SUMMARY OF THE DOCTORAL DISSERTATION

1. INTRODUCTION

Name of Ph.D. candidate: Tran Thi Thu Hien

Dissertation title: Study on chemical composition and evaluation of the anti-cancer effect of stems and leaves of *Stephania dielsiana* Y. C. Wu.

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

Scientific supervisors:

1. Dr. Le Thi Kim Van
2. Assoc. Prof. Dr. Nguyen Quoc Huy

Academic institution: National Institute of Medicinal Materials

2. SUMMARY

2.1. Objectives

- Extraction, isolation, and structural determination of some chemical components from the stems and leaves of *Stephania dielsiana* Y. C. Wu.
- Initial development of a method for isolation oxostephanin from stems and leaves of *Stephania dielsiana* Y. C. Wu. Then development a quantitative method to monitor the oxostephanine content in the medicinal herbs according to the time of collection.
- Evaluation of the cytotoxic effects of some isolated compounds and initial study of the anti-cancer mechanism of oxostephanine.

2.2. Methods

2.2.1. Phytochemical study

+ Extraction of medicinal herbs by soaking with methanol solvent.
+ Isolation of compounds by column chromatography with different stationary phases (silica gel, RP-C_{18}, Sephadex LH-20, and Diaion HP-20) and different elution solvent systems, or crystallization methods in a suitable solvent; fraction monitoring by TLC combined with UV irradiation at two wavelengths 254 and 365 nm or using reagents (Dragendorff, 10% H_2SO_4 solution in 96% EtOH); testing the purity of compounds by TLC and NMR.

+ Structure determination of compounds based on spectroscopic methods: infrared (IR), ultraviolet (UV), mass spectroscopy (ESI-MS and HR-ESI-MS), and one-dimensional (^{1}H-NMR, ^{13}C-NMR, and DEPT) and two-dimensional nuclear magnetic resonance spectroscopy (COSY, HMBC, HMQC, and ROESY).
+ Isolation of oxostephanine, development and verification of quantitative methods, and monitoring of changes in oxostephanine content in medicinal herbs according to the time of collection by high-performance liquid chromatography (HPLC).

2.2.3. Biological evaluation

+ Evaluation of the cytotoxic effect on some experimental cancer cell lines of some isolated compounds by morphological comparison method and MTS test.
+ Study on the mechanism of OVCAR-8 ovarian cancer cytotoxicity of oxostephanine by Real-time cell analysis, immunofluorescence, apoptosis, multicellular tumor analysis, and RT-PCR.
+ Study the effect of oxostephanine on normal cell lines including hUVECs, UC-MSCs, and hFBs by SRB staining, colony formation, growth factor secretion analysis by Luminex, wound healing, and angiogenesis assays.

2.3. Results and Conclusion

2.3.1. Chemical Investigation Results

– Extracted, isolated, and determined the chemical structure of 11 compounds from the stems and leaves of *Stephania dielsiana* Y.C. Wu, including:
  + 08 alkaloids, including 2 new compounds (**stedieltin A and stedieltin B**); aristolactam (**SD6**) was isolated the first time from the genus *Stephania* Lour.; Oxostephanosin (**SD4**) was isolated for the first time from *S. dielsiana* Y.C. Wu 01 alkaloid compound was isolated for the first time from the stems and leaves of this plant (isolated from the tuber, oxocrebanin - **SD5**) and three other alkaloids [oxostephanin (**SD3**), crebanin (**SD7**), and dehydrocrebanin (**SD8**)].

+ 03 non-alkaloid compounds including 4-hydroxybenzaldehyde (**SD9**); benzyl *β*-D-glucopyranoside (**SD10**), and (6*R*,9*S*)-roseoside (**SD11**) were isolated for the first time from *S. dielsiana* Y.C. Wu.

- Developed a method and isolated 4.0 g of oxostephanine (purity 98.5% according to the peak area on HPLC) from 5 kg of the stems and leaves of *S. dielsiana* Y.C. Wu, used as a comparator and as a material for further studies.
- Developed and validated a method for the quantification of oxostephanine in the stems and leaves of *S. dielsiana* Y.C. Wu, which meets the criteria of AOAC and ASEAN Guidelines on the validation of analytical procedures.
- The change of oxostephanine content in the stems and tubers of *S. dielsiana* Y.C. Wu was evaluated according to the time of collection in the range of 0.337 - 0.873%, in which the time of collection for the highest concentration of active ingredients was September and October.

2.3.2. Biological Investigation

- The cytotoxic effects of compounds **SD1 - SD5** were evaluated on HeLa, HepG2, MCF7, N87, and OVCAR-8 cancer cell lines by the MTS staining method. The results showed that compound **SD3** (oxostephanine) had a strong inhibitory effect on HepG2, MCF7, and OVCAR-8 cancer cell lines with IC_{50} in the range of 3.1 - 3.4 μM; compounds **SD4** (oxostephanosine) and **SD5** (oxocrebanin) exhibited moderate to weak effects; compounds **SD1** (stedieltin A) and **SD2** (stedieltin B) did not have cytotoxic effects on all five tested cell lines.
- The mechanism of cytotoxic activity of oxostephanine has been studied. It was an Aurora kinase inhibitor through blocking histone H3 phosphorylation on serin 10, Aurora B mislocalization, and aneuploidy induction. Furthermore, it was selectively cytotoxic to human umbilical vein endothelial cells (hUVECs), while being less cytotoxic to human fibroblasts and umbilical cord-derived mesenchymal stem cell lines. In addition, oxostephanine significantly reduced the migration and angiogenesis

of hUVECs. Oxostephanine plays a dual role in inhibiting Aurora kinase activity and angiogenesis. Therefore, it has the potential used as a drug in cancer treatment.

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

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