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

GP-TCM

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D2.12

Report on workshop and recommendations highlighting the status of the information and identifying non-standard areas. Recommendations for research agenda going forward

Document description	
Name of document	Report on workshop and recommendations highlighting the status of the information and identifying non-standard areas; recommendations for research agenda going forward
Abstract	The deliverable *(i)* takes up problems and non-standard areas that had been previously identified in the D2.10, *(ii)* discusses their relevance for CHM research, *(iii)* gives recommendations towards good practice and *(iv)* addresses particular issues for a research agenda. The most relevant topics are the widely unknown impact of TCM-specific post-harvest treatments on the composition of traditional extracts and the interactions between different herbal drugs in co-extracted multiple component mixtures. Analysis of metabolic fingerprints by multivariate statistics supervised by activity data is suggested as a promising approach to identify the chemical entities responsible for a preparations bioactivity, taking advantage of the rich variability offered by the complex traditional processing techniques. Knowledge about the chemistry responsible for a traditional preparation's activity is an essential prerequisite for the reasonable development of modernised and more convenient application forms.
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TABLE OF CONTENTS

1. INTRODUCTION AND AIM OF D2.12

D2.12 is purposing to identify problems and non-standard areas within the scope of extraction and component analysis of CHM and to give recommendations towards Good Practice related problems as well as recommendations for a future research agenda. In accordance with WP2's 3-year-plan, the previous deliverable D2.10 has been of major importance for the identification of such problems and shortcomings. D2.10 had been planned and executed as a joint action with WP1 and hence aspects are taken up that despite their importance for WP2 coincidence with WP1's scope. This especially applies to the botanical aspects of 2.2, 2.3, 2.4 and 2.5 and we refer to recent WP1 reports for further information about these topics.

In chapter 2, these issues are discussed and recommendations are given towards good practice, typically with regard to reporting experimental data within scientific publications. Chapter 3 includes recommendations for a research agenda based on the findings of chapter 2. This deliverable is closely related to D2.11 which reports about the actions taken towards the preparation of this document.

2. SUMMARY OF IDENTIFIED PROBLEMS AND NON-STANDARD AREAS

2.1. General Introduction

The majority of these shortcomings have been identified in the course of a literature survey related to species from the consortium's priority list of species. About 400 original papers related to the medical use of these species in TCM were analysed for the kind of information provided with regard to the characterisation of the botanical origin, sample processing, extraction and analysis of TCM preparations. We refer to the respective Deliverable D2.10 for more details. Further sources for the identification of problematic issues were discussions during the different GP-TCM meetings and WP2's work towards D2.15.

2.2. Description of botanical identity

The botanical origin of an herbal specimen must be considered as fundamental information as these describe the genetic identity of the specimen, typically on the species level. Hence it is necessary that scientific publications provide sufficient information to leave no doubt about a studied specimen's genetic identity and –more importantly- to allow tracing back the botanical origin by stored reference samples. Botanical descriptors that were expected to be given within a scientific publication are:

1. Valid Latin binominal species name including the authority abbreviation. This is of importance as for example the assignment of different types of the same genus to different species may change in the course of time. Such changes are often only reflected by changes of the authority abbreviation of a species name. As a consequence, the actual comprehension of a binominal name can only be unambiguously realised when the authority name is given.
2. Identification reference (e.g. Recent plant was identified by botanist XY using morphological characteristics). This demonstrates that the specimen's identity was approved by an expert though it does not guarantee that the identification was correct.
3. Reference number of a voucher sample deposited in an accepted scientific herbarium. This can be regarded as the most important reference as it allows tracing back the botanical identity later if deemed necessary.
4. DNA barcode. This is a rather new technique that has some potential advantages compared to a morphological characterisation: *(i)* it potentially allows the clear identification of a species without needing taxonomic expertise *(ii)* it potentially allows distinguishing between different genetic traits within a given species and *(iii)* it potentially allows the identification of strongly processed herbal material that is no longer assessable by morphological characteristics. This technique however is not yet developed to a degree that would allow a broad application in research.

Within our literature survey, we found that 30% of the publications did not provide valid species names because the authority was not given, 50% did not give identification reference and 66% did not provide information about voucher specimen. DNA barcoding was not observed. Especially with regard to the authority name, we recognized that this information was frequently given in the introduction or elsewhere but not in the experimental description of the specimen.

Recommendation: We strongly recommend giving a complete Latin binomen including the authority and a voucher number as a minimum required information about the studied herbal specimen's botanical identity. Considerations for the handling of voucher specimen are published and we refer to these publications (e.g. [1]).

2.3. Description of botanical origin

Besides giving unambiguous information about the plant species, further information about the plant's origin is desirable. In the context of herbal medicines, any circumstance that could impact the herbal metabolome and hence the composition of herbal extracts might be of interest. The metabolome however may be influenced by a multitude of abiotic and biotic factors which probably will never be fully controlled and documented. Literature survey showed that all publications where plant material was harvested by the authors gave some spatial information, while temporal information (harvest date) and information about the plant age was less frequently given with 43% and 24%, respectively. We consider giving these data as reasonable as they are very easily assessable in most of the cases, briefly documentable and may allow linking up to further information, e.g. to climate data from meteorological stations.

Especially the spatial information is of interest for CHM, as herbal drugs of presumably superiour quality, so-called *dao-di* qualities are required to be grown in specific area, hence implying a systematic impact of an herb's spatial origin on the quality of related CHM products [2].

The detail of information given about these issues was variable, typically giving a region or a district for spatial and a season for temporal information. Though a more detailed level of information is not always of interest, widely and openly assessable internet mapping resources like google-maps as well as GPS instruments allow describing the position of a plant collection site very accurately by geographical coordinates in case that the plant collection site is known (probably restricted to studies where the herbal specimen were collected by the authors in most cases). Further, it is well known that plant constituents may be subjected to a diurnal pattern (e.g. [3]) and hence providing very exact information about the collection time is also of interest.

Recommendation: We recommend providing for spatial characterisation *(i)* Geographical coordinates with reasonable precision and *(ii)* a description of the collection site in plain text (city or region). For temporal characterisation we recommend giving information about the harvest date and time, if possible. We discourage to obligatory demand this detail of information for scientific publications as this level of information is not always accessible and enforcing it may result in the publication of unreliable data.

2.4. Origin of commercial samples

About half of the surveyed literature studied commercially obtained samples (herbal drugs or extracts), which were most frequently supplied by a company and less frequently bought on a local market or drug store. In principle, the same information as recommended in 2.3 would be applicable here but exact spatial and temporal information and other details about sample history will probably not always be available. It is however desirable to characterise commercial products as good as possible and especially to rule out major impurities, falsifications and adulterations which have shown to be problematic with TCM preparations in the past. Hence, measures should be taken to support the identity and purity of commercially obtained samples, e.g. based on the respective pharmacopeia monographs. The names and locations of shops and markets should be given and a lot number whenever possible.

Recommendation: Commercially obtained samples should be characterised to support their identity and purity as well as by their source of supply (name and location) and whenever possible by their lot number. Further information as recommended in 2.3 should be given when available.

2.5. Description of herbal drugs

Instead or besides using the botanical species names, the names of herbal drugs originated from the species are frequently given in scientific literature. These names are often given in Latin (e.g. Rhei radix or Radix rhei), TCM also has drug names in Chinese language which can be translated into *pinyin* (phonetic transcript into Latin letters) in order to make them accessible for non-Chinese speaking scientists. Both kinds of drug names are not self-explanatory but require further definition which is given through pharmacopeia monographs; which comprehensively define the drug. Consequently, the use of a drug name may replace giving information about e.g. the plant species, plant parts, post-harvest treatment and other details which are described in the pharmacopeia the drug name relates to. Our literature survey has shown that the Latin drug name was quite frequently (22%) used without giving further information about the drug's identity and even for self-harvested plants without further characterisation. However, different pharmacopeias and even different editions of the same pharmacopeias can have different definitions for the same drug name (e.g. Rhei radix Ph.Eur. 5 and Rhei radix CP2005). Consequently, the sole use of a Latin drug name requires reference to a specific pharmacopeia if no further information is given. Furthermore, an herbal specimen which is referred to with a Latin drug name requires matching any quality criteria of the respective monograph.

Only 3% of the scientific publications probed for this information used the Chinese *pinyin* name to describe an herbal specimen while the Latin drug name was used in 60% of the cases (with regard to the characterisation of herbal specimen in the Material and Methods section). The Chinese pharmacopeia uses both Latin and Chinese names but differentially processed drugs of the same origin can only be distinguished by their Chinese name while they share the same Latin drug name (e.g. Coptidis rhizoma comprises 4 different products with distinct *pinyin* names). Consequently, the Chinese *pinyin* name contains information about an eventual drug processing which is not given by the Latin drug name.

Recommendation: We recommend using drug names with caution and with reference to a specific pharmacopeia. Using a drug name without this reference is ambiguous. Furthermore, even when additional information about e.g. the plant species, post-harvest-treatment or the content of a specific marker component is given, the use of a pharmacopeia drug name implies that the product matches any requirements that are given to the monograph.

2.6. Traditional or non-traditional use?

During our literature survey we were intending to evaluate research papers about the application of a herbal drug in that it should be decided whether the drug-effect was studied in a traditional context or in a non-traditional one. An example for the latter would be that a CHM was tested for e.g. an antiproliferative effect, though the traditional application is restricted to the treatment of obstipation. A major obstacle was that the traditional applications of CHM are defined with TCM-specific descriptors ("strengthens chi"). It was literally impossible for us to decide whether a scientific test system, e.g. a cell culture model would be suitable to support a claim that is described with such descriptors. On the other hand, TCM literature very often refers to a long traditional use.

Recommendation: It should be clearly stated if a particular study is intending to support the traditional use of a CHM and if, how the test system(s) applied link to TCM-specific descriptors.

2.7. Post harvest and *paozhi processing*

On the way from a recent plant to an herbal drug, plant material is processed. The term post harvest processing comprises any procedure that a harvested herbal material is subjected to until its consumption, which typically would be its extraction in case of herbal medicinal drugs [4]. As extraction is separately dealt with further below, we restrict the definition of post-harvest treatment to procedures that are applied between harvest and extraction. These procedures comprise cleaning, cutting, drying, storage and –in case of CHM- very specific procedures referred to as *paozhi* processing. Numerous different *paozhi* processes have been established in TCM for detoxification of toxix herbal materials as well as for, cleansing, aroma correction, preservation of active constituents and other purposes [5, 6]. We will focus on two issues that have been identified as rather specific procedures for TCM while being noticed as non-standard areas: (i) wet cutting and (ii) *paozhi* processing.

Chinese raw herbal drugs are often traded as e.g. whole dried leaves which are remoistened again in order to cut the material in a wet state. This appears to be widely practiced though hardly being

addressed by scientific literature. Drugs which are remoistened again after prior drying must be considered as highly susceptible to changes of their metabolome. When recent plant material is harvested and subjected to drying, the material will continuously loose water with two major consequences *(i)* the cellular compartimentation will collapse at about 30% relative water content (RWC, related to water content of recent material) and *(ii)* the plant enzymes will stop their activity at about 10% RWC. These values are coarsely estimated from few existing studies, but show that there is a time window during drying where enzymatic activity meets a destructed cellular compartimentation and hence has the chance to orderlessly impact on plant metabolites. It is exactly this window where the generation of active sennosides from the inactive genuine anthrone glycosides takes place (activity refers to the desired laxative effect here; anthrone glycosides have other, undesired activities). Further drying leads to a rather stable herbal drug when enzymatic activities can no longer take place due to lack of water. When such a material is remoistened with cold water, enzymes can be reactivated and find the same disorganised environment as described above. This suggests that remoistening of dry plant material can have severe impact on plant secondary metabolites and by that on an herbal drug's therapeutical efficacy. At the present state of research, it is impossible to evaluate whether if and eventually how dry cutting as practiced for CHM impacts their therapeutical efficacy [7, 8].

After wet cutting, Chinese drugs can be dried again or subjected to numerous *paozhi* processing methods which include frying, steaming, calcinating and many other techniques (see D2.5 for further details). The best known example is probably the detoxification of *Aconitum* drugs [9]. In contrast to the great practical importance of *paozhi* methods [6], we hardly found any study during our literature survey where *paozhi* was taken into account. Moreover, it was not clear for a majority of 90% of the studies, if the studied herbal material has been subjected to some kind of *paozhi* processing or not. An especially interesting aspect of *paozhi* is that the same raw drug material can be differentially processed to yield products with distinct therapeutical activities, e.g. Coptidis rhizoma, Angelicae sinensis rhizoma or Aconiti lateralis radix [6]. Taken this traditional claim for granted, the different processing methods should impact those metabolites of an herbal drug that account for its respective therapeutical activity. Metabolites that are influenced by such a process can be identified by metabolomic techniques and constitute a reasonable hypothesis for experimental work that aims at the elucidation of an herbal drug's mode of action.

Recommendation: Generally, any procedure that is likely to influence an herbal drug's metabolome should be described. That is, for example the drying protocol or storage conditions and with regard to CHM especially an eventual *paozhi* processing or wet cutting. As to the already mentioned gap between great practical importance and predominant lack of scientific consideration when studying CHM, we suggest also to explicitly mention if an herbal drug was not processed or cut in wet state.

2.8. Extraction

The most important delivery form of CHM is the water decoction of mixtures of several herbal materials [10] under different modes of preparation. Over thousands of years numerous special instructions for the process have been developed. The methods are related to different physical, chemical and pharmacological characteristics of the active components. Unfortunately, the impact of many parameters in the "simple" process of water extraction on the herbal metabolome of extracts is often completely neglected. Any procedure has a severe impact upon the extract's chemical composition and hence the products' quality with regard to its therapeutic efficacy. Factors which have to be considered and to be given in publications in detail are: application of cold water soaking, duration of soaking and of each following step of extraction, temperature of added water, boiling of the sample in water, addition of some herbal components at different stages of the process, addition of excipients (e.g. honey) at different stages etc.

Approximately half of 135 studies on pharmacological activities or clinical efficacy probed in D 2.10. used the traditional way of preparation but only few include information e.g. about soaking the herbal material in cold water for a variable period of time (30 min to overnight) before heating [e.g. 11, 12, 13]. Most of the examined publications do not clearly state the temperature of the water before addition to the herbal material although it seems likely that in many extraction approaches the water had ambient temperature at the beginning of the process. The potential consequences of bringing a dry herbal drug into contact with cold water have been explained above (2.7).

The use of extraction methods employing modern technology (pressurized hot water extraction, microwave assisted extraction etc.) for the preparation of CHMs is published only rarely until now. Especially pressurised hot water extraction can be regarded as a kind of modernisation. This method has been shown to decrease the extractant's polarity due to the application of high temperature and thus provides the extraction of a wide range of compounds [14]. It has to be underlined that turning away from traditional procedures bears the risk of altering the product's therapeutic properties. An example is the report that the use of pressure cookers for water extraction leads to less active preparations [15]. Therefore an indispensible prerequisite for the consideration of these methods in the preparation of CHMs would be a pharmacological comparison of such a modernized extract with the one prepared traditionally. Nevertheless, scientific investigations linking modern extraction methods to activity are not available until now. Extract optimisation guided by the analysis of single components only cannot be recommended as sufficient due to additive or synergistic effects of ingredients or the improvement of pharmacokinetics (e.g. better absorption due to surface-active water-soluble components).

From a change to less polar extractants such as alcohols, acetone or supercritical CO_2 the partial exclusion of higher amounts of polar, technologically difficult "bulk material" like carbohydrates, proteins, amino acids etc. from the extract can be expected. But this approach decreases the possibility of pharmacokinetic synergism in herbal extracts due to the severe impact on the chemical composition of CHM [e.g. 16]. Additionally, it can be assumed that extraction of very polar constituents with water is essential for the efficacy of the extracts as well. When linking a modernised CHM extract to the long experience of TCM its clinical efficacy should at least match the one of the related traditional preparation.

Recommendation: For best practice and the performance of comparative studies it is essential to include every single step of the extraction with all details (duration, temperature, sequence of addition of herbal components or excipients etc.) in the experimental section of studies. This has to be taken into consideration by authors of studies, referees and editors to improve the comparability of study results. For the development of modern ways of extraction the comparison of the herbal metabolome of the extracts and for their activity in suitable pharmacological models is an important requirement.

2.9. Fingerprinting and quantitative analysis

Of the surveyed publications that were dealing with biological effects of CHM or extracts of TCM drugs, the majority of 53% did not chemically characterize the herbal specimen, 22% measured single marker compounds for characterization, 9% showed a chromatographic fingerprint (mostly HPLC) without further interpretation and 6% showed a chromatographic fingerprint and discussed it with regard to biological activity. The chemical characterization of an herbal specimen is however a critical point, because the chemical composition of herbal drug and hence of an extract prepared from this drug is strongly influenced by the genetic properties and ontogenesis of the plants and the post-harvest treatment (see 2.7). The pharmacopeias account for this by quantitative assays, which measure a drug's content of active markers and more or less targeted fingerprint analysis that often focus on one or two compound classes. However, due to the lack of knowledge about those chemical constituents that account for the therapeutical activity of TCM drugs and especially complex CHM, the analytical assessment of single marker compounds as usually practiced by pharmacopeias seems insufficient. This lack of knowledge is for example illustrated by the TCM drugs Caulis Ionicera, Flos Ionicera and Flos Chrysantemi which are claimed to have distinct pharmacological properties but nevertheless all are quantitatively characterized by their content of the very unspecific secondary metabolite chlorogenic acid. The state of knowledge is even worse when looking at complex CHM preparations, where also those herbal constituents may impact the products therapeutical properties which were hitherto not considered as relevant or even unknown [17]. Consequently, the characterization of an herbal extract by the quantification of single marker compounds is only reasonable in such cases, where the marker compound is known to account for a significant part of the products activity. While this is already often critical for single drugs, TCM further complicates this issue by the use of complex herbal preparations, where one and the same single drug is often used as a part of different complex CHM with distinct therapeutical properties and it is expectable that different metabolites of the same herbal drug can be relevant depending on the complex herbal medicine and therefore its therapeutical intention. Consequently, the substances to be addressed for a herbal drug's quality control can also depend on the drug's intended use (Example of Rhei radix: anthraquinones are relevant for laxative, tannins for adstringent properties).

A chemical fingerprinting analysis is superior when characterizing an effective drug preparation when considering the importance of multiple constituents, as –depending on the methodology- a large percentage of the preparation's metabolites can manifest as analytical signals. The aim of fingerprinting analysis should be to characterize an extract to a maximum possible degree. That is, for example, a chromatographic fingerprint with assignment of identified peaks and –where available- additional analytical data for unidentified peaks, e.g. UV-spectra, mass spectra, exact masses, sum formula or what else is assessable by the analytical method applied. Such a fingerprint may allow to conclude or hypothesize on the impact of an extract's chemical component on its biological activity when two or more extracts with different chemical profiles show different biological activities, taken that both the methods for fingerprinting and for biological activity testing are comparable for the examined extracts. While this is quite unproblematic when the respective analytical data have been acquired within the same study, the often desired comparison of such data between different studies prerequisites an excellent reproducibility of the fingerprinting method. This cannot be expected from the most frequently used HPLC fingerprints [18]. The problem may be partly overcome by presenting additional data [17] but a reproducibility that allows comparing datasets from different studies directly while displaying a broad range of metabolites requires more robust techniques like NMR [18]. NMR fingerprinting has been frequently used in the context of metabolomic studies but not for the characterization of herbal exctracts in the context of activity studies [17]. The latter, however would despite of its limitation (sensitivity, signal complexity, resolution) complement the widely used but less comparable chromatographic fingerprints.

Recommendation: Within any activity study of herbal materials, a chemical characterization of the very same item should be done, that is also used for biological testing. A possible pitfall would be to characterize the drug material, e.g. according to a pharmacopeia monograph while preparing traditional water decocts for biological testing. The chemical characterization should involve a fingerprint analysis which is capable to display a broad range of metabolites, e.g. by gradient HPLC or NMR. Signals of identified components should be labeled and in case of HPLC fingerprints, multiple chromatograms at different detection conditions should be shown and/or prominent peaks should be further characterized by two-dimensional detector data (typically UV-absorbance and or mass spectral data). It must be stated that an examined herbal extract must be considered as unknown without at least some basic characterization of its chemical constituents. Especially with respect to coordinated research approaches, the availability of NMR fingerprints from biologically assessed herbal extracts are desirable because its robustness allows to reuse these data in future studies.

2.10. Granules

As to rather inconvenient use of traditionally prepared herbal water decoctions, CHM are to an increasing amount traded in the form of granules. Generally speaking, these granules are dried water decoctions. However, water extracts contain large amounts of polar substances, especially carbohydrates and proteins, that are technologically difficult and lead to hygroscopic and sticky dry extracts. Excipients are added to the extracts in order to improve its technological properties, or polar extract constituents are removed by e.g. ethanol precipitation. Especially the latter procedure is critical when there is no valid knowledge about those components of the extract that account for its biological activity. Furthermore, the drying process itself may already impact the extract's composition by decomposition of sensitive or removal of volatile components. From a general point of view, the fact that granules are produced from water decoctions rather logically suffices to state that a granule is not the same product as the decoction it is made from. Consequently, it has to be shown that a granule is equivalent to a traditional decoction with regard to its therapeutical efficacy or at least with regard to the content of those chemical entities that predominantly account for the traditional product's therapeutical efficacy. The state of knowledge about these chemical entities however is often poor or doubtful especially when looking at complex herbal mixtures (see 2.9). The distinction between traditionally prepared CHM and granules in literature is often scarce and in some of the surveyed literature it was not even clear if the herbal extract studied was prepared by traditional decoction or by dissolution of granules. Furthermore, granules were more or less implicitly referred to as traditional products, which is not reasonable.

Recommendation: Even though closely related to a traditional decoction, any granule should be treated as new product with presumably altered therapeutical properties unless equivalency could be demonstrated by convincing assessment of their biological activity or by chemical characterisation in

case that the desired efficacy of the traditional product can predominantly be explained by the characterised extract components. Granules should not be referred to traditional preparations.

3. RECOMMENDATION FOR RESEARCH AGENDA

On its way from the plant to the patient, traditional Chinese herbal medicines are subjected to numerous processing steps amongst which the *pao zhi* processing is claimed to alter or positively impact on the products therapeutical properties. Other procedures like wet cutting, soaking in cold water before decoction and the coextraction of multiple herbal drugs within a complex CHM are traditionally established without known or claimed impact on the product's therapeutical efficacy. For example, chemical alterations which are likely to occur during wet cutting might be important of the product's therapeutical quality or not. Generally, the knowledge about the impact of any of the aforementioned processes on the therapeutical properties of a CHM is poor and requires further research in order to elucidate their actual importance. The same can be stated for the use of presumably superior *daodi* qualities which are established for numerous herbal materials [2].

Another important issue is the use of complex herbal mixtures in TCM, while in contrast Western herbal medicine tends to prefer simpler preparations. The complex preparation of the traditional Chinese herbal medicines is usually explained by synergetic effects that are claimed to arise from such mixtures, and there is some evidence that such effects are in fact present and can even very specifically depend on the processing of single ingredients [17].

The chemical background for any of the aforementioned issues is widely unknown and constitutes an interesting field of research by itself, e.g. the impact of wet cutting on herbal material or the interaction of a complex herbal medicine's single ingredients during coextraction. The main ascpect when looking at any herbal medicine's chemistry, however, is to identify those chemical components which directly or indirectly account for the product's therapeutical quality. These comprise those bioactive compounds which finally mediate the therapeutical effect by e.g. interaction with receptors (and possibly show pharmacodynamic synergism with each other) as well as compounds which may improve the therapeutical efficacy by improving the solubility or stability of the actual bioactives (pharmacokinetic synergism) and finally components that may improve the product's organoleptic properties. As illustrated by the aforementioned example of using rather unspecific marker compounds like chlorogenic acid for quantitative assays of different drugs with distinct therapeutical applications, the state of knowledge with regard to an herbal medicine's quality determining chemistry is already poor for single herbal drugs. The state of knowledge is even poorer when considering the frequently claimed synergetic effects of herbal mixtures.

However, the complexity that TCM offers by its rich abundance of different product qualities including intermediate stages arising from the production chain, partly involving claims for altered therapeutical quality, constitutes at the same time an ideal field for metabolomic studies aiming at the identification of those chemical components that account for a CHM's therapeutical efficacy. After assessing a CHM's biological effect by a suitable method, a huge variety of slightly altered recipes can readily be generated aiming to produce CHM with altered chemical profiles and altered therapeutical properties. Based on such a sample set, modern methods of metabolic fingerprinting in combination with methods of multivariate statistics have the potential to generate reasonable hypothesis about the chemistry responsible for the therapeutic properties. For example, taken that a complex CHM's efficacy to a large part veritably depends on the *pao zhi* processing of one of it's herbal constituents, methods of multivariate statistics were capable of detecting the chemical background as well as its correlation to the observed biological activity when fed by metabolic fingerprints of CHM's that were prepared with and without processing of the respective ingredient [for further details see D2.9, 17]. A major advantage of such a metabolomic approach is that it is potentially capable to detect possible synergisms between the chemical components of an herbal extract, while the classical approach of bioguided fractionation will loose track of the biological activity during fractionation, namely when the synergetic components are separated from each other. Still, the result of a metabolomic study must be seen as a hypothesis that remains to be proved by isolation and biotesting of the identified components.

Thorough knowledge about the chemistry that makes a traditional CHM's therapeutical quality allows explaining and predicting a particular product's efficacy and is the key for the reasonable development of modernised extracts and dosage forms as well as for meaningful methods of quality control.

Especially with regard to the frequently stressed synergisms that presumably were developed within thousands of years, metabolomics seem to be an approach that would be capable to verify these claims and to recognise possibly dispensable ingredients and measures.

In summary, WP2 recomments the following topics to be addressed for a research agenda:

(1) Identification of quality determining chemical components from traditionally prepared CHM by activity guided metabolomics taking advantage of the rich variability of TCM herbal drugs.

(2) Elucidation of the impact of traditional post-harvest processing techniques on the product's metabolic profile using metabolomics.

(3) Structural elucidation and biotesting of chemical entities identified within (1) and (2)

(4) If possible reduction of complex traditional preparations by *(i)* removal of dispensable ingredients and *(ii)* skipping of dispensable methods of processing.

(5) Analytically guided development of optimised extracts and dosage forms.

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