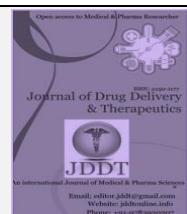
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Review Article

Review on “Standardization an Imp Tool for Herbal Drug Development”

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ABSTRACT

The medicinal plants are important source for pharmaceutical manufacturing. Medicinal plants & herbal medicines account for a significant percentage of the pharmaceutical market. There is increasing awareness and general acceptability of the use of herbal drugs in today's medical practice although most of these applications are not scientific. Herbal medicines are not a simple task since many factors influence the biological efficacy and Reproducible therapeutic effect. So it is necessary to improve safety of herbal drugs by developing certain quality control parameters & by following the WHO guidelines for herbal medicines. This review seeks to enlighten the need to establish quality parameters for collection, handling, processing and production of herbal medicine as well as employ such parameters in ensuring the safety of the global herbal market. It is necessary to introduce measures on the regulation of herbal medicines to ensure quality, safety, efficacy of herbal medicines by using modern suitable standards & GMP. The processes of good quality assurance and standardization of herbal medicines and products using various spectroscopic, chromatographic and electrophoretic methods were also discussed. In fact, the research field of quality control of herbal medicines is really an interdisciplinary research. It needs crossover of chemistry, pharmacology, medicine and even statistics to provide a platform for the quality control of traditional herbal medicines and further to discover the novel therapeutics composed of multiple chemical compounds.

Keywords: Herbal drugs, Adulteration, Standardization, Chromatography, Electrophoresis, HP-LC and GC-MS.

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Introduction

Standardization of herbal drugs is the process of evaluation the quality and purity of crude drug by means of various parameter like morphology, physical, chemical and biological observation.”¹

Need of standardization: - In recent years there is a spurt in the interest regarding survival of ayurvedic forms of medication. In the global perspective, there is a shift towards the use of medicine of herbal origin. As the dangers and the shortcoming of modern medicine have getting more apparent, majority of Ayurvedic formulation are prepared from herbs². It is the cardinal responsibility of the regulatory authorities to ensure that the consumers get the medication, which guarantee. Purity, safety, potency and efficacy. WHO has emphasized the need to ensure quality control of medicinal plant products by using modern technique and by applying suitable parameters and standards³.

Current Regulation for standardization of crude drugs:- In India a great deal of bulk knowledge exists among ordinary people about the traditional use of herbal medicine. It is difficult to quantify the market size of the traditional

Indian system. Since most practitioners formulate and dispense their own recipes⁴. The present annual turnover of product manufactured by large companies is estimated at approximately US\$300 million compared to a turnover of approximately US\$2.5 billion for modern drugs. According to the study on the attitude of modern medicine practitioners are relatively unfamiliar with Ayurvedic product even though some are practiced. They are willing to try an Ayurvedic product if it efficiency disease. Liver and skin disease⁵.

Conventional Methods For Standardization Of Crude Drug :-Standardization of herbal raw drug includes passport data of raw plant drugs. It includes medico-botanical survey, identification, botanical authentication, macroscopic, examination. Testing of drugs as per approved Pharmacopoeial testing protocol- Fully pharmacognostical profile, Identification by various chromatographic techniques, Assessment of purity by physico-chemical profile, Assessment of strength by active marker or assay estimation and Safety by heavy metal profiling, microbiological limit test analysis, aflatoxins analysis, pesticides residue and biological activity⁶. Further, advances

in microscope technology have increased the accuracy and capabilities of microscopy as a mean of herbal crude material identification due to the implication of light and scanning electron microscopes (SEM) in herbal drug standardization⁷⁻⁸.

WHO Guidelines for Quality of Herbal Formulation

- 1) Quality control of crude drugs material, plant preparations and finished products⁹.
- 2) Stability assessment and shelf life¹⁰.
3. Safety assessment; documentation of safety based on experience or toxicological studies¹¹.
- 4) Assessment of efficacy by ethno-medical information and biological activity evaluations¹².

The bioactive extract should be standardized on the basis of active principles or major compounds along with the chromatographic fingerprints (TLC, HPTLC, HPLC, and GC). Generally, all medicines, whether they are synthetic or of plant origin, should fulfil the basic requirement of being safe and effective¹³. The term 'herbal drugs' denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying and storage¹⁴.

Quality Control of Herbal Drugs

Quality control is a term that refers to processes involved in maintaining the quality and validity of a manufactured product¹⁵. In general, quality control is based on three important Pharmacopoeial aspects –

- a. Identity or authenticity- it should have one herb.
- b. Purity – it should not have any contaminant other than herb.
- c. Assay or Content -the active constituents should be within the defined limits¹⁶.

Identity can be achieved by macro and microscopically examinations¹⁷. In addition to this identity tests, which include simple chemical tests, eg. colour or precipitation and chromatographic tests are also necessary. These chemical and chromatographic tests help to provide batch to batch comparability and the chromatogram may be used as a 'fingerprint' for the herbal ingredient by demonstrating the profile of some common plant constituents such as flavonoids, alkaloids and terpenes¹⁸.

Stability Assessment And Shelf Life:- Prolonged and apparently uneventful use of a substance usually offers testimony of its safety. In a few instances, however, investigation of the potential toxicity of naturally occurring substances widely used as ingredients in these preparations has revealed previously unsuspected potential for systematic toxicity, carcinogenicity and teratogenicity¹⁹. Regulatory authorities need to be quickly and reliably informed of these findings. They should also have the authority to respond promptly to such alerts, either by withdrawing or varying the licences of registered products containing suspect substances, or by rescheduling the substances to limit their use to medical prescription²⁰.

Assessment of Quality All procedures should be in accordance with good manufacturing practices²¹.

Crude Plant Material:- The botanical definition, including genus, species and authority, description, part of the plant, active and characteristics constituents should be specified and, if possible content limits should be defined²². Foreign matter, impurities and microbial content should be defined

or limited. Voucher specimens, representing each lot of plant material processed, should be authenticated by a qualified botanist and should be stored for at least a 10-year period. A lot number should be assigned and this should appear on the product label²³.

Plant Preparations The manufacturing procedure should be described in detail. If other substances are added during manufacture in order to adjust the plant preparation to a certain level of active or characteristics constituents or for any other purpose, the added substances should be mentioned in the manufacturing procedures.²⁴ A method for identification and, where possible, assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances to ensure consistent quality of the preparation²⁵.

Finished Product The manufacturing procedure and formula, including the amount of excipients, should be described in detail. A finished product specification should be defined to ensure consistent quality of the product. The finished product should be with general requirements for particular dosage forms²⁶.

Stability The physical and chemical stability of the product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf-life should be established²⁷.

Safety Assessment Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration. However, some of the plants used in herbal medicines can also be highly toxic²⁸. As a whole, herbal medicines can have a risk of adverse effects and drug-drug and drug-food interactions if not properly assessed. Assessment of the safety of herbal products, therefore, is the first priority in herbal research. These are various approaches to the evaluation of safety of herbal medicines. Several reports suggest that many herbal products contain undisclosed pharmaceuticals and heavy metals²⁹. The intentional use of pharmaceutical adulterant is possible. Agrochemicals are used to protect the plant from the crude plant material. More over mechanism of action, pharmacokinetics and drug-drug interactions of many herbs are still in infancy. Clinicians should not prescribe or recommend herbal remedies without well-established efficacy as if they were medications that had been proved effective by rigorous study³⁰.

Assessment of Toxicity investigation will also be required because the analysis alone is unlikely to reveal the contributions to toxicity itself. In assessing toxicity of an herbal medicine, the dose chosen is very important³¹. Toxicity assessment involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern technique, standardization and techniques to adequately model toxicity³².

Assessment Of Efficacy Herbal medicines are inherently different from conventional pharmacological treatments, but presently there is no way to assess their efficacy other than by currently used conventional clinical trial methodologies, in which efficacy is conventionally assessed by clinical, laboratory, or diagnostic outcomes³³. Clinical outcomes include parameters such as improved morbidity, reduced pain or discomfort, improved appetite and weight gain, reduction of blood pressure, reduction of tumor size or

extent, and improved quality of life. Laboratory /other diagnostic outcomes include parameters such as reduction of blood glucose, improvement of haemoglobin status, reduction of opacity as measured by radiological or imaging techniques, and improvement in electrocardiogram ECG findings³⁴.

Microscopic Evaluation: - Full and Accurate characterization of plant material requires a combination of physical and chemical tests. Microscopic analyses of plant are invaluable for assuring the identity of the material and as an initial screening test for impurities. Most manufacturers of herbal products lack the quality control personnel to accurately assess identity and purity microscopically³⁵.

Chemical Evaluation Chemical analysis of the drug is done to assess the potency of vegetable material in terms of its active principles. It covers screening, isolation, identification, and purification of the chemical components. It helps to determine the identity of the drug substance and possible adulteration³⁶.

Biological Evaluation Pharmacological activity of certain drugs has been applied to evaluate and standardize them. The assays on living animals and on their intact or isolated organs can indicate the strength of the drug or their preparations³⁷.

Analytical Methods- It helps in determining identity, quality and relative potency. The most important step in the development of analytical methods for botanical and herbal preparations is sample preparation³⁸. The basic operation includes steps such as pre-washing, drying of plant materials or freeze drying and grinding, to obtain a homogenous sample and often improving the kinetics of extraction of the constituents. In the Pharmacopoeial monographs, methods such as sonication, heating under reflux, Soxhlet extraction, and others are commonly used³⁹⁻⁴⁰. However, such methods can be time-consuming, require the use of a large amount of organic solvent, and may have lower extraction efficiencies.

Chromatography Separation of individual components from the herbal mixture is the key step to enable identification and bioactivity evaluation. TLC is used extensively in the phytochemical evaluation of herbal drugs because it enables rapid analysis of herbal extracts with minimum sample clean-up requirement. It provides qualitative and semi quantitative information of the resolved compounds. In TLC fingerprinting, the data that can be recorded using a high performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, λ max and shoulder inflection/s of all the resolved bands⁴¹. It has been well reported that several samples can be run simultaneously by use of a smaller quantity of mobile phase than in HPLC. HPTLC technique is widely employed in pharmaceutical industry in process development, identification and detection of adulterants in herbal product and helps in identification of pesticide content, mycotoxins and in quality control of herbs and health foods. LC-MS has become method of choice in many stages of drug development⁴².

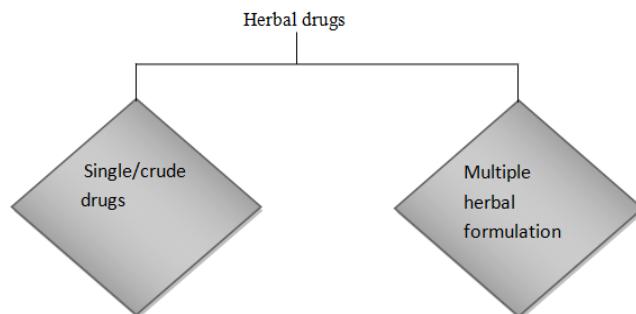
METHODS OF STANDARDISATION OF AYURVEDIC MEDICINES

- 1) Raw material standardization.
- 2) In process standardization
- 3) Finished product standardization.

1. Raw material standardization: This includes authentication process in which following points should be considered. Area of the collection, parts of the plant collection, the regional situation, botanical identity, microscopic and histological analysis, taxonomic identity, Foreign matter, Loss on drying, swelling index, foaming index, ash values and extractive values, Chromatographic and spectroscopic evaluation, Determination of heavy metals, pesticide residues, Microbial contamination, Radioactive contamination⁴³.

2. In process standardization: SOP's it should have the manufacturing procedure in detail, if other substances are added during manufacture in order to adjust the plant preparation. A method for identification and, where possible, assay of the plant preparation should be added. If identification of an active principle is not possible, it should be sufficient to identify a characteristic substance or mixture of substances to ensure consistent quality of the preparation⁴⁴.

3. Final product: Prepared drug should possess standard nature of characteristics. The manufacturing procedure and formula, including the amount of recipients, should be described in detail. A finished product specification should be defined to ensure consistent quality of the product. The finished product should comply with general requirements for particular dosage forms. The processes involve a wide array of scientific investigations, which include physical, chemical and biological evaluation employing various analytical methods and tools. The specific aims of such investigation in assuring herbal quality are as varied as the processes employed⁴⁵.



Standardization of Bhasmas Bhasmas are made from metals like zinc, lead, gold, silver, tin, copper, metal mixtures and alloys as also from gems, coral and mica and some other minerals, etc. These are formed by calcinations of the parents' substances like minerals, etc. in a rigorous, prescribed manner after it has been appropriately purified and emulsified with herbal juices or minerals. These studies also explored intensively the different bhasmas reparation methods of the same metal, chemical nature and crystalline structure of the intermediates and the final products. From results on particle size distribution and crystal structure, one can determine how well the bhasma process has proceeded. With such equipment in hand, it should be possible to standardize and freeze the SOPs for bhasma preparations. A ten points protocol has been suggested for the standardization of bhasmas and the process of their preparations. A standardization programme should also distinguish between bhasmas of metals made of either herbs and non-toxic minerals and those with toxic substances⁴⁶.

Multiple Herbal formulations are obtained by subjecting herbal ingredients to various manufacturing process such as extraction, distillation, expression, fractions, partition, chromatography and formulations⁴⁷.

Toxicity in herbals and their interactions A part from efficacy, FDA is also charged with determining the safety of drug products, and not all botanicals/herbals harmless. In this context the reference to the incidence of 1991 and 1992 in Brussels, Belgium, in which 30 women treated with a Chinese herbal slimming preparation died from renal failure caused by the presence of aristocholic acid in it can be taken into account. One of the herbs had been incorrectly identified as a non-toxic. So the importance on controlling the correct identification of herbal preparations should be taken into account from very beginning. In addition to the problem of incorrect plant identification, some mixtures may be toxic, particularly if they are misused. Important should be given to continuous surveillance and of actively requesting information rather than just collecting reports and even this can be considered as national program⁴⁸.

DNA Fingerprinting Technique DNA analysis has been proved as an important tool in herbal drug standardization. This technique is useful for identification of phytochemically indistinguishable genuine drug from substituted or adulterated drug. It has been reported that DNA fingerprint genome remain the same irrespective of the plant part used while the Phytochemical content will vary with the plant part used, physiology and environment⁴². This concept of fingerprinting has been increasingly applied in the past few decades to determine the ancestry of plants, animals and other micro-organisms. Genotypic characterization of plant species and strains is useful as most plants, though belonging to the same genus and species, may show considerable variation between strains. Additional motivation for using DNA fingerprinting on commercial herbal drugs is the availability of intact genomic DNA from plant samples after they are processed. Adulterants can be distinguished even in processed samples, enabling the authentication of the drug. The other useful application of DNA fingerprinting is the availability of intact genomic DNA specificity in commercial herbal drugs which helps in distinguishing adulterants even in processed samples⁴⁹.

Role Genetic Markers in Standardization: - RAPD based molecular markers have been found to be useful in differentiating different accessions of neem collected from different geographical regions. Germless analysis to study genetic diversity is another important area in which a lot of efforts have been put in. Fingerprinting of crops like rice, wheat, chickpea, pigeon pea, pearl millet etc is being carried out extensively. 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. e.g. *P. ginseng* is often substituted by *P. quinquefolius* (American ginseng). RAPD markers have been successively used for selection of micro propagated plants of *Piper longum* for conservation⁵⁰.

CONCLUSION

The Indian herbal industry is growing in a tremendous rate. With the tremendous increase in traditional herbal therapy several concerns regarding the safety and quality of herbal medicines have also been observed. There is and for more advanced techniques of standardization. The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research, thereby, allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and half-life of herbal drugs. The numerous examples of commercial product variability emphasize the need for continued effort towards more meaningful and effective standardization of herbal

medicines, as well as regulatory programs for ensuring proper botanical identity, quality and strength of commercial products.

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