

Quality Evaluation of Natural and Cultured Cordyceps

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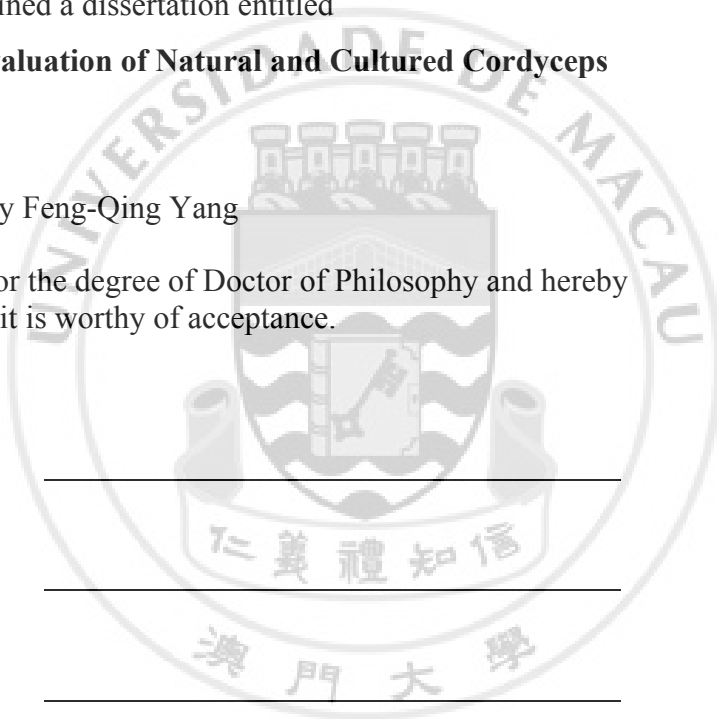
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Quality Evaluation of Natural and Cultured Cordyceps

Abstract

Cordyceps sinensis, known as DongChongXiaCao (winter worm summer grass) in Chinese because of its appearance during different seasons, is a composite consisting of the stromata of the fungus and the dead caterpillar. The fungus *Cordyceps sinensis* (Berk.) Sacc. (Fam. Hypocreaceae) is parasitized on the larva of some species of insects (Fam. Hepialidae). According to record of traditional Chinese medicine, *Cordyceps* sweet in taste and neutral in nature, can replenish the kidney and sooth the lung, arrest bleeding, resolve phlegm, and suppress cough. It is commonly used to replenish the kidney and soothe the lung for the treatment of fatigue, night sweating, hyposexualities, hyperglycemia, hyperlipidemia, asthemia after severe illness, respiratory disease, renal dysfunction and renal failure, arrhythmias and other heart disease, and liver disease.

However, to date, scientific studies on *C. sinensis* are not sufficient to offer understanding of its pharmacological activities and clinical efficacies, while the relationship between the compounds and the pharmacological effects of *Cordyceps* is still partial unclear. Furthermore, most studies have been performed using cultured *Cordyceps* instead of natural one because the latter is rare and expensive. Therefore, in order to developed effective quality control method for *Cordyceps*, systematic chemical separation and several analytical techniques, mainly HPLC-DAD/MS, GC-MS have been applied for the determination of chemical characteristics in natural *C. sinensis*, while bioassay were used for evaluation the relationship of nucleosides in *Cordyceps* and its immune activities. The major achievements of this study are summarized as follows:

(1) Twelve water soluble compounds of *C. sinensis* were separated by sequential medium pressure liquid chromatography (MPLC) and preparing high performance liquid chromatography (Pre-HPLC). Two compounds were separated for the first time from *Cordyceps sinensis*.

(2) The lipid soluble compounds of *C. Sinensis* were studied by using gas chromatography – mass spectrometry (GC-MS). Ten free fatty acids and four sterols were identified and quantified by GC-MS.

(3) Three analytical techniques including capillary electrochromatography (CEC),

capillary electrophoresis – mass spectrometry (CE-MS) and ultra performance liquid chromatography (UPLC) were developed for the analysis of nucleosides and its related compounds in *Cordyceps*, which provide alternative techniques for quality control as well as determination chemical characteristics in *Cordyceps*.

(4) A specific quality control method was developed based on the characteristics of natural *C. sinensis*. Furthermore, liquid chromatography – mass spectrometry (LC-MS) was applied for the determination of nucleotides (the first time identified in *Cordyceps*), nucleosides and nucleobases in *Cordyceps*, while the degradation of nucleotides to nucleosides as well as nucleosides to nucleobases in *Cordyceps* was also studied.

(5) Immunological tests of various nucleosides suggested that different compositions and ratios of nucleosides have significantly different effects on the release of cytokines in normal and activated mice macrophage. Therefore, the nucleosides' variation in natural and cultured *Cordyceps* may result in their difference in immuno-modulating effects.

In summary, the present study broadens the knowledge of chemical compositions in natural *C. sinensis*. Several nucleosides were proven to be bioactive in *Cordyceps*, while different compositions and ratios of nucleosides have different pharmacological activities. The method, using different sample preparation methods coupled with the markers of adenosine, inosine, guanosine and uridine, is useful to control the quality of *Cordyceps*.

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Achievements

Journal Articles

1. **F. Q. Yang**, S. P. Li, P. Li and Y. T. Wang, Optimization of capillary electrochromatography for simultaneous determination of eleven nucleosides and nucleobases in *Cordyceps* using central composite design. *Electrophoresis* 28 (2007) 1681-1688 (Wiley) IF= 3.609 (2007)
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- Sciences and Technology 3rd Prize**, Chinese Pharmaceutical Association, 2008.
2. G. X. Zhong, **F. Q. Yang**, L. J. Zeng and S. P. Li, Determination of 13 components in different parts of *Danshen* from different locations. **Excellent Paper 3rd Prize**, The third PUXITONGYONG Cup of National Pharmaceutical Analysis Symposium, Chinese Journal of Pharmaceutical Analysis, 2008.
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 4. S. P. Li, **F. Q. Yang** and Y. T. Wang, Study on the quality control method of Ezhu. **Excellent Paper 2nd Prize**, The seventh DAOJIN Cup of National Pharmaceutical Analysis Symposium, Chinese Pharmaceutical Journal, 2005.

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2. S. P. Li and **F. Q. Yang**, Quality control of *Cordyceps sinensis*, FIP 2007, Beijing, **Poster presentation**
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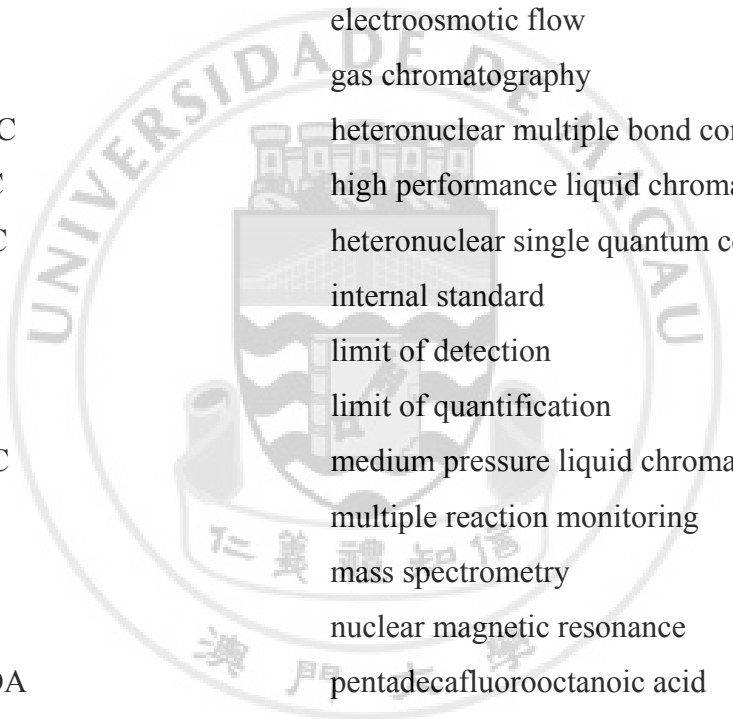
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Abbreviation



BGE	background electrolyte
CCD	central composite design
CE	capillary electrophoresis
CEC	capillary electrochromatography
COSY	correlation spectroscopy
DMSO	dimethyl sulfoxide
DAD	diode array detector
EOF	electroosmotic flow
GC	gas chromatography
HMBC	heteronuclear multiple bond correlation
HPLC	high performance liquid chromatography
HSQC	heteronuclear single quantum coherence
IS	internal standard
LOD	limit of detection
LOQ	limit of quantification
MPLC	medium pressure liquid chromatography
MRM	multiple reaction monitoring
MS	mass spectrometry
NMR	nuclear magnetic resonance
PDFOA	pentadecafluorooctanoic acid
PLE	pressurized liquid extraction
SIM	selected ion monitoring
S/N	signal-to-noise ratio
SRM	selected reaction monitoring
TMS	trimethylsilyl
UPLC	ultra-performance liquid chromatography
UV	ultraviolet visible

Preface

Cordyceps sinensis, a well-known and valued traditional Chinese medicine, is also called DongChongXiaCao (winter worm summer grass) in Chinese. It is found in the soil of a prairie at an elevation of 3,500 to 5,000 meters, mainly in the provinces of Qinghai, Tibet, Sichuan, Yunnan and Gansu. Due to the restricted habitat and over exploration, the ecosystem of *C. sinensis* was terribly destroyed. Although the Ordinance of Resources Protection on Wild Herbal Medicine was issued in 1987, the yield of natural *Cordyceps sinensis* was still decreasing. As the rarity and upstanding curative effects of natural *Cordyceps*, several mycelial strains have been isolated from natural *Cordyceps* and manufactured in large quantities by fermentation technology, and they are commonly sold as health food products and/or drug. Therefore, study on the quality control of natural *C. sinensis* is also helpful to control the quality of cultured *Cordyceps* products due to the bioactive components in natural *Cordyceps sinensis* are still unknown. In the present study, the chemical characteristics of natural *Cordyceps sinensis* were investigated. In brief, the water soluble components of *C. sinensis* were separated and identified, while the lipid soluble compounds were identified and quantified by using GC-MS. Furthermore, the nucleosides and related compounds, contribute to the most biological activities of *Cordyceps sinensis*, in *Cordyceps* were determined by CEC, UPLC and CE-MS. In addition, LC-MS was used to elucidate the transformation of nucleosides in *Cordyceps*.

This thesis includes 10 chapters.

In Chapter 1, the chemical components in natural *C. sinensis* and their biological activities were reviewed and discussed. The markers as well as the analytical techniques for quality control of *Cordyceps* were also summarized.

Chemical separation of water soluble compounds in natural *C. sinensis* was described in Chapter 2. Twelve compounds were obtained from the fraction of MW<3000. The structures of eleven compounds were identified by comparing their UV, MS, ¹H- and ¹³C-NMR data with those of the standards and/or literature. Two compounds were first separated from *Cordyceps*. Furthermore, the lipid soluble compounds including fatty acids

and sterols in *Cordyceps* were determined by GC-MS in Chapter 3. The investigated compounds were converted into their trimethylsilyl (TMS) derivatives after extracting by pressurized liquid extraction (PLE) before GC-MS analysis.

UPLC, CE-MS and CEC methods for the determination of nucleosides and the related compounds in *Cordyceps* were developed in chapter 4, 5 and 6, respectively. Based on the quantitative results, the overall content of nucleosides is much higher in cultured *Cordyceps* than in natural ones. However, the hypoxanthine and inosine are much higher in natural *C. sinensis*. Similar to our previous report, cordycepin, which is abundant in cultured *C. militaris*, is only found in natural *C. sinensis* with very low content and cannot be detected in the cultured ones.

The effects of different sample preparation methods on the quantification of nucleosides from *Cordyceps* were studied in Chapter 7. Three extraction methods, including pressurized ethanol extraction, boiling water extraction and ambient temperature water immersion, were used for sample preparation to analyze nucleosides in *Cordyceps*. The results showed that the chemical characteristics of pressurized ethanol extract and boiling water extract from natural *Cordyceps* were similar. But the nucleosides profile of its ambient temperature water extract was obviously different. Those phenomena were not found in the three extracts of cultured *C. sinensis*, which showed high similarity. Therefore, the approach is easily to discriminate natural and cultured *C. sinensis*. Furthermore, those phenomena were successively studied by LC-MS in Chapter 8. It was found that nucleotides such as AMP, UMP and GMP could be identified in *Cordyceps* for the first time. Those nucleotides can be degraded into adenosine, uridine and guanosine, respectively. In addition, they can be further degraded into their bases adenine, uracil and guanine, respectively.

Based on chapter 4-8, it is obvious that the nucleosides' characteristics are quite different in natural and cultured *Cordyceps* from the analyses results. Therefore, in Chapter 9, the effects of compositions and ratios of nucleosides on the immune system were evaluated by the release of cytokines in normal and activated mice macrophages *in vitro*. The results showed that different compositions and ratios of nucleosides have different or

even opposite effects on the functions of macrophages *in vitro*. The results suggested that the nucleosides at least partially contribute to the immuno-modulating effects of *Cordyceps*, while the compositions and ratios of nucleosides are essential.

A general conclusion was described in Chapter 10. In brief, the chemical compositions in *Cordyceps* were systematically investigated, which is helpful for the understanding of the pharmacological activities and quality control of *Cordyceps*. Nucleosides are one of the bioactive ingredients in *Cordyceps*, while compositions and ratios of nucleosides are the two key factors for their biological activities. Different sample preparation methods coupled with the markers of adenosine, inosine, guanosine and uridine are helpful for the quality control of *Cordyceps*.

