Quality evaluation of *Dendrobium* species based on the analysis of multiple components

by

Xu Jun

Master of Science

2010

Institute of Chinese Medical Sciences
University of Macau

Quality evaluation of *Dendorbium* species based on the analysis of multiple components

by

Xu Jun

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Institute of Chinese Medical Sciences
University of Macau

2010

Approved by _____
Supervisor

_____

_____

_____

Date _____

In presenting this thesis in partial fulfillment of the requirements for a Master's degree at the University of Macau, I agree that the Library and the Institute of Chinese Medical Sciences shall make its copies freely available for inspection. However, reproduction of this thesis for any purposes or by any means shall not be allowed without my written permission. Authorization is sought by contacting the author at

Address:

Telephone:
Fax:
E-mail:

Signature_____

Date _____

碩士學位論文

基于多指标成分分析的石斛质量控制研究

研究生姓名：許 軍

導　　師：李紹平

專　　業：中 藥 學

日　　期：2010.6

澳門大學中華醫藥研究院

University of Macau

ABSTRACT

Quality evaluation of *Dendorbium* species based on the analysis of multiple components

by Xu Jun

Thesis Supervisor: Professor Li Shaoping
Major: Chinese Medicinal Sciences

Caulis Dendrobii (*Shihu* in Chinese), a well-known traditional Chinese medicine (TCM) recorded as first grade in *Shen Nong Ben Cao Jing*, is used for treatment of asthenia pyrosyndrome, dim vision and retching, etc. According to Chinese Pharmacopoeia (2005 edition), the fresh or dried stems from *Dendrobium nobile* Lindl.*, D. officinale* Wall. ex Lindl.*, D. fimbriatum* Hook. var. *oculatum* Hook. and the allied species are all used as *Shihu*. The active components exist in *Dendrobium* are very complex, which mainly include polysaccharides, phenols, alkaloids, coumarins and terpenes. Therefore, quality control is very important to ensure the safety and efficacy of *Dendrobium*. However, current methods usually focus on single compound or one type of componets. In addition, only a few reports concerned the analysis of polysaccharides.

In present study, accurate, quick and convenient methods have been developed for simultaneous determination of three types of active micro-molecular compounds and discrimination of polysaccharides from different *Dendrobium*, respectively.

The thesis consists of five chapters. **Chapter 1** briefly reviewed the advance on the chemitry and quality control of *Dendrobium*. In **Chapter 2**, the parameters of pressurized liquid extraction (PLE) including solvent type, extraction temperature, static extraction time and particle size were optimized using univariate approach. Followed by that, an Ultra performance liquid chromatography-photo diode array

(UPLC-PDA) method has been developed for fast determination of five components including one coumarin (scoparone), two alkaloids (dendrobine and dendronobiline A) and two bibenzyl (gigantol and moscatilin) in 21 samples from 15 *Dendrobium* species. The results showed that the contents of investigated compounds were greatly varied in different species or locations of *Dendrobium*. Gigantol and moscatilin were widely existed in *Dendrobium* species though their amounts were significantly different. Scoparone and dendrobine were the characteristic components of *D. thyrsiflorum* and *D. nobile*, respectively. Dendronobiline A was not detected in any *Dendrobium* species. In **Chapter 3**, a method using PLE and UPLC-PDA was developed for investigating chromatographic fingerprint of 18 samples from 15 *Dendrobium* species**.** Though the common compounds such as moscatilin and gigantol could be found in different species of *Dendrobium*, there were notable distinctions in different species of *Dendrobium*, even the same species from different locations. In **Chapter 4**, a novel method, saccharides mapping, based on enzymatic (carbohydrase) digestion and subsequent chromatographic analysis of enzymatic hydrolysate, was successfully applied to discriminate crude polysaccharides from different *Dendrobium* species, as well as the same species from different locations. According to the digestion response of the selected carbohydrases, polysaccharides from *D. fimbriatum* (Yunnan) and *D. nobile* (Guizhou) could be discriminated out of 8 investigated polysaccharides. Subsequently, the remained six polysaccharides, which could be correspondingly divided into three groups according to their enzymatic hydrolysis characteristics, i.e., the polysaccharides obtained from *D. officinale* (Yunnan) and *D. huoshanense* (Anhui), *D. officinale* (Anhui) and *D. chrysanthum* (Yunnan), as well as *D. officinale* (Zhejiang) and *D. nobile* (Yunnan). They could be distinguished by chracteristic saccharide maps of arabinanase, cellulose or pectinase hydrolysates. **Chapter 5** was a summary of this study.

Key words: *Dendrobium*; multiple components; UPLC-PDA; polysaccharides; saccharides mapping.

摘要

基于多指标成分分析的石斛质量控制研究

碩士生：許軍

導　師：李紹平 教授

專　業：中藥學

石斛（Caulis Dendrobii）是我國使用悠久的名貴中藥之一，始載於《神農本草經》，列為上品。具有滋陰清熱，生津益胃，潤肺止咳的功效，用於熱病傷津，口幹煩渴，病後虛熱等多種病症。《中國藥典》2005年版一部收載的石斛為蘭科植物金釵石斛*Dendrobium nobile* Lindl.、鐵皮石斛*D. officinale* Wall. ex Lindl.或馬鞭石斛*D. fimbriatum* Hook. var. *oculatum* Hook.及其近似種的新鮮或乾燥莖。石斛中的活性成分極其複雜，主要為多糖類、生物鹼類、酚類、香豆素類和萜類等。因此，質量控制對確保石斛的安全有效非常重要。現有的質量控制方法中質控指標的選擇大多都較為單一，且主要集中在小分子類成分，同時分析多類成分的報道較少有關石斛多糖的研究報導較少。

本文在石斛質控現狀的基礎上，分別建立了快速分析石斛中三類活性小分子成分以及區分不同來源石斛多糖的質量評價方法。

全文共分五章。**第一章**對中藥石斛的化學成分和品質控制研究進行了較為詳細的綜述。**第二章**首先對石斛的加壓溶劑提取條件，包括提取溶劑的種類、提取溫度、提取時間和樣品粒徑進行了單因素考察，優化確定了石斛藥材的加壓溶劑提取條件。之後採用超高效液相色譜法建立了石斛中5種活性成分：濱蒿內酯（香豆素類），石斛鹼、dendronobiline A（生物鹼），石斛酚，杓唇石斛素（聯苄類）的快速分析方法，對全國各產地的15種、21批石斛藥材進行了測定。結果表明，該5種成分在21批藥材中的含量差異極大，其中dendronobiline A未在任何藥材中檢測到；石斛鹼和濱蒿內酯分別只能在金釵石斛和球花石斛檢測到；石斛酚和杓唇石斛素在石斛中廣泛存在。**第三章**在第二章研究的基礎上，採用加壓溶劑提取和超高效液相色譜法建立了15種、18批石斛藥材的化學指紋圖譜。結果表明，盡管不同種石斛存在諸如石斛酚和杓唇石斛素等共有成份，但其化學指紋圖譜特徵

仍差異明顯，且同品種不同產地樣品的指紋圖譜亦存在較大差異。**第四章**採用已建立的糖譜方法定性鑒別8種不同來源的石斛粗多糖。先利用特異性糖酶酶解多糖中的相應糖苷鍵初步區分，再用色譜方法分析酶解產物進一步鑒別。實驗結果表明來自不同種或不同產地的石斛多糖酶解後的色譜特徵不盡相同。基於多糖酶解後的響應特徵，可直接區分出來自馬鞭石斛（雲南）和金釵石斛（貴州）的粗多糖，餘下六種石斛的粗多糖，按其酶解響應特徵可分為三組，分別來自鐵皮石斛（雲南）和霍山石斛（安徽）、鐵皮石斛（安徽）和束花石斛（雲南）以及鐵皮石斛（浙江）和金釵石斛（雲南），可通過其阿拉伯糖酶、纖維素酶或果膠酶的酶解產物特徵糖譜來進一步區分。**第五章**總結并討論了研究結果。

關鍵詞：石斛；多成份；超高效液相色譜；多糖；糖譜

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CE	Capillary electrophoresis
DAD	Diode array detector
EC	Enzyme Commission
ELSD	Evaporative light scattering detection
ESI	Electrospray ionization
GC	Gas chromatography
HPLC	High performance liquid chromatography
HPSEC	High performance size exclusion chromatography
LOD	Limit of detection
LOQ	Limit of quantification
MS	Mass spectrometry
MW	Molecular weight
NMR	Nuclear magnetic resonance
PDA	Photo diode array
PLE	Pressurized liquid extraction
PMP	1-Phenyl-3-methyl-5-pyrazolone
RSD	Relative standard deviation
S/N	Signal-to-noise ratio
TCMs	Traditional Chinese medicines
TIC	Total ion chromatogram
TLC	Thin layer chromatography
UPLC	Ultra performance liquid chromatography
UV	Ultraviolet

ACKNOWLEDGMENTS

I would like to express my sincere gratitude to my supervisor, Prof. Shaoping Li, who has given me enlightenment, guidance and inexhaustible patience throughout this two-year research project. I could not do the work well without your carefully consideration and steady support.

Especially, sincere thanks to Director of Institute of Chinese Medical Sciences (ICMS), Prof. Yitao Wang, for giving me the learning opportunity to accumulate speculative knowledge and enhance experimental skills which is significant for my whole life.

Also thanks to Dr. Qingwen Zhang, Prof. Simon Lee, Dr. Ying Zheng, Dr. Ru Yan, Dr. Maggie Hoi, Ms. Hattie U and Ms. Chloe Lao for the support during the learning and living.

This research work was supported by all members of ICMS, University of Macau. Special thanks to Leon Lai for technical supports.

Thanks to Jia Guan, Xiaojia Chen and Zhengming Qian for their guidance on experiment and thesis writing. Thanks to Jing Zhao, Fengqing Yang, Kun Feng, Deqiang Li, Weihua Huang, Jing Li and Lanzhen Meng for their help on my experiments.

Thanks to Dejun Hu, Guangping Lv, Jing Yang and Qiong Meng for offering me help during these two years, and giving me the great memory of happiness to study and work together.

Thanks to my family for offering me great support during this two-year life.