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Date of Award:
2008

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Download date: 02 Oct, 2023

Phytochemical and Pharmacological Studies of the Root of *Ilex pubescens*

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A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

Principal Supervisor: Prof. LIU Liang

Hong Kong Baptist University

March 2008

ABSTRACT

Maodongqing (毛冬青, MDQ), the root of *Ilex pubescens* Hook. et Arn. (Aquifoliaceae), is widely used in southern China for treatment of cardiovascular, peripheral vascular and inflammatory diseases. Owing to its efficacy and safety, this herb has been developed into many single herbal preparations and proprietary products. However, the anti-inflammatory and anti-thrombotic activities and their relevant active principles of MDQ have not been well investigated. In the current work, an activity-guided phytochemical study and pharmacological studies were undertaken in order to clarify the underlying scientific foundations of the therapeutic effects of MDQ, and further provide evidence for exploiting and utilizing MDQ. The results were shown as below:

Anti-thrombotic effects of nine fractions isolated from the *n*-BuOH layer of ethanol extract of MDQ by column chromatography were examined by using Kappa-carrageenan-induced tail thrombosis as a testing model. A phenolic fraction, *Fr. 5,* was identified as the bioactive fraction possessing anti-thrombotic activity. Chemical investigation on *Fr. 5* led to the isolation of two novel hemiterpene glucosides whose structures were elucidated on the basis of spectroscopic and chemical evidences as 2-(*trans*-caffeoyloxy)methyl-3-hydroxy-1-butene-4-*O*-*β*-D-glucopyranoside(pubescenoside A) and 2-hydroxymethyl-3-*trans*-caffeoyloxy-1-butane-4-*O*-*β*-D-glucopyranoside (pubescenoside B). These two hemiterpene glucosides were found to have anti-platelet aggregation activity by using high shear stress-induced platelet aggregation test.

Anti-inflammatory activities of the nine fractions of MDQ were assayed with carrageenan-induced paw edema in rats. A saponin fraction, *Fr.8-2,* was identified as the bioactive fraction with the most potent anti-inflammatory effect among those nine fractions. Two novel triterpene saponins named as pubescenosides C and D together with five known triterpene saponins were isolated from *Fr. 8-2*. Based on spectroscopic and chemical evidences, the structures of pubescenosides C and D were elucidated as 3-*O*-*β*-D-glucopyranosyl(1→2)-*β*-D-xylopyranosyl-urs-12,18-dien-(20β-methyl)-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester and 3-*O*-*α*-L-rhamnopyranosyl (1→2)-*β*-D-glucopyranosyl (1→2)-*β*-D-xylopyranosyl-urs-12,18-dien-(20β-methyl)-28-oic acid 28-*O*-*β*-D-glucopyranosyl ester, respectively. The five known triterpene saponins were elucidated as ilexsaponin B_1, B_2, B_3, A_1 and chikusetsusaponin IVa. HPLC fingerprinting of *Fr. 8-2* demonstrated that more than 70 % of the components in this fraction could be chemically identified.

Subsequently, *in vivo* anti-inflammatory and analgesic effects of *Fr. 8-2* were

comprehensively evaluated. As a result, it showed a significant suppression on the paw edema of rats induced by subplantar injection of carrageenan or histamine when given intraperitoneally at dosages ranging from 12.5 to 100 mg/kg of *Fr. 8-2* in rats, demonstrating its potent anti-inflammatory activity on acute inflammation model. This fraction was also proven to exert analgesic effect, because it could significantly inhibit acetic acid-induced abdominal writhing response of mice and prolong the time required for mice tail flick after exposure to a source of radiant heat at the oral dosages of 100 and 200 mg/kg. Mechanistic studies showed that the anti-inflammatory effect of *Fr. 8-2* was closely related to the selective inhibition on cyclooxygenase-2 (COX-2) expression, and overall attenuation of the ratio of pro-inflammatory /anti-inflammatory cytokines which was resulted from significant inhibition on the pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) and elevation of the anti-inflammatory cytokines (Il-4 and IL-10) production at the sites of inflammation.

In conclusion, this research revealed that MDQ has multiple functions, such as anti-thrombotic, anti-inflammatory and analgesic actions, which may attribute to the unusual hemiterpene glucosides and triterpene saponins in this herb. These results supported the clinical usage of MDQ in treating cardiovascular, peripheral vascular diseases and various inflammatory conditions. Meanwhile, *Fr. 8-2*, a triterpene saponin fraction whose chemical components were largely identified, and pharmacological activities were well verified, is expected to be developed as a botanical drug for anti-inflammation.

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