International Journal of Health Sciences and Research
www.ijhsr.org ISSN: 2249-9571

Review Article

Recent Updates in Ayurvedic Herbs Standardization

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ABSTRACT

Introduction: Herbal medicines are getting more and more popular in recent days with the global trend of people returning to natural therapies. Thus, chance of adulteration and substitution is more in order to meet the demand. Drug standardization is essential to assess the safety, efficacy and quality of drugs[1]. For standardization and quality assurance following three attributes are desirable i) Authenticity ii) Purity iii) Assay. For that macroscopic, microscopic, physiochemical, preliminary phytochemical and chromatographic techniques are well established. [30,31] Nowadays various advanced methods for drug standardization such as Chromatography, Spectrophotometry, Electrophoresis, Chemometrics, Polarography, Molecular biomarkers in fingerprints are currently employed in standardization of herbal drugs. [10]

Methodology: A thorough review of available literatures regarding the recent techniques in drug standardization is done. Relevant e resources are referred to for recent research updates.

Results: Advanced Chromatography, Spectrophotometry, Electrophoresis, Chemometrics, Polarography, Molecular biomarkers in fingerprints are the need of era for proper standardization of herbal drugs and their molecules.

Conclusion: The advancement of analytical technique 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 shelf life of herbal drugs.

Keywords: Standardization, DNA fingerprinting, Chromatography, Herbal medicines, Electrophoresis, Chemometrics

INTRODUCTION

Ayurvedic Herbs are being used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants are the important source of ayurvedic as well as modern drugs. [11] Single plant is capable of synthesizing unlimited number of highly complex and unusual chemical substances so their proper identifying and validation globally is another challenge, [12] this challenge has developed various standardization techniques. There are at least 120 distinct chemical substances derived from plants that are considered as important drugs currently in use in the world, ie. Scopolamine, atropine, Dioxin, Hyoscine, Glucosamin. [13] Ayurvedic medicines include herbs, herbal materials, herbal preparations, Minerals and finished herbal products. Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes, gums, fixed oils, essential oils, resins. [14] Different part of palnts bears some variation in phytochemical. Required concentration of secondary metabolites of Alkaloids, Tannin, Flavanoids, Saponins in the plant species brings the therapeutic effect in the body.

Geographical variation, condition of soil, substitution, adulteration and variation of species alter the phytochemicals. [15-17] During manufacturing, formulating, storing, packaging, transportation and distribution drug may alter the efficacy, safety, stability so for genuine practice, standardization of ayurvedic drugs is the need of era. [18] For Herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application. Increasing reports of adverse reaction from herbal drugs has drawn the attention of many regulatory agencies for the standardization of herbal formulations. [32] The regulatory authorities rigidly follow various standards of quality prescribed for raw materials and finished products in pharmacopoeias, formularies and manufacturing operation through statutory imposed good manufacturing practices. To maintain all these things WHO has developed drug standardizing parameters. [19, 20]

Standardizations protocol of WHO [20-27]

Botanical Parameters:	Sensory evaluation	. Color . Odour . Taste . Texture and Fracture
	Foreign matter	Soil, sands, unnecessary plant parts, insects, etc
	Microscopy	Study of plants tissue(Vascular bundle, parenchyma, collenchyma, sclerenchyma, resin, starch, stone cells, epidermis, cortex, shape size pattern of arrangement of cells, stomatal index, palisade ratio, Vein-islet Number, Vein- Termination Number,

Physiochemical parameters: [2-4]

Physiochemical parameters:	Objectives
Loss on Drying	. It determine the volatile and moisture content of the drugs . Helps in the prevention of decaying of drugs.
Total Ash	It determine the total earthy materials like silica, inorganic and organic impurity present along with authentic drugs
Acid Insoluble Ash	
Water soluble Ash	
Alcohol soluble extractive value	To estimate the amount of Alcohol soluble phytoconstituents as per standarad
Water soluble extractive value	To estimate the amount of water soluble phytoconstituents as per standard

Pharmacological parameters: [2,3,5-7]

Pharmacological parameters	Objectives
• Bitterness value	Bitter properties of the plants materials are determined the comparing the threshold bitter concentration of the materials with that of a dilute solution of quinine HCL. It stimulate the Gastric secretion.
• Haemolytic Property	. Saponin have characteristics of frothing property and have ability to cause haemolysis when added to suspension of blood. . The plants from caryophyllace, Aralaceae, Sapindaceae, primulaceae contain saponin. . it is determined by comparing with reference material saponin which have haemolytic activity in 1000 unit per gram.
• Astringent property	Tannins are present in the cell sap. It has astringent property. Tannin binds with proteins and turns into water insoluble materials and are resist to proteolytic enzymes
• Swelling index	Some medicinal plants have therapeutic effect due to their swelling property. The swelling index is the volume in ml taken up by the swelling of 1gm of plants materials under specified condition.
• Foaming index	Saponin are high molecular weight containing phytoconstituents having detergent activity. The foaming ability of an aqueous decoction of plant materials is measured in term of foaming index.

Toxicological parameters: [1, 8, 9]

Toxicological parameters	Objectives
• Arsenic	Arsenic is danger for health even in trace amount. They have to remove from drugs.
• Pesticide residues	Organochlorine, Organophosphorous carbamet and triazine are major pesticides present in crude drugs which are danger for CNS, Cardiovacular and respiratory system.
• Heavy metals	Metal having high atomic Number, densities and atomic weight ie Cadmium, Mercury, Lead are highly toxic to liver and Kidney.
• Microbial contamination	Determination of total aerobic microbial count, total fungal count, total Entero-bacteriaceae count , test for the presence of E. Coli, Staphylococcus aureus, shigella, pseudomonas aeruginosa, and Salmonella.
• Aflatoxins	Toxin produced by the Staphylococcus aureus which cause hemolysis and tissue damage
• Radioactive contamination	The radioactive substance like Uranium produce the radiation which is mutagenic and carcinogenic.

Chromatographic technique [28, 29, 35]

Thin layer Chromatography (TLC)	Quantification of chemical constituents
High performance Thin Chromatography (HPTLC)	Qualitative and quantitative evaluation of phytoconstituents, Identification and detection of adulterants, pesticide content , mycotoxins.
High performance Liquid Chromatography (HPLC)	Quantitative and qualitative evaluation of phytoconstituents
Liquid Chromatography Mass Spectrometry (LC-MS)	Accurate determination of molecular weight of proteins, peptides. Isotopes pattern can be detected
Gas Chromatography Mass Spectrometry (GC-MS)	Analysis of volatile constituents, Qualitative analysis of the complex constituents.
Liquid Chromatography- Nuclear Magnetic Resonence (LC-NMR)	Useful in the areas of pharmacokinetics, toxicity studies, drug metabolism and drug discovery process.
Supercritical Fluid Chromatography (SFC)	SFC permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography.

Recent Standardization technique:

DNA Finger Printing: [33]

This technique is useful for the 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 environment. 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 samples. DNA markers are helpful to identify cells, individuals or species as they can be used to produce normal functioning proteins to replace defective ones. DNA markers help in treatment of various diseases and helps in distinguishing the genuine herb from adulterated drug.

Capilary electrophoresis (CE) [34]

The methodology of CE was established to evaluate one herb drug in terms of specificity, sensitivity and precision, and the results were in agreement with those obtained by HPLC method. Eg: A characteristic fingerprint of *Flos carthami* established using CE : identifying the raw herb, helping distinguish the substitute or adulterant and further assessing the differences of *Flos carthami* grown in various areas of China.

Differential Scanning Calorimeter (DSC) [37]

DSC is an important technique for measuring the energy necessary to establish a nearly zero temperature difference between a sample and reference substances. DSC is a heat based analytical method used to study the thermal response of the specimen sample.This method gives information about the thermodynamics of any reaction involved.

Spectrophotometry [34]

This technique is to measure light intensity as a function of wavelength.

- **Application:**
  - Concentration measurement
  - Detection of Impurities
  - Structure elucidation of Organic compounds
  - Chemical kinetics
  - Detection of functional groups
  - Molecular weight determination
  - Chemometrics [36]

Chemometrics is the application of mathematical and statistical techniques to retrieve more information from the chromatographic data. It promotes equipment intellectualization and offers new ideas and methods for the construction of new and high dimensional and hyphenated equipments. Chemometrics is used in optimizing experimental procedures, extracting useful information from the chromatographic data and resolution of the mixture into linear components. It is found to be a useful tool in estimating the quality of herbal drugs.

CONCLUSION

The need for standardization of herbals drugs is now very essential in order to make acceptance to herbal products as remedies for various diseases and ailments.

The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research thereby allowing the manufacturing, marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf life of herbal drugs. The assurance of the safety and efficacy of a herbal drug requires monitoring of the quality of the product from collection through processing to the finished packaged product. The various government agencies should follow a more universal approach to herbal quality by adopting the WHO guidelines and also developing monographs using the various quality parameters.

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How to cite this article: Pohtam I, Devraj KC, Yadav MP et.al. Recent updates in ayurvedic herbs standardization. Int J Health Sci Res. 2019; 9(3):252-256.

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