SUMMARY OF DISSERTATION

Name of Doctoral candidate: Nguyen Trung Tuong

Dissertation title: "Botanical properties, phytochemistry and biological activity studies on *Phaeanthus vietnamensis* Ban (Annonaceae)"

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

Name of academic advisors:

1. Prof. Dr. Pham Thanh Ky
2. Dr. Nguyen Xuan Nhiem

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 (anti-inflammatory activity, analgesic activity, cytotoxicity activity) 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 the standard specimens of *Phaeanthus vietnamensis* Ban (National Institute of Medicinal Material, Institute of Ecology and Biological Resources).

- *Anatomical study:* Preparations of specimens (root, stem, leaves) by using the double-dyed method. Photographic documentation of the microscopic characteristics observed under stereomicroscopy were performed.

- *Microscopic study:* Applying microscopic method for the study on microscopy characteristics of root, stem, leaves of *Phaeanthus vietnamensis* Ban.

2.2. Chemical study

- *Extraction and isolation of chemical constituents:*

+ Extraction was carried out using ultrasonic method with methanol as solvent at 50°C. The residue was suspended in 5% acetic acid and extract with *n*-hexane, chloroform, and EtOAc, respectively.

+ Isolation was performed applying column chromatography, preparative thin layer chromatography, and preparative high-performance liquid chromatography. Thin layer chromatography (TLC) was performed on silica gel DC-Alufolien 60 F254, RP18 F254S. Column chromatography (CC) was carried out on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck), RP-18 (30-50 μm), Dianon HP-20. Preparative high-performance liquid chromatography (HPLC) was performed on HPLC Agilent 1200 with J'sphere column (20 × 250 mm, H-80).

- *Structural elucidation of isolated compounds:* Chemical structures were identified base on their physical properties (melting points, rotary polarization) and spectroscopy analysis: Mass spectrometry (HR-MS), One-dimensional Nuclear magnetic resonance spectroscopy and Two-dimensional Nuclear magnetic resonance spectroscopy, in comparison with the published data.

2.3. Biological evaluation

- Evaluation acute toxicity and subchronic toxicity of the extract of leaves and twigs extracts from *P. vietnamensis* Ban according to Ministry of Health, WHO, and OECD Guideline.

- Evaluation of CFA-induced antiarthritis activity according to the method of Newbould; evaluation of acute anti-inflammatory activity by carrageenan-induced acute inflammatory model; evaluation of chronic anti-inflammatory activity by granuloma model of Ducrot.

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

- Evaluation of *in vitro* inhibitory activity on nitric oxide production of isolated compounds.

3. Results and Conclusion

3.1. Botanical properties

- Scientific name of the sample which collected in Hoa Nhon Ward, Hoa Vang District, Da Nang City was identified as *Phaeanthus vietnamensis* Ban (Annonaceae).

- Morphological, anatomical analysis of stem, leaf, root of *Phaeanthus vietnamensis* Ban and microscopy characteristics study were performed.

3.2. Chemical constituents

- Structure of 15 compounds isolated from *Phaeanthus vietnamensis* Ban were identified, including:

+ **3 new compounds**: (7*S*,8*R*,8'*R*)-3,5,3′,5'-tetramethoxy-4,4',7-trihydroxy-9,9'-epoxylignan (**PV1**), 8α-hydroxyoplop-11(12)-en-14-on (**PV5**), and (1*R*,2*S*,4*S*)-2-*E*-cinnamoyloxy-4-acetyl-1-methylcyclohexan-1-ol (**PV12**)

+ **8 compounds were isolated from genus *Phaeanthus* for the first time**: 8*R*,8′*R*-bishydrosyringenin (**PV2**), (+)-5,5'-dimethoxylariciresinol (**PV3**), spathulenol (**PV6**), 1αH,5βH-aromandendrane-4β,10α-diol (**PV7**), 1αH,5βH-aromandendrane-4α,10α-diol (**PV8**), 1βH,5βH-aromandendrane-4α,10β-diol (**PV9**), 3α,4β-dihydroxybisabola-1,10-dien (**PV10**), and nerolidol (**PV11**).

+ **2 new compounds were isolated from *P. vietnamensis* Ban for the first time**: thalifolin (**PV14**) and moupinamid (**PV15**).

3.3. Toxicity and Biological activities

- ***Acute toxicity:***

+ The oral LD_{50} of the extract of leaves from *P. vietnamensis* Ban (**CL1**) was 135.63 (118.97÷154.62) g/kg body weight (p = 0,05).

+ The extract of twigs from *P. vietnamensis* Ban (**CL2**) at the dose of 550.0 g/kg (maximum oral administration) not showed acute toxicity.

- ***Subchronic toxicity***:

The extract of leaves and twigs extracts from *P. vietnamensis* Ban (**CL1**, **CL2**) were safe for use at the doses of 2 and 4 g/kg body weight of mice for 60 days.

- ***Anti-inflammatory activity***

+ At the doses of 1.4 and 2.8 g/kg, **CL1** and **CL2** both showed antiarthritis activity, compare to 10 mg/kg indomethacin as control.

+ At the doses of 1.4 and 2.8 g/kg, **CL1** and **CL2** both showed acute and chronic anti-inflammatory activity, compare to 15 mg/kg diclofenac as control.

- ***Analgesic activity***

+ At the doses of 1.4 and 2.8 g/kg, **CL1** and **CL2** both showed analgesic effect on inflamed tissue, compare to 15 mg/kg diclofenac as control.

+ At the doses of 2.4 and 4.8 g/kg, **CL1** and **CL2** both inhibited analgesic effect in writhing test, compare to 20 mg/kg diclofenac as control.

+ At the doses of 2.4 and 4.8 g/kg, **CL1** and **CL2** both increased reaction time to pain in hot plate model.

- **Activity of isolated compounds**:

+ Spathulenol (**PV6**) showed cytotoxicity activity in Jurkat, HepG-2, and MCF-7 cells with the IC_{50} values of 42.00, 44.64, 53.88 μM, respectively. **PV1**, **PV5**, **PV8-PV11**, and **PV14** inhibited weak effect with an IC_{50} range of 109.11-186.72 μM. Spathulenol (**PV6**) showed the strong inhibitory activity on NO production with the IC_{50} values of 15.7 ± 1.2 μM. **PV2**, **PV8** and **PV9** significantly inhibited inflammatory NO production with IC_{50} values ranging from 22.6 to 25.3 μM.

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ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

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