SUMMARY OF DISSERTATION

1. INTRODUCTION

Name of Ph.D. candidate: Hoang Thai Hoa

Dissertation title: Study on botanical characteristics, chemical constituents, and biological activities of *Physalis angulata* L., Solanaceae.

Specialty: Medicinal Materials - Traditional Pharmacy **Code number**: 9720206

Scientific supervisors:

1. Assoc. Prof. Dr. Tran Thi Oanh
2. Assoc. Prof. Dr. Nguyen Thuong Dong

Academic institution: National Institute of Medicinal Materials

2. SUMMARY

2.1. Objectives

+ Identification of the scientific name and morphological and microscopic characteristics of *Physalis angulata*.
+ Extraction, isolation, and structural determination of compounds from *P. angulata*.
+ Evaluation of the biological activities of extracts and isolated compounds from *P. angulata*.

2.2. Methods

2.2.1. Botanical study

+ Samples were taken from the whole plant, with all parts, made into a dried specimen according to the method recorded in botanical documents.
+ The plant parts were subjected to microsurgery using cross-cutting and double-staining methods. The resulting herbal powder was then examined, observed, and photographed under a microscope.

2.2.2. Phytochemical study

+ Chemical components were detected by applying a specific phytochemical screening test.
+ Medicinal herbs were extracted using the soaking method with 96% EtOH.
+ Compounds were isolated using column chromatography and thin layer chromatography (TLC), with fraction monitoring performed using TLC combined with UV irradiation at two wavelengths (254 and 365 nm).

+ The structures of the compounds were determined using spectroscopic methods, including mass spectroscopy (ESI-MS) and one-dimensional (^{1}H-NMR, ^{13}C-NMR, and DEPT) and two-dimensional nuclear magnetic resonance spectroscopy (HMBC and HMQC).

2.2.3. Biological evaluation

+ To determine the test concentration, samples were evaluated for their effect on the viability of RAW 264.7 and HepG2 cells using the MTT method.
+ The *in vitro* anti-inflammatory effects of extracts and withanolides isolated from *P. angulata* were evaluated by measuring their ability to inhibit PGE2, NO, and IL-1β production and reduce NF-B activity in LPS-stimulated RAW 264.7 macrophage cells using ELISA techniques.
+ The acute anti-inflammatory effect of the 96% EtOH extract from *P. angulata* on a carrageenan-induced paw edema model was evaluated using the Winter - Levy method.
+ The chronic anti-inflammatory effect of the 96% EtOH extract from *P. angulata* was evaluated in the cotton pellet-induced granuloma model of Vogel H. G. et al., 2008.
+ The analgesic effect of the 96% EtOH extract from *P. angulata* on the acetic acid-induced colitis rat model of Koster et al. was evaluated.
+ The activation effects of AMPK, ACC, FAS, and SREBP-1c in HepG2 cells were evaluated using the Western Blot method.
+ The ability of withanolides isolated from *P. angulata* to inhibit lipid accumulation on HepG2 cells was evaluated using the Nile Red test.
+ The cytotoxic effect of extracts on some cancer cell lines *in vitro* was evaluated according to the method of Skehan et al.
+ The cytotoxic effects of withanolides isolated from *P. angulata* on some cancer cell lines *in vitro* were evaluated using the MTT method.

2.3. Results and Conclusion

2.3.1. Botanical properties

− The sample collected in Gia Lam district, Hanoi city was identified as *Physalis angulata* L., Solanaceae.

- The study reported the morphological and anatomical analysis of the stem and leaf of *P. angulata*, as well as the microscopic characteristics of their powders.

2.3.2. Chemical constituents

- The presence of almost all groups of organic substances (flavonoids, carotenes, alkaloids, saponins, coumarins, tannins, organic acids, reducing sugars, amino acids, fats, and polysaccharides) in *P. angulata* has been confirmed.
- We isolated and determined the structures of 15 compounds from *P. angulata*, including 3 phenolics (caffeic acid **PA1**, ferulic acid **PA2**, and 3-*O*-caffeoylquinic acid **PA3**), 5 flavonoids (quercetin **PA4**, quercitrin **PA5**, quercetin 3-*O*-*β*-D-glucopyranoside **PA6**, myricetin 3-*O*-*α*-L-rhamnopyranoside **PA7**, and rutin **PA8**), 2 sterols (stigmasterol **PA9** and daucosterol **PA10**), 4 withanolides (physalindicanol A **PA11**, physalindicanol B **PA12**, physalin B **PA13**, and physalin D **PA14**), and 1 triterpene (oleanolic acid **PA15**). Among them, compounds **PA7** and **PA12** were first reported from *P. angulata*.

2.3.2. Biological activities

❖ **Anti-inflammatory and analgesic activity**

- Among the tested extracts and isolated compounds, the EtOAc extract (**TBE**, 20 μg/mL) and physalindicanol A (10 μM) isolated from *P. angulata* showed the most effective inhibition of PGE2, NO, and IL-1β production and decreased NF-κB activity in LPS-stimulated RAW 264.7 macrophages.
- The 96% EtOH extract from *P. angulata* at a dose of 0.9 g/kg significantly reduced paw edema in white rats induced with inflammation by carrageenan at 4 and 6 hours, with edema rates of only 27.04 ± 2.52% and 25.11 ± 2.17%, respectively, compared to the control group ($p < 0.05$).
- The 96% EtOH extract from *P. angulata* at doses of 0.6 and 1.8 g/kg reduced the number of painful cramps compared to the control group from the first 5 minutes to the 30th minute.

❖ **On fatty acid and glucose metabolism**

- Compared to the tested samples, the EtOAc extract (50 μg/mL) showed the highest increase in the expression of p-ACC and p-AMPK.

- Physalin D (10 μM) had the strongest effect on the expression of p-AMPK, followed by physalindicanol A and physalindicanol B.
- Under the same conditions of high glucose concentration (30 mM), both physalindicanol B and physalin D (10 μM) exhibited strong concentration-dependent inhibition of FAS and SREBP-1c gene expression.
- At 3 dose levels (1, 3, and 10 μM), both physalindicanol B and physalin D inhibited lipid accumulation in a concentration-dependent manner compared to the batch incubated with only a high concentration of glucose.

❖ **Toxic effects on some cancer cell lines**

- The 96% EtOH extract and its *n*-hexane and EtOAc fractions showed cytotoxic activity against various cancer cell lines (4T1, SNU-1, Hep3B, NTERA-2, LLC, and HEK-293A) with IC_{50} values ranging from 3.81 to 13.44 μg/mL.
- Physalindicanol A, physalindicanol B, physalin B, and physalin D exhibited cytotoxic effects on 4T1 and SNU-1 cell lines with IC_{50} values ranging from 1.10 to 3.61 μM. Additionally, physalin B also demonstrated cytotoxic effects on NTERA-2 and LLC cell lines, with IC_{50} values of 4.44 ± 0.40 and 4.59 ± 0.77 μM, respectively.

Hanoi, May 3rd, 2023

THE SCIENTIFIC SUPERVISORS **Ph.D. CANDIDATE**

Assoc. Prof. Dr. Tran Thi Oanh **Hoang Thai Hoa**

Assoc. Prof. Dr. Nguyen Thuong Dong