RGC Ref. No.:
UGC/FDS16/P02/14
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Development of rapid testing methods for the authentication of Chinese Materia Medica (CMM) by mass spectrometry

2. Investigator(s) And Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr SZE Eric Tung-po / Assistant Professor	School of Science and Technology, The Open University of Hong Kong
Co-Investigator	Prof. CHAN Dominic Tak-wah / Professor	Department of Chemistry, The Chinese University of Hong Kong
Co-Investigator	Dr LEE Fred Wang-fat / Assistant Professor	School of Science and Technology, The Open University of Hong Kong

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	1st January 2015	NA	NA
Project Completion Date	31st December 2016	30th June 2017	18th November 2016
Duration *(in month)*	24 months	30 months	18th November 2016
Deadline for Submission of Completion Report	31st December 2017	30th June 2018	18th November 2016

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To compare and identify potential protein markers for authenticity testing between the CMM groups of 1) *Flos Campsis* and *Flos Daturae Metelis* and 2) *Cordyceps sinensis* and its counterfeit species, by the use of proteomic techniques; this part also includes the molecular and microscopic identifications of species.
2. To determine the mass and structural information of the biomarkers identified in point 1, by the use of matrix-assisted laser desorption / ionization time-of-flight mass spectrometer (MALDI-TOF MS).
3. To study the feasibility in direct analysis of CMM extracts by MALDI-TOF MS in authenticity testing
4. To validate the specificity, robustness and limit of detection of the testing method devised in point 3, and to design quality control plans for the method of routine analysis.

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change:

1.

2.

3. ....

5.3 Realisation of the objectives
(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

For objective #1, Chinese Materia Medica (CMM) of 1) *Cordyceps sinensis* and other Cordyceps species with similar morphological features were obtained from local retail market and/or donors. All samples were stored in controlled temperature and humidity. Each sample of was identified genetically and the similarities of all tested CMM were greater than 97% when compared with the sequence in NCBI Genbank.
The Project Team have tried the used of various protein extraction methods for the protein extraction, eventually the results of proteins extracted using lysis buffer were found to be the best among the most commonly used methods. The yields of protein extracted by lysis buffer in this study was also found comparable with result from literatures, where extraction using lysis buffer system was found to have a better protein extraction yield (220% more protein by mass than the use of phenol/SDS method). To optimize the extraction yield and quality of resulting two-dimensional

gel electrophoresis (2-DE) profile of lysis buffer extraction, we have studied the effect of TCA/acetone pre-washing and post-washing of sample in conjunction with lysis buffer extraction. Among the three protocols, lysis buffer extraction without any pre-washing and post-washing of sample was found to be the most effective method in extracting proteins and no significant difference in 2-DE profile quality.

Upon optimizing the extraction protocol, we have successfully analyzed the samples and compared 2-DE profiles. Certain candidate protein marker spots distinct were identified. These candidate protein marker spots were proved to be reproducible from an independent source, demonstrating the feasibility of using protein markers as indicators for authentication.

For objectives #2 and #3, we have then tried to isolate some of the intense protein spots from 2-DE profiles and to analysis by matrix-assisted laser desorption / ionization time-of-flight mass spectrometer. By comparing the mass spectra obtained, certain proteins at the mass range between 2,000 – 3,000 Da were identified to be potential protein markers.

For objective #4, we have conducted extensive method validation to study performance parameters such as specificity, robustness and limit of detection. The specificity and robustness were evaluated by analyze CMM samples obtained from two independent sources. Results were obtained with at least 3 replicates to verify the repeatability. The limit of detections of CMM used in proteomic analysis were achieved by using Bradford assay.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To compare and identify potential protein markers for authenticity testing between the CMM groups of 1) *Flos Campsis* and *Flos Daturae Metelis* and 2) *Cordyceps sinensis* and its counterfeit species, by the use of proteomic techniques; This part also includes the molecular and microscopic identifications of species	✔	100%
2. To determine the mass and structural information of the biomarkers identified in point 1, by the use of matrix-assisted laser desorption / ionization time-of-flight mass spectrometer (MALDI-TOF MS)	✔	100%
3. To study the feasibility in direct analysis of CMM extracts by MALDI-TOF MS in authenticity testing	✔	100%
4. To validate the specificity, robustness and limit of detection of the testing method devised in point 3, and to design quality control plans for the method of routine analysis	✔	100%

6. Research Outcome

6.1 Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

This is the first comparative study of 2-DE profiles between the CMM groups of 1) *Flos Campsis* and *Flos Daturae Metelis* and 2) *Cordyceps sinensis* and its counterfeit species. The research team has tried using various protein extraction systems to optimize protein extraction yield. The research team also studied the effect of pre-wash and post-washing to optimize the extraction yield and quality of resulting 2-DE profile of lysis buffer extraction, we have studied the effect of TCA/acetone pre-washing and post-washing of sample in conjunction with lysis buffer extraction. Among the three protocols, lysis buffer extraction without any pre-washing and post-washing of sample was found to be the most effective method in extracting proteins from CMM samples and no significant difference in 2-DE profile quality.
Upon optimizing the extraction protocol, we have successfully compared the 2-DE profiles with the CMM samples and other similar species obtained in the market and the research team identified certain candidate protein marker spots distinct from with each other. These candidate protein marker spots were proved to be reproducible from independent source, demonstrating the feasibility of using protein markers as indicators for authentication of CMM.
The research team also isolated some of the intense protein spots from 2-DE profiles and to analysis by matrix-assisted laser desorption / ionization time-of-flight mass spectrometer. By comparing the mass spectra obtained, certain proteins at the mass range between 2,000 – 3,000 Da were identified to be potential protein markers.
The findings relating to protein extraction and 2-DE comparison for *Cordyceps sinensis* has been reported in the manuscripts mentioned in items #1 and #2 of Part C Section 8. Besides, an oral presentation of the findings for the protein extraction and 2-DE comparison has been presented in the 6th International Conference on Food Safety & Regulatory Measures (as stated in Part C Section 9). In addition, corresponding results of all findings have been reported in the M.Phil thesis of two research postgraduate students (CHAN Kin-ka and CHAN Chi-ho, corresponding to items #1 and #2 in Part C Section 10) who involved in this project.

6.2 Potential for further development of the research and the proposed course of action
(Maximum half a page)

The results and findings should aid future development of the use of proteomic approach to authenticate other CMM, if substantial protein content is found when extracted from such CMM. Besides, based on the mass analysis results from the isolated protein markers obtained from the 2-DE of *Cordyceps sinensis*, the research team may conduct further study on the identities of the isolated biomarkers and their corresponding amino acid sequence information. In case such amino acid sequences of the isolated biomarkers can be determined, we may be able to develop fast screening diagnostic kits by the producing antibodies and the application of enzyme-linked immunosorbent assay (ELISA) techniques.

7. Layman's Summary

(Describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

The increase in the use of Chinese herbal medicines is a global trend. In Hong Kong, Chinese medicines are not only widely used by the public, but also plays an important role in international trade. According to the Census and Statistics Department, the value of imports and re-exports of Chinese herbal medicines amounted to $3.15 billion and $1.12 billion respectively in 2016. Falsification or mix up of Chinese Material Medica (CMM) has become a big issue in quality and

safety. Examples have been reported such as the adulteration of falsified species in *Cordyceps sinensis*; or substitution of *Flos Campsis* with *Flos Daturae Metelis*.

The Department of Health (DH) has published eight volumes of the Hong Kong Chinese Materia Medica Standards (HKCMMS) since 2002, covering a total of 275 CMM. However, the HKCMMS do not cover *Cordyceps sinensis* and *Flos Campsis*. To address the questions, this project developed new test methods that can identifying between these species. Results of this project can be used by the Chinese medicine industry to effectively identify their purchased products, in order to ensure the products' quality and their brands' value.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project
(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Author(s) *(denote the corresponding author with an asterisk*)*	Title and Journal / Book *(with the volume, pages and other necessary publishing details specified)*	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this Report *(Yes or No)*	Acknowledged the Support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
		✔ reviewers requested for minor amendment		CHAN Chi-ho, LAM, Sai-wo, FONG, Long-yan, CHAN Dominic Tak-wah, LEE Fred, Wang-fat, SZE Eric Tung-po*	Optimization of Protein Extraction and Two-dimensional Gel Electrophoresis Profiles for Identification of Cordyceps sinesis and other Similar Species / PLOS ONE	**No**	**Yes [Attachment 1]**	**Yes**	**Yes (when published)**
		✔ reviewers requested for minor amendment		CHAN Kin-ka, KWOK Celia Sze-Nga, Eric SZE Tung-Po Sze, * and LEE Fred Wang-Fat*	Evaluation of the Use of Trizol-based Protein Extraction Approach for Gel-based Proteomic Analysis on Dried Seafood Products and Chinese Tonic Foods / International Journal of Molecular Sciences	**No**	**Yes [Attachment 2]**	**Yes**	**Yes (when published)**

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered
(Please attach a copy of each conference abstract)

Month / Year / Place	Title	Conference Name	Submitted to RGC *(indicate the year ending of the relevant progress report)*	Attached to this Report *(Yes or No)*	Acknowledged the Support of RGC *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
2017	Authentication of *Cordyceps sinesis* and other counterfeit species by two-dimensional gel electrophoresis	6th International Conference on Food Safety & Regulatory Measures	No	Yes [Attachment 3]	Yes	Yes

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

The research experience and capability built has been used extensively to both undergraduates' final year projects and postgraduate teachings. Besides, to transfer the knowledge to the Industry, a seminar with title of "Authenticity of Chinese Materia Medica" was organized with the Hong Kong Association for Testing, Inspection and Certification on 24 April 2018.

11. Student(s) Trained
(Please attach a copy of the title page of the thesis)

Name	Degree Registered for	Date of Registration	Date of Thesis Submission / Graduation
Chan Kin-Ka [Attachment 4]	Master of Philosophy	1 February 2014	11 January 2018 (Passed the viva)
Chan Chi-ho [Attachment 5]	Master of Philosophy	1 June 2015	Submit thesis in July 2018

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

With the use of research experience in the project, the Principal Investigator has some further collaborations with the industry to identify other Chinese Materia Medica and herbal supplements, such as *Dendrobium Officinale* vs *Dendrobium Devonianum* with some species with similar morphological features in the market.

13. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	**Reasons**
NIL	

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FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report - Attachment
(for completed projects only)

RGC Ref. No.:	UGC/FDS16/P02/14
Principal Investigator:	Dr SZE Eric Tung-po
Project Title:	Development of rapid testing methods for the authentication of Chinese Materia Medica (CMM) by mass spectrometry

Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (Please specify)
No. of outputs arising directly from this research project [or conference]	2	1			2 M.Phil Thesis