SUMMARY OF DISSERTATION

Name of Doctoral candidate: Nguyen Van Linh

Dissertation title: "Phytochemistry and biological activity studies on *Agrimonia pilosa* Ledeb. var. *pilosa*"

Speciality: Medicinal Materials - Traditional Pharmacy **Code of speciality:** 9720206

Name of academic advisors:

1. Prof. Dr. Pham Thanh Ky
2. Assoc. Prof. Dr. Vu Manh Hung

Name of academic institute: National Institute of Medicinal Material **Summary of the dissertation**

1. Objectives

- To identify the scientific name of the sample and analyze botanical, anatomical properties of the sample.

- To isolate pure compounds from the extract and identify their chemical structure.

- To evaluate toxicity and biological activities of extracts and isolated compounds.

2. Methods

2.1. Botanical study

- *Morphological characterization:* Description and analysis were performed on the fresh and dried sample.

- *Scientific name identification:* Morphological characteristics were in comparison with key taxonomy of species, varieties of the genus *Agrimonia* L. in taxonomic reference books (Li Chaoluan et. al. (2003), Flora of China), and the standard specimens depositing in the herbarium (National Institute of Medicinal Material; Vietnam Academic of Science and Technology, Institute of Ecology and Biological Resources). Scientific name of the plant samples proclaimed by Vietnamese taxonomic botanists.

- *Microscopic study:* Applying microscopic method for the study on microscopy characteristics of root, stem, leaves of *A. pilosa* Ledeb. var. *pilosa*.

2.2. Chemical study

- *Extraction and isolation of chemical constituents:*

+ Extraction was carried out using ultrasonic method with methanol as solvent. The residue was suspended in 5 L hot water and extract with dichlomethane, and EtOAc, respectively.

+ Isolation was performed applying column chromatography. Column chromatography (CC) was carried out on silica gel 0.040-0.063 mm (240-430 mesh), RP-18 (30-50 μm, Fuji Silysia Chemical Ltd.), Dianon HP-20 (Mitsubishi Chem. Ind. Co., Ltd.).

- *Structural elucidation of isolated compounds:* Chemical structures were identified base on their physical properties and spectroscopy analysis: Mass spectrometry (ESI-MS, HR-ESI-MS), Nuclear magnetic resonance spectroscopy, and Circular dichroismin, comparison with the published data.

2.3. Biological evaluation

- Evaluation acute toxicity and subchronic toxicity of the extract of aerial parts (CL1) and root (CL2) extracts from *A. pilosa* Ledeb. var. *pilosa* according to Ministry of Health, WHO, and OECD Guideline.

- Evaluation of acute anti-inflammatory activity by carrageenan-induced acute inflammatory model; evaluation of chronic anti-inflammatory activity by granuloma model.

- Evaluation of analgesic activity on inflamed tissue according to the method of Randall and Selitto; evaluation of analgesic activity by acetic acid-induced writhing test and hot plate model.

- Evaluation of antioxidant activity, which may conclude in hepatoprotective action of CL1 and CL2.

- Evaluation of *in vitro* inhibitory activity on lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 murine macrophage cells of isolated compounds.

- Evaluation of *in vitro* cytotoxicity activity of isolated compounds in HepG-2, MCF-7, and SK-Lu-1 cells.

3. Results and Conclusion

3.1. Botanical properties

- Scientific name of the sample which collected in Trung Khanh, Cao Bang was identified as *Agrimonia pilosa* Ledeb.var. *pilosa* (Rosaceae).

- Morphological, anatomical analysis of stem, leaf of *A. pilosa* Ledeb. var. *pilosa* and microscopy characteristics study were performed.

3.2. Chemical constituents

- Structure of 18 compounds isolated from *A. pilosa* Ledeb. var. *pilosa* were identified, including:

+ **2 new compounds**: Agrimopilosid A (**BAP-6**), and Agrimopilosid B (**BAP-13**).

+ **7 compounds were isolated from genus *Agrimonia* for the first time**: naringenin-7-*O*-*β*-D-glucopyranosid (**BAP-4**), leucosid (**BAP-5**), 2*S*,3*S*-(–)-glucodistylin (**BAP-8** = **BAR2**), isolariciresinol-3*α*-*O*-*β*-D-glucopyranosid (**BAP-12**), vanilic acid 4-*O*-*β*-D-glucopyranosid (**BAP-16**), vanillolosid (**BAP-18**); and adenosin (**BAP-20**).

+ **1 new compound were isolated from *A. pilosa* Ledeb. var. *pilosa* for the first time**: quercetin 3-*O*-*β*-D-galactopyranosid (**BAP-1**).

+ **8 know compounds were isolated from *A. pilosa* Ledeb. var. *pilosa***: (–)-aromadendrin 3-*O*-*β*-D-glucopyranosid (**BAP-2** = **BAR1**), quercetin (**BAP-28** = **BAR3**), kaempferol (**BAP-29**), quercetin-3-*O*-rutinosid (**BAP-30** = **BAR4**), (+)-catechin (**BAP-31**); agrimonolid-6-*O*-*β*-D-glucopyranosid (**BAR6**), agrimonolid (**BAR7**), and 1*β*,2*α*,3*β*,19*α*-tetrahydroxyurs-12-en-28-oic acid (**BAR9**).

3.3. Toxicity and Biological activities

- **Acute toxicity*:***

+ The oral LD_{50} of the extracts **CL1** and **CL2** were not determined.

- **Subchronic toxicity**:

CL1 and **CL2** exhibited no subchronic toxicity in rabbits**.** *A. pilosa* Ledeb. var. *pilosa* was safe for use at the experimental doses for a long time.

- **Anti-inflammatory activity**

+ At the doses of 4.2 g/kg in mice, **CL1** and **CL2** both showed anti-inflammatory activity in carrageenan-induced acute inflammatory model, respectively compared to 15 mg/kg diclofenac as control.

+ At the doses of 2.1 and 4.2 g/kg in mice, **CL1** and **CL2** both showed chronic anti-inflammatory activity in granuloma model, compare to control group ($p < 0.05$). At the doses of 4.2 g/kg in mice, **CL1** and **CL2** both showed chronic anti-inflammatory activity respectively compared to 15 mg/kg diclofenac as control.

- ***Analgesic activity***

+ **CL1** and **CL2** both showed *in vitro* analgesic activity in carrageenan-induced acute inflammatory model, writhing test, and hot plate model.

- **Antioxidant activity and the hepatoprotective effect:** CL1 and CL2 at the oral dose of 3.6, and 7.2 g/kg/day both had the free radical scavenging activity (decrease of hepatic MDA content, restored the hepatic GSH content), and the hepatoprotective effect (decrease plasma AST, and ALT levels, also reduced macroscopic and microscopic lesions in liver compared with the positive control group).

- **Activity of isolated compounds**:

+ Naringenin 7-*O*-*β*-D-glucopyranosid (BAP-4) showed the weak inhibitory activity on NO production with the IC_{50} values of 91.07 μg/ml. 8 compounds did not show inhibitory activity on NO.

+ 9 compounds did not show cytotoxicity activity in HepG-2, MCF-7, and SK-LU-1 cells at concentrations 0.8, 4, 20, and 100 μg/ml.

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ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

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