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IMPORTANCE OF MARKERS IN THE STANDARDIZATION OF HERBAL DRUGS: A REVIEW

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ABSTRACT

Standardization and quality control with proper applications of modern scientific techniques and traditional knowledge is the key for conversion of the botanical materials into medicines. The new Pharmacognosy includes all the aspects of drug development and discovery, where bio-technology driven applications play an important role. As Herbal medicinal products are complex mixtures which originate from biological sources, great efforts are necessary to guarantee a constant adequate quality. By carefully selecting the plant materials and a standardized manufacturing process the pattern and concentration of constituents of herbal medicinal products should be kept constant possible. Marker compounds are mainly the chemical constituents in a medicine that can be used to verify its potency or identity. A good marker should be characteristic of the given herbal preparation, be a substance with an established chemical structure and not be present in other herbs contained in the finished product. This review provides a brief account of various aspects of Marker compounds that are useful in genotyping and quick identification of botanicals.

KEYWORDS: *Markers, Standardization, Herbal drugs, Phytoconstituents, DNA markers.*

I. INTRODUCTION

Herbal medicines, also known as botanical medicines or phytomedicines, refer to the medicinal products of plant roots, leaves, barks, seeds, berries or flowers that can be used to promote health and treat diseases. Medicinal use of plants has a long history worldwide. According to the World Health Organization (WHO), traditional herbal preparations account for 30–50% of the total medicinal consumption in China.[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] Herbal drugs, singularly and in combination contains a mydriad of compounds in complex matrices in which no single active constituent is responsible for its overall efficacy. This creates a challenge in establishing quality control standards for raw-materials and standardization of finished herbal drugs.

Botanical extracts made directly from crude plant materials show substantial variation in composition, quality and therapeutic effects. When active principles are unknown, marker compounds are to be established among the known constituents of the extract for standardization purpose. Ideally the chosen marker should be responsible for the therapeutic effect of the herbal drug. Gingko with its 26% gingko flavones and 6% terpenes is a classic example. Such extracts are highly standardized and no longer represent the whole herb and are now considered as phytopharmaceuticals.[3]

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.[4] Chemical fingerprinting has been demonstrated to be a powerful technique for the quality control of herbal medicines.[5] For quantitative studies, specific markers are used .For molecular markers, 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, applied sciences etc.

2. Types of plant marker

There are two types of plant markers

a) DNA markers

b) Chemical markers

DNA markers provide information on polymorphisms as the genetic composition is unique for each species and is not affected by environmental factors, age or physiological factors. The physical form of the sample for assessment does not restrict detection as DNA can be collected from living or dead tissue of the biological origin.[6] Chemical markers include chemical constituents of biological origin, including primary and secondary metabolites and other macromolecules such as amino acids, nucleic acids etc.[7]

2.1 Types of DNA markers

There are three types of marker which are hybridization-methods based markers, polymerase chain reaction (PCR) - methods based markers and sequencing- method based markers.

2.1.1 Hybridization- methods based markers

Hybridization-based methods include restriction fragment length polymorphism (RFLP)[8] and variable number tandem repeats.[9] Labeled probes such as random genomic clones, cDNA clones, probes for microsatellite[10] and minisatellite.[11] sequences are hybridized to filters containing DNA, which has been digested with restriction enzymes. By the presence or absence of bands upon hybridization, detection of polymorphism is done. The various enzymes are used accordingly.

2.1.2. PCR- methods based markers

PCR-based markers involve in vitro amplification of particular DNA sequences or loci, with the help of specific or arbitrary oligonucleotide primers and the thermo stable DNA polymerase enzyme. PCR-based techniques, where the random primers are used, include random amplified polymorphic DNA (RAPD)[12] [13], arbitrarily primed PCR (AP– PCR)**[14]** and DNA amplification fingerprinting (DAF).[15] [16] Inter simple sequence repeats (ISSRs)[17] polymorphism is a specific primer-based polymorphism detection system, where a terminally anchored primer specific to a particular simple sequence repeat (SSR) is used to amplify the DNA between two opposed SSRs of the same type. Polymorphism occurs whenever one genome is missing in one of the SSRs or has a deletion or insertion that modifies the distance between the repeats. A recent approach known as amplified fragment length polymorphism (AFLP)[18], [19] is a technique that is based on the detection of genomic restriction fragments by PCR amplification. Adaptors are ligated to the ends of restriction fragments followed by amplification with adaptor-homologous primers. AFLP has the capacity to detect thousands of independent loci and can be used for DNAs of any origin or complexity.[20]

2.1.3. Sequencing-based markers

DNA sequencing has nowadays being used as a definitive means for identifying the various species. Variations due to transversion, insertion or deletion can be identified directly and the information is obtained on a defined locus. Genetic variation occurs extensively at the single nucleotide level. Direct sequencing can efficiently identify such single nucleotide polymorphisms that usually depend on how closely related are the organisms being compared. Other sequencing-based strategies include analysis of the variable internal transcribed spacer (ITS) sequences of ribosomal DNA (rDNA). The ITS region of 18s–26s rDNA has proved to be a useful sequence for phylogenetic studies in many angiosperm families. The level of ITS sequence variation suitable for phylogenetic analysis is found at various taxonomic levels within families, depending on the linkage. A number of researchers have also sequenced other regions of DNA such as that of chloroplast and spacer region of 5s rDNA as the diagnostic tools for authentication purpose.

2.1.4 Applications of DNA markers

DNA-based molecular markers have proved their utility in fields like applied sciences, agronomy, taxonomy, physiology, embryology, genetics, etc. As the science of plant genetics progressed, researchers have tried to explore these molecular marker techniques for their applications in commercially important plants such as food crops, horticultural plants, etc. and recently in pharmacognostic characterization of herbal medicines.[21] Some of the applications are discussed as follows.

2.1.4.1. Certification of genuine medicinal plants

Techniques such as Sequence Characterized Amplified Region (SCAR), AP–PCR, RAPD and RFLP have been successfully applied for the differentiation of these plants and also 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, *Belladonna leaves* is frequently adulterated or substituted by the cheaper *Ailanthus leaves.*

2.1.4.2. Differentiation among the various species

RAPD-based molecular markers[10] have been found to be useful in differentiating different species of Ashoka, *Taxus baccata, Neem, Juniperus communis L., Codonopsis pilosula, Allium species, Andrographis paniculata* collected from different geographical regions. Interspecies variations have been studied using RFLP and RAPD in different genera such as

Coca,Thea sinensis, Curcuma and Arabidopsis. RAPD has served as a tool for the detection of variability in *Jojoba (Simmondsia chinensis L. Schneider), Citrus sinensis.* and *tea (Camellia sinesis).*

2.1.4.3. Selection of desirable chemo types: AFLP analysis has been found to be useful in predicting phytochemical markers in cultivated *Azadiracta indica* germplasm and some related wild species DNA profiling has been used to detect the phylogenetic relationship among *Trachyspermum ammi* chemotypes differing in their essential oil composition.

2.1.4.4. Breeding in the medicinal plants: Molecular markers have been used as a tool to verify sexual and apomictic offsprings of intraspecific crosses in *Hypericum perforatum*, a well known antihelminthic and diuretic.[22]

2.1.4.5. Industrial Applications in foods and nutraceuticals

DNA-based molecular markers have been used extensively for a wide range of applications in food crops and horticultural plants[23,24,25] These applications include study of genetic variation, genotyping, cross-breeding studies, identification of disease-resistant genes, identification of quantitative-trait loci, diversity analysis of exotic germplasm, sex identification of dioeceous plants, phylogenetic analysis, etc. Recently, the application of DNA-based molecular markers is being explored in the field of nutraceuticals. According to the new European Council legislation[26], the labelling of food or food ingredients produced from, or containing licensed genetically modified organisms must indicate the inclusion of these ingredients where they are present at or above a level of 1%. In compliance with the labeling regulation for GM foods, several countries in Europe such as Germany and Switzerland, have extensively developed PCR methods for both identification and quantification purposes. In response to the reports of unlicensed GM ingredients in food in the international market, the Food Safety Authority of Ireland has completed a survey to determine the levels of GM maize ingredients in tortilla chips and taco shells on sale in Ireland, using the PCR techniques.[27] Where sufficient GM DNA was present in the sample, quantitative analysis was undertaken using real-time PCR. Primers specific for the inserted genes in Roundup ReadyTM soybean have been found to be suitable for detection and discrimination of GM soybean from non-GM products.[28] In another study, 176 maize and Cecropin D Capsicum have been successfully discriminated from non-GM products using primers specific for inserted genes and crop endogenous genes.[29]

2.2. Chemical markers

According to The European Medicines Agency (EMEA), chemical markers are defined as chemically defined constituents or groups of constituents of a herbal medicinal product,based on phytoconstituents which are of interest for quality control purposes regardless whether they possess any therapeutic activity. 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. The quantity of a chemical marker indicates the quality of herbal medicine. Systematic investigations using chemical markers help in the discoveries and development of new drugs.

According to the EMEA, chemical markers are categorized 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.

2.2.1 Applications of chemical markers[22]

- Detection of adulterants- An adulterant of gamboges was differentiated from the authentic sample by an HPLC-UV method using eight caged xanthones as chemical markers
- Authentication of herbal medicines with multiple sources
- Evaluation of processing methods
- Assessment of quality of herbal parts
- Determination of the best harvesting time
- Confirmation of collection sites
- Quantitative determination and identification of proprietary products
- Herbal intoxication diagnosis- 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
- Stability testing of proprietary products- Stability test is used to evaluate product quality over time and to assure the recommended shelf life

CONCLUSION

In order to ensure the quality, safety and efficacy of herbal drugs a check over its quality control parameters is essential. In the current practice of quality control, markers are playing a very important role. 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, the role of markers should be ensured. There are many challenges regarding markers such as non-availability of sufficient markers, no up to the mark chemical and pharmacological data of them are available and also there are lots of technical challenges in the production of markers. There are also factors such as temperature, light, solvents which cause degradation or transformation of the purified markers. Therefore, in order to utilize the markers in their pure form and with full efficacy to attain maximum benefit, there should be serious check over the stability parameters of them. This will also contribute to the easy accessibility to the markers.

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