SUMMARY OF DISSERTATION

The author's name: Vu Duy Hong

Dissertation title: Study on botanical properties, chemical constituents and biological activities of *Hypericum hookerianum* Wight. and Arn., family – Hypericaceae.

Speciality: Medicinal Materials - Traditional Medicine; Code: 9720206

Name of academic advisors:

1. Assoc.Prof.Dr. Nguyen Manh Tuyen
2. Assoc. Prof.Dr. Nguyen Minh Khoi

Name of academic institute: National Institute of Medicinal Materials

Summary of the dissertation:

1. Objectives

1.1. To describe botanical properties, identify the scientific name and microscopic characteristics of *Hypericum hookerianum* Wight. and Arn.

1.2. To extract, isolate and determinate the structure of major compounds from *Hypericum hookerianum* Wight. and Arn.

1.3. To evaluate *in vitro* antioxidant and neuroprotective activities, *in vivo* hepatoprotective effects of total extracts, fractions and potential purified compounds from *Hypericum hookerianum* Wight. and Arn.

2. Methods

2.1. To describe botanical properties, identify the scientific name and microscopic characteristics of *Hypericum hookerianum* Wight. and Arn.

- Describe botanical properties and determine scientific name of *Hypericum hookerianum* Wight. and Arn. on the basis of analysis of plant morphological characteristics, compare to plant taxonomy keys and standard specimens stored at the Plant Template Room of the Department of Medicinal Resources - the National Institute of Medicinal Materials, the Institute of Ecology and Biological Resources and Vietnam Academy of Science and Technology.

- Identify microbiological characteristics through making micro-dissection and powder of leaves, stems, roots of *Hypericum hookerianum* Wight. and Arn., observe and describe the characteristics, take pictures of specimens under the microscope.

2.2. To extract, isolate and determinate the structure of major compounds from *Hypericum hookerianum* Wight. and Arn.

- Extract and determinate major chemical groups in aerial parts of *Hypericum hookerianum* Wight. and Arn. using specific chemical reactions.

- Isolate compounds presenting in aerial parts of *Hypericum hookerianum* Wight. and Arn. using column chromatography, preparative HPLC, monitoring fractions by thin layer chromatography. Determinate chemical structure of isolated compounds by comparing measured spectral of mass spectrometry and nuclear magnetic resonance spectroscopy with published data.

2.3. To evaluate *in vitro* antioxidant and neuroprotective activities, *in vivo* hepatoprotective effects of total extracts, fractions and potential purified compounds from *Hypericum hookerianum* Wight. and Arn.

- Evaluate *in vitro* antioxidant activity through free radical scavenging ability with DPPH assay and Superoxid Dismutase (SOD) assay.

- Evaluate *in vivo* hepatoprotective effect in mice with the paracetamol-induced acute liver injury model.

- Evaluate *in vitro* neuroprotective activity with the glutamate-induced cytotoxicity on rat hippocampal neuronal cells (HT_{22}) model and the 6-hydroxydopamine-induced cytotoxicity on SH-SY5Y neuroblastoma cells model.

3. Main results and conclusions

3.1. Botanical characterization and identification of the scientific name.

- Confirmed that the sample collected in Sa Pa, Lao Cai is *Hypericum hookerianum* Wihgt. and Arn., (Hypericaceae). The microscopic characteristics of leaves, stems, roots, leaf powder, stem powder, and root powder from the research sample have been described.

3.2. Chemical composition

3.2.1. Qualitative

- Based on the qualitative test results, preliminary conclusions that the aerial parts of *Hypericum hookerianum* Wight. and Arn. contains flavonoids, polysaccharides, organic acids, phenolic compounds and tannins.

3.2.2. Extracting and isolating of compounds

- There are 37 compounds (**HH1 – HH37**) isolated by chromatographic method from n-hexane and ethyl acetate fractions. The compounds were identified: chipericumin D (**HH1**), uralione D (**HH2**), uraloidin A (**HH3**), furohyperforin (**HH4**), hypercohin K (**HH5**), multifidol glucoside (**HH6**), 2-(2-methylbutyryl) phloroglucinol 1-O-(6′′-O-β-D-apiofuranosyl)-β-D-glucopyranoside (**HH7**), 1,3,5-trihydroxyxanthon (**HH8**), 1,3,5,6-tetrahydroxyxanthon (**HH9**) , 3-hydroxy-2,4-dimethoxyxanthon (**HH10**), neriifolone A (**HH11**), 4-hydroxy-2,6,4′-trimethoxydihydrochalcon (**HH12**), isolariciresinol 9′-O-β-D-glucopyranoside (**HH13**) , isocubein (**HH14**), sesamin (**HH15**), piperitol (**HH16**), kaempferol (**HH17**), quercetin (**HH18**), (–)-epicatechin (**HH19**), quercitrin (**HH20**), hyperoside (**HH21**), astilbin (**HH22**), engeletin (**HH23**), isorhamnetin-3-O-β-D-glucopyranoside (**HH24**), rutin (**HH25**), nicotiflorin (**HH26**), 3,8′′-biapigenin (**HH27**), caffeic acid (**HH28**), ferulic acid (**HH29**), p-coumaric acid (**HH30**), ethyl-4-methoxy-trans-cinnamate (**HH31**), ethyl-trans-cinnamate (**HH32**), chlorogenic acid (**HH33**), syringic acid (**HH34**), shikimic acid (**HH35**) , 5,7-dihydroxy-2-(1-methylpropyl)chromon-8-β-D-glucopyranoside (**HH36**), piceatannol (**HH37**).

- Among 37 isolated compounds, 11 compounds (**HH6**, **HH7**, **HH11–HH16**, **HH24**, **HH31** and **HH37)** were found for the first time in a species of the genus *Hypericum*. Except for **HH4**, all the remaining compounds were isolated for the first time from *H. hookerianum.*

3.3. Biological effects.

- Ethyl acetate fraction from the aerial part of *Hypericum hookerianum* Wight. and Arn. exhibit the ability to scavenge DPPH and superoxide free radicals with IC_{50} values of 17.92 and 7.39 µg/mL.

- In the experiment to evaluate the hepatoprotective effect, methanol extract from *Hypericum hookerianum* Wight. and Arn. (at 2 dose levels of 250 mg/kg and 500 mg/kg) statistically significantly reduced AST and ALT level compared to the control group. SOD activity in the liver of experimental mice using methanol extract and ethyl acetate fraction (at two dose levels of 250 mg/kg and 500 mg/kg) was statistically significantly higher than in the control group ($p < 0.05$).

- The methanol extract, *n*-hexane, ethyl acetate and *n*-butanol fractions from *H. hookerianum* species exhibited neuroprotective effects on glutamate-induced cytotoxicity on rat hippocampal neuronal cells (HT_{22}) model at all tested concentrations: 5.56; 16.67 and 50.0 µg/mL.

- 4-hydroxy-2,6,4′-trimethoxydihydrochalcon (**HH12**) show the best protection ability against glutamate in HT_{22} cells with EC50 value of 1.48 µM. Compounds **HH18**, **HH31**, **HH36** have EC_{50} values of 9.70; 35.70 and 26.78 µM, respectively.

- **HH1**, **HH15** and **HH16** exhibited a protective effect on SH-SY5Y neuroblastoma cells against 6-OHDA toxicity with EC_{50} values of 47.08; 2.85 and 26.78 µM, respectively.

Ha Noi, May, 2023

ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

Assoc.Prof.Dr. Nguyen Manh Tuyen Assoc. Prof.Dr. Nguyen Minh Khoi Vu Duy Hong