ***Effect of inlet temperature on content of bioactive compounds in GL spray-dried powder***

The results demonstrated that spray-drying under the conditions of 20% maltodextrin, an inlet temperature of 120 °C, and a feed pump rate of 10% yielded the highest combined content of total polyphenols and triterpenoids.

3.3. Determination of ganoderic acid A content in spray-dried powder

The quantification results of ganoderic acid A in spray-dried GL powder are presented in **Table 4**.

Table 4. ***Quantification of ganoderic acid A in spray-dried GL powder***

Sample	Ganoderic A content (mg/g)
1	61.34
2	51.22
3	54.72
Mean ± SD	**55.76 ± 4.19**

IV. DISCUSSION

GL exhibits strong potential as an anticancer, anti-inflammatory, and immunomodulatory agent, with triterpenoids identified as the most important class of bioactive compounds contributing to these pharmacological effects.

Molecular network pharmacology analysis revealed that approximately 69% (191/275 compounds) of the identified compounds in GL have not yet been experimentally confirmed for biological activity against specific targets. This finding highlights the considerable potential for further research on triterpenoids in GL. Additionally, the pharmacological roles of polyphenolic compounds in GL have attracted increasing research attention. Polyphenols in GL comprise a structurally diverse group, including flavonols, flavan-3-ols, flavones, and stilbenes, which contribute significantly to biological activities such as antioxidant and antiproliferative effects [3].

Based on this, simultaneous extraction of triterpenoids and polyphenols from GL has been the objective of several studies worldwide. For example, Taofiq Oludemi et al. (2018) optimized extraction conditions using ultrasound-assisted extraction [6]. This method is recognized for its high extraction efficiency, shorter processing time, and reduced operating cost. However, at an industrial scale, ultrasound-assisted extraction requires higher energy input to maintain efficiency, and achieving consistent ultrasound intensity in large-scale systems remains a significant challenge. Consequently, the number of studies addressing industrial applications of ultrasound-assisted extraction remains limited. In the present study, we optimized extraction using solvent extraction – a conventional, effective, and practically applicable method.

Traditionally, GL is consumed by decocting thin slices or powdered dried fruiting bodies in boiling water (15–30 minutes) for daily use [1]. To enhance convenience, our study further developed a spray-dried powder formulation enriched in triterpenoids and polyphenols. Spray-drying technology has previously been applied to obtain polysaccharide-enriched powders [7]. Thus, applying spray-drying to produce triterpenoid- and polyphenol-rich GL powders aligns with current global research trends.

Finally, the spray-dried powder was quantified for ganoderic acid A, a major triterpene in GL and a recognized quality control marker for Ganoderma species [2]. This result indicated the quality of the spray-dried powder, supporting its potential for

further development as a health-promoting product.

V. CONCLUSION

Through a network pharmacology approach, this study identified triterpenoids as the primary active compounds underlying the pharmacological effects of GL, with polyphenols also confirmed as important bioactive components. An optimized extraction process was established, and spray-drying was applied to develop a powder enriched in triterpenoids and polyphenols. The optimized conditions yielded extracts with high levels of active compounds, while spray-drying ensured compound stability and produced a safe, convenient product.

Quantification of ganoderic acid A in the spray-dried powder further confirmed the presence of triterpenoids, demonstrating the product's suitability for continued research and development of health-promoting formulations.

The findings of this study may serve as a reference for future research on large-scale production of herbal-based products, providing practical solutions to maximize the potential of GL in medical and food applications, and contributing to both public health and socio-economic development.

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