

学位論文の要旨 (論文の内容の要旨)  
Summary of the Dissertation (Summary of Dissertation Contents)

論 文 題 目  
Dissertation title

**Bioactive Compounds Isolated from *Celastrus hindsii* B. and Associated  
Anti-gout, Anti-diabetic, and Anti-tyrosinase Potentials**

広島大学大学院国際協力研究科  
Graduate School for International Development and  
Cooperation,  
Hiroshima University  
博士課程後期 開発科学専攻  
Doctoral Program Division of Development Science  
学生番号 D193261  
Student ID No.  
氏 名 TRAN DUC VIET  
Name Seal

### Background

*Celastrus hindsii* is a species of plant, belongs to the *Celastraceae* family. It distributes widely in many countries in the world. In Vietnam, this plant is found in numerous province such as Hoa Binh, Quang Ninh, Ninh Binh, Quang Nam. For a long time, *C. hindsii* has been used as a medicinal plant in Vietnam to support and treat cancer, ulcers, and inflammation. Many studies have shown the value of this plant because it contains many valuable compounds such as rosmarinic acid, terpenoids, alkaloids, phenolics, and flavonoids. Specifically, it has been shown to have anti-tumor, inflammatory, and ulcerative potential.

### Main objectives

- To evaluate anti-gout potential (Chapter 2).
- To assay anti-diabetic property (Chapter 3).
- To identify anti-tyrosinase capacity (Chapter 4).
- To isolate  $\alpha$ -amyrin and  $\beta$ -amyrin from *C. hindsii* and evaluate their bioactivities (Chapter 5).

### Structure of dissertation

- Chapter 1. General Introduction.
- Chapter 2. Anti-gout Potential.
- Chapter 3. Anti-diabetic Potential.
- Chapter 4. Anti-tyrosinase Potential.
- Chapter 5. Isolation of  $\alpha$ -Amyrin,  $\beta$ -Amyrin and Their Biological Properties.
- Chapter 6. General Discussion.

### Materials

Materials for the experiment were collected in Cao Duong, Son Duong, Hoa Binh, Vietnam. 3kg fresh leaves of *C. hindsii* was dried to obtain 1.12kg. After that, it was milled and soaked in 10 liters of

methanol for 3 weeks at 25°C. And then, it was filtered and concentrated by a rotary evaporator (SB-350-EYELA, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) to have crude extract. This crude extract was continued separating by three solvents (aqueous, hexane and ethyl acetate). The ethyl acetate extract was the most activity test by thin layer chromatography and was chosen to normal phase of column chromatography (20 mm diameter × 500 mm height, Climbing G2, Tokyo, Japan) over silica gel (size A 60, 200-400 mesh particle size, Sigma Aldrich). This process yielded 14 fractions to conduct experiment.

### Methods

In this study, inhibition of xanthine oxidase (XO),  $\alpha$ -amylase,  $\alpha$ -glucosidase, and anti-tyrosinase activities by leave extracts of *C. hindsii* were evaluated for the potential treatment of gout due to hyperuricemia and for diabetes mellitus.  $\alpha$ -Amyrin and  $\beta$ -amyrin had been confirmed by gas chromatography-mass spectrometry (GC-MS), electrospray ionization-mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR). The antioxidant activities of the  $\alpha$ -amyrin and  $\beta$ -amyrin mixture were determined via 2,2-dephenyl-1-picrylhydrazyl (DPPH), and 2,20-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays.

### Main Results

The extract from ethyl acetate (EtOAc) showed the strongest enzymatic inhibition XO ( $IC_{50}$  = 114.06  $\mu$ g /mL),  $\alpha$ -amylase ( $IC_{50}$  = 363.46  $\mu$ g /mL), and  $\alpha$ -glucosidase ( $IC_{50}$  = 689.09  $\mu$ g /mL) was subsequently fractionated by column chromatography to yield active constituents. Among the obtained fractions, P5(C:M=1:1) exerted the maximum inhibition on XO ( $IC_{50}$  = 38.22  $\mu$ g /mL),  $\alpha$ -amylase ( $IC_{50}$  = 68.00  $\mu$ g /mL) and  $\alpha$ -glucosidase ( $IC_{50}$  = 293.22  $\mu$ g /mL) activities. Gas chromatography mass spectrometry (GC-MS) and electrospray ionization mass spectrometry (ESI-MS) were applied to clarify the phytochemical profile of *C. hindsii*. As a result, fucosterol was identified as the principal compound in the leaves of *C. hindsii* (16.22 mg/kg dried weight), which exhibited a significantly stronger anti-gout and anti-diabetes activities than the commercial drug allopurinol used for gout and the drug acarbose prescribed for diabetes mellitus. In addition, a large amount of an  $\alpha$ -amyrin and  $\beta$ -amyrin mixture was isolated from *C. hindsii* (10.75 g/kg dried weight) by column chromatography with applying different solvent systems to obtain maximum efficiency. The mixture has exhibited a high potential for preventing gout by inhibiting a relevant key enzyme, and xanthine oxidase (XO) ( $IC_{50}$  = 258.22  $\mu$ g/mL). Moreover, an important enzyme in skin hyperpigmentation, tyrosinase was suppressed by  $\alpha$ -amyrin and  $\beta$ -amyrin mixture ( $IC_{50}$  = 178.85  $\mu$ g/mL).

### Conclusions

*C. hindsii* is potent to inhibit gout and diabetes in enzymatic activities. This medicinal plant contains rich amounts of  $\alpha$ -amyrin and  $\beta$ -amyrin. *C. hindsii* may be useful to isolate the two compounds for pharmaceutical purposes.  $\alpha$ -Amyrin and  $\beta$ -amyrin are strong in anti-gout and anti-tyrosinase enzymatic activities. This study emphasizes the values of *C. hindsii* in medicinal and pharmaceutical applications.