Doctoral Dissertation

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

（Summary）

TRAN DUC VIET

Graduate School for international Development and Cooperation

Hiroshima University

March 2022

SUMMARY

Background

Celastrus hindsii Benth (*C. hindsii*), a species of Celastraceae family, is appeared in southern America, south of China, and some countries in southeast Asia. In Vietnam, this plant is found in naturally forests. For a long time, *C. hindsii* has been used as a medicinal for treatment of many diseases such as cancer, ulcer, tumor, inflammation, and HIV. The studies have shown the value of this plant because it contains numerous compounds such as rosmerinic acid, terpenoids, alkaloids, phenolics, and flavonoids. In the present study, *C. hindsii* plants were used for the isolation of bioactive compounds and determination of associated biological activities.

In gout disease, xanthine oxidase (XO) is the enzyme which play the key role in the production of uric acid by converting hypoxanthine to xanthine and from xanthine to uric acid. This process is strongly associated with the development of gout. Acute hyperuricemia is the cause of gout, atherosclerosis, diabetes, hypertension, and chronic heart failure. Nowadays, gout become problem that profound threatens the health and development of many countries in the world.

Diabetes is another serious disorder that metabolize carbohydrates, fats, and proteins when the pancreatic insulin hormone is not active or less active. This is a dangerous metabolic disorder leading to serious consequences, both of acute and chronic conditions. α-Amylase and α-glucosidase are the major enzymes to resolute of carbohydrates. α-Amylase play an important role to breakdown the long chain carbohydrates. While α-glucosidase involves in starch separation and glucose desacralization before intestinal absorption. Thus, the inhibition activities of α-amylase and α-glucosidase are studied as the effective methods to treat diabetes.

Tyrosinase is a key enzyme that catalyses the synthesis of melanin. This enzyme has been evaluated extensively for the treatment of malignant cancer. In addition, tyrosinase also involved in neurodegenerative disorders, such as Parkinson's disease. Tyrosinase is an oxidizing enzyme that acts as a catalyst in the formation of melanin pigments by oxidative processes. This enzyme is commonly found in the tissues of plants and animals and detected in the melanosomes of skin melanocytes. In general, tyrosinase involving in melanin-browning reactions is considered as an important object in the development of cosmetic and food industries.

α-Amyrin and β-amyrin are compounds in triterpenes group. Their pharmacological activities have been shown in previous studies, including antitumor, anti-inflammatory, anxiolytic, and hepatoprotective effects. These compounds were detected in plant species such as *Solanum lycopersicum, Liriodendron tulipifera, Olea europaea, Aesculus hippocastanum, Aloe vera, Betula alba, Calendula officinalis, Coffea arabica, Malus domestica, Viscum album* etc.

Main objectives

According to all the above mentions, this study was conducted to (1) Evaluate anti-gout potential (Chapter 2). (2) Assay anti-diabetic properties (Chapter 3). (3) Identify anti-tyrosinase capacity (Chapter 4). (4) Isolation and identification α-amyrin and β-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 α-Amyrin, β-Amyrin and Their Biological Properties.
- Chapter 6. General Discussion.

Materials

Materials for the experiment were collected in Cao Duong, Son Duong, and Hoa Binh, Vietnam. In total, 3kg of the 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^{o}C. And then, it was filtered and concentrated by a rotary evaporator (SB-350-EYELA, Tokyo Rikakikai Co., Ltd, Tokyo, Japan) to create a crude extract. This crude extract was separated by three solvents (aqueous, hexane and ethyl acetate). The ethyl acetate extract was the most active tested by thin layer chromatography and was chosen for the 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 experiments.

Methods

In this study, anti-gout was evaluated by inhibition of xanthine oxidase (XO) capacity. Anti-diabetic property was identified by α-amylase, α-glucosidase inhibition activities. Anti-tyrosinase was assayed for treatment disease such as malignant cancers, skin cancer, or neurodegenerative disorders.

The purification of α-amyrin and β- amyrin were optimized by column chromatography and confirmed by gas chromatography-mass spectrometry (GC-MS), electrospray ionization-mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR). The antioxidant activities, anti-gout, anti-tyrosinase capacities were also determined via 2,2-dephenyl -1-picrylphydrazyl (DPPH) and 2,20-azinobis azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, and enzymatic inhibition XO, α-amylase, α-glucosidase, respectively.

Main Results

The ethyl acetate extract (EtOAc) showed the most activities of enzymatic inhibition XO (IC_{50} = 114.06 µg/mL), α-amylase (IC_{50} = 363.46 µg/mL), and α-glucosidase (IC_{50} = 689.09 µg/mL) were 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 µg /mL), α-amylase (IC_{50} = 68.00 µg/mL) and α-glucosidase (IC_{50} = 293.22 µg/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 α-amyrin and β-amyrin mixture was purified from *C. hindsii* (10.75g/kg dried weight) by column chromatography with applying different solvent system to optimization isolation. This mixture has expressed the high capacity for preventing gout disease by inhibition a relevant enzyme, and xanthine oxidase (XO) (IC_{50} = 258.22 µg/mL). Moreover, anti-tyrosinase activity was evaluated by potential of α-amyrin and β-amyrin mixture (IC_{50} = 178.85 µg/mL).

New Findings of research

- Fraction P5 (CM; 99: 1) (F37-44) is the most potent to inhibit gout and diabetes. Fucosterol is the principal compound in this fraction.
- Fraction P12 (CM; 1:1) (F127-138) is the most potential in anti-tyrosinase activity. Rutin and glycerin may play a role.
- *C. hindsii* is abundant α-amyrin and β-amyrin source.
- α-Amyrin and β-amyrin are anti-gout and anti-tyrosinase compounds potential.

Conclusions

C. hindsii is promising of anti- gout and anti-diabetes source material. This medicinal plant contains abundant amount of α-amyrin and β-amyrin and able to apply for pharmaceutical purposes. α-Amyrin and β-amyrin are strong anti-gout and anti-tyrosinase compounds. This research emphasized the valuable of *C. hindsii* in medicinal and pharmaceutical potential.