SUMMARY OF DISSERTATION

1. INTRODUCTION

Name of Ph.D. candidate: Bui Thi Binh

Dissertation title: Study on chemical components and several biological activities of *Belamcanda chinensis* (L.) DC. collected in Vietnam.

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

Scientific supervisors:

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Academic institution: Vietnam National Institute of Medicinal Materials

2. SUMMARY

2.1. Objectives

- Isolation and structural determination of several chemical contents from *Belamcanda chinensis*
- Study on several biological activities of extract and isolated compounds from *Belamcanda chinensis*.

2.2. Methods

2.2.1. Phytochemical study

- *Quanlification methods:* Chemical group contents in *Belamcanda chinensis* has been qualified by specific chemical reactions.
- *Extraction, Isolation and Structural Determination Methods:*

+ Chemical contents have been extracted with methanol or ethanol 70% at 70^{o}C under reflux.
+ 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, MCI gel (CHP20P, 75 - 150 μm), 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.

+ The structures of isolated compounds have been determined by analysis and comparison of physical and chemical characteristics (melting point, αD) and spectrums (UV-VIS, IR, ESI-MS or HR-ESI-MS, 1D- and 2D-NMR (^{1}H-NMR, ^{13}C-NMR and DEPT) and (COSY, HMBC, HMQC and NOESY).
+ Establishing the quantification method of several main compounds, isolated from *Belamcanda chinensis* by HPLC.

2.2.3. Biological evaluation

- The effect of samples on survival ability of RAW264.7 by MTT assay to determine the concentration test.
- Inflammatory activities have been tested and analyzed on target molecules as COX-2 and PGE2 on RAW264.7 which was induced by LPS, using Western blot, ELISA and RT-PCR.
- Acute inflammation has been evaluated by the carrageenan-induced paw edema model as Winter method.
- Chronic inflammation has been examined by aminant granule ulcer inducing, refrerancing on amiant granule ulcer-induced inflammation model, followed Meier and et.al., (1950).
- Scavering anti-proliferation of cell by cymetry-flow method (11465007001, Sigma).

2.3. Results and Conclusion

2.3.1. Chemical Investigation Results

- *Belamcanda chinensis* has been discovered that it contained 5 main component groups such flavonoid, organic acid, acid amin, polysaccarid và sugar.
- 20 compounds have been isolated from *Belamcanda chinensis* (11 compounds from rhizoma (**BC1 - BC11**) and 9 compounds from air parts (**BC12 - BC20**), among these 4 new compounds (**BC12**, **BC13**, **BC18**, **BC19**) and six compounds which are the first time isolating from *Belamcanda chinensis* (**BC6**, **BC8**, **BC11**, **BC14**, **BC15** and

BC17). Which are iristectorigenin A (**BC1**), acetovanillon (**BC2**), irisflorentin (**BC3**), irilin D (**BC4**), tectorigenin (**BC5**), (7*R*,8*S*)-dehydrodiconiferyl alcohol-γ′-methyl ether (**BC6**), iristectorin A (**BC7**), isorhamnetin-3-*O*-(6″-acetyl-)-*β*-D-glucopyranosid (**BC8**), tectoridin (**BC9**), iridin (**BC10**, **BC20**), 1,3-*O*-diferuloylsucrose (**BC11**), 2′-*O*-acetyl-1,3-*O*-diferuloylsucrose (**BC12**), irigenin 3′-*O*-*β*-glucopyranosid (**BC13**), isoswertisin (**BC14**), 2″-*O*-*α*-L-rhamnosyl-4′-*O*-methylisovitexin (**BC15**), 2″-*O*-rhamnosylswertisin (**BC16**), embinin (**BC17**), 6″-*O*-acetylembinin (**BC18**) và 3″-*O*-acetylembinin (**BC19**).

- Established the quantitative method for together 6 compounds, which have isolated from radix of *Belamcanda chinensis* by HPLC, then given out the concentration of those contents the materials which cultivated Nghe An, Phu Tho, Thai Binh, Thanh Hoa, Vinh Phuc, and Yen Bai. The samples have contained irisflorentine percentage not lower than 0,1%, flavor to Chinese Pharmacopoeia. The percentage of those compounds were examined as tectoridin (**BC9**) 1,66 - 5,27%, iristectorigenin A (**BC1**) 0,17 - 0,40%, irisflorentin (**BC3**) 0,27 - 0,86%, tectorigenin (**BC5**) 0,74 - 1,95%, iristectorin A (**BC7**) 0,12 - 0,38%, and iridin (**BC10**) 0,50 - 1,41%.

2.3.2. Biological Investigation

- At concentration 30 μg/ml of ethyl acetate extract of radix of *Belamcanda chinensis* showed strong inhibitory activity on COX-2 induced by LPS and reduction of PGE2 synthesis.
- At concentration 30 μM, **BC2**, **BC6** and **BC9** also displayed inhibitory ability on reducing COX-2 impression, which induced by LPS in RAW246.7.
- The compounds **BC2** and **BC6** have been examined anti-inflammation pathway *in vitro* via mechanism signals on RAW364.7. The compound **BC6** inhibited genetic expression for COX-2 and reduction of PGE2 synthesis follow dose dependent (3, 10, 30 μM); at 30 μM, these inhibited the miR-146a and miR-155 genetic expression, and COX-2 impression by LPS-induced, miR-146a, and miR-155. The compound **BC2** also inhibited COX-2 genetic expression as dose dependent (3, 10, 30 μg/ml) and

PGE2 synthesis PGE2 as dose dependent (3, 10, 30, 100 μg/ml). **BC2** has inhibitory activity on NF-kB, AP-1, p65, and c-Jun as dose dependent.

- At 225 and 450 mg/kg of body weight of rat, MeOH extract of radix of *Belamcanda chinensis* displayed anti-inflammatory activity on the carrageenan-induced paw edema model, and reduced granule ulcers, comparing with reference groups
- At 20 μg/ml of ethyl acetate extract of air part of *Belamcanda chinensis*, it could inhibit significantly on proliferation of VSMC cells.
- At 30 μM of **BC14**, **BC17 - BC20** could inhibit significantly on proliferation of VSMC cells.

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