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

GP-TCM

D2.10

Comprehensive report on the methodology used in the analysis of 'priority list preparations'

Document description	
Name of document	Comprehensive report on the methodology used in the analysis of 'priority list preparations'
Abstract	This deliverable is an analysis of literature data from within the species of the consortium-wide Priority list. The aim of this deliverable is to identify frequent "Good Practice"-related problems about extraction and component analysis within the monitored literature. The data were acquired by the authors using an Excel-based data acquisition form that provided predefined columns for specific information to be probed and in partly restricted possible entries to predefined categories. In total, more than 400 publications were monitored. The data were analysed using Pivot-tables and revealed a number of "Good Practice"-related problems that give the base for the forthcoming deliverable D2.12.
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1. AIM OF D2.10

For D2.10, a comprehensive review of literature about extraction and component analysis is meant to identify current problems within the Good Practice in research of CHM. In chapter 2, the general procedure is described, chapter 3 gives a comprehensive overview about the data that were analysed; the information presented there were extracted and summarized from the available raw data provided by the contributors. Chapter 4 summarizes problems and that were identified within the information summarised in Chapter 3 and that are to be addressed in D2.12. References are given in Chapter 5.

2. LITERATURE REVIEW PROCEDURE

2.1. Data acquisition

The scope of literature to be reviewed was limited to the plant species from within the priority list of species as provided by WP1. As the survey was partially meant to work as a gap analysis, the review of a representative amount of literature per examined species was desired (in total more than 400 publications). The workload was divided amongst the members of WP2. In order to harmonize the information to be extracted, an Excel-based data acquisition sheet was designed that allowed to enter selected information from revised literature in a controlled manner. As a control mechanism, each data acquisition sheet came along with a mirrored sheet (key word table) that contained exemplary entries that were allowed to enter into the data acquisition sheet by pulldown menus, where applicable. The entries of the key-word table were in part exemplary, because not every necessary entry could be foreseen in advance and hence the keyword table could be expanded by the contributors to match their own needs. The information to be entered had jointly been chosen during the phase III meeting of WP1 and WP2 in December 2010. The following aspects were covered by the acquisition sheet ("descriptors"):

- Primary key: Latin binominal name as used by CP2005[1]
- Identifier of the specimen given:
    - Specimen identifier: term used in literature to describe the herbal specimen. The following identifiers were taken into account and had been provided by WP1 for all species of the priority list:
        - CP2005 latin binominal name
        - Taxinomically accepted latin binominal name
        - Other Latin binominal names (accepted synonyms)
        - Latin drug name as used in CP2005
        - Chinese Pin Yin names
    - Examined as Individual herb or as part of a formula
    - Name of the formula, if given
    - Plant part used
    - Source of the specimen, if given
    - Processing method, if applicable
    - Processing additive, if applicable
- Extraction:

[1] The CP2005 names were collected before CP2010 was available. As recent changes of the terms in CP2010 would not have impacted the outcomes of this deliverable which are based on existing literature, the CP2005 terms were kept.

- Traditional extraction method (e.g. Decoction with water)
- Other extraction method (also includes closely related procedures like lyophilisation)
- Chemistry (coarse description of examined phytochemicals)
- Fingerprint (fingerprint analysis to characterise the herbal specimen)
- Quantitative Analysis used to characterize the herbal specimen)
- Analytical Method used for Fingerprint and/or Quantitative Analysis
- Fractionation method, if applicable
- Fractionation method efficiency, if applicable

- Classification of study:
  - Traditional or non-traditional use
  - Evaluation of activity (by Assay, Clinical study, based on chemical properties etc.)
  - Quality control connection to activity (e.g. targeted on of known active compounds, fingerprints correlated to activity etc.)
- Reference data
  - Full reference
  - Publication URL
- Scoring: Scoring system as suggested in Literature review SOP V1.8
- Free entry comments

The literature research was performed with either Scopus or Pubmed using an OR-conjuncted combination of any botanical identifiers given by the priority list of species (CP2005 latin binominal species name, taxonomically accepted latin binominal species name, Latin drug names, Pinyin names, latin binominal synonyms). The search result was then refined by limiting the hits to the topics “Extraction” and “Chemistry” or adding these terms to the general search term with “AND” conjunctions for pubmed, respectively.

2.2. Data analysis

The acquired data were analysed using Pivot-tables fed by the excel-sheets of the single participants. With this functionality, the entries of an excel-sheet were semi-automatically assorted and counted. Figure 1 shows an example of a Pivot-table that was used to evaluate the entries of some sheets with regard to non-traditional extraction methods. Two smaller problems were included in this procedure:

- The scope of key words that the single contributors used was not completely identical. For example, one contributor did distinguish between alcoholic extractants and organic extractants while the others did not.
- Especially for Pivot-tables based on more than one column of the data acquisition tables, the number of entries suitable for the evaluation of a distinct aspect was limited by the entries or combination of entries that were *(i)* available and *(ii)* consistent enough to allow a semi-automatic evaluation.

This situation had two consequences:

- The number of evaluable entries was different for each aspect, so each aspect was handled with different subsets of data, dependent on how many entries were available with suitable data structure. In order to avoid information bias caused by the different size and composition of data-subsets, the evaluation in part 3.1 is completely given in percentages. The total number of entries used for a subset is always given, so the percentages can be recalculated to total numbers if desired.
- It was not reasonable to merge the different data sets to a single table as originally scheduled, because this procedure would have resulted in loss of information due to harmonisation of

entries to the lowest information level for each column. Instead, each data set was evaluated with separate Pivot-tables and the Pivot-table results were manually evaluated and combined where appropriate.

Fingerprinting	Metabolomics				
Number of Fingerprinting Line captions	**Column captions Assays**	**Chemistry**	**Clinical Study**	**(Empty)**	**Total**
⊞ **Correlated compounds and activity**	**6**				**6**
⊟ **Fingerprint and activity, not correlated**	**10**			**1**	**11**
HPLC	4				4
HPLC-ELSD	1				1
LC-DAD	2				2
LC-MS	2			1	3
TLC	1				1
⊞ **Just fingerprint with no activity**				**10**	**10**
⊞ **Targeted on compounds of known active compounds**	**3**	**7**	**1**		**11**
Total	**19**	**7**	**1**	**11**	**38**

Figure 1: Example of Pivot tables used for data analysis. An exemplary data analysis for this table would be: The data subset fed into this table (138 publications) was analysed for the numbers of publications that contained a metabolomic fingerprint (38 publications AND were categorised by the descriptor "QC connection to activity"). 19 of them did assess the quality of the herbal product by assays. 10 of these 19 publications did not correlate the fingerprint data with the assessed bioactivity. Of these 10 publications, 4 used HPLC (=HPLC-UV), 1 used HPLC-ELSD etc.
The information about 1 publication (highlighted) was not consistent, because it had been categorised as "Fingerprint and activity" but no information about the kind of activity assessment was given. In such cases the respective reference had to be reviewed again if deemed necessary.

2.3. References

In total, more than 400 publications were monitored. The references for these publications were either tracked by full bibliographic reference or URL. Within this deliverable, only those references are given which were considered to be of specific interest for the further work of WP2. Any other reference can be given on demand in full bibliographic reference or URL format.

2.4. Evaluation of the procedure

In general, the data analysis worked very well, with regard to the limitations stated above. Data analysis worked better for descriptors that allowed a very clear categorisation of publications with a limited number of predefined terms. The descriptor "QC connection to activity" is a good example, because more than 80% of the monitored publications could be clearly categorised by one of the five predefined entries (see Figure 1, the predefined entry "Just activity with no fingerprint" is not visible here).

3. DATA ANALYSIS

3.1. Literature scoring

The reviewed literature has been scored by the contributors according to a scoring system that was adapted from the GP-TCM literature review SOP 1.8. The grading system is meant to evaluate the quality of information that is given within the "Material and Methods" section of a publication with regard to botanical origin, processing and extraction of an herbal specimen (Table 1). These scores were analysed in first instance in order to provide a general view on where to search the acquired data in more detail.

The contributors for this deliverable gave in total:

- 355 scores for botanical origin (0-10 points)
- 190 scores for processing (0-5 points)

- 373 scores for extraction (0-10 points)

The number of given scores is not identical for botanical origin, processing and extraction, because not each aspect of the scoring system was not applicable for each reviewed publication. For example, for a publication about NIR analysis of drugs, the scoring system was not applied for the issue of extraction, because no extraction took place. The scoring for processing was frequently not applicable because it was often clear from the context of a publication that no processing took place. In each case, the scores for a specific subject according to Table 1 were either applied completely or not at all so that a comparison of scores between the different subjects is given.

For evaluation, the relative frequencies of each contributor's scores and subsequently the mean value and standard deviation for all contributors' scores were calculated. The standard deviation represents an overall measure for the consistence of scoring between the different contributors while the mean value is unbiased and represents the actual occurrence of the respective scores (Figure 2, Figure 3, Figure 4).

3.1.1. Botanical origin Scores

Botanical origin was rather poorly described in the majority of the papers. As evident from the scores, information about identification and voucher deposition were missing in more than 50% of the reviewed publications, more than 30% were lacking any of the information to be scored. DNA barcoding was found in 2% of the publications probed for this information (see 3.2.1).

As a general impression, information about post-harvest treatment is especially scarce. In many cases it is not even clearly stated if the herbal material was dried at all before further processing. It is evident for a number of herbal materials that the drying conditions can have a major impact on an herb's chemical composition. Prominent examples are Digitalis purpureae folium or any Iridoid-glycoside-bearing material, where the integrity of β-glycosidases has a major impact on the composition of glycosidic components and hence the drying temperature and speed is of major importance, especially when considering that very high drying temperatures will instantly and irreversibly suppress any of those reactions (while heat-labile components may degrade instead). Furthermore, in case of leaves, the subjection to light during the drying process causes the photosynthetic apparatus to produce major amounts of reactive oxygen species, which in turn can give birth to oxidative changes to secondary metabolites (e.g. dimerisation of anthrones or oxidation of nitrile moieties). It is further noteworthy that the practice of wet-cutting (remoistening of dried herbal raw materials before cutting) which seems to be practiced to some extend when preparing Chinese herbal drugs was never mentioned. Through remoistening, residual enzymatic activity can be restarted and such enzymes meet an environment that is completely disorganised through a subcellular and cellular decompartimentation process that took place when drying the material. Hence, changes of the herbal metabolome may occur to an unforeseeable kind and extend. To the best of our knowledge, this issue has never been systematically addressed.

This issue is analysed in further detail at 3.2.1.

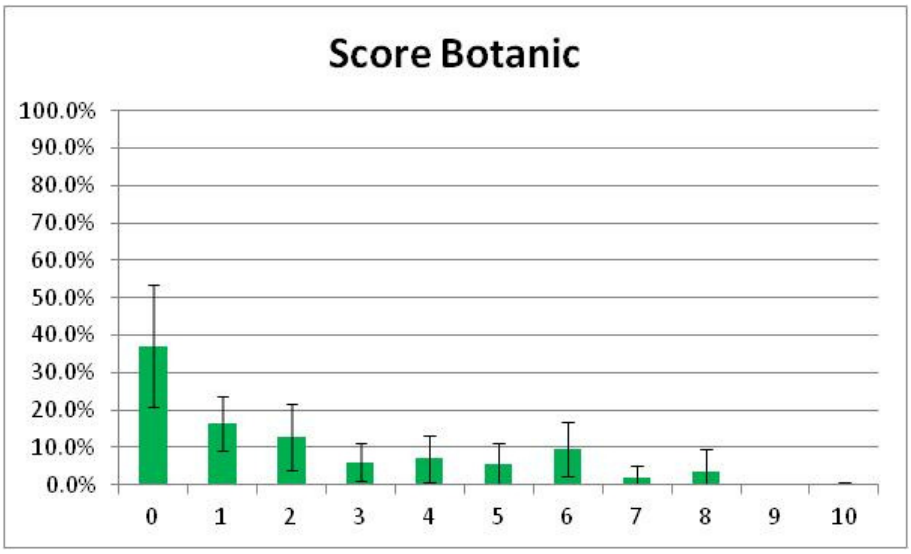

Figure 2: Scores for Botanical Origin that were attributed to the analysed original publications according to the scoring system shown in Table 1.

3.1.2. Processing Scores

The score for processing is related to the different methods of *paozhi*-processing which is a special feature of CHM. Numerous techniques are described and were included in the Chinese pharmacopeia. It is especially noteworthy, that *paozhi*-processing is claimed to affect a drug's bioactivity and that one and the same raw drug can be processed in different ways to yield different products which are claimed to show different therapeutical properties [1].

As clearly visible on the overall scoring for processing, more than 90% of the 190 publications which where scored here got 0 points from the contributors, which means it is not clear whether the examined herbal material has been processed or not.

This issue is analysed in further detail at 3.2.4.

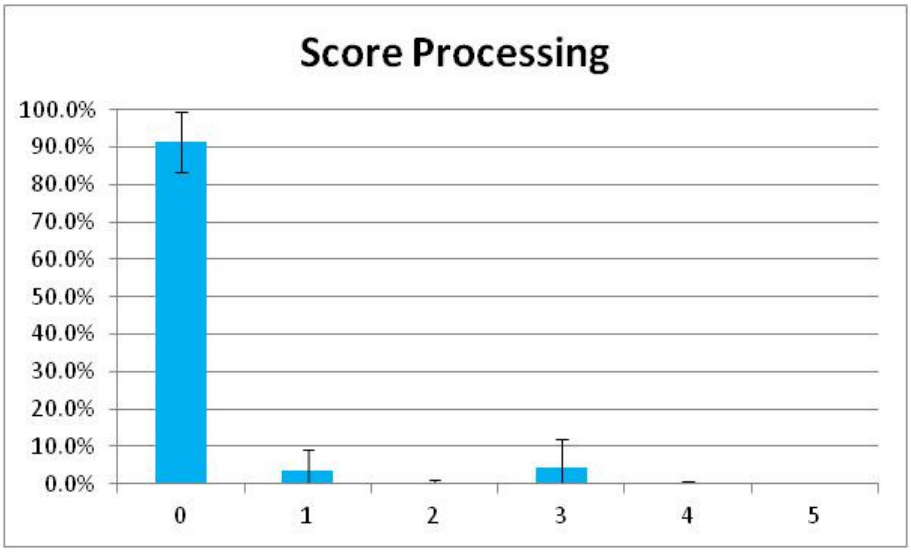

Figure 3: Scores for processing that were attributed to the analysed original publications according to the scoring system shown in Table 1.

3.1.3. Extraction Scores

The overall results of the scoring for extraction procedure (experimental details and extract characterisation) are comparable to the botanical-origin-scores. It is evident from the scores that more than 30% of the scored publications completely lack any characterisation and sufficient experimental details that would allow reproducing the extraction procedure (temperature, duration etc.). Lack of analytical extract characterisation by chromatographic profile or comparable data is even true for at least 66% of the scored publications. Good scores of > 6 points were generally attributed to publications which focused the extraction procedure itself. 3% of the publication received the maximum score; the composition of subjects of these publications did not noticeably differ from the composition of subjects of publications with low scores.

This issue is analysed in further detail at 3.2.5 to 3.2.8.

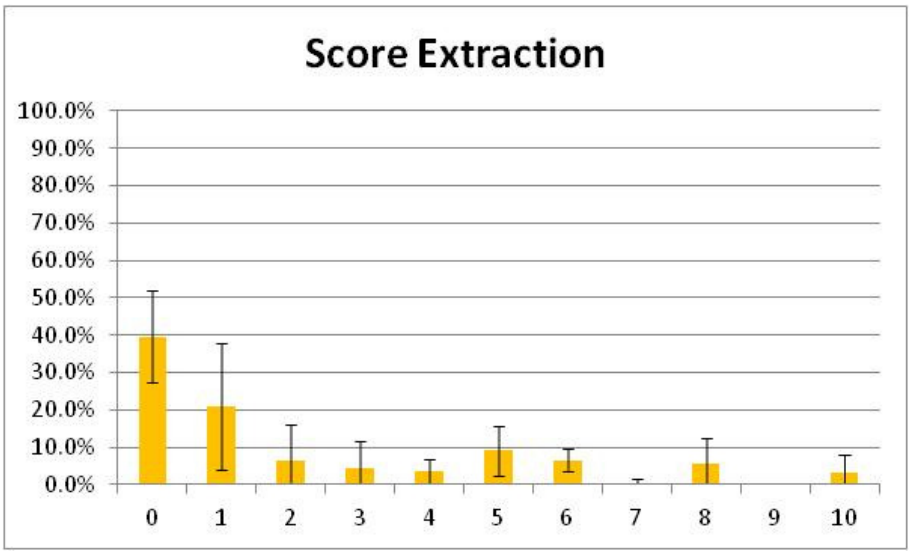

Figure 4: Scores for Extraction that were attributed to the analysed original publications according to the scoring system shown in Table 1.

3.1.4. Correlation analysis

The similarity of scorings between botanical origin and extraction raised the question, in how far the scores for both subjects were correlated. A correlation analysis was performed with a subset of 124 publications. A variance coefficient of 0,191 (r = 0,437) indicated that nearly no correlation can be observed, so it can NOT be concluded that a good botanical characterisation generally comes along with a good extract characterisation. Hence, the scores seem to be rather independent from each other.

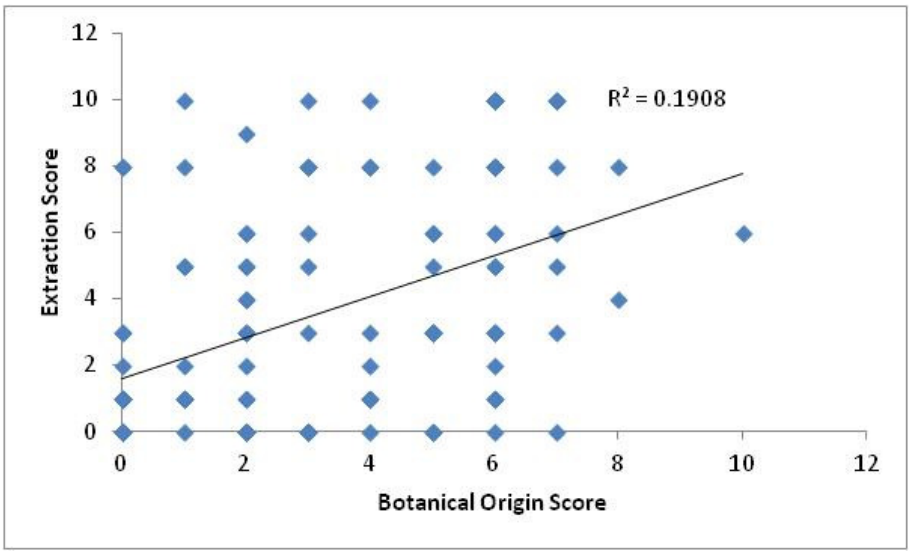

Figure 5: Correlation of Scores for Botanical Origin with Scores for Extraction.

Botanical origin: a mark from 0 to 10

	Score	
	Sufficient information	Insufficient information
Herb provenance indicated?	1	0
Herb harvested:		
Identification information (botanist, reference)?	2	0
Voucher specimens deposited?	3	0
DNA bar-coding	2	0
Post-harvesting treatment?	1	0
Quality and reproducibility of treatment?	1	0
Herb bought in commerce:		
Shop location?	2	0
Voucher specimens deposited?	3	0
DNA bar-coding? Unlikely?	2	0
Post-harvesting treatment (drying, washing, etc.)?	1	0
Quality and reproducibility of treatment?	1	0

Processing: a mark from 0 to 5

	Score	
	Sufficient information	Insufficient information
Herb processing indicated?	1	0
Wet cutting? (highly probable for some herbs, but often not mentioned)	1	0
Eventual processing of the herb?	1	0
Perceived quality and reproducibility of process?	2	0

Extraction: a mark from 0 to 10

	Score	
	Sufficient information	Insufficient information
Detailed extraction procedure?	1	0
Yield?	2	0
Perceived quality and reproducibility of process?	2	0
Are the extracts characterised?		
Chromatographic profile?	3	0
Chromatographic profile with determination of presumed key constituent(s)?	5	0

Table 1: Grading system for original publications as adapted from GPTCM literature review V1.8.

3.2. Data analysis by descriptor

3.2.1. Herbal specimen identifier and plant parts

It was considered that an unambiguous botanical identification of the herbal specimen should be given within the "Material and Methods" section of a publication and reviewed literature was probed for the kind of botanical identifiers used there (**Error! Reference source not found.**). The Latin binominal species names also used by the Chinese Pharmacopeia (2005) and the Latin drug names or a combination of both were used for more than 60% of the 118 publications probed for this information. It is noteworthy that the Chinese pinyin names only occurred in 3% of the examined publications and were always combined with at least one of the above mentioned Latin names. 30% of the examined papers did not give a proper identifier in the "Material and Methods" section. The classification "No proper identifier" was almost exclusively attributed to one of the two following reasons:

1. A Latin binominal name was given without the authority, so the name is not valid and hence not unambiguous. In many cases the complete Latin binominal species name was given elsewhere, typically within the introduction, but a clear link to the specimen that was actually used for the study was not given.
2. A preparation consisting of several herbal drugs was used and the single herbal ingredients were not mentioned within the "Materials and Methods" section. Similar to the first point, some identifiers for the single herbal ingredients were mentioned elsewhere without a clear link to the preparation that was actually used.

Another general observation is that the Latin drug names that were very frequently used are practically never linked to a specific Pharmacopeia, so it is mostly unclear if the specimen that were actually used do indeed match the pharmacopeial quality criteria that are implicated by the use of this term.

It is not clear from 23% of the reviewed publications, which plant part was used, because it was neither explicitly mentioned nor a name was used that includes this information (Latin drug name or Pinyin name).

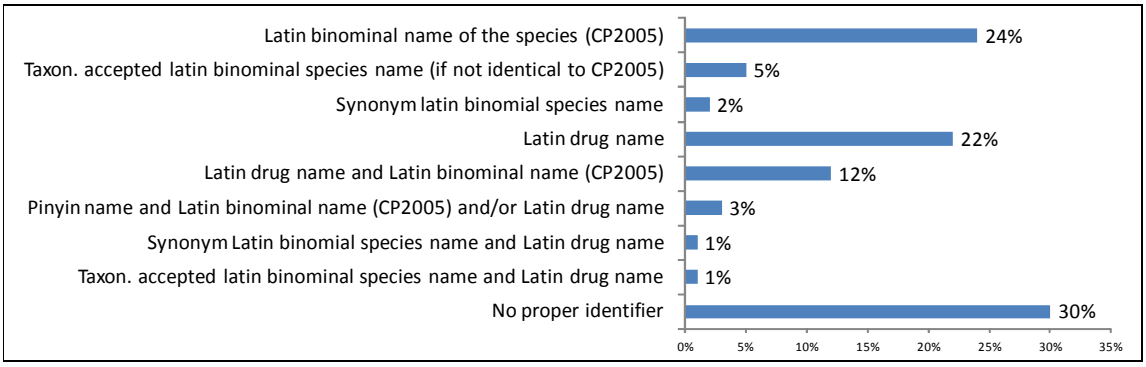

Figure 6: Use of herbal specimen identifiers in reviewed literature, including combinations.

3.2.2. Source of the specimen

The origin of an herbal specimen is considered as part of the characterisation of an herbal product and hence sufficient information should be given within the "Materials and Methods" section of a publication, regardless how a herbal product was obtained. The reviewed literature was probed for the source of the herbal specimen (118 publications), finding that a product's source could in most of the cases be attributed either to commercial supply from a specified provider (Local market, Local drug store, Company), supply by a specified public organisation (academic or clinic) or harvesting of recent plant by the authors and some spatial and/or temporal information were provided. 15% of the papers reviewed here did not give any information about the source of their specimen, including such cases

where no information was given beyond the plain fact that the specimen were either bought or harvested.

Further, the reviewed literature was probed for additional information that was given about the herbal specimens' source. That would be the lot number for commercially obtained specimen, and for harvested products any information that would allow to characterise a specimen's genotype, phenotype or ontogenesis (e.g. DNA-barcoding, morphological characteristics, precise geographic data, weather tracks, plant age etc.). A lot number was given for only 9% of the commercially obtained specimen. In any publication using herbal specimen harvested by the authors, some spatial information was given, 43% had additional information about the harvesting time (temporal information) and 24% had further additional information about the plant age. In no case, information about plant age came without temporal information about the harvesting time.

The quality of spatial and temporal information was different within the probed literature (Figure 9).

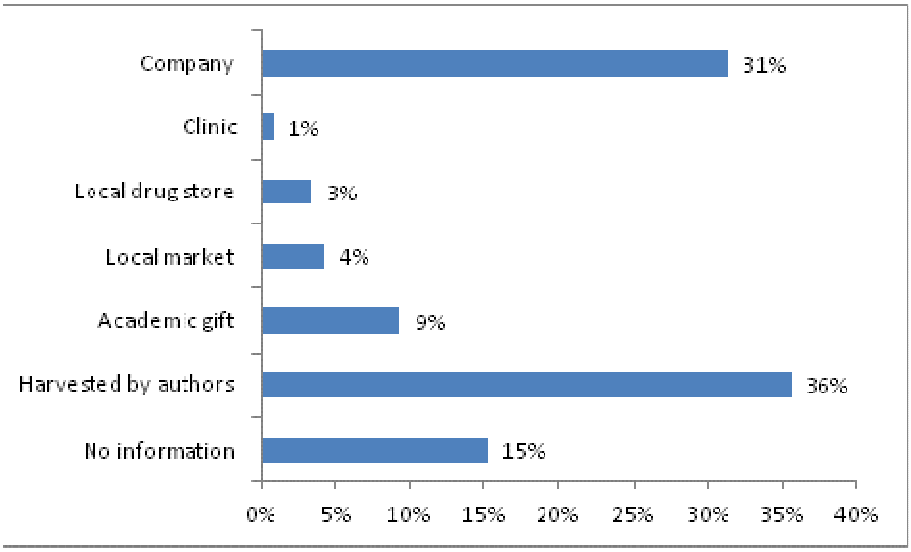

Figure 7: Sources of herbal specimen as assessed from the probed literature.

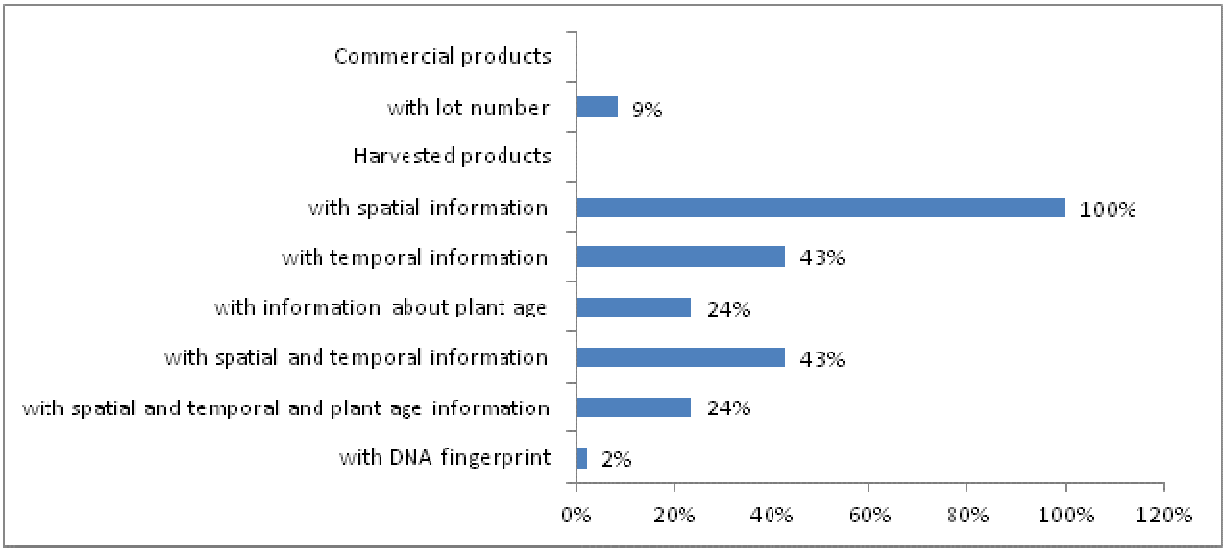

Figure 8: Additional characterisation of herbal specimen as assessed from probed literature.

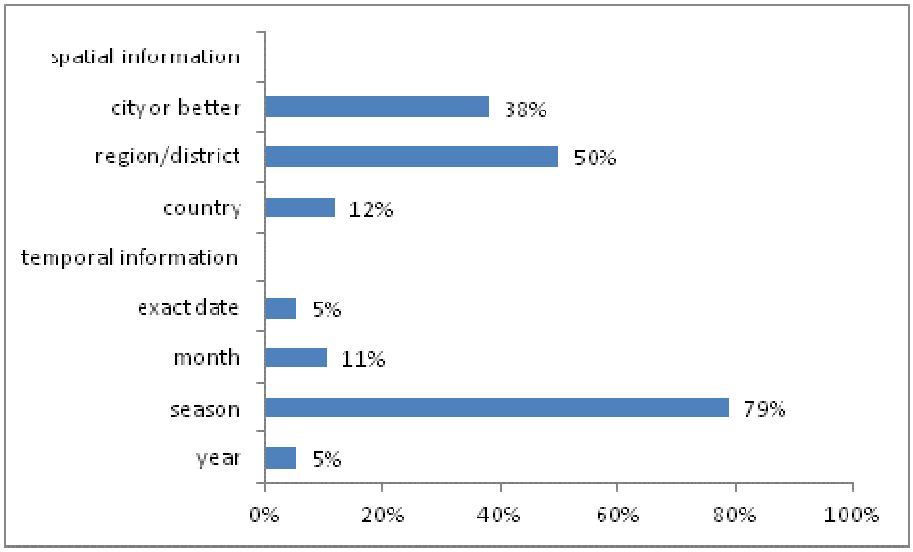

Figure 9: Quality of spatial and temporal information about the herbal specimen used in probed literature.

3.2.3. Traditional or non-tradional use?

In most cases, this question was not easy to answer, as the assays used to assess a products quality in terms of efficacy or safety usually are linked to a western clinical or scientific terminology whereas the traditional application of CHM is given through Chinese descriptors and there is no clear link in-between this terminology. Summed up, the participants of the species survey found 56% of the publications related to a traditional use and 44% to a non-traditional use (326 publications were analysed for this).

3.2.4. Processing (*paozhi*)

Processing was considered as a specific item of CHM and partly other Asian herbal medicinal products and hence the impact of these techniques in scientific literature was of special interest. 400 publications using Chinese herbal material as specimen were probed for information about possible *paozhi* processing of the material. Only 5 of these publications gave some information about a processing, 15 publications described the specimen in the "Material and Method" parts in a way that clearly allowed the conclusion that no *paozhi*-processing was made, whereas no clear information about this issue was given within the other 380 publications. These 95% of the probed literature include studies where the authors harvested the herbal material by themselves (36% expected, see 3.2.2) and for these cases it seems rather unlikely that a *paozhi* processing was done without mentioning that. Also when taking this into account, about 90% of the probed publications give no clear information about a possible *paozhi* processing. This relation is in contrast to the therapeutical, phytochemical and practical relevance of *paozhi* processing [2]. It is noteworthy that the Chinese *pinyin* names that appear to be the only terminology that includes differentiated information about a product's state in terms of *paozhi*-processing, are very rarely listed within the "Material and Methods" section of the probed publications (see 3.2.1).

3.2.5. Extraction

- From the 258 publications that were probed for the description of the extraction procedure, 23% involved a traditional extraction technique.
    - The majority of these traditional extraction techniques were water decoctions (87%), typically involving a second extraction and subsequent merging of the two extracts.
        - 62% of these publications using water decoctions indicated a subsequent drying by lyophilisation, vacuum drying or spray drying (accounting for 34%, 13% and 3% of the publications).

- Granules were also considered as a traditional extraction technique here (this is discussable) and accounted for 9% of the probed publications with traditional extraction techniques. Granules are analysed in further details at 3.2.9.
- One single publication used medicinal wine for extraction.

- 77% of the extraction procedures in the probed literature were considered as non-traditional and involved extraction with organic solvents.
  - Specific procedures like Soxhlet, IP-NPCE [2], matrix solid phase dispersion [3], microwave assisted extraction and supercritical fluid extraction [4] were observed rather seldom, together attributing to 6% of the non-traditional extraction procedures. 25% of them assessed biological activity.
  - The residual 94% of the non-traditional extraction techniques were rather simple extractions with organic solvents (including alcoholic and hydroalcoholic extractants) typically using heat or ultrasonic treatment for acceleration.
  - One subset of probed literature distinguished between alcoholic (methanol or ethanol, pure or as hydroalcoholic mixture) and organic solvents, indicating that the majority (97% of in this subset) of the organic solvents used for extraction were actually alcoholic.
- The analysis of a subset of 135 publications showed that 47% of the publications that tested herbal extracts in a biological test system (assay, animal testing, clinical study, application study) were extracted in a traditional way while 53% were extracted by some other method.

Some specific observations that were made additionally:

Several publications that involve traditional water decoctions state that the herbal material was soaked in cold water for a variable period of time (30 min to overnight) before heating [5; 6; 7]. Most of the publications probed for this issue do not clearly state if the water was already heated up before it came into contact with the herbal material. From a practical point of view, it seems likely that especially for large-scaled extraction approaches the, water had ambient temperature at the beginning of the process.

2 of 7 publications employing Soxhlet extraction made an experimental mistake [8, 9]. Both publications feature a Soxhlet extraction with a non-azeotropic hydroalcoholic mixture (80% ethanol). It must be stated that the actual extractant in this examples has not been 80% ethanol but 96% Ethanol and hence a comparison e.g. with ultrasonic extraction using 80% ethanol is not valid.

A series of publication deals with the extraction of the two-herb formula Danggui Buxue Tang (Astragali radix and Angelicae sinensis radix) and especially with the aspect of coextraction [10; 11; 12, 13; 14; 15; 16].

3.2.6. Fingerprinting and quantitative analysis for extract characterisation

A subset of 108 publications was probed for the question, in how far examined extracts were characterised by fingerprint analysis[2] or quantitative analysis of single analytes indicative for drug

[2] For the analysis of this subset it was necessary to define when an analysis presentation does match the criteria of a fingerprint analysis. In an ideal case, a specimen's complete metabolome should be represented by distinguishable analytical signals. As up to date no analytical method is capable to fulfil this demand and the best suited (hyphenated) techniques based on NMR and/or MS have not been used very frequently, especially in older literature, the demanded properties to accept an analysis result as a fingerprint were set rather low: *(i)* a chromatogram, a spectrum or similar data of an examined extract must be shown; *(ii)* the information content of the chromatogram/spectrum regarding the chemical composition must be significant and *(iii)* enough experimental details must be given to have a chance to reproduce the result. For example, an HPLC-chromatogram that shows the raw data for a quantitative analysis could typically be considered as a fingerprint when a broad gradient was applied and numerous peaks were visible besides the analytes actually addressed while an isocratic approach typically does not give enough additional information about the metabolome (we found examples, where isocratic chromatograms containing literally no information were explicitly used for

quality and –if applicable- in how far the analysis results were correlated to the observed biological activity or other quality parameters.

- 53% of the probed publications were completely lacking a chemical characterisation.
    - In the majority of these studies the biological activity was assessed by cellular assays, *in-vitro*-assays or animal testing (89%), the rest were applications studies and smaller clinical trials.
- 22% of the probed publications characterised the chemical properties of the examined extracts by quantifying compounds of known activity.
    - 42% of these publications did not assess the biological activity of the examined extracts but evaluated their quality based on the results of the quantitative analysis; the purposes of these publications were either *(i)* introduction, optimisation or comparison of methods for analysis, extraction or purification or *(ii)* quality assessment for a range of available products.
        - The majority of these publications (70%) contained chromatographic data with the properties of a fingerprint analysis.
    - One publication correlated the results of the quantitative analysis with genomic fingerprints in order to test the suitability of genomic data for drug quality assessment [17].
    - 54% of these publications assessed the biological activity of the examined extracts by *in-vitro*-assays, cellular assays or animal testing.
        - The majority of these publications (83%) did not contain chromatographic data with the properties of a fingerprint analysis; 17% did.
    - 4% of these publications described a clinical study and contained chromatographic data with the properties of a fingerprint analysis.
- 9% of the probed publications characterised the chemical properties of the examined extracts by providing –mostly chromatographic- pure fingerprint data without quantifying components or relating the fingerprint-data to the biological activity results.
    - All of these publication assessed the quality of the examined extracts based on *in-vitro*-assays, cellular assays or animal testing.
- 6% of the probed publications characterised the chemical properties of the examined extracts by a fingerprint analysis and correlated the results of the fingerprint analysis with the results of the biological activity testing.
    - All of these publication assessed the quality of the examined extracts based on *in-vitro*-assays, cellular assays or animal testing.
    - 83% of these publications additionally gave quantitative date of some components.
    - None of these publications used methods of multivariate statistics to correlate the fingerprint data with the biological activity, but the quantification data of single metabolites were related to the biological activity [11; 12; 13; 15; 18; 19].
- 10% of the probed publication characterised the chemical properties by fingerprint analysis of the examined extracts for other reasons than biological activity.
    - The purposes of these studies were discrimination of drugs from different origins or identifying adulterations by fingerprint analysis and multivariate statistics [20; 21; 22; 23], identification of novel components, demonstrating the potential of a emerging new technology, or pharmacokinetic studies. One publication correlates the quantity of quality-relevant components with the concentration of hexenal, which the author's

fingerprint-characterisation of an extract). The final decision about defining an analysis result as a fingerprint was subjective.

describe to be relevant for the traditional quality control by the drug's organoleptic properties [24].

3.2.7. Validation of quantitative data

A subset of 400 publications was probed for the application of quantitative analysis and the presence of validation data.

- 9% of the probed publications described a quantitative analysis
    - 68% of these publication did not give any validation data
    - 41% of these publications gave some validation data as recommended by ICH guidelines
    - 8% of these publications validated the quantitative analysis according to ICH

3.2.8. Analytical methods used

A subset of 157 publications was probed for the analytical method used for quantitative analysis or fingerprinting, totally accounting for 166 analytical procedures employed.

In total:

- 64% of the procedures were HPLC based (incl. UHPLC), of these were:
    - 62% coupled to a UV or diode array detector (A data subset that distinguished between these detectors gave the result that DAD-detectors accounted for about 50%, though for the majority of these cases no specific DAD-functionality was employed).
    - 27% coupled to some kind of mass spectrometer
    - 8% coupled to an evaporative light scattering detector
    - 1% coupled to a NMR instrument [3]
    - 1% coupled to an electrochemical detector
    - 1% coupled to a non-specified detector
- 7% of the procedures were GC based, of these were
    - 64% coupled to some kind of mass spectrometer
    - 36% coupled to some other detector
- 11% of the procedures were TLC-based
- 11% of the procedures were NMR-based
- 4% of the procedures were IR-based
- 3% of the procedures were UV-Vis assays for component quantification
- 1% of the were based on capillary electrophoresis

A subset of 17 publications allowed analysing which analytical methods were applied for fingerprint analysis aiming at the characterisation of extracts that were tested for biological activity. 94% of these publications describe an HPLC-based method for fingerprint analysis, the other 6% attribute to TLC.

A subset of 24 publications allowed analysing which analytical methods were applied for quantitative analysis of quality related components. 92% of these publications describe an HPLC-based method, the other 8% attribute to GC and photometric methods.

IR-based fingerprint techniques (mainly NIR) found within this survey were exclusively used for the discrimination of drug origins and supported by multivariate statistics [20; 21; 23; 25].

3.2.9. Granules

All present literature (400) was screened for the application of granules. 2% of these publications used granules as specimen. It is assumed, that the production of these granules started with a water decoction, though this information was clearly visible only from 2 of these publications; the same publications described ethanol precipitation [26] and starch addition [27] as procedures applied prior to granulation. It is noteworthy that ethanol precipitation is aiming at the elimination of polymeric carbohydrates from the water extract prepared from Astragali radix; this component has been claimed to be responsible for biological activity [e.g. 28; 29].

One contributor remarked that one publication did not explicitly state that granules were used but the fact that the commercially obtained product was only described to be "suspended as 10% solution in distilled water for gavage" allows to hypothesize that the product was completely soluble[3]. Hence, it is assumed that granules were studied here [30].

To get am impression if this is a significant problem, a subset of 72 publications that studied specimen that were either commercially obtained or that gave no information about the specimen's origin were analysed for their extraction score. 32% of these publications were scored with 0 points for the extraction procedure, which means that no detailed extraction procedure was given. Amongst these, 40% also received 0 points for botanical origin. Of these 8 publications 2 were commented by the contributors indicating that the kind of herbal preparation studied was not clear. The other 6 publications were reviewed again and revealed that one further publication was lacking sufficient information. In total, 6% of the probed publications did not give enough information about the herbal preparation to tell if the preparation was a granule or not.

A further general observation was that granules have been entitled as traditional preparation and that it was not perceptible taken into account that granules could be different from the herbal decoctions they were produced from. Yet, the fact that granules are produced from such a traditional preparation allows the simple logical conclusion that granules are a different product and their entitlement as a traditional preparation seems doubtful, especially when measured by the frequently stressed tradition of thousands of years.

4. SUMMARY OF IDENTIFIED PROBLEMS

Facts that were found within 3 and that were considered as a problem with regard to "Good Practice" in science are briefly summarized below and will be addressed for further discussion in D2.12.

4.1. Characterisation of botanical origin

- Latin binominal species names are frequently (~30%) incomplete because the authority is not given within the "Material and Methods" part. Often, a complete Latin binominal is given elsewhere in the publication, but a clear link to the specimen that was actually studied is not given.
- The use of Latin drug names is practically never linked to a pharmacopeia.
- The pinyin name was only very seldom (3%) given within the "Material and Method" part of the probed publication. Similar to the indication of the full Latin binominal incl. authority acronym, the pinyin name was frequently given elsewhere without providing a clear link to the specimen that was actually used.
- 23% of the probed publications left doubts about the plant part that was used.
- In more than 50% of the probed publications, no identification information (botanist, reference) were given.
- In 66% of the probed publications, no voucher was deposited.
- DNA barcoding was hardly ever observed.

[3] We interpret „to suspend" in a broader sense here, in a pharmaceutical context, a suspension would actually be regarded as a homogenous mixture of undissolved particles in a liquid.

4.2. Source of the specimen

- The presence of information about a herbal specimen's source was generally considered good for specimen that were collected by the authors, though temporal information was found in only 43% of the probed publications. The plant age was given less frequently with 23% but this information is not always accessible. The quality of this information is variable, spatial information is mostly given on a regional scale, temporal information on a seasonal scale. The information level for the majority of the residual cases is better for both temporal and spatial information.
- Products that were commercially obtained on markets, drug stores or pharmaceutical companies were characterised with their lot number in only 9% of the cases.

4.3. Traditional or non-traditional use?

The question whether some assay, animal test or clinical study can be assigned to a traditional use or could not be easily answered in most of the cases, because the traditional terminology is not clearly linkable to the test methods.

4.4. Post-harvest and *paozhi* processing

Anything that happens to an herbal specimen between harvest and extraction is summarised under this heading.

- A vast majority of about 90% of the probed publication left doubts if the studied herbal specimen were processed by a *paozhi*-processing method or not. This in contrast to the traditional, therapeutical, phytochemical and practical importance of this practice [1].
- Post-harvest treatments like drying, cleaning cutting etc. are very seldom mentioned but these procedures can have major impact on the product's chemical composition. Especially the TCM specific practice of wet-cutting was practically never addressed within the publications probed for this information.

4.5. Extraction

- Publications about extraction methods employing modern technology (MAE, SFE, IP-NPCE etc.) were scarce and no publications were found that compared these extraction methods with traditional extraction procedures.
- A number of publications employing water decoctions stated that the herbal materials was soaked in cold water before heating for a variable period of time (up to overnight). Most of the publications probed for this issue do not clearly state if the water was already heated up before it came into contact with the herbal material. Similar to the above mentioned wet-cutting, this procedure is capable to significantly change the herbal metabolome.
- Soxhlet extraction with non-azeotropic solvent mixtures was applied in 2 of 7 publications employing this method and the extracts were compared with e.g. ultrasonic extracts using the same solvent mixture.

4.6. Fingerprinting and quantitative analysis

- 53% of the publications assessing biological activity did no chemical characterisation of the extract at all.
- Only 6% of the publications assessing biological activity discussed the chemical properties of the extracts.
- No publication was found that correlated the biological activity with fingerprint data using methods of multivariate statistics.
- Only 9% of the publications assessing biological activity intentionally presented fingerprint data to characterise the chemical composition of the extract tested.
- Within the publications that chemically characterised extracts by quantifying single quality-related constituents it was discovered that those publications which did additional biotesting

were lacking fingerprint data in the majority of cases (83%) while the publications that did no biotesting did show fingerprint data in the majority of cases (70%). Hence, there is a trend visible that extracts are either tested for biological activity or are well characterised by fingerprint analysis.

4.7. Validation of quantitative data

- The majority of publications that characterised an extract by a quantitative analytical procedure did not provide any validation data.
- The majority of publications that provided validation data did not refer to ICH guidelines.

4.8. Analytical methods used

- The clearly dominant method for both fingerprinting and quantitative analysis was HPLC (incl. UHPLC). 62% of the detectors used were UV-detectors (incl. DAD), 27% were mass spectrometers, 11% other. The application of HPLC is even more dominant (94%) for pure fingerprint analysis of extracts subjected to some kind of biotesting. Due to this strong relevance of HPLC techniques, problems that are generally connected with this method are addressed below.
  With regard to fingerprint analysis applied for the untargeted characterisation of herbal extracts which are frequently restricted to the presentation of a single chromatogram (chromatographic raw data), it should be considered that the reproducibility of the chromatographic separation is problematic because the retention behaviour of the chemical components is affected by factors that are usually not well controllable, e.g. the dwell- and dead-volumina of different instruments, age-dependent changing of column properties or the availability of a specific separation material.
  The presentation of liquid-chromatographic profiles recorded with a mass spectrometer as detector are problematic with regard to the reproducibility of signal intensities which can again be affected by factors, that are not well controllable, like the residual salt concentration of eluents, or instrument properties (e.g. aging or contamination of instruments; different instruments usually have completely different parameter sets so a method transfer to another MS instrument is usually not possible to an extend that would allow for a reproduction of signal intensities).

4.9. Granules

- Procedures like ethanol-precipitation are applied within the production of granules in order to give the extract better technological properties by removing polymeric carbohydrates (and maybe other hydrophilic components). Specifically, this procedure was applied for a decoction of Astragali radix, where polymeric carbohydrates were also discussed as active components.
- In the majority of the observed cases, no noticeable distinction took place between granules and decoctions. In some cases it was not even clear if the studied herbal extract was a decoction or a solution of granules.
- Granules are frequently entitled as traditional preparation.

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