SUMMARY OF DISSERTATION

Name of Doctoral candidate: Do Thi Oanh

Dissertation title: "Botanical properties, phytochemistry and biological activity studies on *Chloranthus japonicus* Sieb. in Vietnam"

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

Name of academic advisors:

1. Prof. Dr. Pham Thanh Ky
2. Assoc. Prof. Dr. Le Viet Dung

Name of academic institute: National Institute of Medicinal Materials

Summary of the dissertation

1. Objectives

- To identify the scientific name of the sample and analyze botanical, anatomical properties of the sample in order to contribute to the herbal material standardization.

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

- To evaluate toxicity, anti-inflammatory activity, antioxidant activity, hepatoprotective activity, and protease HIV-1 inhibitory activity of 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 published data and certificated voucher specimen of *Chloranthus japonicus* Sieb.

- *Microscopic study:* Applying microscopic method for the study on anatomical and microscopy characteristics of aerial parts, root of *Chloranthus japonicus* Sieb.

2.2. Methods

- *Qualitative analysis:* Chemical components were detected applying phytochemical screening test and TLC method.

- *Extraction and isolation of chemical constituents:*

+ Extraction was carried out using 70% ethanol as solvent at room temperature. The residue was suspended in water and extract with *n*-hexane, EtOAc, and *n*-butanol, respectively.

+ Isolation was performed applying column chromatography and preparative thin

layer chromatography. Thin layer chromatography (TLC) was performed on silica gel 60G F_{254}. Column chromatography (CC) was carried out on silica gel (0,040 – 0,063 mm), RP-18, Dianon HP-20, and Sephadex LH20.

- *Structural elucidation of isolated compounds:* Chemical structures were identified base on their physical properties (melting points, rotary polarization) and spectroscopy analysis: Infrared spectroscopy (IR), Mass spectrometry (ESI-MS, HR-ESI-MS), One-dimensional Nuclear magnetic resonance spectroscopy (^{1}H-NMR, ^{13}C-NMR, and DEPT), and Two-dimensional Nuclear magnetic resonance spectroscopy (HMBC, HSQC and NOESY), in comparison with the published data.

2.3. Biological evaluation

- Evaluation acute toxicity of the water extract of aerial parts and root from *Chloranthus japonicus* Sieb.

- Evaluation of acute anti-inflammatory activity of the water extract of aerial parts and root from *C. japonicus* Sieb. by carrageenan-induced acute inflammatory model and mouse peritonitis model.

- Evaluation of acute anti-inflammatory activity of the water extract of aerial parts and root from *C. japonicus* Sieb. by amiant-induced chronic inflammatory model.

- Hepatoprotective activity and antioxidant activity of the water extract of aerial parts and root from *C. japonicus* Sieb. were evaluated by paracetamol-induced liver injury model.

- Protease HIV-1 inhibitory activity of isolated compounds was carried out applying Richards method.

3. Results and Conclusion

3.1. Botanical properties

- Scientific name of the sample was identified as *Chloranthus japonicus* Sieb. (Chloranthaceae).

- Morphological, anatomical analysis of stem, leaf, root of *Chloranthus japonicus* Sieb. and microscopy characteristics study were performed.

3.2. Chemical constituents

- Flavonoid, saponin, organic acid, monosaccharide, polysaccharide, fat, and essential oil were detected in aerial parts of *Chloranthus japonicus* Sieb.

- Flavonoid, saponin, coumarin, organic acid, steroid, carotene, polysaccharide, and

essential oil were detected in aerial parts of *Chloranthus japonicus* Sieb.

- Structure of 14 compounds isolated from *Chloranthus japonicus* Sieb. were identified, including:

+ ***4 compounds was isolated from the aerial extract:*** Shizukolidol **(1)**, 4*α*,8*β*-dihydroxyeudesm-7(11)-en-12,8-olide **(2)**, 4*α*-hydroxy-5*α*,8*β*(*H*)-eudesm-7(11)-en-8,12-olide **(3)**, and linarionoside A **(4)**.
+ ***10 compounds were isolated from the root extract***: Chloranoside A **(5)**, 5-(hydroxylmethyl)fururaldehyde **(6)**, glucosyringic acid **(7)**, vanilloside **(8)**, sarcaglaboside G (**9**), yinxiancaoside C **(10)**, sacaglaboside C **(11)**, calycanthoside **(12)**, shizukanolide F **(13)**, and isofraxidin **(14)**.
+ Compounds **2, 3**, **11**, **13**, and **14** were isolated from *C. japonicus* Sieb. for the first time.
+ Compounds **4**, **6**, **7**, **8**, **9**, and **12** were isolated from genus *Chloranthus* Sw. for the first time.

3.3. Toxicity and Biological activities

- ***Acute toxicity:***

+ The water extract of aerial parts from *C. japonicus* Sieb. at the dose of 150.0 g/kg (oral administration) not showed acute toxicity.
+ The oral LD_{50} of the water extract of root from *C. japonicus* Sieb. was 202.18 (219.86 - 166.82) g/kg body weight.

- ***Acute anti-inflammatory activity***

+ *The rat paw edema model*:
  - ✓ The aerial parts extract of *C. japonicus* Sieb. with the dose 8.4 g/kg exhibited acute anti-inflammatory effects 2 hours after administration through inhibiting carrageenin-induced edema in the hind paws.
  - ✓ The root extract of *C. japonicus* Sieb. with the doses of 2.8 and 8.4 g/kg body weight did not show the acute anti-inflammatory effects.
+ *The peritonitis model:* At the dose of 2.8 g/kg, the aerial parts and root extracts did not reduce inflammatory exudates volume, total number of leukocytes in inflammatory exudates, and protein level in inflammatory exudates compared to the control group. However, the aerial parts extract with the dose of 8.4 g/kg reduced total number of leukocytes in inflammatory exudates compared to the control group ($p < 0.05$).

- Chronic anti-inflammatory activity: The aerial parts and root extracts of *C. japonicus* Sieb. with the doses of 4.0 and 12.0 g/kg body weight reduced the weight of granuloma compared to the control group ($p < 0.05$).

- Hepatoprotective and anti-oxidant activity

+ The aerial parts and root extracts of *C. japonicus* Sieb. at the doses of 4.0 and 12.0 g/kg body weight showed the hepatoprotective effect on a paracetamol-induced liver injury in mice via reducing the activities of ALAT and ASAT.
+ The aerial parts of *C. japonicus* Sieb. at the dose of 4.0 g/kg decreased the concentration of MDA from homogenous liver of mice ($p < 0.05$).
+ The root extract of *C. japonicus* Sieb. did not decrease the concentration of MDA from homogenous liver of mice poisoned by paracetamol.

- **Protease HIV-1 inhibitory activity**: 4α,8β-Dihydroxyeudesm-7(11)-en-12,8-olide, which was isolated from ethyl acetate extract of aerial parts of *C. japonicus* Sieb. showed protease HIV-1 inhibitory activity with IC_{50} = 0,45 μM.

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Academic advisors	**Doctoral Candidate**
Prof. Dr. Pham Thanh Ky	**MSc. Do Thi Oanh**
Assoc. Prof. Dr. Le Viet Dung	