SUMMARY OF DISSERTATION

Name of Doctoral candidate: Ngo Sy Thinh

Dissertation title: Study on botanical properties, chemical constituents and biological activities of *Fissistigma pallens* (Fin. & Gagnep.) Merr., Annonaceae.

Speciality: Medicinal Materials - Traditional Pharmacy

Code of speciality: 9720206

Name of academic advisors:

1. Assoc. Prof. Dr. Nguyễn Thị Bích Thu
2. Assoc. Prof. Dr. Nguyễn Xuân Nhiệm

Name of academic institute: National Institute of Medicinal Materials

Summary of the dissertation

1. Objectives

- To study on botanical properties, identification of the scientific name, identification of micro-anatomy, powder microscopic characteristics of stems and leaves of *Fissistigma pallens* (Fin. & Gagnep.) Merr.

- To study on chemical constituents of the leaves of *Fissistigma pallens* (Fin. & Gagnep.) Merr.

- To evaluate biological activities of isolated compounds from the leaves of *Fissistigma pallens* (Fin. & Gagnep.) Merr.

1. Methods

2.1. Botanical study

- *Scientific name identification:* Morphological characteristics were in compared with the standard specimens of *Fissistigma pallens* (Fin. & Gagnep.) Merr.

- *Anatomical and Microscopic study:* Determination of microscopic characteristics of the leaves, stem and the whole plant powder characteristics by using microscopic method.

2.2. Chemical study

- *Qualitative analysis:* Determination of major chemical groups in *Fissistigma pallens* (Fin. & Gagnep.) Merr. by using specific chemical reactions

- *Extraction and isolation of chemical constituents:*

+ The dried powdered leaves were sonicated twice with hot methanol (MeOH) (each 10 L, 4 h, 50°C) and the solvent was removed in vacuo.

+ Isolation was performed applying column chromatography, preparative thin layer chromatography, and preparative high-performance liquid chromatography.

- *Structural elucidation of isolated compounds:* Chemical structures were identified based on their physical properties (melting points, rotary polarization) and spectroscopy analysis: ESI-MS, HR-EI-MS 1D-NMR, 2D-NMR (HSQC, HMBC, COSY, NOESY) and in comparison with the published data.

2.3. Biological study

- Evaluated cytotoxic activity of the isolated compounds.
- Evaluated the antioxidant activity of the isolated compounds.
- Evaluated the inflammatory acitivity of the isolated compounds.

3. Results and Conclusion

3.1. Botanical properties

- Scientific name of the sample which collected in Pu Hoat Nature Reserve, Tien Phong Commune, Que Phong District, Nghe An Province, was identified as *Fissistigma pallens* (Fin. & Gagnep.) Merr. (Annonaceae).

- Morphological, anatomical analysis of the leaves, stem and the whole plant powder characteristics of *Fissistigma pallens* were performed.

3.2. Chemical constituents

- Identified groups of compounds present in *Fissistigma pallens* (Fin. & Gagnep.) Merr. including: Flavonoids, saponins, reducing sugars, polysaccharides, tannins, amino acids.

- Structure of 23 compounds isolated from leaves of *Fissistigma pallens* (Fin. & Gagnep.) Merr. were identified as followings:

+ Nine new sesquiterpen glucosides (fissispallin A-F) and three new flavonol glycoside (fissflavosid A-C).

+ Twelve compounds were isolated from genus *Fissistigma* Griff. for the first time: alismol; 4*β*,12-dihydroxyguaiane-6,10-dien; alismoxide; 10-*O*-methyl-alismoxide, 1*α*H,5*β*H-aromandendrane-4*β*,10*α*-diol, 15-hydroxy-*α*-cadinol, kaempferol 3-rutinoside, rutin, kaempferol 3-*O*-*α*-L-rhamnopyranosyl (1→6)-*β*-D-galactopyranoside, isorhamnetin 3-robinobioside, kaempferol 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside, rhamnetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside.

+ And two known compounds: fissispallin và spathulenol.

3.3. Toxicity and Biological activities

- ***Cytotoxic activity:***

+ Compounds fissispallin A showed the most potent cytotoxic activity against three HT-29, A-2058, A-549 with IC_{50} values 1.5 ± 0.3 µM, 0.6 ± 0.1 µM, 1.1 ± 0.2 µM respectively .

+ Compounds fissispallin C**,** fissispallin D, fissispallin also showed significant cytotoxic activity against three HT-29, A-2058, A-549 with IC_{50} values ranging from 0.4 to 7.2 µM.

+ Compounds fissispallin E-F exhibited moderate cytotoxic activity against three cancer cell lines with IC_{50} values ranging from 15.5 to 23.9 µM.

- ***Antioxidant activity:***

+ Compounds fissflavosid A-C, kaempferol 3-rutinoside, rutin, kaempferol 3-*O*-*α*-L-rhamnopyranosyl (1→6)-*β*-D-galactopyranoside, isorhamnetin 3-robinobioside, kaempferol 3-*O*-[*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside, rhamnetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside showed significant antioxidant, among three new compounds fissflavosid A-C exhibited antioxidant with troloxequivalents ranging from 6.87 to 10.24 µM.

+ Two compounds rutin and rhamnetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-glucopyranoside having meaningful reducing capacity by measuring the concentration of Cu (I) ions reduced from Cu (II) ion

- ***Inflammatory activity:***

+ Compounds fissispallin A-D displayed significant inhibitory activity against NO production in LPS-stimulated RAW 264.7 macrophages IC_{50} values ranging from 3.04

to 3.68 µM. Compounds fissispallin A-C inhibited potent the production of anti-inflammatory cytokine TNF-α with % inhibition from 47.98% to 59.09 % at the concentrations of 10 µM ($p < 0.01$).

+ Compounds fissispallin B-D showed the inhibitory production of IL-6 with % inhibition from 38.55% to 59.78 % at the concentrations of 10 µM ($p < 0.05$).

Hanoi, February, 2023

ACADEMIC ADVISORS		**DOCTORAL CANDIDATE**
Assoc. Prof. Dr.	**Assoc. Prof. Dr.**	**Ngô Sỹ Thịnh**
Nguyễn Thị Bích Thu	**Nguyễn Xuân Nhiệm**	