Good Practice in Traditional Chinese Medicine Research in the Post-genomic Era

GP-TCM

223154

D.1.8

Identify availability of vouchered collections of different species used in TCM, along with DNA samples and chemical fingerprints associated with different processing and extracting methods, associated substitutes and adulterants

Document description	
Name of document	Identify availability of vouchered collections of different species used in TCM, along with DNA samples and chemical fingerprints associated with different processing and extracting methods, associated substitutes and adulterants
Abstract	This report provides an overview of the information available about vouchers, DNA samples, distribution, processing methods and Good Agricultural Practices for the plants used in the CHM formula Liu Wei Di Huang Wan.
Document identifier	D1.8
Document class	Deliverable
Version	1.0
Author(s)	Monique Simmonds (Kew) et al.
Date of creation	15/11/2011
Date of last modification	4/05/2012
Status	Final
Destination	European Commission
WP number	WP1

TABLE OF CONTENTS

1 WP1 - Identification of samples of species of plants (D1.8)

1.1 Introduction

The overall objectives of WP1 are to:

- Design a standard system for the quality control of Chinese herbal medicines (CHM) in the EU
- Design a system that will provide greater transparency about the complexity of TCM nomenclature, thus clarifying plant identities and improving data gathering on CHM species
- Propose guidelines as a basis for the rational development of research methods to authenticate and monitor the quality of TCM plants entering the trade. Guidelines will aim to avoid duplication of research efforts and maximise the use of modern molecular and chemical techniques, especially a functional genomic approach.

1.2 Documentation about medicinal plants

The aim of this report is to identify the availability of vouchered collections of different species used in TCM, along with DNA samples and chemical fingerprints associated with different processing and extracting methods, associated substitutes and adulterants.

The previous reports from WP 1 (D1.4, D1.6 and D1.7) as well as WP 2 (D2.10) provided information about the scope of information that the WPs considered should be provided to assist define the quality of plants being used in traditional medicine. From the data so far gathered it is clear that a lot more research needs to be undertaken if scientific evidence is to be presented that supports the traditional uses of plants. Although in many cases there are many references in the literature about the medicinal uses of specific species it is often very difficult to relate the information in these papers to the original use of the plant. This is because in some cases it is unclear which species the authors used, what part of plant was studied and whether the extraction method used relates back to the way the plant material would have been prepared traditionally. In addition, very few biological papers provide information about the chemistry (chemical fingerprint) of the material they are testing thus the conclusions from a comparative analysis amongst papers from different laboratories is at best very tentative.

At the 2010 annual meeting of the GP-TCM at Henley, Oxfordshire UK a list of the 50 most commonly used plants in traditional Chinese medicines was presented, along with the number of references for each species obtained using search engines such as Medline. The number of references was over 20,000. It would have been a very large task to go through these references to identify whether they contained information that would further our knowledge about the quality control of each species with respect to the traditional use of the plant. An outcome of the above GP-TCM was to concentrate on gathering information about the 5 plants and one fungal species that are used in the traditional Chinese formula, Liu Wei Di Huang Wan. Table 1 provides an overview of the references available when using the Latin binomial name and traditional Chinese medicine. A greater number of references are obtained from PubMed and Google Scholar when the pharmaceutical name is used. However, these references often relate to publications associated with general screening tests and do not further the knowledge about the traditional uses of the plants.

Table 1: Overview of the number of references available about the species of plants and fungi contained in the traditional Chinese formula Liu Wei Di Huang Wan.

Latin scientific name(s) as stated in the Pharmacopoeia of the PRC (English eds.)	Accepted* Latin scientific name'	Web of science	PubMed	Google Scholar
Alisma orientale (Sam.) Juzep. (note different and incorrect spelling in the 2005 ed. of the Chinese Pharmacopoeia *'Alisma orientalis* (Sam.) Juzep.')	*Alisma orientale* (Samuelsson) Juzepczuk	*37*	*24*	*>2,000 of which less than about 300 contain relevant information (information about use and chemistry)*
Cornus officinalis Sieb. et Zucc.	*Cornus officinalis* Siebold & Zuccarini	*98*	*90*	*>6,000 of which less than about 500 contain relevant information*
Dioscorea opposita Thunb.	*Dioscorea polystachya* Turczaninow	*107*	*32*	*>3,000 of which less than 250 contain relevant information*
Paeonia suffruticosa Andr.	*Paeonia ostii* T. Hong & J. X. Zhang	*150*	*97*	*>4,000 of which less than 300 contain relevant information*
Poria cocos (Schw.) Wolf.	*Wolfiporia extensa* (Peck) Ginns	*220*	*141*	*>4,000 of which less than 300 contain relevant information*
Rehmannia glutinosa Libosch.	*Rehmannia glutinosa* (Gaertner) Liboschitz ex Fischer & C. A. Meyer	*174*	*254*	*>4,000 of which less than 300 contain relevant information*
Liu Wei Di Huang Wan		*7*	*27*	*48*

The publications referred to in Table 1 are still currently being reviewed for their scientific data that could contribute to our understanding of the quality control of the plants. However, the information collated so far it is clear that there is very little published data on the comparative chemical data that would enable the detection of common substitutes and adulterants of the species. There is also very little information about the profile of compounds in material that is considered to be good quality. However, in some species there are papers on what are considered to be the active compounds but it is not always clear from the publications how this

information links to the traditional uses of the plants. To date the best source of basic information about the quality control of the selected plants is found in the respective pharmacopoeias, but they do not contain the comparative chemical and quality information needed to assist with the development of traditional Chinese medicines.

An issue for many researchers working on the traditional Chinese plants is obtaining authenticated material. To collate data presented in Table 2, contact was made with some of the laboratories in China and Europe to identify whether they had verified samples of the selected species as well as verified samples used to obtain DNA data. Searches were undertaken of different DNA databases but it was decided to use the National Center for Biotechnology Information database GenBank, as most journals would expect DNA sequence data about a species to be lodged on this database before publication. Information was also obtained from organisations in China as to the main areas within China that were growing the plants used in Liu Wei Di Huang Wan and whether they were been grown under Good Agricultural Practices. Table 2 provides a summary of the data obtained. More information about the chemistry of the plants as well as the biological activity of the plants is presented in the reports of WP2 and WP4.

Table 2: Sources for scientific data of "Liu Wei Di Huang Wan"

Taxonomically 'accepted' name [Chinese and Pin Yin names]	**Harvest time**	**Methods of process**	**Distribution / Location in China**	**TCM production: Cultivation or Wild collection**	**Voucher specimen in China & Europe**	**GAP monograph availability?** (SFDA approved GAP TCM producer)	**GenBank data**
***Alisma orientale* (Samuelsson) Juzepczuk** [泽泻 'Ze Xie' or 'Zexie']	Harvest in late Dec. [i]	1. Dry with gentle heating, then remove roots and peel, slice rhizome and dry. 2. Soak dry slice with salty water then fry to dryness[ii].	Cultivated mainly in Sichuan, Fujian, Jiangxi provinces, China[i].	GAP production site in Fujian, China (福建省建瓯市吉阳镇Jiyang, Jianou City, Fujian) [iii]	1. RBGK (TCMK 213); 2. Herbarium, Guangxi Institute of Botany, CAS, Yanshan, Guilin, Guangxi, China (IBK-187178) 3. Xi'an Botanical Garden Herbarium (XBGH: 009579)	Yes. (福建农林大学工业原料林研究所 Fujian Agriculture And Forestry University Forestry College) [iv]	9 accessions of nucleotide sequences for
***Cornus officinalis* Siebold & Zuccarini** [山茱萸 'Shan Zhu Yu' or 'Shanzhuyu']	High production reached in 15 years after planted. Fruits were harvested in Sept. to Nov.	1. Some procedures require removal of seeds from fresh fruit before the pulp is dried for use as TCM. 2. The pulp is boiled with yellow rice wine and dried. 3. The pulp is steam cooked then dried [v]	Grows in forest or the edge of forest between altitude 400-1500m, found in Shanxi, Jiangsu, Zhejiang, Anhui, Jiangxi, Shandong, Henan, Hunan, Sichuan, Shaanxi, Gansu provinces in China. Mainly cultivated in Henan, Shaanxi and Zhejiang provinces, China [v]	GAP production site in Anhui and Henan, China(河南省西峡县Xixia county, Henan) [iii].	1. RBGK (TCMK 108, 299, 359, 888);(KEW LCD: 1992-1476) 2. Institute of Medicinal Plant Development (IMPLAD, Beijing): voucher PS1270MT02[vi]; 3. Xi'an Botanical Garden Herbarium (XBGH: 004781) 4. Pharmacy College, Henan University of Traditional Chinese Medicine, Jinshui Road No.1, Zhengzhou, Henan 450008, China (voucher chensq 040815); 5. Plant Biology, North Carolina State University, 2115 Gardner Hall, Raleigh, NC 27695-7612, USA (voucher Xiang 918-85A)	Yes. 安徽地道药材山茱萸、祁术等GAP规范种植示范基地[vii]	18 accessions of nucleotide sequences
***Dioscorea polystachya* Turczaninow** [山药 'Shan Yao' or 'Shanyao']	Harvest in Dec.	1. Clean the rhizome, soak with water, slice and dry when it become soft. 2. Dry fry the slices of the rhizome until yellow. 3. Dry fry with wheat bran until yellow and then	Widely distributed in China, grows on mountain slope, forest, riverside, roadside.[viii]	GAP production site in Henan, China (河南省武陟县西陶镇、大封镇，温县武德镇、招贤乡) [iii]	1. RBGK (TCMK 93); 2. Institute of Plant Protection, Henan Academy of Agricultural Sciences, 1 Nongye Road, Zhengzhou, Henan 450002, China (Xiangningxianshanyao)[ix].	Yes. (南阳张仲景中药材发展有限责任公司 Nanyang Zhongjing medicines Development Co.,	66 accessions of nucleotide sequences for *Dioscorea polystachya*

Taxonomically 'accepted' name [Chinese and Pin Yin names]	**Harvest time**	**Methods of process**	**Distribution / Location in China**	**TCM production: Cultivation or Wild collection**	**Voucher specimen in China & Europe**	**GAP monograph availability?** (SFDA approved GAP TCM producer)	**GenBank data**
		remove bran. 4. Dry fry with clay collected from the chamber of the Chinese cookstove, fry until yellow. 5. Dry fry with rice until the rice is yellow. 6. Fry with wheat bran and honey to dryness and the material is yellow.[viii]			3. Xi'an Botanical Garden Herbarium (XBGH: 005852)	Ltd.)	
***Paeonia ostii* T. Hong & J. X. Zhang** [牡丹皮 'Mu Dan Pi' or 'Mudanpi']	Grow 3-6 years, harvest in Sept. or Oct.[x]	1. Clean with water, cut and dry. 2. Fry with yellow rice wine to dryness. 3. Dry fry to yellow colour 4. Dry fry to dark colour [x]	Cultivated mainly in Sichuan, Hunan, Hubei, Shaanxi, Shandong, Gansu, Guizhou and Anhui provinces, China	GAP production site in Anhui, China (安徽省南陵县何湾镇龙山村Nanling county, Anhui province)[iii]	1. RBGK (TCMK 308, 530, 540, 609); 2. IMPLAD; 3. State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Beijing.	Yes. [安徽省地道药材牡丹皮GAP规范种植示范基地Di Dao TCM "Mu Dan Pi" GAP cultivation centre in Anhui.][xi]	181 accessions of nucleotide sequences for *P. suffruticosa* 7 accessions for *P. ostii*
***Wolfiporia extensa* (Peck) Ginns** [茯苓 'Fu Ling' or 'Fuling']	Harvest after 8-10 months growth.	Clean with water, steam cook then slice and dry[xii].	Grow on the root of pine trees. Found in Jilin, Zhejiang, Anhui, Fujian, Henan, Hubei, Guangxi, Sichuan, Guizhou, Yunnan, Taiwan[xiii].	Production site in Anhui and Hubei, China[xiv]	1. RBGK (TCMK 919); 2. Graduate School of Natural Science and Technology, Kanazawa University, Kakuma, Kanazawa, Ishikawa 920-1192, Japan. 3. Plant molecular biology and evolution (D207) Institution: Department of Life Science, National Taiwan Normal University, Taiwan.	IMPLAD confirmed that there was no SFDA certified GAP producer / supplier of茯苓 'Fu Ling' in China. (Email from Prof. Lin Yu Lin on 09-02-2012)	14 accessions of nucleotide sequences for *Poria cocos* 186 accessions for *Wolfiporia cocos* (no data for *Wolfiporia extensa*)

Taxonomically 'accepted' name [Chinese and Pin Yin names]	**Harvest time**	**Methods of process**	**Distribution / Location in China**	**TCM production: Cultivation or Wild collection**	**Voucher specimen in China & Europe**	**GAP monograph availability?** (SFDA approved GAP TCM producer)	**GenBank data**
***Rehmannia glutinosa* (Gaertner) Liboschitz ex Fischer & C. A. Meyer** [熟地黄 'Shu Di Huang' or 'Shudihuang']	Harvest in Oct. or Nov.	1. Boiled with yellow rice wine 2. Steam cook 3. Steam cook with ginger, spirit and Fructus Amomi. 4. Cook with spirit and Fructus Amomi for 24hrs. 5. Dry fry to dark colour [xv]	Wide plant grows between altitude 50-1100m, found in Hebei,Shanxi, Neimenggu, Liaoning, Zhejiang, Jiangsu, Anhui, Shandong, Henan, Hubei, Hunan and Shaanxi provinces.[xv]	GAP production site in Henan, China (河南省武陟县、温县,Wuzhi and Wen county, Henan province) [iii]	**1. RBGK (TCMK 92, 307);** 2. IMPLAD, Beijing (voucher PS1518MT01); 4. Herbarium, Guangxi Institute of Botany, CAS, Yanshan, Guilin, Guangxi, China (IBK-109785) 3. Xi'an Botanical Garden Herbarium (XBGH: 03057, 02539, 003817, 13396) 4. Institute of Plant Protection, Henan, Academy of Agricultural Sciences (cultivar Dihuang85-5 x Maye).	Yes. (南阳张仲景中药材发展有限责任公司 Nanyang Zhongjing medicines Development Co., Ltd.)	96 accessions of nucleotide sequences for *Rehmannia*

[i] Zhao, G. P.; Dai, S.; Chen, R. S. Zhong Yao Da Ci Dian. 2nd ed.; Shanghai scientific & Technical Publishers: Shanghai, 2006; Vol. 1&2. p2066

[ii] Pharmacopoeia of the People's Republic of China. Chemical Industry Press: Beijing, 2005; Vol. 2, p158

[iii] Information of certified GAP production sites for more than 50 TCM plants is available on SFDA website URL：http://www.sda.gov.cn

[iv] News on website of The Ministry of Science and Technology of the People's Republic of China[福建省中药材GAP示范基地建设取得突出成效16-12-2010]由福建农林大学工业原料林研究所承担的福建省科技重大专项“福建中药材GAP技术平台及示范基地建设”专题“福建省中药材GAP示范基地建设”通过了省科技厅组织的专家验收。完成了泽泻、太子参、南方红豆杉等8种中药材GAP生产示范基地现场实验室的建设。URL: http://www.most.gov.cn/dfkj/fj/zxdt/201012/t20101216_83803.htm

[v] Zhao, G. P.; Dai, S.; Chen, R. S. Zhong Yao Da Ci Dian. 2nd ed.; Shanghai scientific & Technical Publishers: Shanghai, 2006; Vol. 1&2. p246

[vi] Genbank information. URL: http://www.ncbi.nlm.nih.gov/nuccore/GQ435328.1

[vii] Website of science and technology department of Anhui province.
安徽省科学技术厅信息公告：中药现代化项目:
安徽地道药材山茱萸、祁术等GAP规范种植示范基地,
完成单位:神鹿集团九华药业有限责任公司URL: http://www.ahinfo.gov.cn/docs09/kjgk/kjgc/kjly.htm

[viii] Zhao, G. P.; Dai, S.; Chen, R. S. Zhong Yao Da Ci Dian. 2nd ed.; Shanghai scientific & Technical Publishers: Shanghai, 2006; Vol. 1&2. p217

[ix] Genbank information. URL: http://www.ncbi.nlm.nih.gov/nuccore/FJ860114.1

[x] Zhao, G. P.; Dai, S.; Chen, R. S. Zhong Yao Da Ci Dian. 2nd ed.; Shanghai scientific & Technical Publishers: Shanghai, 2006; Vol. 1&2. p1576

[xi] Website of science and technology department of Anhui province.
安徽省科学技术厅信息公告：中药现代化项目：安徽省地道药材牡丹皮GAP规范种植示范基地，合作完成单位：安徽中医学院药学院;安徽技术师范学院;芜湖生物药业研发中心; URL: http://www.ahinfo.gov.cn/docs09/kjgk/kjgc/kjly.htm

[xii] Pharmacopoeia of the People's Republic of China. Chemical Industry Press: Beijing, 2005; Vol. 2, p166

[xiii] Zhao, G. P.; Dai, S.; Chen, R. S. Zhong Yao Da Ci Dian. 2nd ed.; Shanghai scientific & Technical Publishers: Shanghai, 2006; Vol. 1&2. p2152

[xiv] News on Chinese website URL: http://www.chinapharm.com.cn/html/hyyw/23375420041202.html

[xv] Zhao, G. P.; Dai, S.; Chen, R. S. Zhong Yao Da Ci Dian. 2nd ed.; Shanghai scientific & Technical Publishers: Shanghai, 2006; Vol. 1&2. p 3611 and 3704

1.3 Conclusion

Despite the wide use within China of a formula such as Liu Wei Di Huang Wan there is very little information in the western scientific literature that would enable scientists to know the chemical profile associated with good quality plant/fungal material. For the species used in LIU Wei Di Huang Wan there are no available chemical-fingerprint that indicate that this is the chemical profile associated with "quality" or a defined use of the species. This project has illustrated that it is not an easy task to undertake an international overview of all the literature on a small selection of plants. However, over the last three years the amount of information about the plants is increasing, especially from Chinese scientists. It is, however, clear that there are opportunities to take a genomic approach using different chemical and molecular (DNA) analytical techniques to further our understanding about the quality of plants to be used in traditional Chinese medicines.

In this short report we make use of the term taxonomically "accepted name". This is the binomial nomenclature name that has been "agreed" within the botanical community to be the name for a species, that indicates the genus it belongs to and the name for the specific species and the authority (person) that named the species. The application of the scientific Latin binomial name used by taxonomists is governed by the International Code of Nomenclature for Algae, Fungi and Plants (ICN). Other Latinised names are used in the pharmacopoeias but these are not the names used to identify a specific species as a pharmaceutical name can cover more than one species. The project has illustrated the need to get greater clarity on the names of the species being used in TCM to assist link the wealth of information available on the plants. For example, there are many studies that report chemical and biological data about plants the data could be from the correct species but the authors provide no evidence to confirm the identification of the material they have used. Another issue that became very clear when reviewing the literature is that very few authors provide information about how the plant material they are studied has been processed and this is very important to further our understanding of the chemistry of plants as used in TCM.

The availability of the Flora of China provides information about the names of most of the species and these Latin binomial names can be linked to the pharmaceutical names and PinYin names in the Chinese Pharmacopeia. Within China and Europe there are taxonomically verified samples that can be used to assist scientists verify their samples before starting experiments. The fact that the majority of samples are cultivated and being grown under Good Agricultural Practises would indicate that it would be possible to get material from these sources for experimental work.

The next deliverable for this WP will be to bring the botanical, chemical and biological data associated with quality control together so that it is clear as to the main gaps in our knowledge about the quality of the plants and identify the next steps required. This report should be read along side the reports from WP2 and WP4 that provide more information about the chemistry and in-vitro studies on the biological activity of the plants, respectively.

Those members and associated of WP1 and WP2 that have assisted in helping to collate the data presented in this report.

Valerian Bunel (for Pierre Duez, WP1)
Alberto Dias (WP1, WP2)
Rui Fang (WP1)
Svetlana Ignatova (WP2)
Christine Leon (WP1)
Andreas Marmann (WP2)
Jandirk Sendker (WP2)
Monique Simmonds (WP1, WP2)

Ian Sutherland (WP1)
Rob Verpoorte (WP1)
You-Ping Zhu (WP1)

WP1 Contact:

Prof. Monique S. J. Simmonds
Director, Kew Innovation Unit
Deputy Keeper, Jodrell Laboratory
Royal Botanic Gardens, Kew
Richmond
Surrey
TW9 3AB
UK
Tel: +44 208 332 5328
Fax: +44 208 332 5340
E-mail: M.Simmonds@rbgkew.org.uk