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

GP-TCM

D2.9

Report on workshop and recommendations for best practice in information analysis for fingerprinting and component analysis in CHM

Document description	
Name of document	Report on workshop and recommendations for best practice in information analysis for fingerprinting and component analysis in CHM
Abstract	The 2nd Meeting of WP2 was hold as a joint meeting with WP1 on the 3rd-4th of December 2010 in Braga, Portugal. This document contains a brief summary of this meeting and a comprehensive report about best practice in fingerprinting and information analysis.
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1. WORKSHOP

1.1. General

The Phase-III-Meeting of WP2 was planned as a joint meeting with WP1 on 3rd and 4th of December 2010 in Braga. The meeting was hosted by Prof. Dr. Alberto Dias.

1.2. Workshop Program

Table 1 – Workshop Program

Chinese Medicine Research in the Post-genomic Era

Workshop
Braga/Portugal

2rd of December 2010
to
4th of December 2010

WP1 and WP2

Meeting location (Address for Taxi drivers)
(~70€ from Porto airport)
Please don't forget to collect all your travel bills and receipts. Your travel costs will be refunded based on your collected bills

Lamaçães Hotel
Av. D. João II №75 – Nogueiró
Braga, 4710

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Thursday, 2nd of December 2010

20:00
Welcome Dinner
Meeting Point: Hotel Lamaçães

Friday 3rd of December 2010

9:00 – 9:30
Opening
Salutory Speech
Status and Meeting Objectives

9:30 – 10:15 Session 1
Discussion: Problems to approach in WP1 and WP2

10:15 – 10:30 Break

10:30 – 11:15 Session 2 (D2.9)
Presentation of Prof. Dr. R. Verpoorte
Discussion

11:15 – 11:30 Break

11:30 – 12:30 Session 3 (D2.9)
Discussion: Possible Experimental Approaches

12:30 – 14:30 Lunchbreak (University Restaurant)

14:30 – 15:15 Session 4
Discussion: Procedures and recommendations of good practice in quality control of CHM (to be confirmed)

15:15 – 15:30 Break

15:30 – 16:15 Session 5
Discussion: Report on the knowledge gaps about quality control of the priority list of plants (to be confirmed)

16.15 – 16.30 Break

16:30 – 17:30 Session 6 (D2.10)
Discussion: Comprehensive Data collection (D2.10)

18:00 Dinner

Saturday 4th of April 2010

9.00 – 9:45 Session 7
Organisation of forthcoming WP1 deliverables

9:45 – 10:00 Break

10.00 – 10:45 Session 8
Organisation of forthcoming WP2 deliverables

10:45 -11:00 Break

11:00 – 12:00 Session 9
Finalisation of Meeting report (D2.8)

12:00 Lunch (optional)

1.3. List of participants

Valerian Bunel (for Pierre Duez, WP1)

Alberto Dias (WP1, WP2)

Svetlana Ignatova (WP2)

Andreas Marmann (WP2)

Jandirk Sendker (WP2)

Monique Simmonds (WP1, WP2)

Ian Sutherland (WP1)

Rob Verpoorte (WP1)

You-Ping Zhu (WP1)

Some consortium members' arrival was prevented by flight cancellation.

2. PROGRESS

The phase III meeting of WP1 and WP2 hosted by Alberto Diaz brought together participants from Germany, the Netherlands, Belgium, and the United Kingdom at Braga, Portugal.

Just as with the previous WP-meeting that was hampered by force majeure (volcanic activity), the attendees for forced to prolonged journeys and detours while some other attendees' flights were cancelled due to the sudden onset of winter just before the meeting. Blessedly, with luck and patience, nine attendees were able to reach Braga on time.

After a welcome dinner on the 2nd of December, the meeting started with M. Simmonds' presentation on Quality control requirements (WP1) and plant list chosen at Henley meeting as model set for further data collection; followed by J. Sendker's presentation on possible fields for the application of metabolomic methods in TCM extract development (WP2). This led to a detail discussion about how to organize the data collection (D2.10) for forthcoming deliverables within the involved work packages. As a result, a form was drafted based on the insights of previous deliverables of WP1 and WP2, to be amended with some functionality to support a concise data structure in the subsequent week. It was decided to use MS excel for this purpose in order to avoid expectable technical difficulties resulting from the usage of more powerful but less widespread data base software.

The following work concerning the data collection remains to be done and should be finalized in 2010:

- Technical finalisation of data collection (Ignatova, Sendker)
- Amendment of the form with literature review guide and explanations (Simmonds)
- Start of a limited test run with set of scientific papers, a subsequent teleconference (early 2011) about the experiences with the test run will reveal possible problems to be fixed before the actual work begins and choose the best search engines available.

A lecture by Rob Verpoorte about the application of metabolomic technologies in the field of traditional medicines started the second part of the meeting (D2.9). Modern analytical methods (NMR, MS, directly or on-line-coupled with different separation techniques) allow to thoroughly characterize traditional herbal medicines by gaining raw data that bear a high level of information about their chemical composition. With the help of chemometric datamining tools, information about chemical entities with relevance for e.g. bioactivity or any other known sample property may be extracted from

the raw data. For the research on Chinese Herbal Medicines, a number of WP2-relevant issues could be approached by metabolomic techniques:

- Identification of bioactive compounds
- Detection of possible synergisms in multi-component mixtures
- Identification of meaningful analytical markers that may be indicative for e.g. a drug's state of processing
- Optimization of modernized extraction methods

These aspects were to be considered for the forthcoming part of D2.9 which was created after the meeting.

3. RECOMMENDATIONS FOR BEST PRACTICE FOR FINGERPRINTING AND INFORMATION ANALYSIS

3.1. Introduction

The use of medical drugs of any kind requires the best possible knowledge about the chemical entities that are responsible for the drug's effect in order to allow for a meaningful quality control that allows linking chemical characteristics with bioactivity. While this is usually simple for chemically defined drugs that typically consist of a single active chemical component to be assessed by quality control, herbal drugs and extracts of herbal drugs contain hundreds of chemical entities. This complexity often hampers a meaningful quality control because the analytical assessment of any analyte is complicated by the interference of other components ("matrix components") but mainly because the activity of an herbal drug can usually not be linked clearly to a single component. This already applies to well-established herbal drugs as evident from the example of Salicis cortex. Extracts of Salicis cortex are successfully used against arthrosis and rheumatic disorders. The drug contains a number of salicylic alcohol derivatives that are metabolized to the anti-inflammatory salicylic acid and hence are used for the quality assessment of Salicis cortex. However, pharmacokinetic studies revealed that the plasma level of salicylic acid after intake of a standardized Salicis-cortex-extract was significantly lower compared to a therapeutically equivalent amount of acetylsalicylic acid. Thus, it was concluded that further components must contribute to the anti-inflammatory activity of extracts from Salicis cortex [1].

The knowledge about the active constituents of medical plants is often much more scarce and in some cases even clinically well proven effects of herbals drugs –e.g. Valerianae radix- cannot be satisfactorily explained by their chemical composition. However, lacking a substantial knowledge about an herbal drug's active constituents, quality control measures for the drug or its preparations often remain surrogative in terms of activity (e.g. the quantification of Valerianae radix by measuring the *analytical marker* valerianic acid that is required by the European Pharmacopeia).

The examples of Salicis cortex and Valerianae radix show that there is lack of knowledge even for well known herbal medicines that limits the informative value of quality control measures. The development of quality control methods for herbal medicinal products that are actually capable of quantitatively assessing such products' quality requires the identification of both the chemical entities that directly elicit biological activity (*active pharmaceutical ingredients*) and chemical entities that influence the bioavailability of the *active pharmaceutical ingredients* (*active markers)*. Besides controlling the quality of herbal drug material and herbal pharmaceutical products, the development of such improved quality control methods is a prerequisite for the rational development of improved extracts. The traditional approach for the identification of active constituents is the bioguided fractionation which traces an extract's bioactivity throughout its successive fractionation by numerous separation steps until the bioactivity –in an idealized example- can be quantitatively linked to a single chemical entity. This is a promising approach for herbal preparations with highly active (and often toxic) constituents like alkaloids or cardiac glycosides. Many medical plants lack such highly active single constituents but instead a complex combination of several constituents is regarded to be the active principle (synergetic effects mediated by the combination of pharmacodynamic and/or pharmacokinetic

properties of multiple constituents). When such an herbal preparation is subjected to bioguided fractionation, the extract's bioactivity will be progressively lost in the turn of fractionation, so bioguided fractionation is not effectively applicable to identify the active principle(s) here, though the biotesting of recombined fractions can give information about synergetic effects [2].

A modern approach towards the identification of active constituents is the emerging field of metabolomics employing analytical methods that give utmost comprehensive information about the chemical composition (chemical fingerprint) and methods of multivariate statistics that allow reducing this very complex information to a manageable degree. The main advantages of this procedure are that *(i)* multiple chemical entities can be identified as possible actives and hence synergetic components can be readily identified and *(ii)* rather small amounts of sample material are required when compared to bioguided fractionation [3].

3.2. Analytical methods for fingerprinting

The application of chromatographic and spectroscopic fingerprint analysis to the characterisation of the metabolomic profile of single component and multi component TCHM's (Traditional Chinese herbal medicines) is a rational and practical approach that can be used for qualitative and quantitative analysis, quality control and stability studies of test species. These methods can also be used to assist the correlation of pharmacological activity with metabolomic profile. Using metabolome fingerprint analysis (MFA) offers a more robust analysis of TCHM's than methods that are employed to monitor single or selected components.

MFA can utilise a variety of techniques including TLC, HPTLC, CE, GC, HPLC, GC-MS, HPLC-DAD, NIR, LC-MS, LC-MS/MS, online and multidimensional NMR. In many cases fingerprints can be produced by direct infusion of extracts into a chromatographic system coupled with a simple detector (e,g, UV, ELSD, PAD etc.), but the resulting two-dimensional chromatograms will only provide rather limited information, as many substances will not give a signal and consequently cannot be accounted for subsequent statistical analysis. In contrast, hyphenation of chromatographic instruments with analytical instruments providing an additional analytical dimension like MS, DAD or NMR leads to rapid, sensitive and reproducible metabolomic profiles.

Analytical methods that rely on mass spectrometry have the main advantages of *(i)* being very sensitive and hence allowing the analytical assessment of minor components while *(ii)* by coupling with a chromatographic instrument and/or separating distinct signals with an additional mass analyzer (MS/MS), useful structural information of interesting signals can be obtained, especially with modern high-resolution MS-instruments (TOF-MS, FT-MS) that allow a highly specific characterization of interesting metabolites by their exact mass.

DAD instruments coupled to a chromatographic instrument provide a continuous UV/VIS absorption spectrum which allows a limited deconvolution of overlapping signals. The limitations are due to a very low resolution of the absorption spectra (when compared to NMR and especially MS) and the ubiquitous presence of substances which are not detectable by DAD because they lack structural properties that allow interaction with the electromagnetic waves used for detection.

Nuclear Magnetic Resonance (NMR) spectroscopy is less sensitive than mass spectrometry but offers a unique perspective in metabolomic fingerprinting. Analysis can be carried out in several dimensions. NMR can offer a speedy and effective tool for discriminating between groups of related samples and it can be used to identify the most important regions of spectra for further analysis including multivariate analysis that can be used in qualitative analysis, quality control, stability studies. Recently Multivariate Analysis of unassigned 1H NMR spectra has been used to compare TCHM metabolomic profiles. Such profiles can also be used to compare groups of samples. The application of diffusion-ordered spectroscopy (DOSY) NMR analysis for Chemical Mixture Analysis offers another effective tool for TCHM metabolome profiling [4]. Major advantages of NMR are *(i)* the potential of detecting any existing metabolite that bears hydrogen substituents, *(ii)* the well predictable signal intensity which is directly correlated with the signal's molar hydrogen proportion and *(iii)* its reproducibility. The latter is attributable to the fact that NMR is straight assessing the physical properties of a substance. As a consequence, NMR spectra that have been recorded on different machines, different dates etc. are well comparable while any analytical method that relies on physicochemical and/or chemical properties like MS or chromatography lacks an extend of reproducibility (signal intensity and signal distribution with MS; retention behaviour with chromatography) that would allow direct comparison of samples unless the samples have been analysed in a single approach. Important practical

consequences of NMR's excellent reproducibility is that *(i)* a sample set for metabolomic approaches can be continuously expanded with new samples and *(ii)* the prerequisite for identifying secondary metabolites by database comparison is given [5].

When data has been generated on individual or combination TCHM's chemical statistic analysis such as regression analysis (RA) and principal component analysis can be performed, extracting the active metabolites from numerous peaks, which are related to the efficacy of the TCHM, clarifying the therapeutic activity of TCM. Application of RA analysis to MFA and pharmacological screening allows for:

- The assessment of the identity and quality of a TCM.
- The evaluation of synergism or antagonism between the chemical constituents of a TCM.
- The analysis of the relationships between the chemical constituents' therapeutic effects to determine its effective constituents.

However there are limitations, because to date no analytical method is available which is truly capable of detecting a sample's whole metabolome. For example, mass spectrometry instruments may completely miss analytes that are not well suited for the applied ionization technique, NMR is not well suited for minor components and any chromatographic system can only assess analytes that can be eluted from the stationary phase (additional limitations arise from the capability of the detector coupled with the chromatographic instrument).

3.3. Methods of multivariate statistics

After having recorded chemical fingerprints which represent a more or less comprehensive metabolic profile, the embedded information has to be extracted. In any case the objective is the identification of relevant analytical signals (e.g. the NMR signals of an herbal drug's bioactive constituents) from the chemical fingerprint. Having this information, reviewing the identified signals in the original analytical data allows for a more or less (dependent on the analytical method applied) comprehensive characterization of the compounds that originated the analytical signals.

The identification of relevant analytical signals from the chemical fingerprint is possible by different methods of multivariate statistics. Principal component analysis is a widely used and rather simple method. It is used here as an example to demonstrate the general functionality of multivariate statistics:

The analytical data of numerous samples of e.g. a herbal drug that vary in their biological activity are represented by a multidimensional hyperspace in which each distinct analytical signal (e.g. a NMR signal or a HPLC peak) provides a dimension and the whole analytical information (the fingerprint) of each sample is represented by a single point inside the hyperspace. The most widespread technique to analyse such data is **P**rincipal **C**omponent **A**nalysis: the complex data can be reduced by defining a vector through the hyperspace that displays the highest possible variability between the samples' chemical fingerprints (**P**rincipal **C**omponent **1**). In an idealized example, the projections of the data points (each representing a chemical fingerprint) on the vector PC1 would appear ordered by bioactivity and the active constituents could be deduced from the contribution of their analytical signals to vector PC1. Usually, a two-dimensional projection is used employing a second vector PC2 that again displays the highest variability while being orthogonal ($r = 0$) to PC1 [3].

PCA is an unsupervised method, meaning that the data matrix already contains the information that is required for its reduction and that is the variability. With regard to the identification of bioactive constituents it may be possible to get valuable information if it is possible to achieve or generate samples and extracts of the same kind of herbal drug that are highly variable in bioactivity. A PCA analysis of the samples' chemical fingerprints may discriminate the samples along PC1 and/or PC2 (or other PCs) in a way that the bioactive ones are clearly separated from the inactive ones. In that case, relevant chemical entities can be deduced from their contribution to the respective PC. An example where this procedure was used for quality evaluation of Astragali radix has been published [6].

However, there may be other reasons for fingerprint-variation that are not linked to bioactivity. If this applies, the identification of relevant signals is hampered. This problem can be overcome by supervised methods like PLS-DA (Partial least square discriminant analysis) where the independent variables (the chemical fingerprints) are related to a dependent variable, for example the results of a bioactivity assay. With the aforementioned example of a sample set with active and inactive samples,

the application of a discrete class matrix allows the calculation of a PC that is best suited to discriminate the active from the inactive samples. An example for this procedure was published for anxiolytic and sedative drugs from *Galphimia glauca* [7].

A rather new NMR approach based on multivariate statistics that alleviates the identification of associated NMR signals within a multicomponent sample by taking advantage of the associated signals' intensity-correlation (multicollinearity) within a set of spectra is STOCSY (Statistical Total Correlation Spectroscopy) [8]. A similar approach is SHY (Statistical Heterospectroscopy) which allows cross-correlation of signals between fingerprints recorded with different methods. This was demonstrated for ^{1}H-NMR and UPLC-TOFMS data sets, where deconvoluted NMR-spectra were cross-assigned to the related mass spectra [9]. These approaches may facilitate the identification/structural elucidation of unknown metabolites and the development of quality control measures based on NIR.

3.4. Identifying relevant components from CHM with metabolomic approaches

On its way from the plant to the patient, a Chinese Herbal Drug has to undergo a number of production steps that can include storing, washing, rinsing, drying, moistening, remoistening and cutting and numerous processing methods (*pao zhi*, e.g. frying, roasting, steaming, cooking with ginger sap etc., see D2.5 for further details).These methods should guarantee, that a herb can develop its full range of effects and avoid unwanted side effects. Each of these manipulations represents a step in the production chain that can be expected to influence the product's chemical profile. For example, the drying of herbal material results in a disorganization of cellular compartimentation and an oxidative burst both of which is capable to change plant secondary metabolites by oxidation (e.g. Sennae folium) or direct enzymatic activity (e.g. Digitalis-purpureae folium) until further loss of water stabilizes the material. Interestingly, there are examples where the same raw drug material is processed by different methods to yield different products that are claimed to have distinct pharmacological/toxicological properties (e.g. Coptidis rhizome, [15]). Furthermore, the claimed necessity for processing the raw drug material implies that a product's pharmacological/toxicological properties are improved due to the processing. Another aspect that is claimed to impact an herbal drug's therapeutical properties is the occurrence of presumably superior *daodi* qualities. Taken these claims for granted, it should be possible to link these different pharmacological/toxicological properties to differences in the products' chemical profiles, so the rather complex production chain of Chinese herbal medicines may reveal information about pharmacologically/toxicologically relevant plant metabolites that can be identified by metabolomic approaches and subsequently addressed for quality control measures. In case of analytically difficult active components, easier accessible analytical markers may be identified that are at least indicative for the product's pharmacological/toxicological properties.

It is desirable, also from a regulatory point of view, that the abovementioned impacts are well characterized and that the state of an herbal drug material within the production chain can be traced by suitable analytical methods. Currently, the *pao zhi* processing of TCM drugs is usually controlled by experienced, long studies masters called Yi Shi [10], but without objective endpoint definitions. The characterization of a processing method's chemical impact on the product can be assessed by unsupervised metabolomic approaches (e.g. PCA-based) and may aid the definition of suitable objective processing endpoints to connect the TCM with European legislative requirements.

Typically, Chinese herbal medicines are administered as a mixture of several processed herbal drugs, whereupon the mixture is claimed to have synergetic effects. Metabolomic approaches may reveal information about pharmacologically relevant metabolites when correlating the metabolic profiles of variably composed mixtures with their pharmacological activity.

However there are limitations:

1. Any potentially active compound or set of compounds identified by metabolomic approaches should be approved by testing isolated pure compounds or mixtures of pure compounds, respectively.

2. Metabolomic approaches are not well suited for direct quality control in practice because the instrumentation required (e.g. hyphenated methods) is very costly, partly lacking robustness (e.g. LC-MS) and highly qualified personal is required to deal with the rather abstract data processing and evaluation. Currently, the abstractness of metabolomic procedures also hampers a broad

communication of quality control procedures and results and hence undermines its own informative value. As a consequence, it is recommended to use the information obtained from metabolomic approaches to develop meaningful quality control methods based on simple and robust equipment like HPLC-UV, NIR or densitometry. However, quality evaluation based on complex analytical fingerprint data and unsupervised multivariate statistics may become an option for future quality control measures when the required equipment and skills might have become more widespread.

3. None of the analytical methods employed for metabolomic approaches is suitable to detect the whole metabolome, so there is always a chance to miss relevant metabolites. A combination of different analytical methods could reduce this problem.

4. The results of any metabolomic approach that is supervised by bioactivity data can only be as meaningful as the test system that is applied to assess the bioactivity data. In order to identify the active constituents of herbal drugs by metabolomic approaches, meaningful and robust biological test systems with sufficient test capacity have to be established.

3.5. Metabolomic approaches for the development of modernised extracts

Traditionally, CHM are administered as water decoctions of complex mixtures of herbal drugs. Special procedures can be applied dependent on the specific herbal drugs to be administered (see D2.5 for details). It is noteworthy, that the experience of thousands of years in the application of CHM which is surely of great relevance must exclusively be linked to these traditional administration techniques, while any change towards modernised application forms has to measure up with the activity of traditional decoctions. If a modernised application form has not shown to be equivalent to traditional application in terms of activity, any further reference to TCM-tradition is futile. This is especially true if other extractants (organic solvents) or extraction methods are employed, but also dried water extracts or granules (kepi) that are increasingly used cannot readily be regarded to be identical with the original decoction in terms of activity [11, 12] but therapeutical equivalence and safety has to be proven.

The development of modernised extracts and application forms is however desirable for numerous reasons: *(i)* individually prepared water decoctions are more likely to entail quality shortcomings caused by improper herbal drugs when compared to herbal medicines produced in industrial scale under best controllable conditions. *(ii)* Water decoctions are probably the worst possible preparation in terms of stability and may give rise to microbial contaminations, decomposition of constituents by hydrolytic or oxidative reactions or precipitations that may impact the product's quality. *(iii)* TCM Water decoctions are infamous for their unpleasant organoleptic properties. *(iv)* The rather complicated preparation, storage and intake of water decoction may cause compliance problems while modernised application forms based on dry extracts (granules, capsules, tablets etc.) are easily manageable for both patients and practitioners. *(v)* The possibility for standardization of large-scale extracts is a prerequisite for future evidence-based research. *(vi)* The Blinding of Clinical studies employing traditional water extracts is hampered by their strong organoleptic properties.

Though it is possible to produce granules, capsules etc. with dried water extracts, the technological properties of such extracts are impaired by high amounts of hydrophilic constituents, especially carbohydrates, which results in a hydroscopic, sticky and hence hardly processable extract. Rather high amounts of additives are required to overcome these problems [13] but these additives further add on to the extract dose which is already quite large due to the presence of polar "bulk material". With regard to the "Lipinski rule of five" [14], it is generally believed that less polar extractants like lower alcohols, acetone etc. are capable of extracting pharmacologically relevant analytes while excluding higher amounts of polar, technologically difficult "bulk material" like carbohydrates, proteins, amino acids etc. from the extract. However, this is a very reductionistic view which actually applies to a single active compound and especially excludes the possibility of pharmacokinetic synergism in herbal extracts. It cannot be ruled out that the extraction of very polar constituents (needing water as extractant) can be essential for producing active extracts from specific drugs or for specific applications.

Hence, a rational approach for extract optimisation in TCM requires the best possible knowledge about the chemistry that is responsible for a traditional herbal preparation. Having that knowledge, extract optimisation can be led by analytical assessment of the relevant constituents. A general approach for extract optimisation could be:

1. Metabolic profiling and biotesting of numerous varying herbal preparations, taking advantage of the complexity of different qualities (*daodi, paozhi*), intermediates (fresh drugs, raw drugs etc.) and –in case of complex preparations- the combinatorial diversity that can be created by combining the different herbal ingredients in varying proportions. With regard to the long-term experience of herbal drug application in TCM, traditional decoctions should be used for preparing the test material.
2. In case of complex preparations, possible unnecessary ingredients can be identified by the biotesting results using isobolograms.
3. Multivariate analysis of the samples' chemical fingerprints using supervised methodologies which allow correlating the samples' fingerprints with their measured biological activity (e.g. PLSDA) and subsequently the deconvolution of chemical entities relevant to the biological activity.
4. Physical isolation of chemical entities found to be relevant in order to allow for their unambiguous identification and for detailed pharmacological studies that may reveal mechanism of action, synergetic effects or antagonisms. Pure compounds are also required for analytical issue (see 5.)
5. Development of simplified quality control methods addressing the identified pharmacologically relevant components and employing simple and robust techniques that allow for an easy analytical assessment of a product's quality.
6. Extract optimisation guided by simple analytical procedures in order to develop an extract that combines high activity and best possible technological properties. Any resulting extract has to measure up with the original traditional preparation in terms of efficacy and safety.

3.6. Summary and Discussion

Chinese herbal medicines have been used for the treatment of numerous ailments for millenniums and show increasing popularity. However, the traditional water decoction is a disadvantageous application form especially for reasons of stability and compliance; furthermore the highly polar extracts obtained from these decoctions bear technological difficulties that hamper the development of modernised application forms. The use of less polar extractants may overcome these problems but in order to guarantee for an efficacy comparable to that of traditional preparations, knowledge about the active components of a CHM is vital if extract optimisation shall be done in a rational way. Modern metabolomic approaches can correlate metabolic fingerprints that comprehensively characterise an extract's chemical composition with its biological activity and allow identifying a multitude of pharmacologically relevant components. Hence, metabolomic approaches are potentially suitable to reveal synergisms between chemical components that are known to occur in herbal preparations and that are especially claimed to occur in Chinese herbal medicines. TCM offers a unique variety of products and intermediates with presumably distinct quality and hence gives an ideal field for metabolomic research approaches. Besides using the information obtainable by metabolomics for the analytically guided optimisation of extracts, further valuable information can be achieved about the importance of e.g. *daodi*-qualities or *paozhi*-processing and their impact on an herbal product's chemical composition, allowing the development of meaningful quality control measures.

Though such a metabolic approach for the identification of relevant components is a rather holistic approach compared to e.g. bioguided fractionation, it is noteworthy that it still aims at a reduction (namely the reduction to therapeutically relevant components within the extract) like any other scientific procedure does by definition. However, the holistic concept of individualised therapy is not directly compatible with this reduction as standardised pharmacological models and clinical trials with homogenous collectives of test subjects have to be used in order to retrieve the dependent variables for multivariate statistics and to compare the efficacies of traditional and modernised application forms, respectively. Furthermore, is has to be considered that the result of any metabolomic approach aiming at the identification of active components will only be as good as the pharmacological model applied to display their activity.

4. REFERENCES

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