

## The Four Pillars of Best Practice - Ethnopharmacology

In the “Four Pillars of Best Practice” we define in detail what constitutes best practice for manuscripts submitted to the specialty section Ethnopharmacology, within the journal *Frontiers in Pharmacology*. The “Four Pillars of Best Practice” also apply to any manuscript on plant extracts, submitted to any section in *Frontiers in Pharmacology*. These criteria provide a basis for peer-review and build upon the general requirements of Frontiers journals and *Frontiers in Pharmacology*. Biomedical research including pharmacological studies must first and foremost be reproducible and scientifically meaningful as well as relevant (cf. <http://dx.doi.org/10.1016/j.bcp.2015.06.023>).

All manuscripts submitted to the specialty section Ethnopharmacology **must follow** the best-practice assessment criteria defined as the “Four Pillars of Best Practice” to being considered for peer review. Find them below.

### 1. Pharmacological Requirements

- a) *Traditional context* - The traditional context must be described in the introduction and supported with bibliographical primary references. This may be based on modern uses of a plant in general healthcare. This use is the basis for a clearly testable research question (i.e. a hypothesis). Consequently, the experimental approach must focus on the assessment of potential effects and must result in a plausible set of pharmacological/clinical data, which allow an assessment of possible pharmacological effects (or clinical effectiveness).
- b) Therefore, a reasonable and therapeutically relevant dose range must be tested, proper controls must be used and the basic pharmacological data must be reported. The calculations commonly used in drug discovery especially for rodents are based on starting values in humans at nano or microMol levels. Therefore, this conversion factor calculation can only be used if the resulting dose level is within the range defined in the consensus paper (see [here](#)). This covers all extracts from single botanical drugs or multi-herbal preparations. For pure compounds, concentrations must be reported in  $\mu\text{Mol}$  / molar unit.
- c) *Credible experimental models* - methods must be state of the art, or a credible alternative resulting in a better understanding of potential pharmacological effects. The following have specific requirements:

<b>Antioxidant</b>	<ul style="list-style-type: none"> <li>• FRAP, ABTS, DPPH, and Trolox equivalent antioxidant capacity assays are not accepted without other pharmacological experiments. They are chemical assays and as such may only be used to define the chemical profile of a preparation.</li> </ul>
<b>Antimicrobial</b>	<ul style="list-style-type: none"> <li>• Disc diffusion experiments must be followed by <i>in vitro</i> or <i>in vivo</i> experiments.</li> <li>• Specificity must be assessed to rule out general toxic effects, e.g. by including parallel cytotoxicity testing (<a href="#">cf. Cos et al. 2006</a>).</li> <li>• The mechanism of action must be assessed in sufficient detail (for crude extracts, the effects of contaminants should also be addressed).</li> </ul>
<b>Inflammation</b>	<ul style="list-style-type: none"> <li>• Experiments on the rat hind paw oedema model are not acceptable unless they are part of a larger pharmacological – phytochemical study.</li> </ul>
<b>Docking studies</b>	<ul style="list-style-type: none"> <li>• These will not be accepted unless followed by benchwork confirming affinity.</li> <li>• A proposed mechanism of action is required.</li> </ul>

<b><i>In silico</i> network pharmacology studies</b>	<ul style="list-style-type: none"> <li>• In general, network pharmacological studies must be conducted in combination with experimental pharmacology (in vitro or in vivo) or are based on a sound body of experimental pharmacology.</li> <li>• Network pharmacology studies must critically assess the pharmacological evidence to evaluate the potential effects of a preparation / herbal (medical) product and the limitations of the evidence.</li> <li>• The network must be represented in such a way that the underlying mechanism can be understood including a suitable visualisation of the network and the individual data points.</li> <li>• The identification of the compounds must be sound. This information may be derived preferably from benchwork or else from the existing literature. It is essential that the quantities of the compounds in the preparation or plant are stated and are high enough to be of pharmacological relevance.</li> <li>• The bioavailability of the compounds must be assessed based on a brief bibliographic review of its pharmacokinetics.</li> <li>• Ubiquitous or very widely known compounds are highly unlikely to be “active” especially in <i>in vitro</i> assays. Therefore, in these cases, evidence for therapeutic or preventive benefits and mechanism of action is essential.</li> <li>• The major target found by transcriptomics or proteomics need to be validated by other experimental techniques.</li> </ul>
<b><i>Single dose studies</i></b>	<ul style="list-style-type: none"> <li>• These are not accepted unless they focus on a species / compound not yet studied in detail and need to be justified on specific ethical grounds.</li> </ul>

d) All submissions need to comply with the best practice guidelines of the leading journals for pharmacological studies on plant extract / natural products, which was developed by the main editors of seven leading journals including *Frontiers in Pharmacology – Ethnopharmacology* (see Heinrich et al. 2019. [here](#))

## 2. Composition Requirements

Whether the material under investigation is a crude plant extract, a multi-herbal preparation, a single compound from a commercial source or extracted from plants, the botanical and chemical composition must be explicitly stated.

### a) Chemical:

- The process of extraction needs to be described in sufficient detail (solvent, protocol, drug – solvent ratio) and the drug extract ratio must be stated based on the mass (g:g). It is not acceptable to only provide the equivalent of the crude drug that has been used in extraction.

The concentrations of the main compounds need to be stated if these have been identified in previous studies, including dominant impurities if these compounds have been identified in previous studies. Stating the class of compounds present (such as “alkaloids”) is insufficient. We will usually ask for a HPLC or UPLC to establish the compounds present to ensure replicability. If this is not possible, a credible alternative like HPTLC can be used.

- Referring to a previously used preparation in the literature is not acceptable, unless it has come from the same preparation or has the same batch number (in commercial preparations).
- For commercial extracts, the batch number must be given as well as a reference to a source which describes the composition.
- For purchased compounds, purity (%) and the supplier's name must be included.
- For extracted compounds, purity (%) and the method used to determine the purity must be stated.
- The structure of active compounds should be included as figures.
- It cannot be assumed *a priori* that common or ubiquitous compounds like  $\beta$ -sitosterol, or common phenolic acids, flavonoids / flavonoid glycosides are "active". More generally, it is essential to differentiate between marker compounds (for a botanical drug or extract) and compounds which contribute to the activity or are the main actives.

#### **b) Botanical:**

- The species name and plant part (drug) must be stated unambiguously. Drug names from popular source or a pharmacopoeia are insufficient.
- Species names must be fully validated taxonomically (including authorities / families), using the [Kew Medicinal Plant Names Services](#) (MPNS), or an alternative source as stated in this source. Samples must be deposited in a recognised herbarium or collection, and accessible (ideally in an indexed herbarium, please use the [NYBG Steere Herbarium Search tool](#)).
- Collector / sample or voucher numbers from the herbarium must be included in the Methods section of the manuscript.
- It is suggested that, once entered the herbarium, the specimen should be digitized and included as supplementary material.
- For collected specimens, geographical coordinates of plant collecting should also be included, or the commercial source of a preparation, which must include a batch number and details on the preparation's composition.
- For multiherbal preparations, the ratio of the drugs used must be stated in combination with a full detailed description of the extraction and processing procedure.

### **3. Basic Experimental and Ethical Requirements**

#### **a) The study must contribute substantially to the existing literature.**

Authors must state explicitly how the study contributes to the existing knowledge. The most up-to-date surrounding literature should be included and assessed, considering related compounds, to demonstrate the contribution of the study to the field.

#### **b) Compliance with all international ethical standards is essential.**

[The Convention on Biological Diversity and the Nagoya Protocol](#) are of particular relevance. This includes that ethnopharmacological research should benefit the original users and consider their traditions.

**c) The use of animals must be justified.**

If a material is well-characterised pharmacologically, and its chemistry and properties well-known, performing another *in vivo* study is considered an unethical use of animals. A thorough knowledge of the literature is essential to avoid this. Conversely, if herbal preparation is not well characterised, initial experiments in cell-based models should be the first step followed – if needed – by animal experiments.

**d) The effects of traditional medicinal preparations must be testable in scientific terms.**

We acknowledge the importance of understanding medicinal preparations in their cultural context. The treatment of symptoms as defined by traditional practices may form a basis for pharmacological investigations. However, a series of *in vitro* tests will not demonstrate relevant evidence that will contribute to a pharmacological understanding of traditional therapeutic concepts, e.g. “dispelling wind” or “dampness” in Traditional Chinese Medicine. In other words, pharmacological studies generally do not provide evidence for such uses, but rather for the established therapeutic effects based on the molecular targets of the model. Experimental outcomes should be linked to and described in these terms. A justification must therefore be given for choosing a certain model to test a preparation.

#### 4. Article-type Specific Requirements

**a) Field Studies** (*including historical studies*)

- Data must be substantial, original, and based on a sufficiently large set of original data specific to the region of study.
- Studies on the use of herbal medicines in a more biomedical setting (e.g. with participants from urban regions) are encouraged if they are novel and contribute to improved healthcare.
- The study must be discussed critically in the context of previous studies carried out in the region. How the study contributes to the development of the field must be made explicit.
- This journal subscribes to the [ConsEFS standards](#), including any updates.

**b) Reviews**

- We encourage all types of reviews, including general, critical, and systematic reviews, but the approach used must be justified in the context of the research.
- The objective(s) of the review must be clearly defined and provide a testable research question.
- Reviews must provide a specific and critical assessment of the literature. The scientific quality of the original articles must be critically assessed. This includes the experimental design, and reliability of the studies including a sufficiently detailed definition of the material under investigation.
- If the included studies do not use full botanical taxonomic names, this should be highlighted, as must any naming inconsistency between studies. The traditional use must be linked to scientific evidence.
- The authors should also cite the most recent and/or most similar published related manuscripts and briefly explain the scientific advancement of their manuscript as compared to the previous papers.

- A rationale for the methods selected, i.e. for the search strategy, databases, for the method how relevant data was extracted, and for the analysis of the results must be included.
- A description of the limitations and future research needs and priorities must be included.

**c) Systematic Reviews and Meta-Analyses**

- To assure the quality of the studies included, we ask for the inclusion of a summary table describing the composition of the preparation(s) and how these were reported in the original studies (see templates below).
- Systematic reviews, with or without a meta-analysis, must include a flow chart (<http://www.prisma-statement.org>) as a figure in the manuscript.
- We ask that a chemical analysis is included, taken from one of the included studies. The chemical composition of the study material must be well defined. If the composition is poorly characterised, this must be highlighted.
- Quality control measures taken in the original studies, for example, as defined by a pharmacopoeia, must also be included.

**\*\*\*See tables on the next page\*\*\***

**Option 1 – Botanical or multiherbal**

Study	Species, source, concentration	Quality control reported? (Y/N)	Chemical analysis reported? (Y/N)
X et al. (2015)	<ul style="list-style-type: none"> <li>· Leaf of <i>Azadirachta indica</i> A.Juss., [Commercial Supplier Ltd], 8.5g</li> <li>· Root of <i>Vincetoxicum auriculatum</i> (Royle ex Wight) Kuntze [coordinates of the collection site(s)], 7.2g</li> </ul>	Y - Prepared according to x protocol	Y – HPLC or other analytical system
Y et al. (2016)	Dried roots of <i>Panax ginseng</i> C.A.Mey., [Commercial Supplier Ltd] 7.2g	Y - Leaves ground and filtered...	Y – HPLC or other analytical system

**Option 2 – Patented formulations, botanical or chemical**

Study	Formulation	Source	Species, concentration	Quality control reported? (Y/N)	Chemical analysis reported? (Y/N)
X et al. (2015)	[name of preparation]	[Commercial Supplier, Ltd.]	<ul style="list-style-type: none"> <li>· Leaf of <i>Azadirachta indica</i> A.Juss., 8.5g</li> <li>· Root of <i>Vincetoxicum auriculatum</i> (Royle ex Wight) Kuntze 7.2g</li> </ul>	Y - Prepared according to x pharmacopeia	Y – HPLC or other analytical system
Y et al. (2016)	[name of preparation]	Prepared by Y et al. (2016)	<ul style="list-style-type: none"> <li>· Leaf of <i>Azadirachta indica</i> A.Juss., [Commercial Supplier Ltd], 8.5g</li> <li>· Root of <i>Vincetoxicum auriculatum</i> (Royle ex Wight) Kuntze [coordinates of the collection site(s)], 7.2g</li> </ul>	Y - Prepared according to x pharmacopeia	Y – HPLC or other analytical system

**Option 3 – Isolated chemical compound**

The purity must be stated unambiguously

Study	Compound, concentration	Source	Purity (%) (and grade, if applicable)	Quality control reported? (Y/N)
X et al. (2015)	Pure compound, 10mg	[Commercial Supplier]	(≥90%)	Y
Y et al. (2016)	Pure compound, 10mg	Purified by Y et al. (2016)	(≥90%)	Y - HPLC