STUDY ON PHYTOCHEMICAL PROFILE OF LYCII CORTEX AND THE KEY COMPONENTS RESPONSIBLE FOR ANTI-TYPE 2 DIABETES MELLITUS

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ABSTRACT

Lycii Cortex (LyC), the root bark *Lycii Chinese* or *L. barbarum*, is an important folk medicine widely used in East Asian. In Traditional Chinese Medicine (TCM), LyC works as a “heat cleaning” agent to treat chronic diseases like cough, hypertension and diabetes. However, scientific evidence for the effect is still missing. To ensure the efficacy and safety in usage, it is necessary to characterize the functional constituents and to establish quantitative methods for monitoring the function-related quality. The aim of this study covered: 1) qualitative study of the phytochemical profile of LyC with LC-MS; 2) quantitative study of some bioactive components to find out the representative markers for quality assessment; 3) establishment of standard methods for quality assessment; 4) separation of the potentially bioactive constituents; and 5) evaluation of effectiveness in relation to anti-diabetic action.

The phytochemical composition of LyC was achieved by means of a non-targeted profiling. Spermine alkaloids (kukoamine A and B), cyclic-octapeptides (lyciumin A and B) were the major components found in all collected samples. The phenolic constituents, which were commonly existed in most plants, however, are minor compounds for LyC. Then, an LC-MS/MS method coupled with multiple ion monitoring (MIM) and enhanced product ion (EPI) scan was used to structural identification of kukoamine derivatives. Twelve kukoamines were identified based on the proposed method. Except of kukoamines A and B (KA
and KB), other allies are first identified in LyC. With the aim to identify the representative markers for LyC, sixteen bioactive constituent candidates were simultaneously determined by an LC-MS/MS method with multiple reactions monitoring (MRM) scan. Kukoamines, which account for 0.8% (w/w) of the dry mass, were proposed as the representative markers for qualitative assessment of the herb based on their genus-specificity, predominant abundance and notable bioactivities. With the recommended markers, a standard HPLC method was established for quality control in practical usage. Within the investigated samples, the average contents of KA and KB are 3.49 and 10.75 mg g⁻¹, and the proposed limit in contents are 1.45 and 4.72 mg g⁻¹. This result can provide information for quality control of LyC in official compendiums. In order to further improve the feasibility and flexibility of the method for assay, a new separation approach, capillary zone electrophoresis (CZE), was established to replace the HPLC for kukoamine assay. The novel online complexation between phosphate ion and kukoamines during the electrophoresis was discovered to be helpful in greatly enhancing the sensitivity. With the same performance as HPLC in precision, reproducibility and accuracy, CZE was superior in peak resolution, theoretical plate, peak symmetry, separation efficiency, sensitivity and eco-friendliness.

In order to prepare the phytochemicals for biological test, a novel dispersive solid phase extraction (DSPE) method with polyamide resin as adsorbent was developed for purification of KA and KB. The optimal conditions for adsorption happened in an aqueous solution with pH at 6-7 and the condition for desorption
happened in 0.5% acetic solution (v/v). After the DSPE, the kukoamines in the extract can be enhanced for at least 30-fold. It therefore highly improved the separation efficiency of preparative HPLC for the final purification for kukoamine monomers.

A glucose uptake model on human HepG2 cells was utilized to evaluate bioactivities of key components from LyC (i.e., KA, KB, coumarin derivatives, and lyciumins A and B) and the crude extract with regard to anti-T2DM function. KA and KB were effective in enhancing the glucose uptake in hepatic cells, though their potency was weaker than that of the crude extract. While, other purified compounds did not show significant effects on the model. Nevertheless, as T2DM is a complicated disease which involved in multiple pathologies, these compounds might exert actions through other mechanisms, or might produce synergic effects to intervene the syndrome. Kukoamines may also present other activities (i.e., anti-inflammatory effects on metabolic organs) to improve the hyperglycemic situation. All these possibilities need to be verified in further studies.
TABLE OF CONTENTS

ABSTRACT............................................................................................................... i
TABLE OF CONTENT.............................................................................................. iv
ACKNOWLEDGEMENTS............................................................................................ xii
DECLARATION........................................................................................................... xiii
ABBREVIATIONS....................................................................................................... xiv
LIST OF TABLES......................................................................................................... xix
LIST OF FIGURES..................................................................................................... xxi
LIST OF PUBLICATIONS AND PATENTS................................................................. xxv

SECTION A GENERAL INTRODUCTION.........................................................1

Chapter 1 Literature Review and Research Strategy
................................................................................................................................. 2
  1.1 Introduction and Morphological Features of Lycii Cortex............................ 2
    1.1.1 General Information of LyC................................................................. 2
    1.1.1.1 Original plants and harvesting......................................................... 2
    1.1.1.2 Trational usage of LyC................................................................. 3
    1.1.2 Morphological features of LyC.......................................................... 4
    1.1.2.1 Macroscopic features of LyC......................................................... 4
    1.1.2.2 Microscopic features of LyC......................................................... 5
  1.2 Phytochemical Constituents of Lycii Cortex............................................. 8
    1.2.1 Alkaloids............................................................................................... 8
    1.2.1.1 Spermine alkaloids...................................................................... 8
    1.2.1.2 Cyclic peptides........................................................................... 9
    1.2.1.3 Phenolic amides........................................................................ 10
    1.2.1.4 Tropane....................................................................................... 10
    1.2.1.5 Calystegines and Piperdine......................................................... 11
    1.2.1.6 Pyrrolidine................................................................................ 13
    1.2.1.7 Conventional alkaloids existed in LyC........................................... 13
    1.2.2 Flavonoids......................................................................................... 14
2.3 Cell lines and cell culture reagents ................................................. 46
  2.3.1 Cell lines .................................................................................. 46
  2.3.2 Material and reagents used in cell culture .............................. 46
  2.3.3 Preparation of culture medium (DMEM) ................................. 46
  2.3.4 Recovery of cells ..................................................................... 47
  2.3.5 Subculture of cells ................................................................... 47
  2.3.6 Cell counting using Trypan blue .............................................. 48
2.4 Apparatus ..................................................................................... 48

SECTION C RESULTS AND DISCUSSION ............................................. 51

Chapter 3 Non-targeted Analysis of Phytochemical Constituent in LyC by HPLC-DAD-MS ................................................................. 52
  3.1 Introduction .................................................................................. 52
  3.2 Experimental ............................................................................... 53
    3.2.1 Reagents ............................................................................... 53
    3.2.2 Plant material sampling and sample preparation ................. 54
    3.2.3 Apparatus ............................................................................. 54
  3.3 Results and discussion ................................................................. 55
    3.3.1 Preliminary study of constituents in LyC with HPLC-DAD-MS .... 55
  3.4 Conclusion .................................................................................. 63

Chapter 4 Screening of Kukoamine Allies in Lycii Cortex with Precursor Ion Scan Combined Multiple Ion Monitoring Triggered Enhanced Product Ion Scan (MIM-EPI) on QTRAP MS .................................................. 64
  4.1 Introduction .................................................................................. 64
  4.2 Experimental ............................................................................... 67
    4.2.1 Reagents, plant material sampling and sample preparation .... 67
    4.2.2 Apparatus ............................................................................. 67
  4.3 Results and discussion ................................................................. 69
    4.3.1 Fragmentation behavior of kukoamines in tandem MS .......... 69
    4.3.2 Selection of precursor ion and precursor scan ................. 71
    4.3.3 Comparison between EMS-EPI and MIM-EPI for qualitative analysis ...................................................... 76
4.3.4 MIM-EPI for identification of kukoamine allies..................................................79
4.4 Conclusion..................................................................................................................84

Chapter 5 Identification of Kukoamines as the Novel Markers for Quality
Assessment of LyC .............................................................................................................85
5.1 Introduction..................................................................................................................84
5.2 Experimental ..............................................................................................................87
  5.2.1 Reagents..................................................................................................................87
  5.2.2 Plant material sampling..........................................................................................91
  5.2.3 Apparatus................................................................................................................93
  5.2.4 LC-MS/MS methods..............................................................................................93
  5.2.5 Preparation of standard solutions.........................................................................96
  5.2.6 Sample preparation...............................................................................................96
  5.2.7 Method validation..................................................................................................97
    5.2.7.1 Linearity............................................................................................................97
    5.2.7.2 Determination of LOD and LOQ....................................................................97
    5.2.7.3 Precision..........................................................................................................97
    5.2.7.4 Reproducibility...............................................................................................98
    5.2.7.5 Recovery...........................................................................................................98
5.3 Results and discussion..............................................................................................99
  5.3.1 Optimization of sample preparation, chromatographic separation and
      MS/MS conditions......................................................................................................99
    5.3.1.1 Sample preparation.........................................................................................99
    5.3.1.2 HPLC conditions..........................................................................................101
    5.3.1.3 Optimization of MRM conditions in LC-MS/MS.........................................101
5.3.2 Method validation..................................................................................................104
5.3.3 Evaluation for patterns of alkaloids, coumarins, phenolic acids and
      flavonoids in LyC........................................................................................................109
5.3.4 Comparison of the composition for LyC of two species and other
cortex herbs..................................................................................................................111
5.4 Conclusion................................................................................................................114

Chapter 6 Definition of LyC Quality Using Kukoamines A and B by High
Performance Liquid Chromatography.........................................................................115
6.1 Introduction.................................................................................................................114

vii
6.2 Experimental.................................................................117
  6.2.1 Reagents.................................................................117
  6.2.2 Plant materials.......................................................117
  6.2.3 Chromatographic conditions.....................................117
  6.2.4 Sample preparation................................................118
  6.2.5 Method validation..................................................119
  6.2.6 Statistical analysis................................................119
6.3 Results and discussion....................................................120
  6.3.1 Establishment of HPLC conditions for determination of
        kukoamines..................................................................120
    6.3.1.1 Selection of column, mobile phase additive and detection
            wavelength.............................................................120
    6.3.1.2 The influence of pH on sample
            stability.................................................................123
  6.3.2 Optimization of kukoamine extraction procedures.............124
  6.3.3 Method validation....................................................127
  6.3.4 Quantitative determination of kukoamines in LyC samples and the
        proposed content for quality control..............................128
  6.3.5 Application of the HPLC method for quality control of LyC....135
6.4 Conclusion........................................................................136

Chapter 7 Online Complexation between Kukoamines and Dihydrogen
Phosphate ions Enhances Sensitivity Improvement for Kukoamines in Capillary
Zone Electrophoresis.........................................................137
7.1 Introduction.....................................................................137
7.2 Experimental....................................................................139
  7.2.1 Material and Reagents..............................................139
  7.2.2 Apparatus...............................................................139
  7.2.3 Sample preparation..................................................140
  7.2.4 Method for capillary electrophoresis............................140
  7.2.5 Influence of buffer system on electrophoretic behavior and signal
        response.................................................................141
  7.2.6 Method for HPLC.....................................................142
  7.2.7 Method validation....................................................142
7.3 Results and discussion......................................................143
7.3.1 Complexation between dihydrogen phosphate ion (DPI) and kukoamines in CZE.................................................................143
7.3.2 Influence of electrolytes on analytes’ electrophoretic behaviors…..149
7.3.3 Analytical performance of the CZE for kukoamine determination.................................................................151
7.3.4 Determination of kukoamines in real samples with HPLC and CZE ........................................................................156
7.4 Conclusions..................................................................................158

Chapter 8 Dispersive solid phase extraction (DSPE) for enrichment of kukoamines using polyamide as an adsorbent...............................159

8.1 Introduction...............................................................................159
8.2 Experimental.............................................................................162
  8.2.1 Materials.............................................................................162
  8.2.2 Preparation of crude extract...................................................162
  8.2.3 HPLC analysis......................................................................162
  8.2.4 Adsorption experiments..........................................................162
    8.2.4.1 Influence of initial solution pH on adsorption capacity.....162
    8.2.4.2 Influence of methanol concentration on adsorption capacity .................................................................163
    8.2.4.3 Adsorption kinetics and isotherm......................................164
    8.2.4.4 The mode of column solid phase extraction and dispersi ve solid phase extraction.................................166
    8.2.4.5 Influence of number of DSPE times on transfer rate........166
  8.2.5 Desorption procedures...........................................................167
    8.2.5.1 Influence of solvents on desorption rate.........................167
    8.2.5.2 Contents of kukoamines in concentrated extracts.............168
    8.2.5.3 Desorption ratio of kukoamines from polyamide to elution........................................................................168
  8.2.6 Regeneration of polyamide resins.............................................169
  8.2.7 Separation of kukoamines A and B by preparative HPLC.........169
8.3 Results and discussion...............................................................170
  8.3.1 Adsorption behavior between polyamide resin and kukoamine.....170
    8.3.1.1 The pH-dependent adsorption between kukoamines and polyamide resin..............................................170
    8.3.1.2 Influence of methanol concentration on adsorption capacity
8.3.1.3 Adsorption curves, first-order kinetics and isotherms........174
8.3.1.4 Comparison between CSPE and DSPE..........................177
8.3.1.5 Influence of DSPE cycles on transfer rate.......................179
8.3.2 Optimization of desorption procedures..............................180
  8.3.2.1 Influence of eluting solvent on desorption rate................180
  8.3.2.2 Influence of eluting solutions on kukoamine contents in the
        enriched fraction........................................182
  8.3.2.3 Influence of numbers of desorption times on desorption rate
        ................................................................182
  8.3.3 Reutilization of the resin.................................................182
  8.3.4 Influence of DSPE on performance of preparative HPLC in further
        purification.........................................................184
8.4 Conclusions..................................................................187

Chapter 9 Effects on Lycii Cortex Extract and Kukoamines on Glucose
Consumption in Human HepG2 cells........................................188

  9.1 Introduction..................................................................188
  9.2 Experimental..............................................................190
    9.2.1 Chemicals and reagents..............................................190
    9.2.2 Preparation of crude extract.......................................190
    9.2.3 Purification of the key components from the sample........190
    9.2.4 Sample preparation for cell experiments.......................191
    9.2.5 Cell culture and glucose consumption assay on HepG2 cells....191
    9.2.6 Statistical analysis..................................................192
  9.3 Results.........................................................................193
    9.3.1 Investigation on reprehensive phytochemical composition for LyC
          ........................................................................193
    9.3.2 Time dependent effects of the isolated compounds and extract for
          improving glucose consumption in HepG2 cells..................193
    9.3.3 Dose dependent effects of the isolated compounds and extract for
          improving glucose adsorption in HepG2 cells......................196
  9.4 Discussion.....................................................................198
  9.5 Conclusions...............................................................200
SECTION D: OVERALL CONCLUSIONS & SUGGESTIONS FOR FUTURE STUDIES

Chapter 10 Overall Discussion, Conclusion and Future Study

10.1 Overall discussion
10.2 Breakthrough of this study
10.3 Overall conclusion
10.4 Suggestion of Future study

SECTION E: REFERENCES