Role of Markers in Standardization of Herbal Medicines

As per WHO definition, there are three kinds of herbal medicines: raw plant material, processed plant material and medicinal herbal products. Herbal drugs are finished labeled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combination thereof, whether in the crude state or as plant preparations. The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, teas, extracts and fresh or dried plants.

Herbal medicinal products may vary in composition and properties, unlike conventional pharmaceutical products, which are usually prepared from synthetic, chemically pure materials by means of reproducible manufacturing techniques and procedures. 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.

Factors that influence the identification and quality of herbal drugs

- Drug of plant origin are usually mixtures of many constituents.
- The source and quality of the raw material are variable.
- The active principle in some cases is unknown.
- Selective analytical methods or reference compounds may not be available commercially.
- Plant materials are chemically and naturally variable.
- The required herb is deliberately or in- deliberately substituted with the other low quality and morphologically similar medicinal or non-medicinal plants e. g. *Belladonna* leaves are substituted with *Ailanthus* leaves, papaya seeds in place of *Piper nigrum* L., mother cloves and clove stalks are added to clove, beeswax is adulterated with Japan wax.
- The exact time and methods of harvesting, drying, storage, transportation and processing have an effect, eg. *Datura strumarium* L. leaves should be collected during the flowering stage and wild cherry bark in autumn.
- Common vernacular name for different plants or different vernacular names in different states of the same medicinal plants. e. g. *Centella asiatica* L and *Bacopa monnerri* L. are interchangeably known as Brahmi and Mandukparni.

Standardization

Herbals are traditionally considered harmless and increasingly being consumed by people without prescription. However, some can cause health problems, some are not effective and some may interact with other drugs. Standardization of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles

Standardization is the application of product knowledge, good agricultural or good manufacturing practices to minimize inherent variations in the composition of natural substances in order to ensure a consistent product from one batch to the next.

OR

Standardization is a system that ensures a predefined amount of quantity, quality & therapeutic effect of ingredients in each dose.

Standardization of herbal drugs for Global competitiveness such as raw materials needs to be authentic, physico-chemical standards, storage conditions, size and shape. Processing of raw material include material, energy inputs, operational uniformity, safety and occupational health, intermediate quality whereas finished product include physicochemical properties, biological assay, storage stability, user safety *etc*.

Markers

Marker compounds are constituents that occurs naturally in the material and that is selected for special attention (for identification or standardization purposes).

OR

Markers are chemically defined constituents of a herbal drug which are of interest for quality control purposes independent of whether they have any therapeutic activity or not. Markers may serve to calculate the amount of active component of herbal drug or preparation in the finished product.

Marker compounds are pure, single isolated compounds, secondary metabolites mostly with terpenes, steroid, alkaloid, flavonoid aromatic hetero aromatic frameworks and glycosides having alcoholic, carbonyl, olefinic, acid, ester and amide functionalities highly useful for single / crude drugs: may or may not survive in multiherbal. For quantitative studies, use of specific markers that can be easily analyzed to distinguish between varieties, remains a preferred option

Types of Markers

Molecular or **DNA Markers**

They 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.

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: Random Amplified Polymorphic DNA (RAPD) - RAPD-based molecular markers have been found to be useful in differentiating different accessions of *Taxus wallichiana, Neem, Allium schoenoprasum , Andrographis paniculata* collected from different geographical regions.

Interspecies variation has been studied using RFLP(Random Fragment Length Polymorphism) and RAPD in different genera such as Glycerrhiza, Echinacea, Curcuma and . RAPD has served as a tool for the detection of variability in *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:

Amplified Fragment Length Polymorphism (AFLP) - 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 essentialoil 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.

Foods and nutraceuticals: Soybeans, maize and capsicum have been successfully discriminated from non-GM products using primers specific for inserted genes and crop endogenous genes.

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

Generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids 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.

Types of chemical markers.

***Active principles*-** Well defined chemicals with known clinical activity

e.g Ephedrine in Ephedra sinensis, Silymarin in Silybum marianum

Active markers - They are the constituents or groups of constituents with known pharmacological activity that contribute to *efficacy.* May or may not have proven clinical efficacy.

e.g Allin in Allium sativum, Hypericin in Hypericum perforatum

Analytical markers - They are the constituents or groups of constituents that serve solely for analytical purposes & *have no clinical or pharmacological activities*. Aid in the positive identification of raw material and extracts or used to achieve standardization.

e.g Different alkylamides found in roots of Echinacea angustifolia and Echinacea purpurea but totally absent in Echinacea pallid.

Negative markers *– Demonstrate allergenic or toxic properties* or those which interfere with bioavailability.

e.g Gingolic acids in ginkgo preparations (allergenic agent).

Applications of chemical markers

Identification of adulterants.

· *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.

Boswellia serrata also known as Salai Guggul is extensively used in Ayurveda for joint support and provides an overall sense of well being. It offers broad health and immunomodulating benefits, anti-inflammatory, antiatherosclerotic and anti-arthritic activities. It also improves circulation of blood and also used in cosmetic products. In gum resin of *Boswellia serrata* , the marker compound is Boswellic acid and the standardized extract of *Boswellia serrata* is fixed to contain a consistent level of this compound (usually a few percent

Biochemical markers

Biochemical markers are either proteins or isoenzymes. Isoenzymes, also known as alloenzymes are the enzymes which are functionally similar but differ from each other in the location, structure, net charge, electrophoretic mobility, types of activators and inhibitors and heat stability. The isoenzyme pattern has been used in many population genetics studies; including

measurements of out crossing rates, sub population structure and population divergence. These are particularly valuable to distinguish closely related species and therefore useful to study diversity and identification of crops.

Example: The isoenzyme pattern of glucose 6 phosphate dehydrogenase has been used for the identification of *Eclipta prostrate* L. The peroxidase isoenzyme pattern is used to distinguish the three *Gastrodia elata* variants.

Advantages of DNA markers over chemical markers

Analysis of DNA markers is more reliable than the chemical markers. They provide an efficient and accurate means of testing the authenticity of several hundreds of samples simultaneously whereas the conventional chemical based methods usually takes several days for verification. In order for any compound to act as a chemical marker, it should be unique to that particular species. Not all plants have a unique chemical compound and also the same chemical marker is used for the identification of two or more plants. Moreover the concentration of secondary metabolites and other biochemical markers may change due to environmental factors and hence correct identification of the botanicals is difficult, whereas genetic markers are unique and are not affected by age, physiological conditions and environmental factors.

DNA based techniques and molecular markers in identification of medicinal plants.

Botanical name of medicinal plant	Therapeutic uses	Chemical marker	DNA based techniques	Purpose of using molecular marker
Abrus precatorius	Parturition, Dysuria	Abruquinones	RAPD ,ISSR	To study genetic diversity
Andrographis paniculata	Hepatoprotective, antipyretic,	Andrographolid	RAPD	To study genetic diversity
Azadirachta indica	Helminthiasis, pyrexia,	Epicatechin	AFLP, SAMPL,	To find out intra-population
Acorus calamus	Epilepsy, dyspnoea,	*Beta- asarone, methyl*	RAPD, ISSR,	To develop population genetic structure,
Bacopa monnieri	Anti leprotic,	Bacoside A	RAPD, ISSR	For authentication,
Carthamus tinctorius	Constipation	Carthamin	EST-SSR,	To carry out genetic analysis, and

Chenopodium ambrosioides	Helminthiasis	Z-& E-ascaridoles	RAPD	To find out genetic Relationship.
Moringa oleifera	Inflammatory condition,	Niazinin B	AFLP, RAPD	To find out genetic variability, out
Ocimum tenuiflorum	Dysuria, burning micturition,	Linoleic acid	RAPD, AFLP, ISSR	Genetic diversity, phylogenetic
Ricinus communis	Root: inflammatory disorders, abdominal colic. Leaf: Lower	Rutin, Ricinoleic acid	EST- SSR, microsatellite ,RAPD,	Genetic purity testing of hybrids, Genetic diversity and

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