MASTER'S THESIS

絞股藍含量測定與指紋圖譜研究

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摘　要

絞股藍 *Gynostemma pentaphyllum* (Thunb.)Makino 爲葫蘆科絞股藍屬的多年生草質藤本植物的全草。《中藥大辭典》名七葉膽，味苦、寒、歸肺、肝、脾經。具有清熱解毒、止咳祛痰、益氣養陰等作用。用於胸膈痞悶、痰阻血瘀、心悸氣短、眩暈頭痛、健忘耳鳴、自汗乏力、高血脂症、疲勞綜合症、慢性氣管炎等。

本文對廣西，四川等省份絞股藍藥材的品質進行了初步研究，建立了新的品質評價方法。包括：HPLC-ELSD 法對絞股藍水解產物人參二醇進行含量測定，HPLC-DAD 法建立絞股藍藥材黃酮類成分的指紋圖譜。

在絞股藍含量測定方面，採用高效液相-蒸發光散射檢測器法對絞股藍水解產物進行含量測定，以人參二醇作爲指標性成份，對不同產地 9 批絞股藍進行了定量研究。研究中分別考察了不同色譜條件和提取條件，確立了最佳的方案：固定相爲 Alltech C_{18} 色譜柱（150×4.6 mm，5 μm，Alltima），流動相爲甲醇-水(88:12)，柱溫 30℃，流速 1.0 mL/min，漂移管溫度：75℃；以甲醇超聲提取 30 分鐘，共 3 次，用 3 mol/L　HCL 70℃加熱回流 4 小時，再以二氯甲烷萃取 3 次。含量測定結果得出，暫定本品按乾燥品計算，人參二醇平均含量爲 1669.53 mg/kg（SD=444.43 mg/kg）。

在 HPLC 指紋圖譜的建立，本文研究了 9 批絞股藍藥材黃酮類成分 HPLC 色譜指紋圖譜。通過指紋圖譜分析，確定了 6 個峰爲指紋圖譜的共有峰，構成該藥材特有的色譜指紋圖譜特徵，以 6 號峰(商陸素)爲參照物，對其它色譜峰的相對保留時間和相對峰面積進行計算，利用相似度計算軟體，計算了 9 批藥材指紋圖譜的相似度，爲制定藥材標準指紋圖譜提供了可靠的理論依據。

關鍵字：絞股藍；絞股藍皂苷；人參二醇；高效液相色譜-蒸發光散射檢測器；黃酮類化合物； HPLC 指紋圖譜。

Abstract

Gynostemma pentaphyllum (THUNB.) MAKINO (Jiaogulan) is a perennial creeping herb, which belongs to the *Cucurbitaceae* family. In the most recent dictionary of Chinese Materia Medica, it names qiyedan. According to traditional Chinese medicine (TCM) principles, the taste and nature of *G. pentaphyllum* is slightly bitter, cool, and the plant is prescribed in TCM benefiting for lung, liver, spleen, heat clearing, detoxification, antitussive, enhancing 'Qi' and supporting'Yin'. It indicated for chest congestion, palpitation and shortness of breath, stagnation of phlegm and blood, headache, dizziness, forgetfulness, hyperlipidaemia, fatigue syndrome and chronic bronchitis.

In this thesis, a new method of quantitative analysis and liquid chromatographic fingerprinting of *Gynostemma pentaphyllum*(THUNB.) MAKINO was developed. Including determination of panaxadiol by High Performance Liquid Chromatography with Evaporative Light Scattering Detector and flavones Fingerprinting by High Performance Liquid Chromatography with Diode-Array Detection .

The panaxadiol was used as the internal standard in quantitative analysis of the extracts of Gynostemmatis Herba from guangxi and sichuan provinces .The optimized extraction method are: firstly, The *G. pentaphyllum* powder was sonicated for three times with methanol for 30 mins and then total saponins were hydrolyzed by 3mol/L HCL for 4 hrs in 70℃ water-bath and cooled down with running water. Finally, partitioning the panaxadiol from hydrolysis solution by dichloromethane for three times. the optimized of HPLC conditions are: Column: Alltech C_{18}, 4.6 x 150 mm, 5 μm,Alltima; Mobile phase: water(A):methanol(B) 12:88 ; Column temperature: 30℃; Flow rate: 1.0 mL/min; Detector:ELSD; Drift tube temperature:75℃.The conclusion of determination is that the average concentration of panaxadiol in dried Gynostemmatis Herba is 1669.53 mg/kg ,Standard Deviation is 444.43 mg/kg.

Besides, the method to produce HPLC fingerprints for Gynostemmatis Herba was established. By analyzing the fingerprints of nine samples from various sources, 6 peaks had been identified as the common peaks. The relative retention time and relative peak

areas of the others were calculated by setting the Ombuin as the marker compound. The similarity of HPLC fingerprints of the nine samples were compared with the Similarity Evaluation System of Chromatographic Finger Prints of TCM (Version2004A).

The quality standard of Gynostemmatis Herba was set up to establish quality controlling standard of international market.

Key words Gynostemma pentaphyllum; gypenoside; panaxadiol; HPLC-ELSD; flavones; Fingerprint

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