SUMMARY OF DISSERTATION

1. INTRODUCTION

Name of Ph.D candidate: Hoang Minh Chau

Dissertation title: Study on the major compounds and chemovariation of bioactive substances in *Gymnema sylvestre* (Retz.) R. Br. ex. Schult "

Speciality: Medicinal Material - Traditional Pharmacy

Code number: 9720206

Scientific supervisors:

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Academic institution: Vietnam National Institute of Medicinal Materials

2. SUMMARY

2.1. Objectives

- Isolation and structure elucidation of the main compounds from the leaves of Vietnamese *Gymnema sylvestre*.

- Identify the seasonal accumulation of the bioactive compounds of Vietnamese *G. sylvestre*.

2.2. Methods

2.2.1. Phytochemical study

- Maceration and sonication extraction of the leaves of Vietnamese *Gymnema sylvestre* using 60% ethanol. Isolation and purification of compounds by various chromatographic methods including silica gel, reverse-phase, Sephadex LH20 column chromatography and preparative high-performance liquid chromatography (HPLC).

- Structure elucidation of isolated compounds based on the physical properties (specific optical rotation) and spectroscopic data including ultraviolet (UV), Liquid chromatography - electron spray ionization mass spectrometry (LC-ESI-MS), high-resolution electron spray ionization mass spectrometry (HRESI-MS) and nuclear magnetic resonance (NMR), as well as by comparison with the literature data.

2.2.2. Biological evaluation

- The leaves of Vietnamese *G. sylvestre* was extracted with ethanol 60% thrice by sonication at 50 °C in 3 hours, and the extract was concentrated in vacuo at 50°C. The dried extract was suspended in water to test its blood glucose lowering effect in streptozotocin-induced diabetic rats.

- Hypoglycemic activity of the Vietnamese *G. sylvestre* extract was carried out on the high-fat diet and streptozotocin-induced diabetic mice.

- All the isolated compounds were tested for their PTP1B inhibitory activity. Furthermore, their effects on glucose uptake of 3T3-L1 adipocytes were carried out using a fluorescent derivative of glucose - 2- (*N* - (7-Nitrobenz-2-oxa-1,3-diazol-4-yl) Amino) -2-Deoxyglucose (2-NBDG).

2.2.3. *Bioactive compound accumulation of Vietnamese G. sylvestre*.

- The chemical marker of Vietnamese *G. sylvestre* was collected by isolation of single compound from hydrolysis product of the total extract after intensive column chromatographic methods and preparative high-performance liquid chromatography (HPLC). The structure of gymnemagenol was elucidated by using spectroscopic data (LC-ESI-MS, HR-ESI-MS, 1D-NMR, and 2D-NMR) in addition to comparison with the literature data.

Quantification study and evaluate the chemical accumulation of gymnemagenol by preparing the isolated standard gymnemagenol at different concentrations 0.08; 0.04; 0.02; 0.01; 0.005; 0.0025 mg/ml and compared with the hydrolysis products of *G.sylvestre* extract collected in 12 months checking in the same LC-MS condition Analysis was performed using an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA, USA) with INNO C18 (4.6 × 250 mm inner diameter, 5 μm particle size) at a column temperature of 30 °C. Chromatographic condition was carried out using a gradient mobile phase of 10% to 100% MeCN/H_2O elution at a flow rate 0.6 mL/min in 60 minutes. The retention time of gymnemagenol was detected at 31.5 minutes. The area under the curve (AUC) of standard gymnemagenol was collected to establish the linear regression for gymnemagenol AUC = 161,662,666 × concentration of gymnemagenol + 2,407,421 (r^2=0.99). The following formula calculated the amounts of gymnemagenol in all the samples:

$$\%\text{Gymnemagenol} = \frac{\text{AUC} - 2{,}407{,}421}{161{,}662{,}666} \times \frac{1}{2} \times \frac{\text{The weight of dried extract}}{\text{The weight of raw material}} \times 100(\%)$$

The analysis was carried out on 3 separated samples collected for every month and the percentage of gymnemagenol for each month was calculated by $\bar{X} \pm$ SD (%).

2.3. Results and conclusion

2.3.1. Phytochemicals

- 8 compounds were isolated and elucidated from Vietnamese *Gymnema sylvestre*, including 6 previously undescribed compounds : Compound **1**: 3*β*,16*β*,28-trihydroxyolean-12-en-29-oic acid 3-O-*β*-D-glucopyranosyl(1→3)-*β*-D-glucuronopyranoside; Compound **2:** Sitakisogenin 3-*O*-*β*- D-glucopyranosyl (1→3)-*β*-D-glucuronopyranoside; Compound **3**: Sitakisogenin 3-*O*-*β*-D-glucuronopyranoside; Compound **4**: 29-*O*-(*β*-D-glucopyranosyl) gymnemagenol 3-*O*-*β*-D-glucuronopyranoside; Compound **5**: Gymnemagenol 3-*O*-*β*-D-glucuronopyranoside; Compound **8**: 3-*O*-[*β*-D-xylopyranosyl(1→6)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] oleanolic acid 28-[*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] ester, and 2 known compounds: Compound **6**: 3-*O*-[*β*-D-xylopyranosyl(1→6)-*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] oleanolic acid 28-*β*-D-glucopyranosyl ester. Compound **7:** 3-*O*-[*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] oleanolic acid 28-[*β*-D-glucopyranosyl(1→6)-*β*-D-glucopyranosyl] ester.

- None of the 8 isolated compounds was reported in the Indian sample.

2.3.2. Biological activities

- On high-fat diet and streptozotocin-induced diabetic mice, administration of Vietnamese *G.sylvestre* extract in two weeks at dose 2.88 g extract/kg/day and 8.64 g extract/kg/day showed significant hypoglycemic activity comparing with negative control. There was no significant difference in the blood glucose lowering effect in the group treated with gliclazide and group treated with Vietnamese *G.sylvestre* extract ($p>0,05$).

- Vietnamese *G. sylvestre* extract treatment in two weeks at dose 2.88 g extract/kg/day and 8.64 g extract/kg/day also improved the pancreas microstructures of streptozotocin-induced diabetic mice

- Among 8 compounds isolated from *G.sylvestre*, compound 1-5 showed PTP1B inhibitory effects, while compound 6-8 exhibited no activity. Compound 5 showed the most potent PTP1B inhibitory activity and also significantly enhanced glucose uptake in 3T3-L1 adipocytes.

2.3.3. Bioactive compound accumulation

- Compound 4 and 5 isolated from Vietnamese *G. sylvestre* showed most potent PTP1B inhibition. Since gymnemagenol was identified as their aglycon, it was selected as a chemical marker to evaluate the seasonal chemical variation for this plant. An analytical method for the validation of gymnemagenol in Vietnamese *G. sylvestre* was established.

- The accumulations of gymnemagenol were identified to be highest in May and October. These months are also the most suitable time of year for the development of Vietnamese *G. sylvestre* when the plant has the highest collection yield. On the other hand, winter season (December- February) afforded the lowest qualities concerning both collection yield and the amount of gymnemagenol.

Hanoi, November 8th, 2018

ON BEHALF OF THE SCIENTIFIC SUPERVISORS
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