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Role of Markers in the Standardization of Herbal Drugs: A Review

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Abstract

The efficacy and safety of any pharmaceutical product is determined by the compounds (desired and undesired) which it contains. The purpose of quality control is to ensure that each dosage unit of the drug product delivers the same amount of active ingredients and is, as far as possible, free of impurities. As herbal medicinal products are complex mixtures which originate from biological sources, great efforts are necessary to guarantee a constant and adequate quality. By carefully selecting the plant material and a standardized manufacturing process the pattern and concentration of constituents of herbal medicinal products should be kept as constant as possible as this is a prerequisite for reproducible therapeutic results.

Key Words: Herbal medicinal products, Quality, Safety, Efficacy, Marker

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Introduction

Within the context of increased herbal medicines use and lack of effective regulatory control, the safety of herbal medicines has become a key priority issue. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The new Pharmacognosy includes all the aspects of drug development and discovery, where biotechnology-driven applications play an important role.

Scientifically validated and technologically standardized herbal medicines may be derived using a safe path of reverse pharmacology approach based on traditional knowledge database. This may play a vital role in drug discovery, development and therapeutics, in addition to dealing with a typical Western bias against Ayurveda [1].

Correct identification and quality assurance of the starting material is, therefore, an essential prerequisite to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy [2].

Selection of chemical markers is crucial for the quality control of herbal medicines, including authentication of genuine species, harvesting the best quality raw materials, evaluation of post harvesting handling, assessment of intermediates and finished products, and detection of harmful or toxic ingredients [3]. Chemical fingerprinting has been demonstrated to be a powerful technique for the quality control of herbal medicines [4].

According to regulatory guidelines and pharmacopoeias macroscopic and microscopic evaluation and chemical profiling of the botanical materials is used for quality control and standardization [5, 6]. Thin layer chromatography (TLC) and High Performance Thin Layer Chromatography (HPTLC) are valuable tools for qualitative determination of small amounts of impurities. Also many analytical techniques such as Volumetric Analysis, Gravimetric Determinations, Gas Chromatography, Column Chromatography, High Performance Liquid Chromatography and Spectrophotometric methods are also frequently used for quality control and standardization [7].

For quantitative studies, specific markers is used .For molecular marker chemo type of plant is necessary. For example, *Withania somnifera* is reported to have three chemotypes depending upon the presence of a class of closely related steroidal lactones like withanolides, withaferin A etc. The content of withanolides, withaferin A and other biologically active compounds may vary depending upon the environment, genotype, time of collection of plant material, etc. Hence selection of the right chemo type having therapeutic efficacy is important.

The use of chromatographic techniques and marker compounds to standardize botanical preparations has limitations because of their variable sources and chemical complexity. DNA-based molecular markers have utility in the fields like taxonomy, physiology, embryology, genetics, etc.

Markers are categorized in to two classes:

(1) **DNA markers** are reliable for informative polymorphisms as the genetic composition is unique for each species and is not affected by age, physiological conditions as well as environmental factors. DNA can be extracted from fresh or dried organic tissue of the botanical material; hence the physical form of the sample for assessment does not restrict detection [8].

(2) **Chemical markers** generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids [9].

DNA Markers:

Various types of DNA-based molecular techniques are utilized to evaluate DNA polymorphism. These are hybridization-based methods, Polymerase Chain Reaction (PCR)-based methods and sequencing-based methods.

Applications of molecular markers in herbal drug technology:
DNA-based molecular markers have proved their utility in fields like taxonomy, physiology, embryology, genetics, etc.

- **Genetic variation/genotyping:** RAPD-based molecular markers [10] have been found to be useful in differentiating different accessions of *Taxus wallichiana*, *neem*, *Juniperus communis L.*, *Codonopsis pilosula*, *Allium schoenoprasum L.*, *Andrographis paniculata* collected from different geographical regions. Interspecies variation has been studied using RFLP and RAPD in different genera such as Glycerrhiza, Echinacea, Curcuma and Arabidopsis. RAPD has served as a tool for the detection of variability in Jojoba (*Simmondsia chinensis* L. Schneider), *Vitis vinifera* L. and tea (*Camellia sinesis*).

- **Authentication of medicinal plants:** Sequence Characterized Amplified Region (SCAR), AP–PCR, RAPD and RFLP have been successfully applied for differentiation of these plants and to detect substitution by other closely related species. Certain rare and expensive medicinal plant species are often adulterated or substituted by morphologically similar, easily available or less expensive species. For example, *Swertia chirata* is frequently adulterated or substituted by the cheaper *Andrographis paniculata*.

- **Marker assisted selection of desirable chemo types:** AFLP analysis has been found to be useful in predicting phytochemical markers in cultivated *Echinacea purpurea* germplasm and some related wild species DNA profiling has been used to detect the phylogenetic relationship among *Acorus calamus* chemotypes differing in their essential-oil composition.

- **Medicinal plant breeding:** Molecular markers have been used as a tool to verify sexual and apomictic offspring of intraspecific crosses in *Hypericum perforatum*, a well known antihelminthic and diuretic.

- **Applications in foods and nutraceuticals:** Roundup ready soybeans, maize and cecropin, capsicum have been successfully discriminated from non-GM products using primers specific for inserted genes and crop endogenous genes.

DNA markers as new pharmacognostic tool. These markers have shown remarkable utility in quality control of commercially important botanicals like Ginseng, Echinacea, Atractylodes. Although DNA analysis is currently considered to be cutting-edge technology, it has certain limitations due to which its use has been limited to academia another important issue is that DNA fingerprint will remain the same irrespective of the plant part used, while the phytochemical content will vary with the plant part used, physiology and environment

Chemical markers
The European Medicines Agency (EMEA) defines chemical markers as chemically defined constituents or groups of constituents of an herbal medicinal product which are of interest for quality control purposes regardless whether they possess any therapeutic activity.

The quantity of a chemical marker can be an indicator of the quality of an herbal medicine. The study of chemical markers is applicable to many research areas, including authentication of genuine species, search for new resources or substitutes of raw materials, optimization of extraction and purification methods, structure elucidation and purity determination. Systematic investigations using chemical markers may lead to discoveries and development of new drugs. The EMEA categorizes chemical markers into analytical markers and active markers.

Analytical markers- are the constituents or groups of constituents that serve solely for analytical purposes.

Active markers- are the constituents or groups of constituents that contribute to therapeutic activities.

Srinivasan proposed the following four categories:
a. Active principles,
b. Active markers,
c. Analytical markers and
d. Negative markers.

Active principles possess known clinical activities; active markers contribute to clinical efficacy; analytical markers have no clinical or pharmacological activities; negative markers demonstrate allergenic or toxic properties.

Applications of chemical markers

- *Identification of adulterants*- An adulterant of gamboges was differentiated from the authentic sample by an HPLC-UV method using eight caged xanthones as chemical markers.
- *Differentiation of herbal medicines with multiple sources*
- *Determination of the best harvesting time*
- *Confirmation of collection sites*
- *Assessment of processing methods*
- *Quality evaluation of herbal parts*
- *Identification and quantitative determination of proprietary products*
- *Stability test of proprietary products*- Stability test is used to evaluate product quality over time and determine recommended shelf life.
- *Diagnosis of herbal intoxication*- Toxic components may be used as chemical markers in screening methods, e.g. rapid diagnosis of acute hidden aconite poisoning in urine samples by HPLC-MS.
- *Lead compounds for new drug discovery*- The components responsible for the therapeutic effects may be investigated as lead compounds for new drug discovery.

Conclusion

Quality control of herbal medicines aims to ensure its quality, safety and efficacy. Chemical markers are pivotal in the current practice of quality control. Chemical markers should be used at various stages of the development and manufacturing of an herbal medicine, such as

authentication and differentiation of species, collecting and harvesting, quality evaluation and stability assessment, diagnosis of intoxication and discovery of lead compounds. Lack of chemical markers remains a major problem for the quality control of herbal medicines. In many cases, we do not have sufficient chemical and pharmacological data of chemical markers. Furthermore, there are many technical challenges in the production of chemical markers. For example, temperature, light and solvents often cause degradation and/or transformation of purified components; isomers and conformations may also cause confusions of chemical markers.

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