SUMMARY OF DISSERTATION

Name of Doctoral candidate: Doan Thi Huong

Dissertation title: "Study on chemical constituents and biological effects in Alzheimer's disease-oriented treatment of *Huperzia phlegmaria* (L.) Rothm..

Speciality: Medicinal Materials - Traditional Pharmacy

Code of specciality: 9720206

Name of academic advisors:

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Name of academic institute: National Institute of Medicinal Materials

Summary of the dissertation

1. Objectives

- To isolate pure compounds from the extract and identify their chemical structure.
- To evaluate toxicity and biological effects in Alzheimer's disease-oriented treatment of methanol, fractional extracts and isolated compounds.

2. Methods

2.1. Botanical study

- *Scientific name identification:* Morphological characteristics were in comparison with the standard specimens of *Huperzia phlegmaria* (L.) Rothm.
- *Anatomical and Microscopic study:* Determination of microscopic characteristics of the arial part, leaves, stem and the whole plant powder characteristics by using microscopic method.

2.2. Chemical study

- *Extraction and isolation of chemical constituents:*

The dried aerial parts of *H. phlegmaria* (1.5 kg) were extracted with MeOH (3 times, 5.0 L each) at room temperature to yield 75 g of a dark solid extract. This was then dissolved in 3% tartaric acid (1.0 L) and filtered to separate the solid residue. The remaining acidic solution was adjusted to pH 10 with saturated aqueous Na_2CO_3, and partitioned with

dichloromethane (CH_2Cl_2) (3 times, 2.0 L each) to obtain total alkaloidal fraction (HC, 8 g) after removal of the solvents *in vacuo*. The solid residue (57 g) was washed with distilled water until neutral, and extracted with EtOAc to give EtOAc fraction (HE, 38 g).

+ 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 base on their physical properties (melting points, rotary polarization) and spectroscopy analysis: FT-IR, UV-Vis, ESI-MS, HR-EI-MS 1D-NMR, 2D-NMR, CD, and in comparison with the published data.

2.3. Biological study

- Evaluation acute toxicity and subchronic toxicity of the alcaloidal extract of dried aerial parts of *Huperzia phlegmaria* according to Ministry of Health, WHO.

- The AChE inhibitory assay was performed by the colorimetric method reported by Ellman G.

- Evaluate the ameliorating effects of the alkaloid extract of *Huperzia phlegmaria* on memory and cognitive dysfunction in scopolamine-treated mice through the Y maze test, the Morris water maze and the novel object recognition tests.

- Evaluate the Anti-aging effects of alkaloid extract of *Huperzia phlegmaria* on the D-galactose-induced brain ageing model.

4. Results and Conclusion

3.1. Botanical properties

- Scientific name of the sample which collected in Huong Son Commune, Huong Hoa District, Quang Trị Province was identified as *Huperzia phlegmaria* (L.) Rothm (Lycopodiaceae).

- Morphological, anatomical analysis of the arial part, leaves, stem and the whole plant powder characteristics of *Huperzia phlegmaria* were performed.

3.2. Chemical constituents

- Identified groups of compounds present in *Huperzia phlegmaria* (L.) Rothm including: Flavonoids, saponins, coumarins, tannins, alcaloids, steroids, carotenoids, reducing sugars, amino acids and polysaccharides.

- Structure of 15 compounds isolated from stem and leaves of *Huperzia phlegmaria* (L.) Rothm were identified as followings: 5 alkaloids (fawcettidin, huperphlegmin A, huperphlegmin B, phlegmariurin B, huperzin A), 3 diterpenoids (huperphlegmarin A, huperphlegmarin B, lycoxanthol), 4 triterpenoids (21β-hydroxyserrat-14-en-3β-yl acetat, 21α-hydroxyserrat-14-en-3β-yl acetat, 21α-hydroxyserrat-14-en-3β-ol, lycophlemariol A), and 3 furan derivatives (5-hydroxymethyl-2-furaldehyd, rehmanon C, loliolid). In which,

+ 4 new compounds including 2 alkaloids named huperphlegmine A and huperphlegmine B, 2 abietane diterpenoids named huperphlegmarine A and huperphlegmarine B.

+ 3 compounds were isolated from genus *Huperzia* Bernh. for the first time: phlegmariurine B, lycoxanthol and 21β-hydroxyserrat-14-en-3β-yl acetate.

+ 3 compounds were isolated from *Huperzia phlegmaria* (L.) Rothm for the first time: 5-hydroxymethyl-2-furaldehyde, rehmanone C and loliolide.

3.3. Toxicity and Biological activities

- ***Acute toxicity:***

+ The oral LD_{50} of the alkaloid extract of *Huperzia phlegmaria* in mice was identified as 1170 mg/kg (the 95% confidence interval of LD_{50} was 1148 mg/kg - 1192 mg / kg), equivalent to 219.38 g of dry medicinal material/kg body weight.

- ***Acetylcholinesterase inhibitory activity in vitro:***

+ Methanol and fractional extracts from *Huperzia phlegmaria* were tested for AChE inhibitor activity. The methanol, dichloromethane and residue extracts have weak AChE inhibitory effects with IC_{50} values from 49.81 ± 0.80 to 433.07 ± 7.16 µg/ml. The alkaloidal extract showed the most potent inhibitory activity against the AChE *in vitro* with an IC_{50} value of 1.54 ± 0.10 µg/mL.

+ The two new alkaloids, huperphlegmin A and huperphlegmin B, have AChE inhibitory activities with IC_{50} values of 65.50 ± 1.83 and 73.55 ± 1.94 µM, respectively. Huperzin A has strong *in vitro* AChE inhibitory activity with IC_{50} = 0.74 ± 0.04 µM, stronger than the positive control under the same test conditions.

- The ameliorating effects of the alkaloid extract of *Huperzia phlegmaria* on memory and cognitive dysfunction in scopolamine-treated mice through the behavioral experiments: The alkaloid extract of *Huperzia phlegmaria* improved working memory on the Y-maze

model at a dose of 150 mg/kg, improved long-term spatial memory in Morris water maze model at dose of 150 mg/kg, and improved the cognition on object recognition model at 100 mg/kg and 150 mg/kg.

- The *Huperzia phlegmaria* alkaloid extract at dose of 150 mg/kg significantly inhibited AChE activity in the hippocampus compared to the scopolamine-treated group.

- Anti-aging effects of alkaloid extract of *Huperzia phlegmaria* on the D-galactose-induced brain ageing model: the alkaloid extract of *Huperzia phlegmaria* at dose of 100 and 150 mg/kg increased the concentration of GSH-Px, increased the SOD level at dose of 150 mg/kg; all three doses of 50, 100 and 150 mg/kg reduced plasma MDA concentration in tested model.

Hanoi, March, 2021

ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

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