SUMMARY OF DISSERTATION

Name of Doctoral candidate: Nguyen Thi Hong Anh

Dissertation title: "Study on botanical properties, chemical constituents and anti-imflammatory activity of *Balanophora laxiflora* Hemsl."

Speciality: Medicinal Materials - Traditional Pharmacy

Code of specciality: 9720206

Name of academic advisors:

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Name of academic institute: National Institute of Medicinal Materials

Summary of the dissertation

1. Objectives

- Identification of the scientific name, identification of main morphological characteristics and microscopic identification of *Balanophora laxiflora*
- To isolate pure compounds from the extract and identify their chemical structure.
- To evaluate anti-imflammatory effect of ethanol, fractional extracts and isolated compounds from *Balanophora laxiflora*

2. Methods

2.1. Botanical study

- *Scientific name identification:* Morphological characteristics were in comparison with the standard specimens of *Balanophora laxiflora* Hemsl.
- *Anatomical and Microscopic study:* Determination of microscopic characteristics of the arial part, leaves, stem and the whole plant powder characteristics by using microscopic method.

2.2. Chemical study

- *Qualitative analysis:* Determination of major chemical groups in *Balanophora laxiflora* by using specific chemical reactions
- *Extraction and isolation of chemical constituents:*

+ Chemical contents have been extracted with ethanol 80% at 70^{o}C under reflux and methanol under ultrasonic.
+ Fraction and Isolation methods have been open column chromatography method, using station phases silica gel (0,04 - 0,063 mm, Merck), YMC RP-18 (30-50 μm, Fuji Silysia Chemical Ltd.), Sephadex LH20 and preparative HPLC method. Determination the

similar fractions has used TLC, which have been stained by 10% H_2SO_4 in 96% ethanol, then heated and see bands under UV light at 254 nm and 366 nm.

- *Structural elucidation of isolated compounds:* Chemical structures were identified base on their physical properties (melting points, rotary polarization) and spectroscopy analysis: ESI-MS, HR-EI-MS 1D-NMR, 2D-NMR, CD, and comparison with the published data. Determination of sugar configuration by hydrolysis method, absolute configuration by Mosher method and theoretical CD spectrum calculation method.

2.3. Biological study

- The effect of samples on the survival ability of RAW264.7 by MTS assay to determine the concentration test.
- Inflammatory activities have been tested and analyzed on target molecules such as COX-2 and IL-1β, IL-6, INF-β, iNOS, TNFα on RAW264.7 which was induced by LPS, using Western blot, ELISA, and RT-PCR. The relative AP-1 promoter activity was calculated based on ratio of Firefly to Renilla luciferase activity. NOXs activity was examined by measurement of the luminescence emitted upon the cleavage of luminogenic substrate lucigenin, cellular reactive oxygen species (ROS) measurement acquired using BD FACSCalibur™ (BD Biosciences, San Jose, CA, USA).
- Acute inflammation has been evaluated by the *carrageenan-induced paw edema* model as the Winter method
- Chronic inflammation has been examined by aminant granule ulcer-inducing, referencing on amiant granule ulcer-induced inflammation model, followed by Meier et.al., (1950).
- Determination of radical scavenging activities against DPPH and superoxide radicals.

3. Results and Conclusion

3.1. Botanical properties

- Scientific name of the sample which collected in Sa Pa District, Lao Cai Province was identified as *Balanophora laxiflora* Hemsl. (Balanophoraceae).
- Morphological, anatomical analysis of the arial part, leaves, stem and the whole plant powder characteristics of *Balanophora laxiflora* were performed.

3.2. Chemical constituents

- Identified groups of compounds present in *Balanophora laxiflora* Hemsl. including: Flavonoid, coumarin, tannin, acid hữu cơ, acid amin, sterol and fatty acid.

Structure of 27 compounds isolated from *Balanophora laxiflora* Hemsl. were identified, in which,

- 5 new compounds including 3 lignans named ((*8S,8′S*)-secoisolariciresinol-9′-*O*-*β*-D-glucopyranosid, balanophorosid B, balanophoron), a phenyl propanoid (balanophoroside A) and an iridoid (balanolaxin)

- 10 compounds were isolated from genus *Balanophora* for the first time: salicifoliol, *(8S,7′R,8′S)*-isolariciresinol 9-*O*-*β*-D-glucopyranosid, *Trans*–*p*–coumaryl aldehyd, 6-*O*-galloyl-1-*O*-*E*-caffeoyl-*β*-D-glucopyranose, deacetyl asperulosidic acid, (21*β*)-22-hydroxyhopan-3-on, (21*α*)-22-hydroxyhopan-3-on, *p*-hydroxybenzaldehyd, piceol (p-hydroxy acetophenon) and 1-*O*-(3-methylbutyl)-6-*O*-*β*-D-xylopyranosyl- (1→6)-*β*-D-glucopyranose

- 4 compounds were isolated from *Balanophora laxiflora* Hemsl. for the first time: (*8R,8′R*)-secoisolariciresinol-4-*O*-*β*-D-glucopyranosid, *(8R,7′S,8′R)*-lariciresinol-4′-*O*-*β*-D-glucopyranosid, coniferyl aldehyd *β*-D-glucopyranosid and 4-*O*-galloyl-1-*O*-*E*-caffeoyl-*β*-D-glucopyranose.

- And 9 known compounds: *(8S,7′R,8′S)*-isolariciresinol 4-*O*-*β*-D-glucopyranosid, coniferin, ferulic aldehyd, ethyl caffeat, 1-*O*-*E*-caffeoyl-*β*-D-glucopyranose, lupeol, *β*-amyrin, 5-(hydroxymethyl)-2-furaldehyd.

3.3. Biological activities

- ***Anti-imflammatory activity in vitro:***

+ Ethyl acetate extract (30 µg/ml), (21α)-22-hydroxyhopan-3-one (10 µM) and piceol (10 µM) shown inhibitory effect on COX-2 expression in LPS-stimulated Raw 264.7 macrophages. Furthermore, **BL23** suppressed the expression of the inflammatory mediators iNOS, IL-1β, INFβ, and TNFα in activated Raw 264.7 macrophages.

+ Mechanistically, the anti-inflammatory effects of **BL23** were mediated *via* decreasing cellular reactive oxygen species (ROS) levels by inhibiting NADPH oxidases and free radical scavenging activities. By downregulating ROS signalling, **BL23** reduced the activation of MAPK signalling pathways, leading to decreased AP-1-dependent transcription of inflammatory mediators.

- Antioxidation activity in vitro:

+ Ethanol extract exhibited DPPH, superoxide radical scavenging activity with IC_{50} values of 36.41 và 27.44 µg/ml, respectively.

+ Ethyl acetate extract exhibited DPPH, superoxide radical scavenging activity with IC_{50} values of 19.12 và 5.69 µg/ml, respectively.

+ Compound **BL23** exhibited DPPH, superoxide radical scavenging activity with IC_{50} values of 4.48 và 0.53 µg/ml, respectively.

- ***Anti-imflammatory activity in vivo:***

+ Ethanol, ethyl acetate extract at dose of 150 mg/kg and 300 mg/kg; compound **BL23** at dose of 40 mg/kg and 80 mg/kg displayed acute anti-inflammatory activity on the *carrageenan-induced paw edema* model.

+ Ethanol, ethyl acetate extract at dose of 150 mg/kg and 300 mg/kg; compound **BL23** at dose of 40 mg/kg and 80 mg/kg displayed chronic anti-inflammatory activity on the *Cotton pellet–induced chronic inflammation* model.

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