



REVIEW ON QUALITY CONTROL PARAMETERS FOR STANDARDISATION OF HERBAL DRUG**Pooja Gupta*, Akriti pal, Swati Trivedi, Rajneesh Kumar Gupta***Kanpur Institute of Technology and Pharmacy, Rooma, Kanpur***Corresponding author: poojasolus9@gmail.com*

ABSTRACT

The rise in the use of herbal product has also given rise to various forms of abuse and adulteration of the products leading to consumers' and manufacturers' disappointment and in some instances fatal consequences. The word "standardized" on a product label is no guarantee of higher product quality, since there is no legal definition of the word "standardized." Consumers are often left on their own to decide what is safe and effective for them and the lack of consistent labelling on herbal products can be a source of consumer frustration. Certain information such as "the product has been manufactured according to Pharmacopoeia standards," listing of active ingredients and amounts, directions such as serving quantity (dosage) and frequency of intake of the drug, must be in the label. Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The quality control standards of various medicinal plants used in indigenous system of medicine are becoming more relevant today in view of commercialization of formulations based on medicinal plants. This review seeks to enlighten techniques involved in standardization of crude/finished compound drugs so far, e.g. macroscopic methods, microscopic methods, physical methods, chemical methods, biological methods. Standardization of herbal drugs means confirmation of its identity, quality and purity.

Keywords: Herbal drug, Standardization, Adulteration, Quality, Safety, Efficacy.

1. INTRODUCTION

Herbal drug includes herbs, herbal materials, herbal preparations and finished herbal products. In some countries herbal medicines may contain, by tradition, natural organic or inorganic active ingredients that are not of plant origin (e.g. animal and mineral materials). Herbs include crude plant material, such as leaves, flowers, fruit, seeds, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered. Herbal materials include, in addition to herbs, fresh juices, gums, fixed oils, essential oils, resins and dry powders of herbs. In some countries, these materials may be processed by various local procedures, such as steaming, roasting or stirbaking with honey, alcoholic beverages or other materials. Herbal preparations are the basis for finished herbal products and may include comminuted or powdered herbal materials, or extracts, tinctures and fatty oils of herbal materials. They are produced by extraction, fractionation, purification, concentration, or other physical or biological processes. They also include preparations made by steeping or heating herbal materials in alcoholic beverages and/or honey, or in

other materials. Finished herbal products consist of herbal preparations made from one or more herbs. If more than one herb is used, the term "mixture herbal product" can also be used. Finished herbal products and mixture herbal products may contain excipients in addition to the active ingredients. However, finished products or mixture herbal products to which chemically defined active substances have been added, including synthetic compounds and/or isolated constituents from herbal materials, are not considered to be herbal. Herbal medicines are used very commonly in various health practices or therapies of Traditional Medicines like Chinese medicine, Ayurveda, Unani, Naturopathy, Osteopathy and Homeopathy [1].

In order to have a good coordination between the quality of raw materials, in process materials and the final products, it has become essential to develop reliable, specific and sensitive quality control methods using a combination of classical and modern instrumental method of analysis. Standardization of herbal drugs is not an easy task as numerous factors influence the bioefficacy and reproducible therapeutic effect. In order to obtain quality oriented herbal

products, care should be taken right from the proper identification of plants, season and area of collection and their extraction and purification process and rationalizing the combination in the case of polyherbal drugs [2].

2. NEED OF STANDARDIZATION

In olden days Rishis, Vaidyas and Hakims used to treat patients on individual basis, and prepare drug according to the requirement of the patient. In almost all the traditional systems of medicine, the quality control aspect has been considered from its inspection of its Rishis, Vaidyas and Hakims. Unlike in olden times where traditional practitioners prepared and tested the qualities of herbal medicines, the problem faced today are these of economics of industrial scale production, shelf life and distribution to long distances. These have necessitated development of modern and objective standards for evaluating the safety, quality and efficacy of these medicines. People are also becoming aware of the potency and side effect. To gain public trust and to bring herbal product into mainstream of today health care system, the researchers, the manufacturers and the regulatory agencies must apply rigorous scientific methodologies to ensure the quality and lot to lot consistency of the traditional herbal products [3]. Need of quality control and standardization of herbal products is required because when traditional medicines were developed technology and concept of standardization was quite different and during past thousand years dynamic process of evolution may have changed the identity of plant material and due to commercialization, supply of genuine raw material has become a challenge or may have properties of botanicals undergone change due to time and environmental factors [4].

3. HERBAL DRUG STANDARDIZATION

Standardization is a system to ensure that every packet of medicine that is being sold has the correct amount and will induce its therapeutic effect [5]. The overuse of synthetic drugs with impurities, resulting in higher incidence of adverse drug reactions in more advanced communities, has motivated mankind to go back to Nature for safer remedies. Therefore, quality control standards of various medicinal plants used in indigenous system of medicine are becoming more relevant today in view of commercialization of formulations based on medicinal plants, unlike in the past when the traditional doctors would themselves dispense the medicines. Due to varied geographical locations where these plants

grow, coupled with the problem of different vernacular names these plants are known by, a great deal of adulteration or substitution is encountered in the commercial markets. Therefore, reproducible standards of each plant are necessary for effective quality control. Although some of these plants are covered in various pharmacopoeias, their standards especially in terms of chemical markers and TLC fingerprinting have not been covered [6].

An important factor, which can contribute to the consistent quality of Herbal products, is to have adequate control on the quality of medicinal plants. Due to the natural heterogeneity, the quality of herbal starting materials obtained from wild collections shows more and more fluctuations. Thus cultivation of the most important medicinal plants has been considerably promoted during the last years. It seems to be the only way to meet the increasing demand of consistent qualities of herbal materials taking account of controlled environmental conditions.

3.1. Standardization of herbal crude drugs- process and procedure

The subject of herbal drug standardization is massively wide and deep. The guidelines set by WHO can be summarized as follows:-

1. Reference to the identity of the drug. Botanical evaluation- sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements etc.
2. Refers to the physicochemical character of the drug. Physical and chemical identity, chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil and alkaloidal assays, quantitative estimation protocols etc.
3. A reference to the pharmacological parameters, biological activity profiles, bitterness values, hemolytic index, astringency, swelling factor, foaming index etc.
4. Toxicity details- pesticide residues, heavy metals, microbial contamination like total viable count, pathogens like *E. coli*, *Salmonella*, *P. aeruginosa*, *S. aureus*, *Enterobacteria* etc.
5. Microbial contamination.
6. Radioactive contamination.

According to WHO [7-9], standardization and quality control of herbals is the process involved in the physicochemical evaluation of crude drug covering aspects, such as selection and handling of crude material, safety, efficacy and stability assessment of

finished product, documentation of safety and risk based on experience, provision of product information to consumer and product promotion. Attention is normally paid to such quality indices such as:

1. Macro and microscopic examination: For Identification of right variety and search of adulterants.
2. Foreign organic matter: This involves removal of matter other than source plant to get the drug in pure form.
3. Ash values: These are criteria to judge the identity and purity of crude drug - Total ash, sulphated ash, water soluble ash and acid insoluble ash etc.
4. Moisture content: Checking moisture content helps reduce errors in the estimation of the actual weight of drug material. Low moisture suggests better stability against degradation of product.
5. Extractive values: These are indicative weights of the extractable chemical constituents of crude drug under different solvents environment.
6. Crude fibre: This helps to determine the woody material component, and it is a criterion for judging purity.
7. Qualitative chemical evaluation: This covers identification and characterization of crude drug with respect to phytochemical constituent. It employs different analytical technique to detect and isolate the active constituents. Phytochemical screening techniques involve botanical identification, extraction with suitable solvents, purification, and characterization of the active constituents of pharmaceutical importance.
8. Chromatographic examination: Include identification of crude drug based on the use of major chemical constituents as markers.
9. Quantitative chemical evaluation: To estimate the amount of the major classes of constituents.
10. Toxicological studies: This helps to determine the pesticide residues, potentially toxic elements, safety studies in animals like LD50 and Microbial assay to establish the absence or presence of potentially harmful microorganisms.

The standardization of crude drug materials includes the following steps:

3.1.1. Authentication

- a) Stage of collection.
- b) Parts of the collected plant.
- c) Regional status.
- d) Botanical identity like phytomorphology, microscopical and histological analysis (characteristic of

cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibers, vessels etc.) [6].

3.1.2. Histological parameter studies

- a) Leaf constant: - Palisade ratio, Vein islet number, Vein termination, Stomatal number and Stomatal index.
- b) Trichomes.
- c) Stomata.
- d) Quantitative microscopy.
- e) Taxonomical identity.
- f) Foreign matter.
- g) Organoleptic evaluation.
- h) Ash values and extractive values.
- i) Moisture content determination.
- j) Chromatographic and spectroscopic evaluation.
- k) Heavy metal determination.
- l) Pesticide residue.
- m) Microbial contamination.
- n) Radioactive contamination.

3.1.3. Stability parameters

The stability parameters for the herbal formulations which include physical, chemical and microbiological parameters are as follows:

Physical parameters include color, odor, appearance, clarity, viscosity, moisture content, pH, disintegration time, friability, hardness, flowability, flocculation, sedimentation, settling rate and ash values.

Chemical parameters include limit tests, chemical tests, chemical assays etc.

Chromatographic analysis of herbals can be done using TLC, HPLC, HPTLC, GC, UV, GC-MS and fluorimetry etc. Microbiological parameters include total viable content, total mold count, total enterobacterial and their count. Limiters can be utilized as a quantitative or semiquantitative tool to ascertain and control a number of impurities like the reagents used during abstraction of various herbs, impurities coming directly from the manufacturing vessels and from the solvents etc.

4. QUALITY CONTROL AND VALIDATION OF HERBAL DRUGS

Quality control of herbal drugs has traditionally been based on the appearance and today microscopic evaluation is indispensable in the initial identification of herbs, as well as, in identifying small fragments of crude or powdered herbs, and detection of foreign matter and adulterants. A primary visual evaluation, which seldom

needs more than a simple magnifying lens, can be used to ensure that the plant is of the required species, and that the right part of the plant is being used. At other times, microscopic analysis is needed to determine the correct species and/or that the correct part of the species is present. For instance, pollen morphology may be used in the case of flowers to identify the species, and the presence of certain microscopic structures such as leaf stomata can be used to identify the plant part used. Although this may seem obvious, it is of prime importance, especially when different parts of the same plant are to be used for different treatments. Herbal drugs should be made from the stated part of the plant and be devoid of other parts of the same plant or other plants. They should be entirely free from moulds or insect, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matters such as insects and "invisible" microbial contaminants, which can produce toxins, are also among the potential contaminants of herbal medicines [10-12].

The validation of herbal products is a major public health concern both in developed and resource-poor countries, where fakers selling adulterated herbal medicines are common. In this regard, there is no control by the government agencies, despite the existence of certain guidelines in some individual countries and those outlined by the WHO. If the herbal products are marketed as therapeutic agents, and irrespective of whether the products really have any positive effects to cure and reduce the severity of the disease, it is necessary to ensure scientific validation and periodic monitoring of the quality and efficacy by drug control administrators. It is feasible that the introduction of scientific validation would control the production of impure or adulterated herbal products and would eventually ensure their rational use. This could also lead to the regulation of the industry so that only qualified physicians and health providers are allowed to prescribe the medication. Several of the principal pharmacopoeias contain monographs outlining standards for herbal drugs. The major advantage of an official monograph published in a pharmacopoeia is that standards are defined and available, and that the analytical procedures used are fully validated. This is of major importance, since validation can be a rather time-consuming process. Generally, validation investigations must include studies on specificity, linearity, accuracy, precision, range, detection, and quantitative limits, depending on whether the analytical method used is

qualitative or quantitative [13].

5. RECENT APPROACHES IN HERBAL DRUG STANDARDIZATION

5.1. DNA fingerprinting

Correct identification and quality assurance of the starting material is an essential prerequisite in herbal medicine to ensure reproducible quality of herbal medicine, which contributes to its safety and efficacy [14-16].

5.2. Molecular markers

Molecular markers generally refer to biochemical constituents, including primary and secondary metabolites and other macromolecules such as nucleic acids. Secondary metabolites as markers have been extensively used in quality control and standardization of botanical drugs. DNA markers 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 [17]. DNA can be extracted from fresh or dried [18] organic tissue of the botanical material; hence the physical form of the sample for assessment does not restrict detection.

Various types of DNA-based molecular techniques [19] are utilized to evaluate DNA polymorphism. These are hybridization-based methods, polymerase chain reaction (PCR)-based methods and sequencing based methods.

5.3. Hybridization-based methods

Hybridization-based methods include restriction fragment length polymorphism (RFLP) [20] and variable number tandem repeats [21]. Labelled probes such as random genomic clones, cDNA clones, probes for microsatellite [22] and mini satellite [23] sequences are hybridized to filters containing DNA, which has been digested with restriction enzymes. Polymorphisms are detected by presence or absence of bands upon hybridization.

5.4. PCR-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 thermostable DNA polymerase enzyme. PCR-based techniques where random primers are used, include random amplified polymorphic DNA (RAPD) [24, 25], arbitrarily primed PCR (AP-PCR) [26] and DNA amplification fingerprinting (DAF) [27, 28]. A recent

approach known as amplified fragment length polymorphism (AFLP) [29] 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 [30].

5.5. Chromatographic fingerprinting

The use of chromatographic fingerprinting for herbal drugs tends to focus on identification and assessment of the stability of the chemical constituents observed by chromatography. Chemical and chromatographic techniques may also be used to aid in identification of a herbal material or extract. Chromatographic techniques such as HPLC, thin layer chromatography (TLC), gas chromatography (GC), and capillary electrophoresis have been used for identity tests. Examples have been found in the literature where marker compounds and chromatographic profiles ("fingerprints") are used to help in identification of herbals, and in assessment of their potency and stability [31].

5.6. Fluorescence quenching

When a plant extract is spotted on a fluorescent silica gel layer and exposed to UV light, it appears as a spot on a fluorescent background, thus causing quenching and is directly proportional to the concentration of the extract. The silica gel GF plate was used as an adsorbent for fluorescence quenching. Solvents took hexane toluene, ether, ethyl acetate, butanol, methanol and water [32].

5.7. Good manufacturing practices

For standardization and quality assurance purposes, following three attributes are desirable i) Authenticity, ii) Purity and iii) Assay. Authenticity as the name suggests relates to proving that the material is true, i.e. it corresponds to the right identity. Authentication in itself involves many parameters including gross morphology, microscopy, chemical analysis and DNA fingerprinting. Purity pertains to evaluating that there are no adulterants present in the plant material. Assay part of standardization is chemical and biological profiling which could assess the chemical effects and curative values get established. Safety for use could also be assessed through this parameter. In biological assays, the drug activity is evaluated through a pharmacological model. Chemoprofiling is a versatile technique and can

be made to good use in standardization. Fingerprinting in essence is chemo profiling, which means establishing a characteristic chemical pattern for the plant material or its cut or fraction or extract [33].

Quality control and the standardization of herbal medicines also involve several other steps like source and quality of raw materials, good agricultural practices and good manufacturing practices. These practices play a pivotal role in guaranteeing the quality and stability of herbal preparations [34, 35]. The quality of a plant product is determined by the prevailing conditions during growth, and accepted Good Agricultural Practices (GAP) can control this. These include seed selection, growth conditions, fertilizers application, harvesting, drying and storage. In fact, GAP procedures are integral part of quality control.

Factors such as the use of fresh plants, age and part of plant collected, period, time and method of collection, temperature of processing, exposure to light, availability of water, nutrients, drying, packing, transportation of raw material and storage, can greatly affect the quality, and hence the therapeutic value of herbal medicines. Apart from these criteria, factors such as the method of extraction, contamination with microorganisms, heavy metals, and pesticides can alter the quality, safety, and efficacy of herbal drugs. Using cultivated plants under controlled conditions instead of those collected from the wild can minimize most of these factors [36, 37]. Sometimes, the active principles are destroyed by enzymic processes that continue for long periods from collection to marketing, resulting in a variation of composition. Thus, proper standardization and quality control of both the raw material and the herbal preparations should be conducted.

6. FUTURE PERSPECTIVES

Herbal drug technology is used for converting botanical materials into medicines, where standardization and quality control with proper integration of modern scientific techniques and traditional knowledge is important. The routine methods of herbal drug standardization address quality related issue using botanical and organoleptic parameters of crude drugs, and chemoprofiling assisted characterization with spectroscopic techniques but the new era of herbal drug standardization includes pharmacognostical, chemical, biological, biopharmaceutical and molecular approaches. The subject of herbal drug standardization is massively wide and deep. There is so much to know and so much seemingly contradictory theories on the subject of

herbal medicines and its relationship with human physiology and mental function.

Nowadays newer and advanced methods are available for the standardization of herbal drugs like fluorescence quenching, the combination of chromatographic and spectrophotometric methods, biological assays, use of biomarkers in fingerprinting etc. Bioassay can play an important role in the standardization of herbal drugs and can also become an important quality control method as well as for proper stability testing of the product [38].

India can emerge as the major country and play the lead role in the production of standardized, therapeutically effective Ayurvedic formulation. India needs to explore the medicinally important plants. This can be achieved only if the herbal products are evaluated and analyzed using sophisticated modern techniques of standardization such as UV- visible, TLC, HPLC, HPTLC, GC-MS, spectrofluorimetric and other methods [39].

Drug development from herbal medicine should take into account the diversity of species and ecological ethics. Ideally, during the drug development, one first isolates and identifies target compounds from herbs or plants, which is followed by total chemical synthesis of the compound(s), as was the case for aspirin [40].

7. CONCLUSION

The need for standardization of herbals is now very essential considering the global acceptance of herbal products as remedies for various diseases and evidence is emerging on the dangers of indiscriminate use of certain herbs. The assurance of the quality, safety and efficacy of an herbal drug requires monitoring of the quality of the product from collection through processing to the finished packaged product. It is recommended that 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 outlined above. This will strengthen the regulatory process and minimize quality breach. Unlike standard chemically-defined drugs, herbal products have often had substantial human use prior to clinical trial evaluation. To capitalize on the use of this information in protocols to evaluate these products, it is important that the chemistry, manufacturing, and control of the product to be used mimic that for the traditionally-used formulation.

Conflict of interest

None declared

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