RECENT ADVANCES IN HERBAL FORMULATION STANDARDIZATION – A REVIEW

ABSTRACT

Recently peoples are getting attracted towards traditional medicines due to many advantages. Herbal formulations have reached substantial acceptability as therapeutic agents for a number of diseases. Although, most of these applications are rare, it is however a known fact that over 80% of the community depends on Ayurveda and herbal medicines for healthy lifestyle. This raise in the use of herbal product has also given rise to various forms of misuse and falsification of the formulation leading to consumer's and manufacturer's despondency and in some cases harmful effects.(1) The development of genuine analytical techniques which can reliably profile the phytochemical configuration, including quantitative analysis of marker/Biologically active compounds and other major constituents, is a major challenge to Researcher. Standardization is an important step in creating a consonant biological activity, a standardized chemical profile or simply a quality assurance system for herbal Formulation manufacturing. In present review article various traditional methods as well as conventional approaches are discussed. Recent advancements includes DNA fingerprinting, sophisticated analytical techniques, Chromatographic Markers Analysis etc are observed. Sophisticated Analytical Techniques and Chromatographic technique contributions towards standardization of polyherbal formulation is also reported.(2)

Key Words: Standardization, herbal Formulation, DNA fingerprinting, chromatographic techniques

INTRODUCTION

Standardization of polyherbal formulations is imperative in order to measure of satisfactory herbs, especially based on the amount of their lively principles, Physicochemical, phyto-chemical, standardization, and in-vitro, in-vivo research. The quality categorisation of herbal formulations is of predominant essentiality in order to confirm their acceptability in traditional system of medicine. One of the major problems faced by the herbal industry is the inconvenience of stiff quality control profiles for herbal formulations. In India, the department of Ayush, Government of India, launched a central scheme to develop a standard operating procedures for the manufacturing process to develop pharmacopeial standards for ayurveda medicines. The subject of herbal drug standardization is considerably broad and deep. There is lot of to know and lot of apparently opposed theories on the subject of herbal medicines and their relationship with human physiology. India needs to survey the medicinally consequential plants. This can be attain only if the herbal medicines are asses and examine using sophisticated modern approach of standardization. World Health Organization (WHO) motivate, suggest and encourage traditional/herbal remedies in natural health care programmes because these medicines are easily available at low cost, safe and people have belief in them. WHO specific recommendations for assessments of the safety, efficacy and best of natural drug treatment as a prerequisite for global harmonisation are utmost importance. Hence, standardization is a most important aspect for establishing the quality and/or efficacy of ayurvedic formulation or any other multiple ingredient herbal formulations.

Standardization of raw materials includes the following steps:-

Authentication- Each and every step has to be validated, location of the collection, parts of the plant collection, the regional circumstances, as phytochemistry, microscopic and histologic analysis(characteristic appearance of cell walls, cell content, starch grains, calcium oxalate crystals, hairs, fibers, vessels etc.)

Evaluation of a drug ensures the identity of a drug and determines the quality and purity of drugs. The main reasons behind the need for evaluation of crude drugs are biochemical variation in the drug, effect of treatment and storage of drugs, and the adulterations and substitutions. Improvements in analytical system have certainly show to advance in harvesting scheme, cultivation schedule, storage, activity, stability of active blend, and product virtue. All of these gains have resulted in tremendous improvements in the quality of herbal preparations now available. Methods currently employed in evaluating herbs are organoleptic, microscopic, physical, chemical, and biological parameters.

Organoleptic Evaluation

Organoleptic assessment method is the study of medicament using system. It refers to the techniques of analysis like physical, organoleptic and special features, such as: touch, texture, etc. evidently, the starting sight of the plant or plant extract is so specific that it tends to identify itself. If this is not sufficient, may be the plant or extract has a specific odour or taste. Organoleptic analysis constitute the effortless, yet the most human form of analysis.(3)

Microscopical Evaluation

Microscopic assessment is necessary in the initial identification of herbs, as well as in identifying small piece of crude or powdered herbs, and in the noticing of adulterants (e.g. insects, animal Waste matter, mold, fungi, etc.) as well as identifying the plant by specific tissue features. Every plant having a characteristic tissue structure, which can be performed by study of tissue arrangement, cell walls, and configuration when properly mounted in stains, reagents, and media.

Chemical Evaluation

The chemical evaluation includes qualitative chemical tests, quantitative chemical tests, chemical assays, and instrumental analysis. The segregation, purification, and identification of active constituents are chemical methods of evaluation. Qualitative chemical tests include identification tests for various phytoconstituents like alkaloids, glycosides, tannins, etc.

Chromatographic and Spectroscopic Analysis

It includes TLC, HPLC, HPTLC, GC, UV, IR, FT-IR, AAS, LC-MS, GC-MS, fluorimetry etc.

Physical Evaluation

Physical methods are mostly used in crude plant evaluation for determination of the solubility, specific gravity, optical rotation, viscosity, refractive index, melting point, water content, degree of fibre elasticity, and other physical character of the crude material.

Biological Evaluation

The plant or extract can then be evaluated by various biological methods to determine pharmacological activity, potency, and toxicity. The biological evaluation would serve better than the physical and chemical evaluation for drugs that could not be satisfactorily assayed by these last two methods. Moreover, this is an important method, the crude drugs are considered important only because of their biological effects and this evaluation would conclude the effect

These assays are conducted by determining the amount of drug of known potency required to produce a definite effect on suitable test animals or organs under standard conditions. Reference standard are used in certain bioassay procedures to minimize errors.

Toxicity studies are performed in suitable animal models to decide the lethal dose and effective dose of crude drags. Mice are used to test the effects of various vaccines.

Oxytocic activity of vasopressin injection is tested on guinea pigs, and oxytocic injection is assayed on young domestic chickens by injecting into an exposed crural or brachial vein and noticing the changes in blood pressure.

WHO GUIDELINES FOR QUALITY STANDARDIZED HERBAL FORMULATIONS

1) Quality evaluation of crude drugs material, plant preparations and finished formulation.

2) Stability evaluation and shelf life study.

3) Safety evaluation; documentation of safety or toxicological studies.

4) Evaluation of efficacy by pharmacognostic information's and biological activity evaluations. The herbal formulation must be standardized on the basis of active principles along with the chromatographic fingerprints (TLC, HPTLC, HPLC, and GC).

1. Potential hazardous contaminants and residues in herbal medicines

Chemical contaminants: Toxic metals and non-metals: contamination of crude material with toxic substances such as arsenic can be attributed to many causes. These include environmental pollution , soil composition and fertilizers. This contamination of the raw material leads to contamination of the formulation during various stages during the manufacturing process.

Radioactive Contaminants Dangerous contamination may be the consequence of a nuclear accident or may arise from other sources. WHO, in close collaboration with numerous other worldwide organisations, has developed guidelines for use in the event of worldwide contamination by means of radionuclides resulting from a primary nuclear accident. Example of such radionuclides include long-lived and short-lived fission products, actinides and activation products. In general the nature and the intensity of these Radionuclides.

Biological Contaminants:

Microbiological contamination: Herbs and herbal material normally carry a large number of bacteria and moulds, often originating in soil or derived from manure. While a large range of bacteria and fungiform the naturally occurring microflora of medicinal plant. Aerobic spore forming bacteria frequently predominate. Current practice of harvesting, production, transportation and storage may cause additional contamination and microbial growth. Proliferation of microorganism may results from failure to control the moisture level of herbal medicines during the transportation and storage, as well as from failure to control the temperature of liquid forms and finished herbal products. Microbial contamination may also occur through handling of materials by personnel who are infected with pathogenic bacteria during harvest/ collection, post-harvest processing and the manufacturing process. This should be controlled by implementing best practice guideline such as GACP and GMP.

Parasitic Contamination: parasites such as protozoa and nematode, and their ova, may be introduced during cultivation and may cause zoonosis, especially if uncomposted animal excreta are used. Contamination with parasites may also arise during processing and manufacturing if the personnel carrying out these processes have not taken appropriate personal hygiene measure.

Agrochemical residues: The main agrochemical residues in herbal medicines are derived from pesticides and fumignats.

2. Guiding principles for assessing safety of herbal medicines with reference to contaminants and residues.

Foreign matter: foreign matter found in sample of herbs and herbal materials should not exceed limits set in national, regional or international pharmacopoeias. Foreign matter includes insects and other animal contamination including animal faeces, as well as other species of plants. In general, any substance other than the acceptable sample of good quality medicinal plants material is regarded as foreign matter. A pure sample is seldom found and there is always some foreign matter present. However no poisonous, dangerous or otherwise harmful foreign matter should be allowed. Thus the following GACP should help to ensure that contamination is kept to a minimum.

Pesticides Residues: The toxicological evaluation of pesticides in herbal materials should be bases on the intake of the material by patients. In the absence of a full risk assessment and for practical reasons. It is recommended that, in general, the intake of residues from herbal materials should account for no more than 1% of total intake from all sources. Including food and drinking water. since the level of pesticides residues may change during the production process, it is vital to determine the actual quantity of residues consumed in the final dosage form.

Physical parameters: It includes colour, odour, appearance, clarity, viscosity, pH, and ash values.

Chemical parameters: It includes limit tests, chemical tests, chemical assays etc.

Chromatographic analysis: chromatographic analysis of herbals can be done using TLC, HPTLC, HPLC, GC, UV, GCMS, Fluorimetry etc.

Pharmacological Evaluation: The said formulation should be evaluated for its pharmacological activity.

1. Quality Control of Crude Drugs

   Quality control for safety and efficacy of herbal drug is of supreme importance. Quality can be report as status of drug that is determined via identification, purification, content, and other physicochemical or biological properties, or by the in process quality control. Quality control is a term that refers to processes involved in maintaining the standard and shelf life of a finished product. The term “Herbal Drug” denotes Herbs or plant material that have been covered into authentication by means of simple processes involving harvesting, drying, and storage at its favourable condition that does not affect its phytoconstituent. Hence they are capable of variation. This variability is also caused by differences in cultivation, geographical location, and time of harvesting. In general, quality control is based on three principal pharmacopeial definitions

   - Identity- it should have one herb
   - Purity – Herb should not have any contaminant.
   - Content or assay-the active constituents should be within the defined limits. It is clear that the data is the most hard one to assess, since in most herbal medicine the active constituents are unspecified. Sometimes markers can be used which are, by definition, chemically defined component that are of attentiveness for control purposes, independent of whether they have any pharmacological activity or not. Identity can be achieved by physical examinations. Voucher specimens are reliable reference sources. Purity is closely linked with safe use of crude drugs and deals with factors such as ash values, adulterants and heavy metals. However, due to the implementation of improved analytical methods, modern purity assessment also includes microbial contamination, contamination, aflatoxins, radioactivity, and pesticide residues. Analytical methods such as spectrophotometric analysis, Thin layer chromatography (TLC), High performance liquid chromatography (HPLC), High performance thin layer chromatography (HPTLC), and Gas chromatography (GC) can be employed in order to demonstrate the constant composition of herbal preparations.

Stability evaluation and Shelf Life

Stability evaluation and Shelf Life The past decade has seen a significant increase in the use of herbal drugs. As a result of WHO‟s encourage of Ayurveda medicine, countries have been seeking the support of the organization in identifying safe and effective herbal medicines for use in national health care systems.

Evaluation of quality

Quality assessment of crude drugs: representation of macroscopic characters of crude drugs and records of test results. All these records, production plans and their details, contracts or agreements etc. should be filed and kept properly by a designated person. All planof action should be in comformity with good manufacturing practices.

Finished product

The manufacturing procedure and formula, including the amount of additives, 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.

Stability

The physicochemical stability of the finished product in the container in which it is to be marketed should be tested under defined storage conditions and the shelf-life should be established

Safety assessment

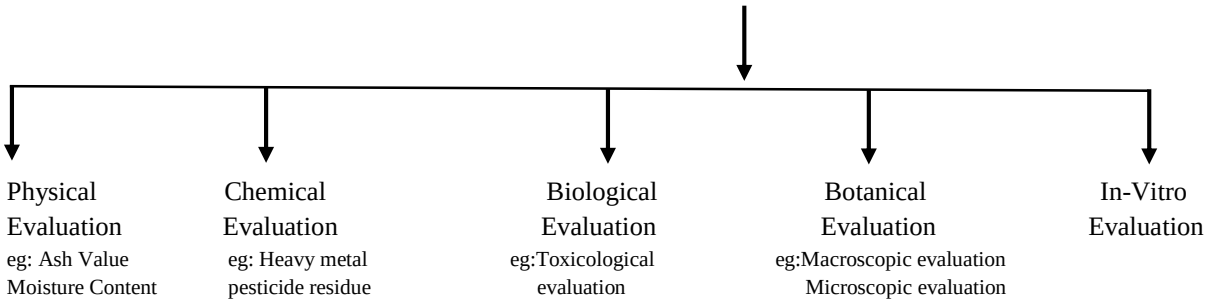
Herbal medicines are generally regarded as safe based on their long-standing use in various cultures. However, there are case reports of serious adverse events after administration of herbal products. In a lot of cases, the toxicity has been traced to contaminants and adulteration. However, some of the plants used in herbal medicines can also be highly toxic. As a whole, herbal medicines can have a risk of adverse effects and drug and drug-food interactions if not properly assessed.(1) 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. The toxic effects of herbal preparation may be attributed mainly to the following: Inherent toxicity of plant constituents and ingredients and Manufacturing malpractice and contamination. Evaluation of the toxic effects of plant constituents of herbal formulation requires detailed phytochemical and pharmacological studies. It is, however, safe to assume that, based on human experiences in various cultures, the use of toxic plant ingredients has already been largely eliminated and recent reports of toxicity could largely ne due to misidentification and overdosing of certain constituents. Substitution and misidentification of herbal substances, documented or regulatory approaches, development of monitoring and surveillance systems, assessment of toxicity, risk assessment approach. The evaluation of new herbal products consists of six steps, which define the following: Characteristics of new substances, history and pattern of use, any adverse reaction, biological action, toxicity and carcinogenicity, and clinical trial data. The presence of impurities is either an intended addition, or accidental contamination via processing. The substitution of plants arises because of similar plants/ wrong identification, or the use of cheaper alternatives.

Assessment of toxicity

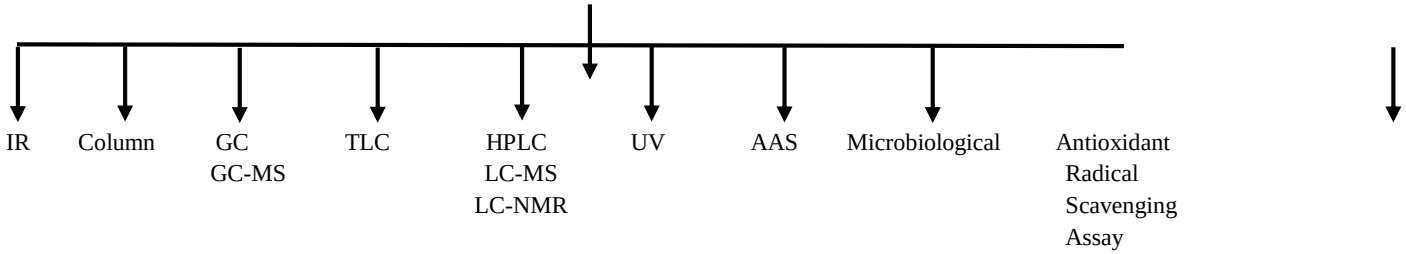
Toxicity investigation will also be required because the analysis alone is unlikely to reveal the contributions to toxicity itself. In assessing toxicity of an herbal medicine, the dose chosen is very important [12]. Toxicity assessment involves one or more of the following techniques- In vivo techniques, in vitro techniques, cell line techniques, micro- array and other modern technique Standardization techniques to adequately model toxicity.(4)

CONVENTIONAL METHODS FOR STANDARDISATION OF HERBAL FORMULATION

STANDARDISATION OF HERBAL DRUG(5)



CHROMATOGRAPHIC TECHNIQUES OF STANDARDISATION OF HERBAL FORMULATION



INFRARED SPECTROSCOPY

Infrared spectroscopy utilize the fact that molecules absorb specific frequencies that are characteristic of their structure. These absorptions are resonant frequencies, i.e. the frequency of the absorbed radiation matches the frequency of the bond or group that vibrates. The energies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms, and the associated vibronic coupling.(6)

Thin Layer Chromatography

In 1958 Stahl demonstrated the wide applicability of TLC, a technique which had been known in principle for many years but was never developed. It has now achieved remarkable success in the separation of mixtures of all classes of natural products and is established as an analytical tool in modern pharmacopoeias. In outline the method consists of preparing, on a suitable glass plate, a thin layer of material, the sorbent, which may be either an adsorbent as used in column adsorption chromatography or an inert support which holds an aqueous phase as in column partition chromatography.(7) The mixtures to be resolved are dissolved in a suitable solvent and placed as a series of spots on the film towards one end of the plate; this end is then dipped in a suitable solvent mixture and the whole enclosed in an airtight container. The solvent front travels up the film and after a suitable time the plate is removed, the solvent front is marked, the solvent is allowed to evaporate and the positions of the separated compounds are determined by suitable means. TLC has certain advantages over paper chromatography. Fractionations can be effected more rapidly with smaller quantities of the mixture.(8)

Gas Chromatography

- GC is also recognized as Vapor phase chromatography or gas-liquid partition chromatography.
- In which mobile phase is gas and stationary phase either solid or liquid.
- Solute have higher diffusivities in gases as compared to liquids.
- GC is specially designed techniques for chemical analysis of volatile substances.

Principle:

- In GC, sample is vaporized and injected onto the column head and elution is brought about by the flow of an inert gaseous mobile phase.
- Basic principle of GC is that the greater the affinity of compound for the stationary phase, the more the compound will be retained by the column.
- The column through which the gas phase passes is located in an oven.

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) is basically a upgraded form of column chromatography. As an alternative of a solvent being allowed to drip out through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes speedy. All chromatographic separations, including High Performance Liquid Chromatography run under the same fundamental principle; separation of a sample into its essential parts because of the difference in the relative affinities towards the mobile phase and the stationary phase used in the separation.(9) It having a four type

1) Normal Phase HPLC
2) Reverse Phase HPLC
3) Size-Exclusion HPLC
4) Ion-Exchange HPLC

Ultra Violet Spectrophotometry

Ultraviolet-visible spectroscopy or ultraviolet-visible spectrophotometry mention to absorption spectroscopy in the ultraviolet-visible spectral region. This way it uses light in the visible and adjacent and near-infrared ranges.(9) UV spectrophotometer is used in the quantitative estimation of concentrations of the absorber in the solutions of transition metal ions and highly associate organic compounds. The Beer-Lambert law says that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, Ultra Violet spectroscopy can be used to determine the concentration of the absorber in a solution. The absorbance changes with concentration. This can be taken from references standards, or more precisely, determined from a calibration curve.(10)

AAS

Atomic Absorption Spectrometry is a technique in which free gaseous molecule absorb electromagnetic waves at a specific wavelength to produce a measurable signal. The absorbing signal is directly proportional to the concentration of those free absorbing atoms in the optical path. Therefore, for AAS measurement the analyte must be first converted into gaseous atoms, usually by application of heat to a cell called atomizer. The type of automizer defines the two main Atomic Absorption Spectrometry based analytical techniques: Flame Atomic Absorption Spectrometry that provides analytical signals in a discontinuous mode. In both cases, liquid sample are easily introduced into the analyzer, as an aerosol in the case of FAAS or as well-defined low microliter volume in ETAAS.(11)

Antioxidant Radical Scavenging Assay

Diphenyl- -picryl-hydrazyl radical scavenging (DPPH) Assay. The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. This method is based on the reduction of Diphenyl-picryl-hydrazyl in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non-radical form DPPH-H. (2)This conversion results change in colour from purple to yellow, which is measured spectrophotometrically. The disappearance of the purple color is monitored at 517 nm. The free radical scavenging activity can be measured by using 2, 2-diphenyl-1-picryl-hydrazyl by the method of McCune and Johns (2002). The reaction mixture (3.0 ml) consist of 1.0 ml of DPPH in methanol, 1.0 ml of the extract and 1.0 ml of methanol. It is incubated for 15 min in dark background, then the absorbance is measured at 517 nm. In this assay, the positive controls can be Ascorbic acid, Gallic acid, BHA, BHT, Tocopherol, Quercetin. The percentage inhibition can be calculated using the formula:

Inhibition (%) = (A0 – A1 / A0) × 100

Where; A0 is the absorbance of control and A1 is the absorbance of test.(12)

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