The Four Pillars of Best Practice - Ethnopharmacology

In the "Four Pillars of Best Practice" we define 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 manuscripts on plant extracts, submitted to any other 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. article here). Frontiers Media has established clear general guidelines on what constitutes valid research (see here).

All manuscripts submitted to the specialty section Ethnopharmacology **must follow** the best-practice assessment criteria defined as the "Four Pillars of Best Practice" for being considered for peer review. They cover pharmacological, analytical-chemical, and general aspects of research on medicinal plants and other products derived from natural sources like fungi.

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.

b) The experimental approach must result in a plausible set of pharmacological data. Therefore, a reasonable and therapeutically relevant dose range must be tested, proper controls must be used and the basic pharmacological data must be reported. For in vivo studies and as a general rule, the dose tested should not exceed 1g/kg & day of the extract. This covers all extracts from single botanical drugs or multi-herbal preparations. For pure compounds, concentrations must be reported in µMol / 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:

Antioxidant	• FRAP, ABTS, DPPH, and Trolox equivalent antioxidant capacity assays are not acceptable in the context of pharmacological experiments. They are chemical assays and as such may only be used to define the chemical profile of a preparation.
Antimicrobial	• Disc diffusion experiments must be followed by in vitro or in vivo experiments. • Specificity must be assessed to rule out general toxic effects, e.g. by including parallel cytotoxicity testing (cf. Cos et al. 2006 - DOI: 10.1016/j.jep.2006.04.003). • The mechanism of action must be assessed in sufficient detail (for crude extracts, the effects of contaminants should also be addressed).

Inflammation	• Experiments on the rat hind paw oedema model are not acceptable unless they are part of a larger pharmacological – phytochemical study.
Docking studies	• These will not be accepted unless followed by benchwork confirming affinity. • A proposed mechanism of action is required.
In silico network pharmacology studies	• In general, network analysis must be conducted in combination with experimental pharmacology (in vitro or in vivo) or must be based on a sound body of experimental pharmacology that is referenced in the article. Network studies on complex extracts are generally not considered. • Network analyses must critically assess the pharmacological evidence to evaluate the potential effects of a preparation / herbal (medical) product and the limitations of the evidence. • 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. • 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. In case of identification using mass spectrometry, the data must be derived from high resolution experiments. • The bioavailability of the compounds must be assessed based on a brief bibliographic review of its pharmacokinetics. • Ubiquitous or very widely known compounds such as quercetin or curcumin are highly unlikely to be "active" and "specific", especially in in vitro assays. Therefore, in these cases, evidence for therapeutic or preventive benefits and mechanism of action is essential. • The major target found by transcriptomics or proteomics need to be assessed using other experimental techniques.
Single dose studies	• 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. Exceptions can be made if the study is an early-stage exploratory study, but this then needs to be spelled out clearly in the discussion and conclusion.

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) Botanical /Pharmacognostic

- The composition incl. preparation must be stated unambiguously (including the amount of each drug in a polyherbal preparation and the extraction procedure) and the complete species and drug name must be included. Here an example of how it should be presented: *Salvia miltiorrhiza* Bunge [Lamiaceae; Salviae miltiorrhizae radix et rhizoma]. All species must be validated taxonomically (e.g. Kew Medicinal Plant Names Services (MPNS) or Kew Plants of the World Online) and the full species name including authorities and family needs to be included, as well as the drug name, if one has been assigned in a pharmacopeia (see Rivera, D., et al., 2013; DOI: 10.1016/j.jep.2013.12.022) For fungi, Index Fungorum should be used. In addition, the inclusion of common names is strongly encouraged. Normally it is best to have these aspects covered as a section at the start of 'Material and Methods'.
- For samples collected in the field, collector / sample or voucher numbers from the herbarium must be included in the Methods section of the manuscript.
- Once the specimen has entered the herbarium it 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.

b) Chemical

- All manuscripts investigating extracts need to be checked with the ConPhyMP tool. A chemical profile following the standards established in the ConPhyMP statement is essential: Front. Pharmacol. 13:953205. https://doi.org/10.3389/fphar.2022.953205. Here three types of extracts are defined based on the regulatory status of the botanical drugs investigated. They can either be a listed/registered or licensed medicine, or a food supplement used widely, or a lesser-known botanical drug, which is at an early stage of research and development. For each different levels of detail are needed.
- 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. The full composition must be reported as for other preparations.
- For purchased compounds, purity (%) and the supplier 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 using an internationally accepted programme to draw them.
- It cannot be assumed *a priori* that common or ubiquitous compounds like β-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.

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. Authors must consider and fully respect their traditions.

c) The use of animals must be justified.

Complying with the 4R rule (Reduce, refine, replace – responsibility) is strongly encouraged. 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. In vivo studies using rodents or other mammals as models need to be relevant in the context of developing evidence-based practices or novel therapeutic approaches for specific and significant improvements in the treatment of major diseases in humans or animals. 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.

If drugs derived from animals are included in a preparation used experimentally, detailed evidence on ethical sourcing is essential. If a submission is believed or found to contain such a product, the editorial office may request further evidence to guarantee that these conditions have been met. Such evidence may include but is not limited to, signed ethical approval documents and proposals submitted to the Ethics committee from the authors affiliated institution(s).

Frontiers in Pharmacology will generally not consider manuscripts which use materials sourced by methods which are known to cause unacceptable levels of suffering to animals, or where there are concerns about the sustainability of its sourcing. Specifically, this includes, but is not limited to, preparations derived from the following: tiger bones, rhino horns, deer antlers, bear and snake bile. Manuscripts using such drugs will be rejected at desk review on ethical grounds'.

In the event that a protected species (e.g., plants, fungi animals or microorganism covered under CITES, the IUCN Red List, or are considered to be a risk of extinction or are under serious decline) are included in a submission, such a study must be fully justified either based on ethical sourcing (cultivated plants etc.) or be the basis for searching for alternative sources to replace protected species.

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, there needs to be a plausible link between these uses and the pharmacodynamic experiments conducted. For example, 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. Please consult ConsEFS JEP 2017.

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 assessed critically. This includes the experimental design, and reliability of the studies including a sufficiently detailed definition of the material under investigation.
- A critical assessment of the validity of the studies is an essential part of a review, identifying, for example, methodological and conceptual shortcomings of the original studies reviewed. 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 relevant related publications 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 discussion of the limitations and future research 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 (as outlined above) covering the composition of the preparation and how this is reported. The pharmaceutical producer must be included. Problems identified with the original studies reviewed need to be assessed and reported.
- Systematic reviews, with or without a meta-analysis, must include a flow chart (http://www.prisma-statement.org) as a figure in the manuscript.
- Quality control measures taken in the original studies, for example, as defined by a pharmacopoeia, must also be included.