RGC Ref.: M-HKUST604/13
(please insert ref. above)

The Research Grants Council of Hong Kong
SRFDP & RGC ERG Joint Research Scheme
Completion Report

(Please attach a copy of the completion report submitted to the Ministry of Education by the Mainland researcher)

Part A: The Project and Investigator(s)

1. Project Title

Quality control and mechanism study of Guizhi-Fuling-Capsule, An ancient herbal formulation for primary dysmenorrhea, by a systemic biology approach

2. Investigator(s) and Academic Department/Units Involved

	Hong Kong Team	Mainland Team
Name of Principal Investigator *(with title)*	Prof. Karl WK TSIM	Prof. Ping LI
Post	Professor and Director	Professor and Director
Unit / Department / Institution	Division of Life Science, and Center for Chinese Medicine, HKUST, Hong Kong	State Key Laboratory of Natural Medicines, China Pharmaceutical University, China
Contact Information	botsim@ust.hk	liping2004@126.com
Co-investigator(s) *(with title and Institution)*	Dr. Guizhong XIN Research Associate Division of Life Science, and Center for Chinese Medicine, HKUST, Hong Kong	Prof. Huijun LI Professor State Key Laboratory of Natural Medicines, China Pharmaceutical University, China Prof. Xiaodong WEN Professor Pharmacognosy, School of Traditional Chinese Medicines, China Pharmaceutical University, China
PhD student(s) (with period of involvement)	Name: Xuan ZHENG (Zoey) Institution: Division of Life Science, and Center for Chinese Medicine, HKUST, Hong Kong	

	Period from Aug 2014 to Aug 2018	

Note: The Hong Kong project team must involve at least one research postgraduate student pursuing a Doctor of Philosophy degree at the UGC-funded university (PhD student) at any time throughout the project period.

3. Project Duration

	Original	Revised	Date of RGC/ Institution Approval *(must be quoted)*
Project Start date	01 Jan 2014		
Project Completion date	31 Dec 2016		
Duration *(in month)*	36		
Deadline for Submission of Completion Report	31 Dec 2017		

Part B: The Completion Report

5. Project Objectives

5.1 Objectives as per original application

1. To develop the chemical profiling of Guizhi-Fuling-Capsule (GFC) for quality control;
2. To reveal and identify the molecular targets of GFC in endometrium cell, uterine stromal cell, and uterine smooth muscle cell;
3. To develop a biological multi-target evaluation system and identify the active ingredients of GFC;
4. To delineate the integration mechanisms of GFC for the treatment of PD by the holistic approach.

5.2 Revised Objectives

Date of approval from the RGC: Nil

Reasons for the change: Nil

1.
2.
3. ....

6. Research Outcome

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

Objective 1: To develop the chemical profiling of GFC for quality control

Here, an analytical strategy combining two approaches was established to provide a global qualitative analysis of complex medicinal mixtures such as Guizhi Fuling capsule (GFC), which was investigated through collaborations.

One approach, tailored for volatile and semi-volatile compounds, was to use GC-MS with AMDIS. Using automated mass spectral deconvolution, 161 compounds within GFC were detected, but only 35 of them were tentatively identified using the AMDIS database, and another 13 compounds were identified by the manual search. Unfortunately, the rest of the unknown compounds was not able to identify because of relatively low abundance owing to the lack of matched spectra in the database and reference compounds. A total of 48 components, including four acetophenones, one alkaloid, two alkanes, two aromatic aldehydes, three fatty acid esters, one fatty aldehyde, two monoterpenes, one phenolic compound, 13 phenylpropanoids and 19 sesquiterpenes were identified in GFC.

The other approach, suitable for nonvolatile compounds, was to use RRLC-ESI-Q-TOF MS/MS. By virtue of the high resolution and high speed of RRLC and the accurate mass measurement of TOF/MS, a total of 70 components in GFC, including six acetophenones, 12 galloyl glucoses, 31 monoterpene glycosides, three phenols and 12 triterpene acids, were separated within 30 min, and identified or tentatively characterized after comparison with available references.

Objective 2: To reveal and identify the molecular targets of GFC in endometrium cell, uterine stromal cell and uterine smooth muscle cell

The etiology of primary dysmenorrhea (PD) is not precisely understood, but most of the symptoms can be explained by the action of uterine prostaglandins. Cyclooxygenase (COX) is traditionally believed to be the major generator of the prostaglandins. Pharmacological inhibition of COX could provide relief from the symptoms of inflammation and pain. Two isoforms of COX have been identified: COX-1 and COX-2. The mRNA levels of the two isoforms of COX, COX-1 and COX-2, were determined in cultured human umbilical vein endothelial cells (HUVECs). HUVECs were well established for studying primary dysmenorrhea by others. The GFC samples were provided by our collaborator: Prof. Ping LI's group. The cells were applied with different concentrations of GFC for 48 hours. Our results showed that the application of GFC could significantly down-regulate the mRNA levels of COX-1 and COX-2 in dose- and time-dependent manners. These results suggested that GFC possessed the ability to relief the symptoms of PD.

Prostaglandin 2α ($PGF_{2\alpha}$) is responsible for the control of a myriad of essential biological processes such as pain, inflammation, menstruation and constriction of blood vessels. $PGF_{2\alpha}$ is derived from arachidonic acid and transformed by prostaglandin synthetase into a number of structurally related carbocyclic molecules. According to literatures, $PGF_{2\alpha}$ could be produced directly from prostaglandin H via prostaglandin F synthase (PGF synthase). The mRNA level of PGF synthase was determined after the application of different concentrations of GFC in cultured HUVECs for 48 hours. The mRNA level of PGF synthase was significantly decreased after the application of GFC in HUVECs in a dose-dependent manner, which suggested that GFC possessed the ability to regulate essential biological processes such as pain.

Objective 3: To develop a biological multi-target evaluation system and identify the active ingredients of GFC

Dissolution is a vital first step when medicinal drugs are taken in the form of capsules. Rate of dissolution is an important property of a medicine as it indicates how quickly the drug in a formulation is released in the body and made available for absorption. The effectiveness of capsule relies on the drug dissolving in the fluids of gastrointestinal tract prior to absorption into systemic circulation. The rate of dissolution of GFC capsule is therefore crucial. After the dissolution analysis of GFC, 9 chemicals were identified: paeoniflorin, amygdalin, paeonol,

gallic acid, benzoylpaeoniflorin, cinnamic aldehyde, cinnamic acid, benzoic acid and pachymic acid. These chemicals were separated by our collaborator: Prof. Li's group. As previous described, the mRNA level of PGF synthase, COX-1 and COX-2 were determined after the application of the 9 chemicals mentioned above in cultured HUVECs for 48 hours. Paeoniflorin, amygdalin, paeonol, benzoylpaeoniflorin, cinnamic aldehyde, cinnamic acid, benzoic acid and pachymic acid could significantly decrease the mRNA level of PGF synthase, COX-1 and COX-2; these results indicated that these chemicals are the active ingredients in GFC.

The application of GFC in HUVECs could down regulate the mRNA expressions of COX-1 and COX-2. Nine major components from GFC were tested in inflammatory system, and three compounds, including paeoniflorin, benzoylpaeoniflorin and amygdalin, exhibited robust activation of COX-1 and COX-2 expressions in HUVECs. The combination of paeoniflorin, benzoylpaeoniflorin and amygdalin, showed over 80% of the anti-inflammatory activation. Our study supports that GFC showed a promising role in anti-dysmenorrhea function by decreasing COX expression. Besides, paeoniflorin, benzoylpaeoniflorin and amygdalin could be considered as the major regulators for the anti-dysmenorrhea effects of GFC.

Objective 4: To delineate the integration mechanisms of GFC for the treatment of PD by the holistic approach.

Using UPLC-MS/MS-based metabonomics method, we identified the metabolic profiles of blood stasis (BS)- and uterine hypercontractility (UC)-based PD models. We successfully clarified the linkage between animal-based model and PD. Both models altered the levels of glycerophospholipid, biosynthesis of unsaturated fatty acids, steroid hormone biosynthesis, amino acid metabolism and TCA cycle. Using metabolomics approach, these PD models had also been utilized to reveal the potential action mechanism of GFC. As a result, four altered metabolites for UC model and eleven for BS model were successfully restored back to the control-like level after GFC treatment. Interestingly, our findings confirmed the metabolic profiles of UC and BS bio-samples, suggesting similar metabolic pathways in the pathogenesis of PD. However, different dosage regimens resulted in different reversal effect on the altered endogenous metabolites. Based on our results, we conjectured that GFC ameliorated PD depending on a long-term administration. Further studies will be conducted to demonstrate this conjecture, and to clarify the potential action mechanism of GFC.

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

Guizhi Fuling capsule (GFC) is a famous and effective Chinese herbal formula utilized in the treatment of gynecological blood stagnation such as dysmenorrhea, oophoritic cyst and endometriosis.Through analyzing metabolic alterations after the intervention by GFC, its functioning pathway could be predict. The study of potential action mechanism of GFC could provide valuable data for clinical trial progress in drug development for the treatment of PD.

7. The Layman's Summary

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

Dysmenorrhea, a common gynaecological symptom experienced by 50% of all menstrual women, can be divided into primary dysmenorrhea (PD) and secondary dysmenorrhea.

Among them, PD is a more common gynecologic complaint, which torments adolescent girls and women of reproductive age greatly. Clinically, Guizhi-Fuling Capsule (GFC) has been successfully used in the therapy of gynecological diseases, including PD and endocrine disorders with a great success. In 2007, the US FDA approved the phase II clinical trial of GFC against PD. However, the clinical usages of GFC in treating this disorder are still greatly hindered due to the deficiency of quality control of the herbal extract, as well as the limited knowledge of its action mechanism.

This project is aiming to establish a systems biology approach in finding multi-target bioactive compounds and action mechanism of GFC for the treatment of PD, which will benefit for the further development of this ancient herbal formula. Besides, the study will not only provides sufficient scientific data support for GFC phase II clinical trials approved by the US FDA, but also promotes the internationalization process of traditional Chinese medicines (TCMs).

Part C: Research Output

8. Peer-reviewed journal publication(s) arising directly from this research project

(Please attach a copy of each 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) *(**bold** the authors belonging to the project teams and 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 this Joint Research Scheme *(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)*						
2016				Miernisha A, Bi CW, Cheng LK, Xing JG, Liu J, Maiwulanjiang M, Aisa HA, Dong TT, Lin H, Huang Y, **Tsim KWK***	Badiranji Buya Keli, a Traditional Uyghur medicine, induces vasodilation in rat artery: signaling mediated by nitric oxide production in endothelial cells/Phytotherapy Research, 2016, 30(1):16-24	2015	Yes	Yes (Page 23)	Yes

2016				Lau KM, Gong AGW, Xu ML, Lam CTW, Zhang LML, Bi CWC, Cui D, Cheng AWM, Dong TTX, **Tsim KWK**, Lin H*	Transcriptional activity of acetylcholinesterase gene is regulated by DNA methylation during C2C12 myogenesis/ Brain Res, 2016, 1642:114-123.	2017	Yes	Yes (Page 122)	Yes
2016				Lam CT, Gong AG, Lam KY, Zhang LM, Chen JP, Dong TT, Lin HQ, **Tsim KW***	Jujube-containing herbal decoctions induce neuronal differentiation and the expression of anti-oxidant enzymes in cultured PC12 cells/ J Ethnopharmacol, 2016, 188:275-283	2017	Yes	Yes (Page 282)	Yes
2016				Gong AG, Lau KM, Xu ML, Lin HQ, Dong TT, Zheng KY, Zhao KJ, **Tsim KW***	The estrogenic properties of Danggui Buxue Tang, a Chinese herbal decoction, are triggered predominantly by calycosin in MCF-7 cells/ J Ethnopharmacol, 2016, 189:81-89	2017	Yes	Yes (Page 88)	Yes

2016				Xiong A, Yan AL, Bi CW, Lam KY, Chan GK, Lau KK, Dong TT, Lin H, Yang L, Wang Z, **Tsim KW***	Clivorine, an otonecine pyrrolizidine alkaloid from Ligularia species, impairs neuronal differentiation via NGF-induced signaling pathway in cultured PC12 cell/Phytomedicine, 2016, 23(9):931-938	2017	Yes	Yes (Page 938)	Yes
2016				Lou JS, Yan L, Bi CW, Chan GK, Wu QY, Liu YL, Huang Y, Yao P, Du CY, Dong TT, **Tsim KW***	Yu Ping Feng San reverses cisplatin-induced multi-drug resistance in lung cancer cells via regulating drug transporters and p62/TRAF6 signalling /Sci Rep, 2016, 6:31926. doi: 10.1038/srep31926.	2017	Yes	Yes (Page 14)	Yes
2016				Yan L, Hu Q, Mak MS, Lou J, Xu SL, Bi CW, Zhu Y, Wang H, Dong TT, **Tsim KW***	A Chinese herbal decoction, reformulated from Kai-Xin-San, relieves the depression-like symptoms in stressed rats and induces neurogenesis in cultured neurons/Sci Rep, 2016, 6:30014. doi: 10.1038/srep30014.	2017	Yes	Yes (Page 14)	Yes

2016				Lam KY, Chen J, Lam CT, Wu Q, Yao P, Dong TT, Lin H, **Tsim KW***	Asarone from Acori Tatarinowii Rhizoma Potentiates the Nerve Growth Factor-Induced Neuronal Differentiation in Cultured PC12 Cells: A Signaling Mediated by Protein Kinase A/PLoS One, 2016, 11(9):e0163337. doi: 10.1371	2016	Yes	Yes (Page 17)	Yes
2016				Lam KY, Ku CF, Wang HY, Chan GK, Yao P, Lin HQ, Dong TT, Zhang HJ, **Tsim KW***	Authentication of Acori Tatarinowii Rhizoma (Shi Chang Pu) and its adulterants by morphological distinction, chemical composition and ITS sequencing/ Chin Med, 2016, 11:41 DOI 10.1186/s13020-016-0113-x	2017	Yes	Yes (Page 10)	Yes
2016				Gong AG, Zhang LM, Lam CT, Xu ML, Wang HY, Lin HQ, Dong TT, **Tsim KW***	Polysaccharide of Danggui Buxue Tang, an Ancient Chinese Herbal Decoction, Induces Expression of Pro-inflammatory Cytokines Possibly Via Activation of NFκB Signaling in Cultured RAW 264.7 Cells/Phytother Res, 2017, 31(2):274-283.	2017	Yes	Yes (Page 282)	Yes

2016				Gong AG, Huang VY, Wang HY, Lin HQ, Dong TT, **Tsim KW***	Ferulic Acid Orchestrates Anti-Oxidative Properties of Danggui Buxue Tang, an Ancient Herbal Decoction: Elucidation by Chemical Knock-Out Approach/PLoS One, 2016, 11(11):e0165486. doi: 10.1371/journal.pone.0165486	2017	Yes	Yes (Page 13)	Yes
2017				Gong AGW, Wang HY, Dong TTX, **Tsim KWK**, Zheng YZ*	Danggui Buxue Tang, a simple Chinese formula containing Astragali Radix and Angelicae Sinensis Radix, stimulates the expressions of neurotrophic factors in cultured SH-SY5Y cells/Chin Med, 2017, 12:24. doi: 10.1186/s13020-017-0144-y.	2017	Yes	Yes (Page 7)	Yes
		2017		Yu-zhong Zheng, **Gui-Zhong Xin**, Ping Yao, Kelly YC Lam, Amy GW Gong, Lu Yan, Tina Ting-Xia Dong, **Karl WK Tsim**, **Ping Li***, Li-Fang Liu*	Guizhi Fuling Capsule, an ancient Chinese herbal formula, induces inflammatory response in human umbilical vein endothelial cells: identification of active components / Journal of chromatography B	2017	Yes	Yes (Page 13)	No

		2017		**Gui-Zhong Xin**, Dan-Dan Wang, Rui Li, Jian-Qun Liu, Tina Ting-Xia Dong, **Karl WK Tsim**, **Ping Li***, Li-Fang Liu*	Distinct metabolic profiles of two models of primary dysmenorrhea and the effects of Guizhi Fuling capsule intervention / Journal of chromatography B	2017	Yes	Yes (Page 12)	No

9. Recognized international conference(s) in which paper(s) related to this research project was/were delivered *(Please attach a copy of each delivered paper. All listed papers must acknowledge RGC's funding support by quoting the specific grant reference.)*

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 this Joint Research Scheme *(Yes or No)*	Accessible from the institutional repository *(Yes or No)*
Aug/2015/ Hong Kong	The estrogenic properties of Danggui Buxue Tang, a Chinese herbal decoction, are triggered predominantly by calycosin in MCF-7 cells	2015 11th International Postgraduate Symposium on Chinese Medicine	2015	No	Yes (In the poster)	No
Aug/2015/ Hong Kong	Chemical and biological studies of Chinese date decoctions	2015 11th International Postgraduate Symposium on Chinese Medicine	2015	No	Yes (In the poster)	Yes
Aug/2015/ Hong Kong	Establishment of quality control standard for edible bird's nest by monoclonal antibodies and fingerprint	2015 11th International Postgraduate Symposium on Chinese Medicine	2015	No	Yes (In the poster)	Yes

Aug/2015/ Hong Kong	Protective functions of flavonoids in nervous system via EPO regulation	2015 11th International Postgraduate Symposium on Chinese Medicine	2015	No	Yes (In the poster)	Yes

10. 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
ZHENG Xuan	MPhil/PhD	20 Aug 2014	2018
LOU JianShu	PhD	1 Feb 2015	2018

11. Other impact *(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, etc.)*

This project collaborates with the former post-doctoral staff (Prof. Yuzhong ZHENG (Hanshan Normal University, Guangdong) and Dr. Guizhong XIN (China Pharmaceutical University, Nanjing) to establish the quality control parameters and the mechanistic studies of the active ingredients. Some potential active ingredients in GFC have been identified as the major regulators for the anti-dysmenorrhea effects. The results and findings have been applied to the quality control system in the production process as well as supporting evidence for drug registration.