SUMMARY OF DISSERTATION

1. INTRODUCTION

Name of Ph.D candidate: Nguyen Thi Phuong

Dissertation title: Study on chemical constituents and biological activities of the medicinal plant *Leea rubra* Blume ex Spreng., (family Leeaceae).

Speciality: Traditional Pharmacy **Code number:** 62720406

Scientific supervisors:

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

Academic institution: Vietnam National Institute of Medicinal Materials

2. SUMMARY

2.1. Objectives

- *Botanical properties:* Identification of main morphological characteristics and microscopic identification of *Leea rubra*.
- *Chemical constituents:* Determination of organic compound groups, isolation and structural elucidation of constituents from aerial parts and underground parts (roots) of *L. rubra.*
- *Biological activities:* Evaluation of some biological activities of ethanol extract and isolated compounds from *L. rubra.*

2.2. Methods

2.2.1. Botanical study

- Identification of the scientific name of the plant samples on the basis of the morphological characteristics by comparison with key taxonomy of species, varieties of the genus *Leea* (family Leeaceae) in taxonomic reference books and the standard specimens depositing in the herbarium (Institute of Ecology and Biological Resources). Scientific name of the plant samples proclaimed by Vietnamese taxonomic botanists.
- Investigation of phytotomical characteristics including those of stems, rhizomes, leaflets, and roots. Preparations of specimens by using the double-dyed method (carmine and methylene blue). Photographic documentation of the microscopic characteristics observed under stereomicroscopy.

2.2.2. Phytochemical study

- *Qualitative analysis:* Determination of major chemical groups in *Leea rubra* by

using specific chemical reactions and thin layer chromatography.

- *Extraction and isolation of chemical constituents:*
    + Extraction of plant materials using ethanol, and subsequently successive partitioning of the extract using increasing polarization solvents (*n*-hexan, ethyl acetate, *n*-buthanol).
    + Isolation and purification of compounds by column chromatography using silica gel, reverse-phase YMC RP-18 as adsorbents and Sephadex LH-20; using preparative high performance liquid chromatography (preparative HPLC).
- *Structural elucidation of isolated compounds*

    Structural elucidation of isolated compounds based on the analyses of physical properties (appearance, melting point, specific optical rotation), spectroscopic data (UV, IR, MS, CD, NMR), and comparison with the literature data.

2.2.3. Biological evaluation

- Sample preparation: total extract of leaves, stems and roots of *Leea rubra*.
- Evaluation of antioxidant activity, scavenging free radicals DPPH and superoxide by optical measurement method.
- Evaluation of inhibitory activity against xanthinoxidase enzyme by optical measurement method.
- Evaluation of 5-lipoxygenase inhibitory activity by optical measurement method.
- Evaluation of cyclooxygenase inhibitory activity by optical measurement method, according to Cayman company protocol, code mumber 560131.
- Evaluation of acute anti-inflammation in carrageenan-induced paw edema in rats according to Winter method.
- Evaluation of chronic anti-inflammatory activity in the cotton pellet-induced granuloma model.
- Evaluation of protease HIV-1 inhibitory activity by optical measurement method.

2.3. Results and Conclusion

2.3.1. Botanical properties

- The plant samples was identified as *Leea rubra* Blume ex Spreng., (Leeaceae).
- Morphological characteristics including those of flowers, fruits, seeds of *L. rubra* were documented and microscopic characteristics of stems, leaflets, and roots were described.

2.3.2. Chemical constituents

- The groups of organic compounds contained in the leaves of *Leea rubra* were identified as flavonoid, tannin, triterpenoid, sterol, carbohydrate, and organic acid. The stems and roots contain sterol, tannin and organic acid.
- Twenty-one compounds were isolated from leaves of *Leea rubra* and identified as kaempferol (**1**), quercetin (**2**), myricitrin (**3**), europetin-3-*O*-α-L-rhamnopyranoside (**4**), rhamnetin-3-*O*-α-L-rhamnopyranoside (**5**), juglanin (**6**), artabotrysid B (**7**), acid gallic (**8**), protocatechuic acid (**9**), *p*-hydroxybenzoic acid (**10**), arctiin (**11**), β-sitosterol (**12**), daucosterol (**13**), ursolic acid (**14**), oleanolic acid (**15**), maslinic acid (**16**), β-amyrin (**17**), huzhangoside D (**19**).
- Compounds from roots were identified as gallic acid (**8**), β-sitosterol (**12**), daucosterol (**13**).
- Compounds from stems were identified as gallic acid (**8**), protocatechuic acid (**9**), β-sitosterol (**12**), daucosterol (**13**), lup-20(29)-en-3β,6α-diol (**18**), stigmast-4-en-3,6-dion (**20**), goniothalamine (**21**).
- Ten compounds were isolated for the first time from *Leea* genus are compounds number **4**, **5, 6, 7, 11, 16, 18,19, 20** và **21**. 18 compounds were firstly isolated from species *Leea rubra*: **1, 2, 4, 5, 6, 7, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21**.
- Development of a method for quantitive analysis of gallic acid from stems and roots and a method for simultaneous quantification of gallic acid and europetin-3-*O*-α-L-rhamnopyranoside from *Leea rubra* leaves. The contents of gallic acid (**8**) in roots, stems, and leaves were determined as 0.236%, 0.116%, and 0.142%, respectively. The content of europetin-3-*O*-α-L-rhamnopyranoside (**4**) in *Leea rubra* leaves were identified as 0.097%.

2.3.3. Biological activities

- The ethanol extracts of GHR, GHL, GHT showed the potential radical scavenging effect on DPPH with the IC_{50} values of 8.72; 19.71 and 25.94 μg/mL, respectively. The isolated compounds also exhibited potential activity (IC_{50}< 5 μg/mL), except for *p*-hydroxybenzoic acid.
- GHR showed moderate $O_2^{-\bullet}$ radical scavenging effect with an IC_{50} of 28.05 μg/mL.
- GHR showed supressive activity against xanthine oxidase (XO) with an IC_{50} value of 18.39 μg/mL.
- *Leea rubra* extracts showed inhibitory effects on some enzymes related to inflammatory process:
    + GHR, GHT, GHL showed inhibitory activity against lipoxygenase (LO) with IC_{50} values of 14.37; 24.95 and 21.50 μg/mL, respectively.

+ GHL and GHR showed inhibitory activity against both COX-1 and COX-2 with IC_{50} values of 35.35 and 40.78 µg/mL (on COX-1); and 26.89 and 30.10 µg/mL (on COX-2), respectively.

- The ethanol extracts of GHL, GHR showed peripheral analgesic effect but not central analgesic effect on mice at the doses of 200 mg/kg and 400 mg/kg body weight.
- Ethanol extract from underground parts and the leaves of *L. rubra* displayed both acute and chronic anti-inflammatory activities at the doses of 100 mg/kg and 400 mg/kg body weight in rat models.
- Ethanolic extract, EtOAc-soluble fraction of leaves and stems, as well as *n*-hexane-soluble fraction from leaves showed inhibitory effect on enzyme HIV-1 protease. Three triterpenoid compounds, including ursolic acid (**14**), maslinic acid (**16**) and lup-20(29)-en-3β,6α-diol (**18**) showed relatively strong activity against HIV-1 protease with IC_{50} values of 6.2 µM; 4.3 µM and 33.1 µM, respectively.

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