SUMMARY OF DISSERTATION

Name of Doctoral candidate: Tran Thi Thuy Linh

Dissertation title: "Study on chemical constituents and some biological effects of *Pogostemon auricularius* (L.) Hassk. - Lamiaceae"

Specialty: Medicinal Materials - Traditional Pharmacy

Code of specialty: 9720206

Name of academic advisors:

1. Prof. Dr. Nguyen Thi Hoai
2. Assoc. Prof. Dr. Le Viet Dung

Name of academic institute: National Institute of Medicinal Materials

Summary of the dissertation

1. Objectives

- To determine the chemical structure of compounds isolated from the fraction had anti-inflammatory effects *in vitro*.

- To evaluate the acute toxicity of the fraction had anti-inflammatory effects *in vitro* and investigate some biological effects of the crude extract, fractions, and some isolated compounds.

2. Methods

2.1. Botanical study

- *Scientific name identification:* Morphological characteristics were compared to the standard specimens of *Pogostemon auricularius* (L.) Hassk.

2.2. Chemical study

- *Extraction and isolation of chemical constituents:*

P. auricularius collected in Huong Hoa district, Quang Tri province, were washed, chopped, and dried at 50-60^{o}C. The dried material was then ground into a coarse powder. The crude extract was obtained by immersing the raw powder in methanol at room temperature and soaking for 07 days each time. After combining the extracts and conducting solvent removal, 207 g of methanol extract was obtained. This extract was

then suspended in water and successively partitioned with n-hexane, CH_2Cl_2, and EtOAc (3 times, 5.0 L each) to obtain the n-hexane (65 g), CH_2Cl_2 (51 g), EtOAc (47 g), and water (44 g) fractions after removal of the solvents in vacuo.

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

- *Structural elucidation of isolated compounds:* Chemical structures were identified based on their spectroscopy analysis: HR-ESI-MS 1D-NMR, 2D-NMR, CD, and in comparison with the published data.

2.3. Biological study

- Evaluation of acute toxicity of ethyl acetate fraction according to the guidance of the Ministry of Health of Vietnam

- Evaluation of the *in vitro* anti-inflammatory effect via inhibiting NO production of mouse macrophages RAW 264.7

- Evaluation of the effect on the release of IL-6, IL-10 and TNF-α on RAW 264.7 mouse macrophages.

- Evaluation of cytotoxicity by SRB staining on Hep-G2 (human hepatocellular carcinoma), AGS (human gastric carcinoma), KB (human epidermoid carcinoma), LU-1 (human lung carcinoma), and SW-480 (human colon adenocarcinoma).

- Evaluation of apoptosis induction by Hoechst 33342 dye and flow cytometric method on LU-1 cell line.

- Evaluation of caspase-3 induction ability on LU-1 cell line.

3. Results and Conclusion

3.1. Botanical properties

- Scientific name of the sample collected in Huong Hoa District, Quang Trị Province, was identified as *Pogostemon auricularius* (L.) Hassk. (Lamiaceae).

The morphology of the leaves, stem, and flower of *Pogostemon auricularis* was described.

3.2. Chemical constituents

- Identified groups of compounds in *Pogostemon auricularis* including alkaloids, flavonoids, saponins, tannins, steroids, cardiac glycosides, reducing sugars, and fats.

- Structure of 11 compounds isolated from *Pogostemon auricularius* (L.) Hassk. were identified, in which,

+ 9 new compounds including 3 meroterpenoids (Pogostemin A, Pogostemin B, and Pogostemin C), 5 phloroglucinol derivatives (Pogostemonon A, Pogostemonon B, Pogostemonon C, Pogostemonon D, and Pogostemon D), and 1 triterpen (Pogostem).

+ 2 compounds were isolated from *Pogostemon auricularius* (L.) Hassk. for the first time: geranyllinalool and stigmasterol

3.3. Toxicity and Biological activities

- ***Acute toxicity:***

The oral LD_{50} of the ethyl acetate extract of *Pogostemon auricularius* in mice was identified as 9.18 g/kg

- ***In vitro* anti-inflammatory effects:**

+ Ethyl acetate and dichloromethane extracts showed strong inhibitory activity on NO production with IC_{50} of 25.28 ± 1.52 and 28.68 ± 1.49 µg/mL. Meanwhile, the n-hexane extract has not shown activity at the studied concentrations. The crude methanol and aqueous extracts exhibited moderate activity, with IC_{50} in the range of 60.54 - 64.73 µg/mL.

+ Pogostemin C and Pogostemon D can inhibit NO production strongly, with IC_{50} of 3.76 ± 0.15 and 7.29 ± 0.74 µM, respectively. Other samples, such as Pogostemonon A, Pogostemonon C, and Pogostemin B showed good activity with IC_{50} in the range of 26.77 - 40.78 µM.

+ At the concentration of 50.5 µM, Pogostemin C can activate IL-10 production and inhibit TNF-α production compared with negative controls ($P < 0.05$).

- ***Cytotoxic effects on cancer cells:***

+ Pogostemin A showed average activity on Hep-G2, AGS, KB, LU-1 and SW-480 cell lines, with IC_{50} in the range of 18.75 - 33.18 µM. Pogostemin B-C, Pogostemonon A-C, and Pogostemon D exhibited weak activity with IC_{50} in the range of 77.28 - 236.08 µM in 5 tested cell lines.

+ At the concentration of 52.08 µM, Pogostemin A can induce apoptosis on LU-1 lung cancer cells, causing the concentration or fragmentation of the cell nucleus at the rate of

34.30%; and inducing caspase-3 production with an increase of 1.22 times compared to the negative control ($P < 0.05$); the ratio of both early and late apoptotic cells increased at 14.9 % and 12.6%, respectively through the flow cytometry technique.

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ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

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