SUMMARY OF DISSERTATION

Name of Doctoral candidate: Nguyen Thi Hang

Dissertation title: "Study on botanical characteristics, chemical constituents and *in vitro* anti-inflammatory activities of *Gouania leptostachya* DC. (Rhamnaceae)"

Speciality: Medicinal Materials - Traditional Pharmacy

Code of specciality: 9720206

Name of academic advisors:

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2. Assoc. Prof. Dr. Tran Van On

Name of academic institute: National Institute of Medicinal Materials

Summary of the dissertation

1. Objectives

- Identification of the morphological and microscopic characteristics of *Gouania leptostachya.*

- Extraction, isolation, and structural determination of compounds from *G. leptostachya*.

- Evaluation of the *in vitro* anti-inflammatory activities of extracts and isolated compounds from *G. leptostachya* in LPS-stimulated RAW 264.7 macrophage cells.

2. Methods

2.1. Botanical study

- Samples were taken 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. Chemical 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, HR-EI-MS) and one-dimensional (1D-NMR) and

two-dimensional nuclear magnetic resonance spectroscopy (2D-NMR). Determination of sugar configuration by hydrolysis method.

2.3. Biological study:

- The *in vitro* anti-inflammatory effects were evaluated base on the inhibition of PGE_2, NO, IL-1β, IL-6 production, mRNA and protein COX-2 expression, and COX-2 luciferase activity in LPS-stimulated RAW 264.7 macrophage cells.

3. Results and Conclusion

3.1. Botanical properties

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

3.1. Chemical constituents:

- The presence of almost all groups of organic substances (flavonoids, carotenes, saponins, reducing sugars, sterol) in *G. leptostachya* has been confirmed.

- Structure of 15 compounds isolated from *Gouania leptostachya* were identified, in which:

- ✓ 5 new saponin triterpenoides named gouaniaside VII-IX, joazeiroside C, and gouanioside A.
- ✓ 5 compounds were isolated from genus *Gouania* for the first time: 4 flavonoids (quercitrin, isoquercitrin, catechin, and kaempferol-3-*O*-[(6-*O*-*E*-caffeoyl)-*β*-D-galactopyranosyl]-(1→2)-*α*-L-rhamnopyranoside), and 1 fructoside (*n*-butyl-*β*-D-fructopyranoside).
- ✓ 3 compounds were isolated from *Gouania leptostachya* for the first time: lupeol, alphitolic acid, and epigouanic acid.
- ✓ 2 known compounds: acid ceanothenic and daucosterol.

3.2. Biological activities:

- ***Anti-imflammatory activities of G. leptostachya extracts:***

- The EtOAc extract (**GLE)** at 20 µg/mL showed the most effective inhibition of PGE_2, IL-1β and IL-6 production in LPS-stimulated RAW 264.7 macrophages 49.2 ± 5.6 %, 55.2 ± 4.0 %, and 38.8 ± 5.5 % ($p < 0.01$), respectively; decreased expression of mARN COX-2, protein COX-2, and COX-2 luciferase activity 41.5 ± 5.6 %, 44.4 ± 7.4 % và 25.9 ± 1.7 % ($p < 0.01$), respectively. **GLE** at 1, 5 và 25 µg/mL inhibited NO production in LPS-stimulated RAW 264.7 macrophages ($p <0,05$ và $p < 0,001$).

- The 96% EtOH extract (**GLT)** inhibited PGE_2 production (at 20 µg/mL) and NO production (at 25 µg/mL).

- The n-butanol extract (**GLB)** inhibited NO production (at 25 µg/mL) and IL-1*β* production (at 20 µg/mL).

- The water extract (**GLW)s** inhibited NO production (at 25 µg/mL); IL-1β and IL-6 production (at 20 µg/mL).

➢ ***Anti-imflammatory activities of isolated compounds:***

- **Alphitolic acid** (**GL7**) at 3, 10, and 30 µM showed effective inhibition of PGE_2 production, decreased mARN COX-2 and protein COX-2 expression concentration dependent, the inhibition rate at the highest concentration were 64.5 ± 3.3 %; 58.3 ± 4.9 %, and 47.5 ± 4.4 % ($p < 0.001$), respectively. **GL7** (10 µM) decreased COX-2 luciferase activity 55.4 ± 3.5 % ($p < 0.001$) and inhibited IL-1β, IL-6 production 45.1 ± 3.4 % and 39.8 ± 3.6 % ($p < 0.001$), respectively; inhibited of NO production concentration dependent at 2, 10, and 50 µM.

- **Epigouanic acid** (**GL9**) at 10 µM inhibited PGE_2, IL-1β, and IL-6 production 52.8 ± 7.3 %, 61.5 ± 3.2 % và 57.1 ± 3.3 % ($p < 0.001$), respectively; decreased mARN COX-2 expression, COX-2 luciferase activity 54.4 ± 2.1 % and 42.1 ± 5.7 % ($p < 0.001$), respectively.

- **Gouaniosid A** (**GL5**) at 10 µM inhibited PGE_2 production, decreased mARN COX-2 expression, COX-2 luciferase activity 52.3 ± 6.0 %, 48.7 ± 5.7 %, and 41.0 ± 4.6 % ($p < 0.001$), respectively; inhibited NO production at 5 and 25 µM.

- **Ceanothenic acid** (**GL8**) and **lupeol** (**GL6**) inhibited IL-1β, IL-6 production (at 10 µM). Besides, **lupeol** inhibited NO production (at 25 µM).

- **Gouaniasid VII** (**GL1**) inhibited PGE_2, IL-1β, and IL-6 production (at 10 µM), and NO production (at 5 and 25 µM).

- **Gouaniasid VIII** (**GL2**) inhibited NO production at 5 and 25 µM.

- **Gouaniasid IX** (**GL3**) inhibited NO production (at 5 and 25 µM) and IL-6 production (at 10 µM).

Hanoi, July 05th, 2023

ACADEMIC ADVISORS **DOCTORAL CANDIDATE**

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