SUMMARY OF DISSERTATION

Name of Doctoral candidate: **Bui Thi Thu Ha**

Dissertation title: **"Study on the experimental anti-tumor effects of the roots of *Panax Notoginseng* (Burk.) F.H. Chen, Araliaceae) grown in Vietnam before and after steaming process".**

Specialty: **Pharmacology - Clinical Pharmacy**

Code of specialty: **9720205**

Name of the advisors:

1. Assoc. Prof. Vu Manh Hung, PhD.

2. Prof. Nguyen Thanh Hai, PhD.

Name of academic institute: **National Institute of Medicinal Materials**

Summary of the dissertation

1. Objectives

- Study on the effect of steaming processing method on saponin content of the roots of the *Panax Notoginseng.*

- Study on experimental anti-tumor effects of NP(H) and NP(O) and some saponins isolated from the roots of *Panax Notoginseng.*

- Evaluate acute toxicity, subacute toxicity of NP(H) and NP(O) after steaming.

2. Methods

2.1. Study on the effect of steam processing method on saponin content of the roots of Panax Notoginseng.

- Extracting, isolating and determining the structure of the main saponins present in the steaming and non-steaming samples.

- Investigate the change of active ingredient content in saponins before and after steaming at different conditions using HPLC.

2.2. Studying the effects of 6 saponins isolated from the roots of the Panax Notoginseng and the experimental antitumor effects of NP(H) and NP(0).

- Evaluate *in vitro* the antitumor effects of 6 saponins isolated by the method Monks A et al. (1991). - *In vivo* assessment of cytotoxicity and ability to stimulate programmed cell death (apoptosis) of highly quantified NP(H) on the mice

bearing solid sarcoma TG 180. Activation and propagation of the TG180 sarcoma cell line. Evaluation of cytotoxic potential of NP(H) on TG180 sarcoma cell line and cultured by MTT method. Evaluation of the ability to induce apoptosis by high level of the of NP(H) on the mouse bearing solid sarcoma TG 180.

- Successfully created a solid tumor of TG 180 sarcoma in mice according to Lapis et al. Evaluation of tumor growth inhibitory effects of NP(H) and NP(O); anti-tumor effect according to Itokawa; Determination of tumor growth ratio (GR); Determination of tumor suppression ratio (IR, Inhibition Ratio);

- Evaluate the effect of NP(H) and NP(O) on the immune system of mice bearing solid sarcoma TG 180 by Stefanova T.H method; Evaluation of antioxidant effects according to Zhao et al; Determination of survival time of mice bearing solid tumors sacoma TG 180 according to Geran et al.

2.3. Evaluation of acute, subchronic toxicity of NP(H).

- Evaluation of acute oral toxicity of NP(H) in white mice according to the regulations No 141/2015 of the Ministry of Health of Vietnam and WHO.

Main parameters: General condition of rats, signs of poisoning, number of mice that died in each group within 72 hours after giving the mice the NP(H) and continue to follow up within 14 days.

- Evaluation of subchronic toxicity of NP(H) in rats according to regulation 141 of the Ministry of Health of Vietnam and WHO;

Criterias: General condition of rats, signs of poisoning, at least 2 times per day, early morning and late afternoon. Rat body weight, hematology and blood biochemistry were evaluated at depart time, at days on 30, 60 and 90. At the end of day 90, animals were killed and dissected for gross evaluation. and making HE-stained slides for microscopic assessment of liver, spleen, and kidney tissues of mice in each group ones.

3. Main results and conclusions

3.1. Effect of steam processing method on saponin content of root of panax Notoginseng.

From the n-buthanol extract of the 80% MeOH extract, four compounds were isolated from the roots of the tuber, which have not been steaming, namely ginsenoside Rg1 (1), ginsenoside Re (2), ginsenoside Rd (3) and ginsenoside Rb1 (4) and 2 compounds of ginsenoside Rg3 (5) and ginsenoside Rh1 (6) from the roots of Panax Notoginseng tubers after steaming at 120oC for 8 hours.

Surveying the steaming time and temperature (100^0C, 120^0C) , with fresh or dried samples, the content of the main saponins in the untreated Panax Notoginseng roots by steaming is Rg1, Rb1 and Rd, Re decreasing significantly; the content of new saponins such as Rh1 and Rg3 increased markedly.

Selected conditions for steaming to give the highest Rh1 and Rg3 content are: dry samples, steaming at 120oC for 8 hours.

Two types of NP(O) from unsteamed Panax Notoginseng and of NP(H) from steamed Panax Notoginseng have been prepared, with high extraction efficiency and high saponin content.

3.2. Experimental anti-tumor effects of high-quantitative forms and some saponins isolated from the roots of the Panax Notoginseng.

- Anti-cancer effects of 6 isolated saponins and 2 NP(O), and NP(H) on some human cancer cell lines.

Comparing the *in vitro* activity of six human cancer cell lines HT29, HepG2, RD and MCF7, SK LU-1 and A549, the activity of two saponins Rg3 and Rh1 obtained from heat-treated *Panax Notoginseng* was more potent. compared with 4 saponins Rg1, Re, Rd and Rb1 obtained when the material was not subjected to steaming. Totally show the same trend when comparing the activity of the NP(H) steaming with effective steaming.

- Proven ability to induce apoptosis by quantitatively NP(H) on the mice bearing solid tumor sarcoma TG 180.

NP(H) has a toxic effect on sarcoma TG180 , with IC50 values obtained: IC50=206.65±10.11µg/ml.

NP(H) at a concentration of 103.3 µg has the effect of inducing and stimulating TG180 sarcoma cells to die by apoptosis at an early stage, showing a very high positivity rate: at 24h, an increase of 134.4% and 48h was 193.4 % respectivenly, approximately 200% increase compared to the control ones.

- Anti-tumor effects on in vivo experiments

In mice bearing sarcoma solid tumor TG 180 of NP(H) has good anti-cancer effect, reduces tumor size, enhances immunity, protects liver, and prolongs survival time of mice bering tumors. These effects of NP(H) were stronger than those of NP(O).

3.3. Acute toxicity, subchronic toxicity of NP(H)

- *Acute toxicity:* LD_{50} of oral NP(H) has not been found in white mice. With the highest dose that can be given to mice for 24 hours, 6000 mg/kgwb did not show acute toxicity.

- *Subchronic toxicity*: When given to white rats orally with of NP(H) with 2 doses of 200mg/kg/day and 900mg/kg/day belong for 90 days, there was no effect on weight growth, weight off body and activity of rats, did not affect hematological parameters (red blood cell count, hemoglobin, hematocrit, mean red blood cell volume, white blood cell count and platelet count); biochemical parameters (AST, ALT, creatinine, albumin and cholesterol) and gross and microscopic morphology of liver, spleen and kidney.

Ha Noi, 1st November, 2022

SUPERVISORS **DOCTORAL CANDIDATE**

Assoc. Prof. Vu Manh Hung, PhD. **Prof. Nguyen Thanh Hai, PhD.** **Bui Thi Thu Ha**