

## MASTER'S THESIS

### 紋股藍含量測定與指紋圖譜研究

盧燕華

*Date of Award:*  
2011

[Link to publication](#)

#### General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

# 絞股藍含量測定與指紋圖譜研究

盧燕華

09427848

中藥學碩士學位課程

指導老師：姜志宏教授

香港浸會大學

二零一一年六月

## 摘 要

絞股藍 *Gynostemma pentaphyllum* (Thunb.) Makino 為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，以人參二醇作為指標性成份，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：固定相為 Alltech C<sub>18</sub> 色譜柱（150×4.6 mm，5 μm，Alltima），流動相為甲醇-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，人參二醇平均含量為 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 個峰為指紋圖譜的共有峰，構成該藥材特有的色譜指紋圖譜特徵，以 6 號峰(商陸素)為參照物，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，為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 *Gynostemmis 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°C 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<sub>18</sub>, 4.6 x 150 mm, 5 μm,Alltima; Mobile phase: water(A):methanol(B) 12:88 ; Column temperature: 30°C; Flow rate: 1.0 mL/min; Detector:ELSD; Drift tube temperature:75°C.The conclusion of determination is that the average concentration of panaxadiol in dried *Gynostemmis Herba* is 1669.53 mg/kg ,Standard Deviation is 444.43 mg/kg.

Besides, the method to produce HPLC fingerprints for *Gynostemmis 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 *Gynostemma Herba* was set up to establish quality controlling standard of international market.

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

# 目 錄

致 謝.....	1
聲 明.....	2
摘 要.....	3
Abstract .....	4
目 錄.....	6
一 絞股藍的研究概況.....	10
1 植物資源概況.....	11
2 化學成分研究.....	12
2.1 皂苷成分 .....	12
2.2 黃酮類成分 .....	14
2.3 多糖 .....	14
2.4 其他化成成分 .....	15
3 藥理活性與臨床應用.....	15
3.1 降血脂、抗動脈硬化作用 .....	16
3.2 降血糖作用 .....	16
3.3 降血壓作用 .....	17
3.4 調節免疫功能 .....	17
3.5 抗腫瘤作用 .....	17
3.6 抗衰老作用 .....	18
3.7 保護肝臟的作用 .....	18
3.8 其它作用 .....	18
3.9 毒性作用 .....	19
4 產品開發 .....	19
二 絞股藍品質評價方法研究現狀 .....	19
1 絞股藍鑒別方法的研究進展.....	19
2 絞股藍定量方法的研究進展.....	19
3 絞股藍藥材指紋圖譜的研究現狀.....	20
三 立題依據及研究意義.....	21
1 絞股藍含量測定研究.....	21

2 絞股藍指紋圖譜的建立.....	22
第二部分 HPLC 測定絞股藍水解產物人參二醇的含量.....	23
一 儀器與試藥.....	23
1 儀器.....	23
2 試藥.....	24
3 色譜條件.....	24
二 實驗方法與結果.....	25
1 色譜條件與選擇.....	25
1.1 流動相的選擇.....	25
1.2 色譜條件的確定.....	26
2 供試品溶液製備方法的考察.....	27
2.1 提取次數的考察.....	27
2.2 水解條件的考察.....	28
2.2.1 水解酸濃度的考察.....	28
2.2.2 水解時間的考察.....	29
2.2.3 水解溫度的考察.....	30
2.3 萃取次數的考察.....	31
2.4 供試品溶液製備的確定.....	32
3 對照品溶液的製備.....	32
4 方法學驗證.....	33
4.1 系統適用性試驗.....	33
4.2 線性與線性範圍的考察.....	34
4.3 精密度試驗.....	35
4.4 重複性試驗.....	34
4.5 加樣回收試驗.....	36
4.6 最低檢測限試驗 (Method Detection Limit, MDL).....	37
4.7 定量限 Limit of Quantitation (LOQ)回收率試驗.....	37
4.8 絞股藍藥材水解產物中人參二醇含量測定 HPLC/ELSD 典型色譜圖.....	38
5 含量測定.....	39
6 討論.....	41

6.1 對照品的選擇研究 .....	41
6.2 色譜條件的研究 .....	41
6.3 提取條件的研究 .....	42
6.4 絞股藍水解產物人參二醇的含量測定 .....	42
<b>第三部分 絞股藍黃酮類成分 HPLC 指紋圖譜的研究 .....</b>	<b>43</b>
<b>一 儀器與試藥 .....</b>	<b>43</b>
1 儀器 .....	43
2 試藥 .....	43
3. 色譜條件 .....	44
<b>二 實驗方法與結果 .....</b>	<b>45</b>
1 色譜條件與選擇 .....	45
1.1 檢測波長的選擇 .....	45
1.2 色譜柱的選擇 .....	45
1.3 柱溫的選擇 .....	46
1.4 流動相的選擇 .....	46
1.5 梯度洗脫比例的選擇 .....	48
1.6 色譜條件的確定 .....	48
2 供試品溶液製備方法的考察與結果 .....	48
2.1 提取溶劑的考察 .....	48
2.2 提取時間的考察 .....	50
2.3 供試品溶液製備方法的確定 .....	50
3 對照品溶液的製備 .....	51
4 方法學的驗證 .....	51
4.1 系統適用性的考察 .....	52
5 絞股藍黃酮類化合物 HPLC 指紋圖譜的建立 .....	54
5.1 指紋圖譜測定 .....	54
5.2 參照物的選擇 .....	54
5.3 指紋圖譜的技術參數 .....	54
5.4 指紋圖譜的相似度計算 .....	58
6 討論 .....	59



6.1 檢測波長的研究 .....	59
6.2 提取方法的研究 .....	59
6.3 不同產地絞股藍藥材指紋圖譜分析與評價 .....	59
<b>第四部分 結論</b> .....	60
1 人參二醇的含量測定.....	61
2 絞股藍黃酮類成分 HPLC 指紋圖譜的研究 .....	61
參考文獻 .....	62