# SUMMARY OF DISSERTATION

**Name of Doctoral candidate**: Le Quoc Hung

**Dissertation title**: Study on chemical constituents and antiproliferative activity against cancer cell lines of the roots of *Salvia miltiorrhiza* Bunge (Lamiaceae)

**Speciality**: Traditional Pharmacy **Code of speciality**: 9720206

**Name of academic advisors:**

1. Assoc. Prof. Dr. Phuong Thien Thuong
2. Assoc. Prof. Dr. Nguyen Huu Tung

**Name of academic institute**: Vietnam National Institute of Medicinal Materials

**Summary of the dissertation:**

## 1. Objectives

- Isolation and structural elucidation of constituents and determination of main tanshinones content in roots of *S. miltiorrhiza*.

- Evaluation of *in vitro* anticancer activity of extracts and isolated compounds from the roots of *S. miltiorrhiza*.

## 2. Methods

### *2.1. Scientific name identification*

Identification of the scientific name of the plant samples on the basis of the morphological characteristics comparison with key taxonomy of species, varieties of the genus *Salvia* (family Lamiaceae) in taxonomic reference books and the standard specimens. Scientific name of the plant samples was expertised by Vietnamese taxonomic botanists.

### *2.2. Phytochemical study*

- *Extraction and isolation of chemical constituents*:

+ Extraction of plant materials using ethanol, and subsequently successive partitioning of the extract using increasing polarization solvents (*n*-hexan, ethyl acetate, *n*-butanol).

+ Isolation and purification of compounds by column chromatographic method using silica gel, reverse-phase RP-$C_{18}$ as adsorbents.

- *Structural elucidation of isolated compounds*: On the basis of the analyses of physical properties (morphology, melting point), spectroscopic data (MS, NMR), and comparison with the literature data.

- Simultaneous quantification of main tanshinones in roots of *S. miltiorrhiza*: by High performance liquid chromatography (HPLC).

### *2.3. Biological study*

* Research samples: ethanol extract, and fraction extracts of *S. miltiorrhiza* roots.

* Determination of antiproliferative activity by MTT assay.

* The mechanisms of apoptosis

- Cell nuclear morphology analysis by using Hoechst 33258 staining

- DNA fragmentation analysis as indicated by DNA laddering detected using agarose gel electrophoresis.

- Western blot analysis.

- Analysis of the mitochondrial membrane function.

## 3. Results and Conclusion

### *3.1. Scientific name identification*

- The plant samples were identified as *Salvia miltiorrhiza* Bunge (Lamiaceae).

### *3.2. Chemical constituents*

- Seventeen compounds were isolated from roots of *S. miltiorrhiza* and identified as dihydrotanshinone I (**1**), trijuganone C (**2**), trijuganone B (**3**), cryptotanshinone (**4**), tanshinone IIA (**5**), tanshinone I (**6**), 7$\beta$,24-dihydroxy ursolic acid (**7**), 24-hydroxy corosolic acid (**8**), ursolic acid (**9**), oleanolic acid (**10**), maslinic acid (**11**), asiatic acid (**12**), iriflophenone-2-*O*-$\alpha$-L-rhamnopyranoside (**13**), rosmarinic acid (**14**), methyl rosmarinate (**15**), ethyl rosmarinate (**16**) và ethyl salvianolate A (**17**). Among the isolates, **7**, **8** and **13** have been found in genus *Salvia* for the first time. Four compounds (**11**, **12**, **16** and **17**) have been firstly isolated from species *S. miltiorrhiza*.

- A method was developed for the simultaneous quantification of main tanshinones including tanshinone I (**6**), tanshinone IIA (**5**) và cryptotanshinone (**4**) in roots of *S. miltiorrhiza*. total content of 03 Tan is Sapa 3,695 mg/g, respectively; Ha Giang 4,607 mg/g, Lai Chau 3,490 mg/g; Lam Dong 3,402 mg/g, China 2,052 mg/g..

### *3.3. Biological activities*

- Ethanol and *n*-hexan extracts showed antiproliferative effects on human leukemia cells HL-60.

- Tanshinones (**2-6**) inhibited the growth of three human leukemia cells HL-60, Jurkat, and U937. Among them, compound **2** exhibited the most potent antiproliferative activity with $IC_{50}$ values of 6.1; 8.9 and 13.4 $\mu$M, respectively.

- Compounds **7**, **9**, **10**, **13**, **15-17** significantly suppressed the growth of human leukemia cells HL-60 with $IC_{50}$ values ranging from 8.9-26.8 $\mu$M. Compound **13** showed the strongest inhibitory effect with $IC_{50}$ value of 8.9 $\mu$M.

- Compound **2** exhibited potent antiproliferative activity with $IC_{50}$ values less than 10 $\mu$M against the colon cancer cells DLD-1 ($IC_{50}$ 6.1 $\mu$M), COLO 205 ($IC_{50}$ 7.2 $\mu$M), and Caco-2 ($IC_{50}$ 8.4 $\mu$M). In addition, **2** significantly inhibited cell growth of colon cancer HCT-15 ($IC_{50}$ 13.2 $\mu$M), prostate cancer PC-3 ($IC_{50}$ 11.2 $\mu$M), LNCap FGC ($IC_{50}$ 13.7 $\mu$M), breast cancer MCF-7 ($IC_{50}$ 16.7 $\mu$M). Compound **2** had no effect on the proliferation of two normal cell lines WRL 68 and NB1RGB.
- Compound **2** exerted antiproliferative effects on HL-60 cells *via* apoptosis induction mediated by mitochondrial dysfunction and caspase activation.

*Hanoi, July th, 2022*

| **ACADEMIC ADVISORS** | **DOCTORAL CANDIDATE** |
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