



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

GP-TCM

D2.14

**Final report including complete database of experts and
distribution of skills**



Document description	
Name of document	D2.14 – Final report including complete database of experts and distribution of skills
Abstract	Final report including complete database of experts and distribution of skills (internal use only)
Document identifier	D2.14
Document class	Deliverable
Version	1.0
Author(s)	Dr. Jandirk Sendker
Date of creation	26/05/2012
Date of last modification	26/05/2012
Status	Final
Destination	Coordination Office
WP number	WP2

1 OBJECTIVES OF WP2

- Describe and evaluate traditional extraction methodologies for the preparation of Chinese Herbal Medicines
- Describe problems connected with traditional extraction techniques that can potentially be solved by the application of modernised extraction techniques
- Describe and evaluate current approaches for the modernisation of Chinese Herbal Medicines
- Describe and evaluate analytical methods used for the fingerprint analysis of herbal extracts
- Describe and evaluate methods of multivariate statistics that can be applied for the identification of pharmacologically relevant components and/or marker substances that are indicative for a products quality and/or processing status
- Describe and evaluate techniques that aid the isolation and unambiguous identification of pharmacologically relevant components and marker substances

2 BRIEF HISTORY OF WP2 MEMBERSHIP AND LEADERSHIP DEVELOPMENT

2.1 WP2 members at the time of proposal submission

- Peter Proksch (Germany): Coordinator
- Brigitte Kopp (Austria): Deputy Coordinator
- Jandirk Sendker (Germany): Assistant Coordinator
- Gabriele König (Germany): Beneficiary Member
- Helen Sheridan (Ireland): Beneficiary Member
- Liselotte Krenn (Austria): Beneficiary Member
- Werner Knoess (Germany): Beneficiary Member
- Monique Simmonds (UK): Beneficiary Member
- Yanjiang Qiao (China): Beneficiary Member
- Alberto Dias (Portugal): Beneficiary Member
- Kirsi-Marja Oksman (Finland): Beneficiary Member

2.2 Membership changes of WP2 during the first year

- Xinmiao Liang (China) becomes non-beneficiary member.
- Derek Fisher (UK) becomes non-beneficiary member.
- Wenhan Ling (China) becomes non-beneficiary member.
- Kirsi-Marja Oksman (Finland) leaves the consortium.
- Ren-wang Jiang (China) becomes non-beneficiary member.
- Mirko Bayer (Germany) is appointed as local assistant of Peter Proksch.
- Kirsten Knapp (Germany) is appointed as local assistant of Gabriele König.
- Ingrid Hook (Ireland) becomes beneficiary member.
- Annika Orland (Germany) is appointed as local assistant of Gabriele König.
- Derek Fisher (UK) is replaced by Svetlana Ignatova (UK)

2.3 Membership changes of WP2 during the second year

- Ling Dong (China) becomes beneficiary member.
- Shao-ping Li (China) becomes non-beneficiary member.
- Andreas Marmann (Germany) is appointed as local assistant of Peter Proksch.



- Yun Wang (China) becomes beneficiary member.
- Jandirk Sendker (Germany) is appointed as Co-coordinator.

2.4 Membership changes of WP2 during the third year

- Moustapha Ouedraogo (Burkina Faso) becomes non-beneficiary member.
- Jean-Baptiste Simbi Lumbu (D. R. Congo) becomes non-beneficiary member.
- Wei-dong Zhang (China) becomes non-beneficiary member.

3 WP2 ACTIVITIES

- WP2 held three workshops. As the need for a particular close collaboration with the thematically closely related WP1 had been identified early in 2009, each workshop was held as a joint workshop with WP1. The workshops were used for general discussions around the recent topics as given by the deliverable schedule from Annex I and the 3-year plan which had been drafted in late spring of 2009.

- 13. – 14.09.2009 London (UK) **D2.1**
- 18. – 19.04.2010 Düsseldorf (Germany) **D2.6**
- 3. – 4.12.2010 Braga (Portugal) **D2.8**

The latter two workshops have been hampered by force majeure: the Düsseldorf-Meeting coincided the eruption of the Volcano Eyjafjallajökull and the Braga meeting by snow storms and a strike of the Spanish flight controllers. As a consequence, both meetings took place with a reduced number of participants.

- Telecommunication mainly based on multi- and bilateral teleconferences and E-Mail and to a less extent on web-based resources was used to discuss and coordinate the WP2 objectives in detail. For reasons explained in chapter 5, the final workshop scheduled for fall 2011 (**D2.11**) was replaced by telecommunication.
- WP2 members actively participated on the consortium's annual general meeting (AGM) and the GP-TCM Final Conference presenting WP2 progress and seeking input from other work packages.
 - 1st AGM 28. – 30.07.2010 Henley-on-Thames (UK)
 - 2nd AGM 22. – 24.07.2011 Braga (Portugal)
 - 3rd AGM 12. – 13.04.2012 Kerkrade (Netherlands)
 - Final Conference 16. – 19.04.2012 Leiden (Netherlands)
- WP2 members have partaken in the electronic Management and Science Meetings and Literature-Review-Good-Practice-Panel Meetings organised by the Coordination office.
- Two major coordinative literature surveys have been performed in order (i) to gather information about methodologies currently in use in the research of Chinese Herbal Medicines (CHM) and (ii) to review the overall current research and Good Practice status on the examples of the consortium's priority list of species as well as to discover gaps of knowledge and reasonable fields for a future research agenda. Each literature survey resulted in the analysis of several hundred original research papers and was directly related to the deliverables **D2.4**, **D2.5**, **D2.10**, **D2.12** and **D2.15**. The latter survey was extensively planned in order to prepare the technical means necessary for the targeted data extraction and analysis of the probed literature. WP1 members have been involved in both surveys.
- 15 Deliverables have been prepared and submitted.
- The main results of WP2 have been published [1]. The extended lifetime of the GP-TCM project will be used for further dissemination.

4 WP2 DELIVERABLES

4.1 D2.1 – Kick-off WP meeting and report (month 6)

- The report describes the workshop's program, participants and course.
- The workshop took place on 13. – 14.7.2009 in London (UK) and was hosted by Monique Simmonds.

4.2 D2.2 – Report of findings from workshop including priority list of species (month 6)

- The report summarizes the workshop's outcomes.
 - General coordination needs
 - Utilisation of web-based resources
 - 3-year plan
 - Task allocation
 - Priority list of CHM to be used for **D2.4** (see 4.4). This list was a surrogate as it became clear that a consortium-wide priority list would be reasonable and that its selection and general agreement would take more time.

4.3 D2.3 – Establishment of an interactive website containing evolving information produced during the project and open to the consortium (month 6)

- The webpage has been technically established by the Coordination office. WP2 has used the website's functionalities to report on workshops and for discussing particular issues in the forum.

4.4 D2.4 – Report summarising techniques and standards employed for the analysis of simple and complex extracts currently in use in reference laboratories (month 6)

- The report shows the outcome of a literature survey summing up analytical techniques applied for the analysis of CHM and TCM drugs.

4.5 D2.5 – Report summarising techniques and standards employed for the preparation of extracts used in TCM (month 12)

- The report describes techniques of extraction and post-harvest processing as traditionally applied for the preparation of CHM.

4.6 D2.6 – Workshop targeting selection of minimum acceptable standards for extract preparation, purification and component analysis (month 12)

- The report describes the workshop's program, participants and course.
- The workshop took place on 18. – 19.4.2010 in Düsseldorf (Germany) and was hosted by Peter Proksch.
- The workshop was hampered by the eruption of the Volcano Eyjafjallajökull. This prevented a number of participants from reaching reach the workshop.

4.7 D2.7 – Report on findings from workshop including minimum acceptable standards for extract preparation, purification and component analysis (month 12)

- The report summarises the outcomes of **D2.6** (see 4.6).
 - Extraction and post-harvest techniques applied to CHM
 - The application and potential of Counter Current Chromatography
 - Regulatory view on CHM
 - Draft for a data assessment table for the literature survey to be done for **D2.10** (see 4.10).

- 4.8 D2.8 – Workshop reviewing best practice methods used for information analysis (month 18)**
- The report describes the workshop's program, participants and course.
 - The workshop took place on 3. – 4.12.2010 in Braga (Portugal) and was hosted by Alberto Dias. It was used to discuss general procedures of analytical methodologies and information analysis and their potential for the research of traditional herbal medicines.
 - The MS-Excel based procedure for the data handling of the forthcoming literature survey for **D2.10** (see 4.10) was planned on this workshop and realised in the weeks afterwards.
 - The workshop was hampered by heavy snowfalls in large parts of Europe and a strike of the Spanish air traffic controllers which prevented a number of participants from reaching the workshop.
- 4.9 D2.9 – Report on workshop and recommendations for best practice in information analysis for fingerprinting and component analysis (month 24)**
- The report describes modern methodologies for non-targeted chemical fingerprint analysis and methods of multivariate statistics that can be applied for the analysis of such fingerprint data. The potential of these methods for the research and extract development of CHM is also discussed.
- 4.10 D2.10 – Comprehensive report on the methodology used in the analysis of 'priority list preparations' (month 24)**
- About 400 original scientific research papers which were reviewed by WP2 and WP1 members constituted the base for this report. The contributors screened the papers for particular information on botanical or commercial origin, post-harvest treatment and methods used for extraction, chemical analysis, data analysis and assessment of biological activity and extracted this information to a preformed MS-Excel spreadsheet that allowed analysing the literature data semiautomatically (see 4.8). The report describes the outcomes of this data analysis, indicating gaps of knowledge and Good-practice-related problems.
- 4.11 D2.11 – Workshop reviewing the correlation of methodology used in the analysis of priority list with best practice standards recommended in this study (month 30)**
- The report describes the means that were used to achieve D2.12. The workshop was replaced by telecommunication (see 5).
- 4.12 D2.12 – Report on workshop and recommendations highlighting the status of the information and identifying non-standard areas. Recommendations for research agenda going forward (month 30)**
- The report summarizes the previously identified problems and gaps of knowledge related to CHM research (see 4.10). The identified topics are addressed to Good-Practice recommendations and fields for a future research agenda, respectively. See 6 for further details.
- 4.13 D2.13 – Presentation of findings at the conference planned in work package (month 36)**
- The major findings of WP2 were presented on the GP-TCM Final Conference which was organised by WP9 on 16.04.2012. See 6 for further details.
- 4.14 D2.14 – Final report including complete database of experts and distribution of skills (month 36)**
- This is the report at hand.

4.15 D2.15 – Review papers published in scientific journals (month 36)

- The major findings of WP2 have been published [1]. See 6 for further details.

5 EXPERIENCES GAINED AND LESSONS LEARNED

- Delayed progress. Time limitations effected in deficient coordination (leads) and deficient support (participants). This is effecting synergetically and is further complicated by coordination needs with other WPs and the CO. This has been a problem throughout the project runtime which unlike other problems could not be completely overcome. We tried to reduce the impact of this problem on the progress in that preliminary deliverable contents were generated by a limited number of persons and to be approved or modified by the WP instead of generating the deliverable contents in a full-coordinated approach by the whole WP.
- Use of web-based resources. Contrary to our initial expectations and despite repeated approaches, web-based resources like the wiki-system or the forum have not proven to be useful in generating contents, collect data or jointly compiling deliverable reports within WP2. This is related to the above mentioned time limitations which prevented the participants in familiarising with new platforms and/or monitoring additional communication channels. The coordination worked best with conventional personal (tele)communication and E-Mail which consequently persisted as the main communication techniques throughout the project. Experiencing the problems with communication technologies at an early stage also led to the application of MS-Excel for analysing literature data instead of raising a web-based data base for this purpose.
- Disruption of the 3-year plan. The originally scheduled workflow was disrupted twice by superordinate coordination needs.
 - The generation of a consortium-wide priority list of species prevented a continuous collection of data for **D2.10** from the beginning of the project. Instead, the initial deliverables, which were actually supposed to be based on a constant priority list, were achieved using a surrogate list (**D2.4**) and more general approach focusing on the methodology itself (**D2.5**).
 - The scheduled preparation for **D2.15** was predrawn in order to contribute to the JEP special issue on TCM which was published in April 2012. As a consequence, large parts of the discussions which were scheduled for the month 30 workshop **D2.11** had already been done so that a personal meeting was skipped and the residual discussions were done remotely.
- Importance of personal meetings. Regular face-to-face meetings have proven an important prerequisite to establish a mutual understanding and collaboration between the participants. This was essential for this project to succeed, especially with regard to the assembly of a network.
- Suggestions to utilize the experiences in future coordination projects:

6 SUMMARY OF WP2 RESULTS

6.1 First year

- In the first year, WP2 worked in studying analytical methods and standards employed for the analysis of simple and complex extract. A literature analysis of publications dealing with the analysis of herbal extracts from about 20 plant species demonstrated that different chromatographic methods are dominating the established techniques but that metabolomic techniques for evaluating fingerprint data were scarcely used, usually for discriminating material of different geographical origin.

6.2 Second Year

- In the second year, WP2 dealt with extraction and processing techniques that were traditionally established within the production of CHM. At this stage it became clear that the widely applied traditional decoctions with water can be very complex, involving the joint coextraction of some herbal drugs. Beyond that, herbal drugs may be added at a later stage or even after separate extraction. Further, it became clear that the

paozhi processing constitutes a widely applied and very characteristic procedure within the production of CHM. Especially the *paozhi* processing was considered as an important issue because it is linked to traditional claims implying changes of an herbal drug's therapeutic properties due to processing and consequently changes within the herbal drug's chemical composition must be expected. The knowledge about the consequences of *paozhi* seemed to be rather limited for many herbal drugs. Further, a first draft for the pattern of a targeted literature survey was created that was intended to identify gaps of knowledge and non-standard areas in CHM research from the analysis of scientific literature. The reporting period solely consisted of a WP meeting in Braga (joint meeting in Braga). During that workshop an MS-Excel based system for the targeted literature survey was composed and finalised in the following time.

6.3 Third year

- During the third year, WP2 dealt with the identification of gaps of knowledge and non-standard areas in CHM research by a comprehensive literature review and the potential of metabolomic methods to approach these areas. The literature review resulted in the analysis of selected data from about 400 scientific publication about extraction and component analysis of priority list species and revealed some general gaps of knowledge, non-standard areas or shortcomings:
 - The naming or identification of plant species was often deficient (30-60%)
 - Often no information or doubts about the used plant part was given. (23%)
 - Post-harvest treatment of the examined herbal material was hardly ever mentioned. This is in contrast to the widespread practices of wet-cutting and *paozhi* processing of TCM drugs and left doubts about the quality of the herbal drugs used.
 - It was very often impossible to evaluate if the bioassays frequently used to assess a CHM's biological activities are in compliance with the traditional activity which is described by specific Chinese descriptors.
 - For traditional water decoctions, herbal drugs were often soaked with cold water before heating or the decoction was started at ambient temperature. These procedures are likely to allow enzymatic activity in the drug material to impact on secondary metabolites and hence might also influence the quality of the product.
 - There was lack of studies comparing the biological activity or chemical composition of traditional water decoctions with modernised extracts.
 - Only 47% of the studies assessing an extract's biological activity did chemically characterise the examined extract, 9% using chromatographic fingerprint and only 6% of the studies included results of the chemical characterisation in their discussion. In no case, chemical profiles were correlated to bioactivities by methods of multivariate statistics.
 - Extraction with water can show significant interactions between the different herbal drugs co-extracted during the preparation of a traditional decoct and influencing the extract's chemical composition. Several studies on the (co-)extraction behaviour of the two-herb mixture Danggui buxue tang impressingly demonstrate that already such rather simple preparations show complex interactions.

7 WP2 PLANS FOR FUTURE ACTIVITIES TOWARDS THE EXTENDED LIFESPAN OF THE GP-TCM PROJECT

- Further dissemination, partly based on already existing WP2 results:
 - One or two reviews about the impact of Co-extraction on extract composition and/or pharmacokinetic and/or pharmacodynamic synergism in herbal medicines.
 - Review(s) utilizing the plant species data collected by WP1 and WP2 (e.g. for D2.10). We consider publishing the overall results of this survey (related to Good Practice) and/or reviews related to particular plant species.

- Eventually Review about the impact of drying, wet-cutting and soaking of drug material in cold water prior to extraction.
- WP1 and WP2 have agreed to organize another joint meeting in 2012 to draft a detailed work plan for these publications including allocation of tasks and input from other WPs.
- Seeking funding opportunities for scientific collaboration on WP-level

8 WP2 FINAL CONCLUSIONS

- The water decoctions traditionally prepared from mixtures of Chinese herbal drugs are a disadvantageous formulation especially with regard to stability and compliance. Hence, the development of modernised application forms is reasonable. As shown on the example of Danggui buxue tang the combination of only two herbs during the extraction process with water already can involve complex interactions between the ingredients. Hence, moving away from the complex traditional procedures towards modern herbal extracts may cause complications by distorting the compositions and activities of CHM.
- Comprehensive knowledge about the chemical compounds accounting for a CHM's biological effect is an important prerequisite for the reasonable development of modernised extracts which can then be analytically controlled. However, the marker compounds currently used for the quality control of herbal drugs are often rather unspecific and sometimes the same compound is used for the quality assessment of different herbal drugs with distinct application areas. Consequently, the active and other relevant components of many single herbal drugs and especially complex herbal mixtures must be regarded as widely unknown.
- When assessing the biological activity of an herbal extract, a chemical characterisation of the extract is vital, but was completely missing in about half of the studies we surveyed. Quantitative analysis of known marker compounds was most frequently used for characterisation in the other half of the studies. However, unless the extract activity can be widely explained by known markers, a fingerprint analysis is recommendable because it represents a broad spectrum of known and unknown metabolites, amongst them the (unknown) components accounting for the extract's activity.
- Fingerprint data can also be analysed by methods of multivariate statistics (metabolomics). These methods allow correlating the biological activities of a number of comparable but not identical preparations with a limited number of extract components which can be seen as responsible or at least indicative for the extract's quality.
- TCM offers a huge variety of different qualities for herbal drugs. Presumably superior *daodi* qualities of many herbal are produced from plants grown in a specific region and one and the same drug can be modified in different ways by *paozhi* processing methods to yield products with presumably distinct therapeutical properties. Furthermore, the production chains of many herbal drugs involve steps that may have significant effect of the herbs chemical composition where the meaning of these steps for the product quality is poorly examined (e.g. dry cutting, soaking in cold water). Hence, TCM offers a broad spectrum of different products of the same origin but with altered chemical profiles and presumably altered activities. By this variety, the complexity of TCM drugs in their different processing stages constitute an ideal sample set for the identification of the active components by metabolic fingerprinting combined with multivariate statistics.

9 DATA OF EXPERTS AND DISTRIBUTION OF SKILLS

This section of the report has been removed in order to protect the details of members/experts. The information given under this section is available within the "internal version" of this report.



10 REFERENCES

- [1] Sheridan, H., Krenn, L., Jiang, R., Sutherland, I., Ignatova, S., Marmann, A., Liang, X., Sendker, J., The potential of metabolic fingerprinting as a tool for the modernisation of TCM preparations. *Journal of Ethnopharmacology* 2012, 140, 482-491.