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PRELIMINARY RESEARCH ON ESTABLISHING THE QUALITY STANDARDS FOR ALCOHOL-PROCESSED *RHIZOMA LIGUSTICI WALLICHII*

Pham Anh Minh, Do Thi Thuy Linh, Nguyen Thi Ha Ly, Nguyen Thi Kim Anh, Le Xuan Duy, Tran Anh Quang, Nguyen The Chung, Do Thi Ha, Nguyen Tuan Hiep*

National Institute of Medicinal Materials (NIMM), Hanoi, Vietnam;

*Corresponding author: nguyentuanhiep.2710@hotmail.com

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Summary

Preliminary Research on Establishing the Quality Standards for Alcohol-Processed *Rhizoma Ligustici wallichii*

A foundational standard for medicinal material *Ligusticum wallichii* Franch. processed with alcohol was developed. The evaluation criteria include sensory evaluation, qualitative and quantitative analysis, moisture content, total ash and extractable substance content. These results form the basis for evaluating the quality of *Rhizoma Ligustici wallichii praeparatum* available on the market. Additionally, the research results suggest upgrading the monograph of *Rhizoma Ligustici wallichii praeparatum* in the promulgated Vietnamese Pharmacopoeia V. In this study, the acid ferulic content was proposed to contain not less than 0.06%, the moisture content should not exceed 12%, total ash content of no more than 5% was proposed, and the extractive compounds content in the alcohol-processed *Rhizoma Ligustici wallichii* sample should not be less than 8%.

Keywords: *Acid ferulic, Alcohol-processed Rhizoma Ligustici wallichii, Rhizoma Ligustici wallichii praeparatum.*

1. Introduction

Currently, traditional medicine is emerging as a rapidly growing trend. People favor traditional medicine due to its specific strengths, such as its effectiveness in treating diseases, safety, mild nature, and fewer side effects compared to Western medicine. The processing methods of medicinal materials and traditional medicines significantly impact their chemical composition and therapeutic effects. Therefore, applying the standards of unprocessed medicinal materials to processed traditional medicines is not entirely appropriate. Consequently, the standardization of traditional medicinal ingredients has been addressed in the pharmacopeia of several countries. In the fifth edition of the Vietnamese Pharmacopoeia, there are monographs on processed Vietnamese Codonopsis (Dang Sam) and Rehmannia (Thuc Dia), but other processed medicinal materials have not yet been included. Thus, standardizing the raw materials is the most fundamental step.

Ligusticum wallichii Franch., commonly known as "Xuyen khung", is one of the prominent species in the genus *Ligusticum*. This plant is mainly cultivated in Sichuan province, China and has been introduced to Vietnam. The rhizomes of *Ligusticum wallichii* Franch. have been identified to contain various chemical constituents such as phthalides, terpenes, polysaccharides, alkaloids and phenolic acids, among others [1],[2],[3],[4]. Despite the numerous chemical constituents in *Ligusticum wallichii*, ferulic acid, senkyunolide I, senkyunolide H, senkyunolide A, *Z*-ligustilide, and levistolide A are commonly used as chemical markers [5]. These chemical components contribute to *Ligusticum wallichi*'s significant biological activities, including antioxidant, neuroprotective, and anti-inflammatory effects on organs and systems such as the brain, blood, and cardiovascular system [1]. To enhance its therapeutic effects, *Ligusticum wallichii* Franch. is often processed by alcohol decoction. The processed *Ligusticum wallichii* Franch., characterized by its pungent taste and warm nature, is effective in promoting Qi circulation, activating blood flow, dispelling wind, and relieving pain, and is used for treating menstrual disorders, colds, and wind-cold-damp conditions [6]. However, the processing of medicinal materials can alter the chemical compositions or physicochemical properties of *Ligusticum wallichii* Franch.[7]. In Vietnam, research on the standards for alcohol-processed *Rhizoma Ligustici wallichii* is limited, this limitation results in inadequate quality management and assurance for this medicinal product. Therefore, this study aims to establish a foundational standard for alcohol-processed *Rhizoma Ligustici wallichii* derived from *Ligusticum wallichii* Franch. that meets the Vietnamese Pharmacopoeia V standards, serving as a basis for evaluating the quality of this medicinal product within the Vietnamese market.

2. Material and Method

2.1. Materials, Chemicals, and Equipment

Materials: The subject of the study is alcohol-processed *Rhizoma Ligustici wallichii.*, which has been processed at the Center for the Application of Medicinal Technology - National Institute of Medicinal Materials, following the guidelines of Circular 30/2017/TT-BYT [6] on the processing methods of traditional medicines. The raw material samples are cut into cross-sections 1-1.5 mm thick, dried, and ground into coarse powder. The raw material samples are stored at the Department of Extraction Technology, National Institute of Medicinal Materials.

The *Ligusticum wallichii* samples which have met Vietnamese Pharmacopoeia V quality standards used in this study were supplied by Indochina Company Vietnam (DL). The alcohol-processed sample was derived from the DL sample.

Processing Method*: Ligusticum wallichii*, is cleaned, drained, and cut into slices 1-2 mm thick. Approximately 1 kg *Ligusticum wallichii* is soaked with 150 mL of alcohol, then roasted over low heat until the alcohol aroma is released, and the slices turn light brown. The slices are then cooled and packaged.

2.2. Chemicals and reagents

All solvents and chemicals used in the study meet the analytical pure (PA) chemical standards. Solvents used for chromatographic analysis (TLC, HPLC) are purchased from Merck (or equivalent). The reference substance, ferulic acid, is provided by Chemfaces, batch number: CFN92394, with a purity of 99%.

2.3. Research methods

The standards for alcohol-processed *Rhizoma Ligustici wallichii* (Chuanxiong) are established based on the general criteria specified in the Vietnamese Pharmacopoeia V and the National Standard TCVN 11776-16:2017 for processed medicinal materials, including description, moisture content, total ash, qualitative analysis, and quantitative analysis.

• *Description*: A sensory description, observing the shape, texture, color, smell, and taste of the medicinal material [8].

• *Moisture Content*: Determination of water content by distillation with solvent according to the Vietnamese Pharmacopoeia V, Appendix 12.13 [9].

• *Total Ash:* Tested according to the Vietnamese Pharmacopoeia V, Appendix 9.8 [9].

• *Extractable Matter in Medicinal Materials:* Conducted according to the Vietnamese Pharmacopoeia V, Appendix 12.10, using the hot extraction method with 96% ethanol as the solvent [9].

• *Qualitative Analysis:*

Thin-Layer Chromatography (TLC): For identifying *E*-Ferulic and Z-ligustilide acid by thin-layer chromatography [10].

* **TLC Plate:** *Silica gel* 60 F_{254} (Merck);

Developing solvent: Dichloromethane: diethyl ether (2:1, v/v);

Test solution Weigh 1.0 g of the powdered sample and put it into a 50-mL centrifugal tube, then add 10 mL of methanol. Sonicate (490 W) the mixture for 30 min. Centrifuge at about 1800 x *g* for 10 min. Transfer the supernatant to another tube. Evaporate the solvent to dryness with a gentle stream of nitrogen. Dissolve the residue in 2 mL of ;

Reference solution:

Standard solution of ferulic acid. 1 gram of ferulic acid was dissolved in 1 mL methanol. Weigh 1.0 mg of *Z*-ligustilide and dissolve in 1 mL of methanol.

- **Spray reagent:** Add slowly 10 mL of sulphuric acid to 90 mL of ethanol.

- **Procedure:** Carry out the method by using an HPTLC *silica gel* F254 plate and a freshly prepared developing solvent system as described above. Apply separately *Z*-ligustilide standard solution, *E*-ferulic acid standard solution and the test solution (1 µL each) to the plate. Develop over a path of about 5 cm. After the development, remove the plate from the chamber, mark the solvent front, and dry it in air. Spray the plate evenly with the spray reagent, then heat at about 70˚C until the spots or bands become visible (about 10 min). Examine the plate under UV light (366 nm). Calculate the R_f values. For positive identification, the sample must give spots or bands with chromatographic characteristics, including the colour and the *Rf* values, corresponding to those of *Z*-ligustilide and *E*-ferulic acid.

***Quantitative Analysis:* Quantification of ferulic acid by High-Performance Liquid Chromatography (HPLC):**

+ *System, equipment, and HPLC analysis conditions*:

HPLC system: Shimadzu (Japan), consisting of an LC-20AD pump, SIL-20AHT auto-sampler, UV-VIS detector, and Labsolution software for data recording and analysis on the HPLC. Excel software is used for result calculation.

HPLC conditions: Shimpack C_{18} reversed-phase column (250 mm x 4.6 mm; 5 µm), UV-VIS detector at 321 nm wavelength; mobile phase consisting of 0.085% phosphoric acid and acetonitrile (ratio 17:83), isocratic elution mode; elution rate is 1.3 mL/min; injection volume is 10 µL.

+ *Sample solution*: 0.5 g of powdered sample (sieved through a 355-mesh screen) was accurately weighed into a 100 mL conical flask with a ground glass stopper. Exactly 50.0 mL of 70% methanol (reagent) was added. The flask was covered, weighed, and the mass was determined. The mixture was refluxed in a water bath for 30 minutes, then cooled, weighed again, and any lost mass was replenished with 70% methanol (reagent) if necessary. The mixture was then mixed well and filtered through a 0.45 µm membrane.

+ *Standard solution*: Standard ferulic acid was dissolved in 70% methanol (reagent) to obtain a solution with an exact concentration of about 10 µg/mL.

Formula for calculating content (X%):

$$X(\%) = \frac{Ct \times V \times 100 \times 100 \times P}{m \times 1000 \times 100 \times (100 - a)} = \frac{Ct \times V \times P}{m \times 10 \times (100 - a)}$$

where Ct: The concentration of analyte in sample solution (µg/mL);

V: The volume of test solution (mL).

m: The mass of medicinal materials (mg)

a: The sample moisture (%);

P: The purity of standard (%).

3. Results and discussion

3.1. Description of the alcohol-processed Rhizoma Ligustici wallichii:

Color: The herb slices exhibit a brown or brownish-yellow color.

State: The slices are thin, yellow, and pliable when moistened with water.

Odor and Taste: They possess a distinctive aromatic smell and a spicy taste.

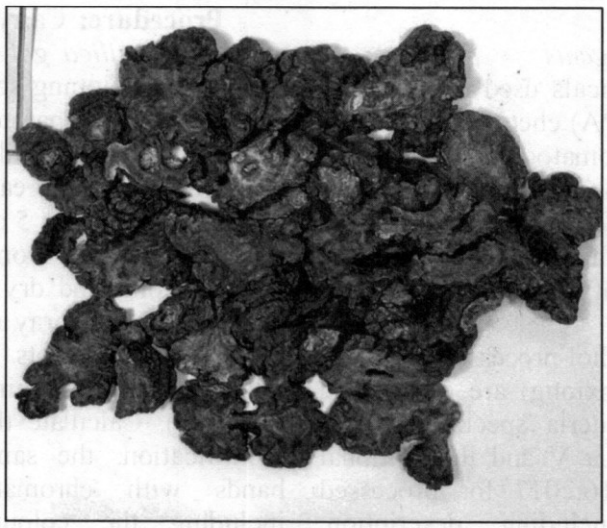


Fig. 1. Image of the alcohol-processed *Rhizoma Ligustici wallichii*

3.2. Results of moisture and ash content analysis

The results for the moisture and ash content of the alcohol-processed *Rhizoma Ligustici wallichii* are presented below.

Table 1. Moisture and Ash content of the alcohol-processed alcohol-processed *Rhizoma Ligustici wallichii*

No.	Sample Name	Moisture (%)	Ash (%)
1	VT Xuyên Khung_1	9.05 ± 0.05	3.92 ± 0.04
2	VT Xuyên Khung_2	9.15 ± 0.03	4.01 ± 0.03
3	VT Xuyên Khung_3	8.96 ± 0.06	4.05 ± 0.05

(Samples were analyzed in triplicate, n=3).

Proposed standards: Moisture content should not exceed 10%, and ash content should not exceed 5%.

3.3. Results of extractable compounds analysis

The results of the extractable substances of the alcohol-processed *Rhizoma Ligustici wallichii* are presented in the following table:

Table 2. Percentage of extractable compounds in the alcohol-processed *Rhizoma Ligustici wallichii* samples

No.	Sample Name	Moisture (%)	Extractive (%)
1	VT Xuyên Khung_1	9.05 ± 0.05	8.772 ± 0.004
2	VT Xuyên Khung_2	9.15 ± 0.04	8.581 ± 0.006
3	VT Xuyên Khung_3	8.96 ± 0.06	9.645 ± 0.003

(Samples were analyzed in triplicate, n=3).

Proposed standard: Extractable compounds content should not be less than 8%.

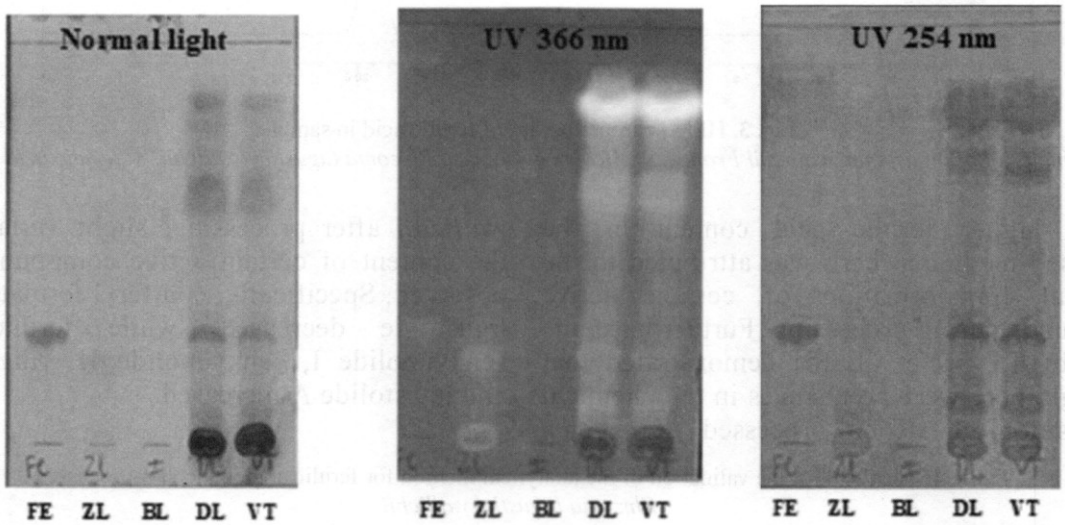
3.4. Qualitative analysis results

Thin Layer Chromatography (TLC).

Result:

When observed under UV light at 254 nm, both the standard solution and the sample solutions exhibited spots. After spraying the plate with ethanol-sulfuric reagent and heating, the sample solutions showed spots with the same color and an Rf value of 0.38 as the standard solution of ferulic acid and 0.25 as the standard solution of Z-ligustilide.

When observed under UV light at 366 nm, both the standard solution and the sample solutions exhibited spots. After spraying the plate with ethanol-sulfuric reagent and heating, the sample solutions showed spots with the same color and an Rf value of 0.25 as the standard solution of Z-ligustilide.



(DL): Sample solution from the extract of raw Ligusticum wallichii, (VT): Sample solution from the extract of alcohol-processed Rhizoma Ligustici wallichii, (FE): Reference standard solution (ferulic acid), BL (Blank): MeOH, (ZL): Reference standard solution (Z-ligustilide)

Fig. 2. TLC for qualitative analysis of acid ferulic and Z - ligustilide in raw andalcohol-processed *Rhizoma Ligustici wallichii*

Evaluation results: On the chromatogram of the alcohol-processed sample, there are spots with the same color, intensity, and Rf values as the spots on the chromatogram of the standard solution (R_f = 0.38).

On the chromatogram of the alcohol-processed sample, there are spots corresponding in color and R_f values to the spots on the chromatogram of the reference medicinal material solution (R_f = 0.1, 0.25; 0.38; 0.66; 0.76; and 0.85).

The chromatogram of the blank sample does not show spots corresponding in color and R_f values to the spots on the chromatogram of the standard solution and the reference medicinal material solution.

3.5. Ferulic acid content analysis

The quantitative analysis of ferulic acid

indicated that all test samples showed peaks with retention times corresponding to the ferulic acid standard. The ferulic acid content in alcohol-processed *Rhizoma Ligustici wallichii* was found to be above 0.05%. Hence, the proposed standard for ferulic acid content should not be lower than 0.05%.

Table 3. Result of ferulic acid content analysis

No.	Sample Name	Content (%)
1	*Rhizoma Ligustici wallichii*	0.062 ± 0.004
2	VT Xuyên Khung_1	0.072 ± 0.003
3	VT Xuyên Khung_2	0.079 ± 0.005
4	VT Xuyên Khung_3	0.069 ± 0.004

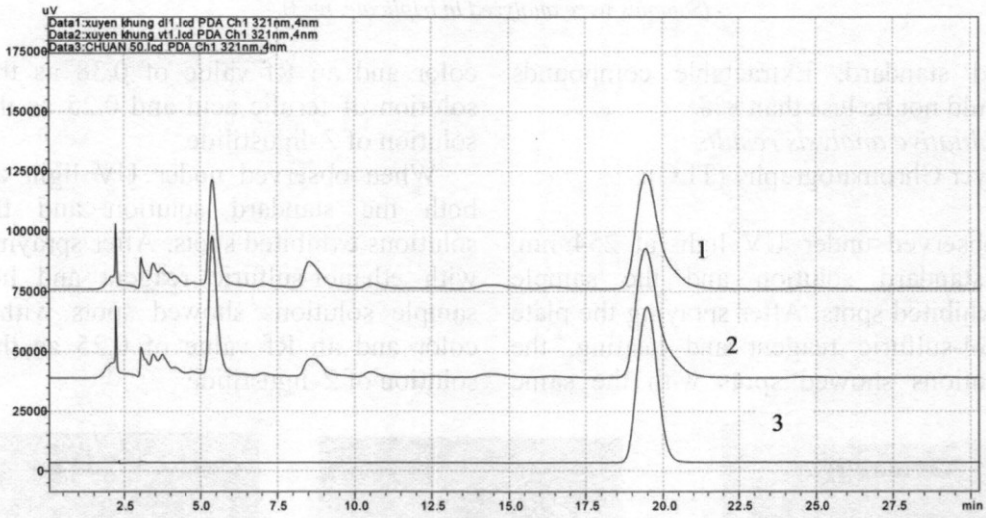


Fig. 3. HPLC chromatogram of ferulic acid in samples
(1: Raw material of Ligusticum wallichii Franch, 2: Alcohol-processed Rhizoma Ligustici wallichii, 3: ferulic acid standard).

The higher ferulic acid content in the processed medicinal herb was attributed to the chemical transformation of certain active compounds during processing. Furthermore, the study by Li SL et al. [5] demonstrated that although there were no changes in the chemical composition of alcohol-processed *Ligusticum wallichii* after processing, slight variations in the content of certain active compounds were observed. Specifically, coniferyl ferulate and *Z*-ligustilide decreased, while ferulic acid, senkyunolide I, senkyunolide H, riligustilide, and levistolide A increased.

Table 4. The result of the validation of the analytical method for ferulic acid in alcohol-processed *Rhizoma Ligustici wallichii*

No	Validation characteristics	Experiment [11,12]	Results
			Method for analyzing oleanolic acid
1	System suitability	Repeat analysis of the standard sample 6 times on the HPLC system	RSD (t_R) = 0.503% RSD (S) = 0.138%
2	Selectivity (Specificity)	Analysis of blank sample (Methanol), standard sample, and test sample	High specificity t_R (AO) = 18.725
3	Linearity	Analysis of standard solutions of different concentrations	Linear range: 5- 200 µg/mL Calibration curve: y = 56012x - 12138 R^2 = 0.9996 x is the concentration of acid ferulic (µg/mL); y is the peak area (mAu)
4	Precision	Repeat analysis of a test sample within a day and on different days (6 times/day) The concentration of ferulic acid in standard solution was 50.125 µg/mL.	RSD = 1.143%

No	Validation characteristics	Experiment [11,12]	Results
			Method for analyzing oleanolic acid
5	Accuracy	Standard addition method (The accuracy was evaluated by adding standards to the sample at 3 levels 80%, 100% and 120%). The concentration of the ferulic acid sample was 51.575 µg/mL.	Recovery rate (%) = 98.462%
6	LOD	Determined based on the signal-to-noise ratio (S/N) of standard solutions.	0.09 ppm
7	LOQ	Determined based on the signal-to-noise ratio (S/N) of standard solutions.	0.3 ppm

4. Discussion

Currently, the standardization of traditional medicinal herbs has received attention and has been addressed in the pharmacopeias of several countries worldwide. Among the 103 herbs regulated by Circular 30/2017/TT-BYT, the Vietnamese Pharmacopoeia V currently specifies monographs for only three processed herbs: Thuc dia, Dang sam and Ha thu o do. Therefore, establishing foundational standards for processed herbs is essential.

Ligusticum wallichii, is known for its numerous applications in traditional medicine such as antioxidant, neuroprotective and anti-inflammatory on organs and systems such as the brain, blood, and cardiovascular system [1],[2]. While the raw herb has its own monograph in the Vietnamese Pharmacopoeia V, the processed herb does not yet have a specific monograph. Moreover, the current quality criteria are primarily qualitative and lack quantitative active ingredient content standards. Thus, the results of this study contribute to creating a database for research and the development of quality evaluation standards for processed herbs.

Description: The alcohol-processed *Rhizoma Ligustici wallichii* was found to exhibit a brown or brownish-yellow color, with a firm texture, a distinctive aromatic smell, and a spicy taste. In contrast, the raw material of *Ligusticum wallichii* in its root form was observed to appear distorted, with a diameter of 2 cm to 5 cm and numerous irregular protrusions. The exterior was earth-brown, wrinkled, and rough. The texture was hard and difficult to break, with a cross-section that was yellowish-brown. The raw material was noted for its aromatic smell and mildly spicy taste, making it easily distinguishable.

Qualitative Analysis: The qualitative reactions observed were consistent with the general qualitative reactions for the raw material of *Ligusticum wallichii* as described in the Vietnamese Pharmacopoeia V.

Quantitative Analysis: According to the Vietnamese Pharmacopoeia V, the raw material of *Ligusticum wallichii* was required to contain no less than 0.05% ferulic acid in the dried herb. The results presented in Table 1 showed that the ferulic acid content in the processed sample was 0.073%, which was not significantly different from the raw sample. Therefore, a quantitative standard for the alcohol-processed *Rhizoma Ligustici wallichii* was proposed to contain not less than 0.06% ferulic acid in the dried material.

Moisture Content: The Vietnamese Pharmacopoeia V specified that the moisture content for the raw material of *Ligusticum wallichii* should not exceed 13%. The moisture content in the processed sample was determined to be 9.04%. Therefore, it was proposed that the moisture content for the alcohol-processed *Rhizoma Ligustici wallichii* should not exceed 12%.

Total Ash Content: According to the Vietnamese Pharmacopoeia V, the total ash content for the raw material of *Ligusticum wallichii* should not exceed 6%. The total ash content in the alcohol-processed *Rhizoma Ligustici wallichii* sample was determined to be 3.95%. Therefore, a total ash content for the alcohol-processed *Rhizoma Ligustici wallichii* of not more than 5% was proposed.

Extractive compounds: The Vietnamese Pharmacopoeia V required that the extractable compound for the raw material of *Ligusticum wallichii* should not be less than 9%. The extractive compounds content in the processed sample was determined to be 8.978%. Therefore, it was proposed that the extractive content in the alcohol-processed *Rhizoma Ligustici wallichii* sample should not be less than 8%.

5. Conclusion

The quality standards for the alcohol-processed *Rhizoma Ligustici wallichii* included the descriptive criteria according to the Vietnamese Pharmacopoeia V, qualitative analysis by thin layer chromatography using a solvent system of dichloromethane and diethyl ether (2:1, v/v), quantitative analysis with ferulic acid content not less than 0.05%, moisture content not exceeding 12%, total ash content not exceeding 5%, and the extractive compounds not less than 8%. Additionally, further quantitative analysis of prominent markers was recommended to clarify the differences between the raw material and the alcohol-processed *Rhizoma Ligustici wallichii* samples, providing a precise basis for establishing comprehensive standards. The initial results of this research contributed to the evaluation of post-processing herbal quality and laid the groundwork for further studies on the chemical composition and pharmacological effects of processed material.

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HEPATOPROTECTIVE ACTIVITY OF CRUDE EXTRACT AND GANODERIC ACIDS IN *GANODERMA NEO-JAPONICUM* FRUIT BODY AND BIOMASS

Nguyen Quoc Cuong[1,*], Vo Huy Sang[2], Hoang Anh Tuan[2], Pham Van Phuc[3], Bui Thi Minh Dieu[1]

[1]Institute of Food and Biotechnology, Can Tho University, Vietnam;

[2]Bu Gia Map Nation Park, Binh Phuoc, Vietnam;

[3]Stem Cell Institute, University of Nature Sciences, Ho Chi Minh City, Vietnam

*Corresponding author: nguyenquoccuong.ma@gmail.com

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Summary

Hepatoprotective Activity of Crude Extract and Ganoderic Acids in *Ganoderma neo-japonicum* Fruit Body and Biomass

The mushroom *Ganoderma neo-japonicum* (GNJI) is a new mushroom recently found in Bu Gia Map National Park on the decomposing bamboo stump (*Schizostachyum* sp.) which has been cultured and studied in recent years. In this study, the hepatoprotective activity of ganoderic acids (GAs) and crude extract (Ext) from GNJI fruit body (Frb) and biomass (Mas) were determined. The mice were administered GAs and crude extract with one dose of 16 mg/kg or 32 mg/kg body weight (bw) per day for 21 days before hepatic damage induced by paracetamol (acetaminophen) 400 mg/kg bw. The results of relative liver weights, liver histopathological observation (surface parenchyma, histology of lobules), and serum SGOT/SGPT levels showed that GAs from fruit body (FrbGAs) and biomass (MasGAs) had hepatoprotective activity compared to silymarin (16 mg/kg bw). After 21-day administration of FrbGAs or MasGAs at a dose of 16 mg/kg or 32 mg/kg bw, the parenchyma, lobules of the liver, and serum SGOT/SGPT levels restored nearly normal as same as physiological control. Specially, FrbGAs and MasGAs at 32 mg/kg bw showed the best hepatoprotective activity.

Keywords: *Ganoderma neo-japonicum, Hepatoprotective, Ganoderic acids, Biomass.*