SUMMARY OF DISSERTATION

1. INTRODUCTION

Name of Ph.D. candidate: Nguyen Van Lieu

Dissertation title: Study on the chemical constituents and *in vitro* immunomodulatory and anti-inflammatory effects of the twigs and leaves of *Wikstroemia indica* (L.) C.A.Mey.

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

Scientific supervisors:

1. Assoc. Prof. Dr. Do Thi Ha
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Academic institution: National Institute of Medicinal Materials

2. SUMMARY

2.1. Objectives

+ To extract, isolate, and elucidate the chemical structures of compounds from the stems and leaves of *Wikstroemia indica* (L.) C.A.Mey. via bioactivity-guided isolation.

+ To screen and evaluate the *in vitro* activity of isolated compounds on inflammation- and immunity-related cytokines.

2.2. Methods

2.2.1. Phytochemical study

+ Extraction: The total crude extract was prepared by reflux extraction with 70% ethanol, followed by liquid–liquid partition to yield sub-fractions.

+ **Isolation:** Compounds were isolated using open column chromatography (CC) monitored by thin-layer chromatography (TLC). TLC chromatograms were visualized using 10% H_2SO_4/EtOH reagent and inspected under UV light at 254 nm and 366 nm. Purification was performed using semi-preparative high-performance liquid chromatography (semi-preparative HPLC) combined with recrystallization from suitable solvent systems.

+ Structure Elucidation: Structural determination of isolated compounds was performed using spectroscopic methods, including mass spectrometry (ESI-MS, HR-ESI-MS/MS), 1D-NMR (^{1}H-NMR, ^{13}C-NMR, ^{13}C-APT NMR), 2D-NMR (HMBC, HSQC, COSY, etc.), and experimental/calculated electronic circular dichroism (ECD) spectra in comparison with literature data.

+ Theoretical ECD Calculation: Theoretical ECD spectra were calculated based on conformers for each potential stereoisomer identified using MAESTRO software.

+ Absolute Configuration of Sugar Moieties: The absolute configurations of sugar units were determined according to Tanaka's method: following acid hydrolysis and

derivatization, sugar moieties were analyzed by HPLC and compared against standard reference sugars.

2.2.2. Evaluation of activity on inflammation- and immunity-related cytokines

+ **Cytotoxicity Assay**: The effects of extracts and isolated compounds on RAW 264.7 and CD4+ T cells were assessed using the MTT assay.

Screening for cytotoxicity against cancer cell lines *in vitro* using the MTT and SRB methods.

+ **Cytokine Quantification**: Screening and activity evaluation on inflammation- and immunity-related cytokines were based on cytokine concentrations measured by ELISA according to the manufacturers' instructions.

+ Statistical Analysis: Data with normal distribution are presented as mean ± SEM and analyzed using GraphPad Prism® 9.5.0 software. Student's *t*-test was applied to compare two independent groups; one-way analysis of variance (one-way ANOVA) followed by Dunnett's post-hoc test was used to compare treatment groups with the control group. Differences were considered statistically significant at $p < 0.05$.

2.3. Results and Conclusion

2.3.1. Chemical constituents

A total of 19 compounds were isolated and structurally elucidated from the ethyl acetate (**WIE**) and aqueous (**WIW**) fractions of the stems and leaves of *Wikstroemia indica*, including:

+ 05 phenolic derivatives: Methyl 4-hydroxybenzoate (WI1), syringin (**WI2**), genkwanin (WI9), kaempferol-3-*O*-rhamnoside (**WI10**), and yuankanin (**WI11**). Among these, kaempferol-3-*O*-rhamnoside was isolated for the first time from the genus *Wikstroemia*.

+ 06 coumarins: Umbelliferone (**WI3**), adicardin (**WI4**), daphnoretin (**WI5**), daphnorin (**WI6**), wikstronutin (**WI7**), and triumbelletin-7-*O*-*β*-D-glucoside (**WI8**). Among these, adicardin was isolated for the first time from the genus *Wikstroemia*, and wikstronutin was isolated for the first time from *W. indica*.

+ 08 lignans:

- 04 compounds isolated for the first time from *W. indica*: (7*S**,8*R**)-5-methoxydehydrodiconiferyl alcohol 4-*O*-*β*-D-glucopyranoside (**WI17**), pinoresinol di-*O*-*β*-D-glucopyranoside (**WI18**) and liriodendrin (**WI19**)

- 02 compounds isolated for the first time from the genus *Wikstroemia*: *erythro*-syringylglycerol-8-*O*-4'-sinapyl ether 4-*O*-*β*-D-glucopyranoside (**WI12**) and *erythro*-citrusin B (**WI13**).
- 02 new lignans: *threo*-citrusin B (**WI14**) and isonitidanin-4-*O*-*β*-D-glucoside (**WI15**).

2.3.2. In vitro activity on inflammation and immunity-related cytokines

+ The fractions exhibiting potent activities on both IL-1β and IL-10 pathways were **WIE** and **WIW**.

+ The compounds demonstrating significant activities on both IL-1β and IL-10 pathways included: umbelliferon (**WI3**), daphnoretin (**WI5**), wikstronutin (**WI7**), triumbelletin-7-O-β-D-glucoside (**WI8**), genkwanin (**WI9**) and liriodendrin (**WI19**).

+ The compounds showing superior inhibitory activity on IL-1β production in RAW 264.7 cell lines compared to other tested compounds were umbelliferon (**WI3**) with an IC_{50} value of 6.4 ± 1.23 μM and liriodendrin (**WI19**) with an of IC_{50} value of 9.73 ± 1.26 μM.

+ The compound demonstrating superior inhibitory activity on IFN-γ and IL-17 production in CD4+ T cells compared to others was umbelliferon (**WI3**), with IC_{50} values of 9.28 ± 1.23 μM and 12.62 ± 2.57 μM, respectively.

+ The compound demonstrating superior inhibitory activity on Foxp3 production in CD4+ T cells compared to others was liriodendrin (**WI19**), with an IC_{50} value of 13.67 ± 1.85 μM.

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

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