

## SUMMARY OF DISSERTATION

**Name of Doctoral candidate:** Ha Thi Thanh Huong

**Dissertation title:** “*Study on botanical properties, chemical constituents and biological activities of the medicinal plant Phong quy sa pa (Anemone chapaensis Gagnep., Ranunculaceae)*”

**Speciality:** Medicinal Material - Traditional Pharmacy

**Code of speciality:** 9720206

**Name of academic advisors:**

1. Assoc. Prof. Dr. Phuong Thien Thuong
2. Assoc. Prof. Dr. Sc. Nguyen Minh Khoi

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

**Summary of the dissertation:**

### 1. Objectives

- Identification of the scientific name of the research sample *Anemone chapaensis* Gagnep., Ranunculaceae, description of the botanical morphology and microscopic characteristics of this species.
- Elucidation of the chemical constituents of the medicinal plant Phong quy sa pa: qualification of groups of substances, isolation of main compounds and identification of chemical structures of compounds.
- Evaluation of some biological activities on in vitro models of compounds isolated from *Anemone chapaensis*.

### 2. Methods

#### 2.1. Botanical study

- Identification of the scientific name of the plant samples on the basis of the morphological characteristics comparison with key taxonomy of species, varieties of the genus *Anemone* (family Ranunculaceae) in taxonomic reference books and the standard specimens with the scientific name *Anemone chapaensis* Gagnepain, which are currently stored in the Medicinal Plant Template Room of the Department of Medicinal Resources, National Institute of Medicinal Materials. Scientific name of the plant samples was expertised by Vietnamese taxonomic botanists.

- Investigation of microscopic characteristics including leaves, stems, and roots, powder characteristics of the upper and underground parts of the research sample. Photographic documentation of the microscopic characteristics was observed under microscope Axioskop 40.

#### 2.2. Phytochemical study

- Qualitative analysis: Determination of major chemical groups in the roots of *Anemone chapaensis* by using specific chemical reactions.

- Extraction and isolation of chemical constituents:

+ Extraction of plant materials using ethanol, and subsequently successive partitioning of the extract using increasing polarization.

+ Isolation and purification of compounds by column chromatographic method using silica gel, reverse-phase RP-C18 with suitable solvent systems.

- Structural elucidation of isolated compounds: On the basis of the analyses of physical properties (morphology, melting point), spectroscopic data (UV, IR, MS, NMR ( $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , DEPT, HMBC, HSQC, COSY)).

- Method of determining the sugar units: configuration of sugar units of a new saponin compound was determined by gas chromatography mass spectrometry (GC-MS), obtained after hydrolyzing saponin .

### **2.3. Biological study**

- Evaluation of inhibitory activity on production of LPS-stimulated NO in RAW264.7 macrophages by Griess reagent assay. Anti-inflammatory activity was carried out after cytotoxicity test by MTT colorimetric method.

- Evaluation of effect of inhibiting COX-2 protein in LPS-stimulated RAW264.7 macrophages by compound ACR2 was conducted by Western Blot method.

- Evaluate toxic effects to cancer cells by MTT method.

## **3. Results and conclusion**

### **3.1. Botanical properties**

- The plant samples collected at Hoang Lien Pass, Sa Pa District, Lao Cai Province were identified as *Anemone chapaensis* Gagnep., (Ranunculaceae).

- The morphological characteristics of the medicinal plant have been fully described, in which micro-anatomy and medicinal powder characteristics of the parts of Phong Quy Sa Pa were described for the first time.

### **3.2. Chemical constituents**

15 compounds from the research sample have been isolated, including 11 compounds (**ACL10-ACL6, ACL3-ACL1**) from the upper part and 04 compounds (**ACR1-ACR4**) from the underground part. The compounds identified were trans-tilirosid (**ACL10**), arctiin (**ACL9**), arctigenin (**ACL8**), 5-hydroxymethylfurfural (**ACL11**), blumenol A (**ACL5**), ethyl caffeate (**ACL7**), caffeic acid (**ACL6**). , huzhangosid D (**ACL3**), hupehensis saponin F (**ACL4**), clemastanosid D (**ACL2**), 3-O- $\beta$ -D-ribofuranosyl- (1  $\rightarrow$  3) - $\alpha$ -L-rhamnopyranosyl- (1  $\rightarrow$  2) - $\alpha$ - L-arabinopyranosyl-hederagenin-28-O- $\beta$ -D-glucopyranosyl- (1  $\rightarrow$  6)--D-glucopyranosid (**ACL1**, new compound), prosapogenin CP6 (**ACR1**), huzhangosid A (**ACR2**), huzhangosid C (**ACR3**), 3-hydroxy-4-methyl- $\gamma$ -butyrolactone (**ACR4**).

Among the 15 compounds, there is a new saponin compound (**ACL1**), 6 compounds **ACL5**, **ACL8-11**, and **ACR4** were first found in the Anemone genus. This is the first announcement of the chemical composition of *A. chapaensis* Gagnep.

### **3.3. Biological activities**

The compounds isolated from the research sample were tested for anti-inflammatory and toxic effects on cancer cells with the following results:

- The compounds **ACL8**, **ACL7**, **ACL3**, and **ACR2** inhibit the production of NO in the LPS-stimulated RAW264.7 macrophage cells with IC<sub>50</sub> values of 23.19 ; 47.86; 32.36; and 3.68  $\mu$ M, respectively.

- Compound **ACR2** at concentrations 1; 5 and 10  $\mu$ M reduced the expression of COX-2 protein in the LPS-stimulated RAW 264.7 macrophage cells according to the concentration-effect correlation. At 10  $\mu$ M, **ACR2** demonstrated the inhibitory effect on COX-2. Thus, compound **ACR2** has anti-inflammatory effect by inhibiting COX-2 in the LPS-stimulated RAW264.7 macrophage cells.

- The compound **ACR1** was capable of poisoning 08 cancer cell lines HepG2, A549, MCF7, OvcAR-8, NCI-N87, RD, Panc-1, MIA Paca-2 with IC<sub>50</sub> values of 16.7; 13.2; 24.1; 11.8; 5.4; 7.5; 7.5; and 2.7  $\mu$ g / ml, respectively, while compound **ACR2** was active on 05 cancer cell lines HepG2, OVCAR-8, NCI-N87, RD, and PANC-1 with IC<sub>50</sub> values of 11.3; 10.6; 18.2; 12.2; and 19.6  $\mu$ g / ml, respectively.

*Hanoi, 21<sup>st</sup> August, 2020*

**Academic advisors**

**Doctoral Candidate**

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**Ha Thi Thanh Huong**