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STANDARDIZATION OF HERBAL DRUGS – A REVIEW
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ABSTRACT
In the past few decades, the utilization of herbal drugs has been increased all over the world due their enormous therapeutic effects and less adverse effects as compared to the synthetic drugs. The emergent use of herbal drug by the human is forcing moves to evaluate the health claims of these agents and to develop standards of quality, purity, safety and efficacy of the drug. The mostly herbal drugs are effective but they lack of standardization. So there is a need to develop standardization parameters. Standardization of herbal drug is necessary to confirm its identity and to determine its quality, purity, safety, content, physical, chemical and biological properties. But the most important challenges faced by these herbal drugs are the lack of complete standardization by physiochemical. The standardization of the herbal drug, the macroscopic characters, microscopic characters, physico-chemical properties, moisture content, ash values, extractive values, volatile oil content and fluorescence study were carried out. Chromatography study, heavy metal content, microbial contamination and pesticide residues were also carried out to assure the quality, purity and safety of the herbal drugs.
1. INTRODUCTION:
Nature always stands as a golden mark to represent the outstanding phenomena of symbiosis. Natural plants are source of raw material for both traditional and modern system of medicine. Now-a-day about 80% of the world’s population, living in developing countries still relay on traditional medicine based largely on the different species of plants for their primary health care. About five hundred of plants with medicinal uses are mentioned in ancient literature and eight hundred plants have been used in indigenous system of medicine. The various indigenous systems such as Ayurveda, Siddha and Unani use several plant species to treat different diseases. Herbal medicines make up an important part of the trend toward alternative medicine. In many countries herbal drugs are lunched into the market without standard scientific evaluation. There is no effective way to regulate herbal drugs manufacturing process in herbal pharmacy industry. Therefore, standardization plays an important role for the herbal industry and authentication of herbal drugs [1, 2, 28].

Herbal Drugs
Traditionally, herbal drugs have played a significant role in the management of both minor and major medical illnesses. Herbal drugs means a dosage form consisting of one or more herbs or processed herb in a specified quantities to provide specific nutritional, benefits, and/or other benefit meant for use to diagnosis treat, mitigate diseases of human beings or animal and/or alter the structure or physiology of human beings or animals. Herbal drugs are obtained by whole plant, cut plant part, powdered plant part, extract, essential oil, expressed juices and plant parts to treatment such as distillation, extraction, expression, fractionation, purification and concentration [16,18-20,24].

Advantages of Herbal Drugs
- They have low risk of side effects.
- They have more effective than any synthetic drug.
- They have lower cost.
- They have large amount of availability.
- They have better patient tolerance as well as acceptance.
- They have large therapeutic activity.
- The medicinal plants have renewable source of cheaper medicines.

Need of Standardization
The quality control of herbal drug is important in justifying their acceptability in modern system of medicines. Modern system of medicine offers no problem with very well defined parameters of analysis and based on experimental data, toxicity studies and human clinical
studies. It is not uncommon to have as many as five or more different herbal ingredients in one single product. The batch to batch variation starts from the collection of the raw materials itself in absence of any reference standard for identification. These variations multiply during dry, storage and further processing. Pharmacopoeial standards on herbal drugs are not available. cGMP for the herbal industry are not well defined quality control parameters of herbal drugs are maintained or regulated. The lack of quality standards has result serious adverse effect. The pharmaceutical industry has shown interest to development of standardized plant drugs with proven safety, efficacy and quality. World Health Organization (WHO) set specific guideline for the quality control of medicinal plants products by using modern techniques and by applying suitable standards and parameters. Standardization brings important benefits to business including a solid foundation upon which to develop new technologies and an opportunity to share and enhance existing practices. Standardization also plays an essential role in assisting governments, administrations, regulators and the legal profession as legislation, regulation and policy initiatives are all supported by standardization of herbal drugs [7-9,11,12,17].

Pharmacopoeial Guidelines for Standardization of Herbal Drugs

Several pharmacopoeias such as Indian Pharmacopeia, The Ayurvedic Pharmacopeia of India, British Pharmacopeia, Chinese Herbal pharmacopoeia, British Herbal Pharmacopeia, British Herbal Compendium, Pharmacopeia Committee, Japanese pharmacopoeia, Japanese Standards for Herbal Medicine and USP do cover monographs and quality control tests for medicinal plants used in their respective countries. Internationally, several pharmacopoeias have provided monographs stating quality parameters and standards of many herbs and their herbal products. Brief review of WHO Guidelines concerning the quality control of the herbs, herbal material and herbal drugs is narrated below [5,6,13,14].

WHO Guidelines for Standardized Herbal Drugs

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

- **Botanical evaluation-** sensory characters, foreign organic matter, microscopical, histological, histochemical evaluation, quantitative measurements etc.
- **Physicochemical parameters-** Physical and chemical identity, chromatographic fingerprints, ash values, extractive values, moisture content, volatile oil content, quantitative estimation protocols etc.
- **Pharmacological parameters-** biological activity profiles, bitterness values, swelling factor, foaming index etc.
WHO Guidelines for Herbal Drug Standardization and Evaluation

WHO developed guidelines for the assessment of herbal drugs and same were ratified by the sixth international conference of DRA held at Ottawa. The WHO guidelines for quality control of herbal drugs can be summarized as follows [3,4,10,15,21-23,25-30]:

Foreign organic matter
Collected drugs from plants should be free from soil, stone, dust, insect parts or animal excreta etc. Weigh a sample of plant material, taking the quantity indicated above unless other-wise specified in the test procedures for the plant material concerned. Spread it in a thin layer and separated the foreign organic matter into groups either by visual inspection, using a magnifying lens (6x or 10x). After complete separation foreign organic matters was weighed and calculate the content of each group in grams per 100 g of air-dried sample.

Macroscopic and microscopic examination
In the case of herbal drugs, the macroscopic and sensory characters are usually sufficient to identify the drug. Visual inspection provides the simplest and quickest means to establish identity, purity and quality. In these include colour, consistency, odour, taste, size, shape, surface characteristics, texture, fracture characteristics and appearance of the cut surface. Microscopy inspection of the herbal drug is valuable both powder and crude drugs. The types of certain factors such as epidermal parenchyma, stomata, trichomes, fibers, vessels and calcium oxalate crystals, help identification of drugs. In quantitative microscopy determination such as veinislet number, veinlet termination number, palisade ratio, stomatal number, stomatal index and determination of size of trichomes, fibers, and vessels help in the identification of the herbal drugs.

Ash values
The ash values are useful for detecting the excess of soil or earthy elements. The presence of ash is determined as total ash, acid-insoluble ash, water-soluble ash and sulphated ash.

Total ash
The test is designed to measure the amount of material remaining after ignition. In the determination of total ash values the carbon must be removed at as low temperature as possible, since alkali chlorides which may be volatile at high temperature, would otherwise may be lost. Total ash usually consists of carbonates, phosphates, silicates and silica to get more consistent ash.
About 2-4 g of the graduated material was accurately weighed in to previously ignited and tarred crucible the material was placed in the crucible and ignited by gradually increasing the heat to 500-600°C for 5 hours in a muffle furnace until it was white, indicate the free from carbon. It was cooled in desiccators and weighed. Total ash content was calculated in mg per g of air dried material.

**Acid-insoluble ash**

Acid-insoluble ash is the residue which was obtained after boiling the total ash with dilute hydrochloric acid and igniting the washed insoluble matter left on the filter, the determination measures the presence of silica, especially sand and siliceous earth. 25 ml of hydrochloric acid was added to the crucible containing the total ash and covered with a watch glass and boiled gently for 5 minutes. The watch glass was rinsed with 5ml of hot water and this liquid was added to the crucible. The insoluble matter was collected on an ash less filter paper and washed with hot water until filtrate was neutral. The filter paper containing the insoluble matter was transferred in the crucible, dried on a hot plate and ignited by gradually increasing the heat to 550°C for 3 hours in a muffle furnace to constant weight. Acid-insoluble ash content was calculated as mg per g of air-dried material.

**Water-soluble ash**

Water-soluble ash the difference in weight between the total ash and the residue left after treatment of the total ash with water. 25 ml of water was added to the crucible containing the total ash and boiled gently for 5 minutes. The insoluble matter was collected in a sintered glass crucible. The insoluble matter was collected on an ash less filter-paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C in a muffle furnace. The weight of the residue in mg was subtracted from the weight of total ash. Water-soluble ash content was calculated as mg per g of air-dried material.

**Sulphated ash**

Placed a suitable quantity of the substance being examined, accurately weighed, in the crucible, added 2ml of 1M sulphuric acid heated first on a water bath and then continuously over a flame of about 600°C. Continued the heating until all black particle have disappeared and then allowed to cool. Added a few drops of 1M sulphuric acid, heated to ignition as before and allowed to cool. Added a few drops of 16% w/v solution of ammonium carbonate, evaporated to dryness and continuously ignited. Cooled, weighed, ignited for 15 minutes and repeated their procedure to constant weight.

**Loss on drying (LOD)/ Moisture content**

Moisture is an expected component of crude herbal drug, which must be eliminated as far as
practicable. Drying of crude drug is important during collection of drug and is also important for preservation, preventing hydrolytic degradation of active constituents and for easy size reduction of crude drug. Excess moisture or insufficient drying is responsible for spoilage of drug due to growth of microbes. There for drying process should reduce the moisture content of drug below the critical level. Dried petridish was weighed. The samples are kept in the petridish and accurately weighed the petridish and their contents. The samples were dried in an oven at a temperature of 105°C, until content weight of sample was obtained. After drying was completed the oven was opened, the petridish was allowed to cool to room temperature in a desiccator and weighed for LOD calculation.

**Extractive values**

The determination of water soluble or ethanol soluble extractive matter is used as a means of evaluating drugs. Extraction of any crude drug with a particular solvent yields a solution containing different phytoconstituents. The composition of these phytoconstituents in that particular solvent depends upon the nature of the drug and solvent used. Extractive value determined the amount of active constituents in a given amount of medicinal plant material when extracted with solvent. It is employed for that material for which no chemical or biological assay method exist.

**Water soluble extractive**

About 4 gm of coarsely powder air-dried drug was accurately weighed into glass stopper conical flask. It was macerated with 100 ml of water for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Filter and evaporates 25 ml of the filtrate to dryness at 105°C and cooled in a desiccator for 30 minutes and weight was calculated in percentage of extract with reference to the air-dried materials.

**Alcohol soluble extractive**

About 4 gm of coarsely powder air-dried drug was accurately weighed into glass stopper conical flask. It was macerated with 100 ml of ethanol of specified strength in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Filter and evaporates 25 ml of the filtrate to dryness at 105°C and cooled in a desiccator for 30 minutes and weight was calculated in percentage of extract with reference to the air-dried materials.

**Fluorescence analysis**

Herbal drugs undergo to UV light. The original molecules of herbal drugs absorb light usually over a specific range of wavelength, get excited to a high level and many of them emit such radiation while coming back to the original state. Such a phenomenon of re-emission of
absorbed light that occurs only when the substance is receiving the excited rays is known as fluorescence. Fluorescence can also occur on treatment with certain reagents. This can be useful in certain cases for identification of medicinal plants. The fluorescence analysis for the drug sample was done under lights of wave length 254 nm, 365 nm and visible light.

**Chromatography**

Chromatography is the technique used of separation of compounds based on their difference in their structure and/or composition. Chromatographic separation can be carried out using a verity of supports, including immobilized silica on glass plates which simply known as thin layer chromatography (TLC), very sensitive High performance thin layer chromatography (HPTLC), volatile gases (gas chromatography), paper (paper chromatography) and liquids which may incorporated hydrophilic, insoluble molecules (liquid chromatography). These are techniques used for standardization and to control the quality of herbal drugs. The results from these sophisticated techniques provide a chemical fingerprint as to the nature of chemicals or impurities present in herbal drugs.

**Volatile oil content**

Pharmaceutical significance of aromatic herbal drugs is due to their odoriferous principles, such as volatile constituent. These drugs are standardized on the basis of volatile oil content. Volatile oil is characterized on their basis odour and ability of volatilization at room temperature. In order to determine the volume of volatile oil in herbal drugs, place plant material distilled with water at specific time period the distillate is collected in graduated tube. Volatile oil content was calculated as ml per g of herbal drug.

**Crude fiber content**

Estimation of crude fiber indicates the measurement of the content of cellulose, lignin and cork cell in the plant tissue of the herbal drugs. The crude fiber consists of the material other than ash which cannot be dissolved in water and cannot be digested by boiling H₂SO₄ or NaOH. Thus it indicate the more resistant part of the plant cells as well as some less resistant cell wall component like cellulose and pectin. The presence of adulteration containing sclerenchyma or other resistant tissue than is permissible for the crude drug under examination may be determined by ascertaining the crude fiber of that sample. About 2 g of the drug sample accurately weighed is extracted with ether. 200 ml of 1.25 % H₂SO₄ is added to the extracted drug and the whole mixture boiled for 30 minutes under reflux in a 500 ml flask. The mixture is then filtered through a hardened filter and the residue washed with boiling water until free of acid. The entire residue is rinsed back into the flask with 200 ml of 1.25 % NaOH and gained
boiled under reflux for 30 minutes. The liquid is then quickly filtered and residue with boiling water until neutral, dried at 110°C to constant weight and weigh. After that it is incinerated in crucible and form ash. The difference in weight indicated weight of crude fiber content of drug.

**Bitterness values**

Those herbal drugs that have strong bitter taste are used as appetizing agent and bitter tonic. The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride. After rinsing the mouth with safe drinking water, taste 10 ml of the most dilute solution swirling it in the mouth mainly near the base of the tongue for 30 seconds. If the bitter sensation is no longer felt in the mouth after 30 seconds, spit out the solution and wait for 1 minute to ascertain whether this is due to delayed sensitivity. Then rinse with safe drinking-water. The next highest concentration should not be tasted until at least 10 minutes have passed. The threshold bitter concentration is the lowest concentration at which a material continues to provoke a bitter sensation after 30 seconds. After the first series of tests, rinse the mouth thoroughly with safe drinking water until no bitter sensation remains. Wait for at least 10 minutes before carrying out the second test. The bitter value expressed in unit equivalent to the bitterness of a solution containing 1 g of quinine hydrochloride.

**Swelling index**

Most of herbal drugs are of specific therapeutic properties or pharmaceutical utility because of their swelling properties, especially gums and those containing an appreciable amount of mucilage, pectin or hemicelluloses. Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant materials. Using a glass stopper measuring cylinder, the plant material is shaken repeatedly for 1 hour and then allowed to stand for a required period of time. The volume of the mixture in ml is then read. The specified quantity of the plant material concerned is introduced previously reduced to the required fineness and accurately weighed, into a 25 ml glass stopper measuring cylinder. The internal diameter of the cylinder should be about 16 mm, the length of the graduated portion about 125 mm, marked in 0.2 ml division from 0-25 ml in an upwards direction. Unless otherwise indicated in the test procedure, 25 ml of water is to be added. The mixture is shaken thoroughly every 10 minutes for 1 hour. Allow to stand for 3 hours at room temperature or as specified. The volume in ml has be measured which is occupied by the plant materials, including any sticky mucilage. Swelling index was determined as the volume in ml of taken up by the swelling of 1 g of plant material.
Foaming index

The saponins are high molecular weight containing phytoconstituents having the detergent or soap like property. Many herbal drugs contain saponins that can cause persistent foam when shaken with water. The foaming ability of herbal drugs and their extracts is measured in terms of foaming index. 1 g of the plant materials to a coarse powder (Sieve size No. 1250), weigh accurately and transfer to a 500 ml conical flask containing 100 ml of boiling water. Maintain at moderate boiling for 30 minutes. Cool and filter into a 100 ml volumetric flask and add sufficient water through the filter to dilute. Pour the decoction in 10 ml stoppered test tube (height 16 cm, diameter 16 mm) in successive portion in 1 ml, 2 ml, 3 ml and adjust the volume of the liquid in each tube with water to 10 ml and stopper the tubes and shaken them in a length wise motion for 15 seconds, two shakes per second. Allow standing for 15 minutes and measured the height of the foam.

Pesticide residue

Pesticide residues produce toxic effects like irritation of eye, sweating breathlessness, hypotension, cardiac arrhythmia, respiratory paralysis and convulsion. Limits for pesticide residues should be set by World Health Organization (WHO) and FAO (Food and Agricultural Organization), which are usually present in herbal drugs. These pesticides are mixed with the plants during the time of cultivation. Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues in the herbal drugs. Samples are extracted by a standard procedure, impurities are removed by partition and/or adsorption, and the presence of a moderately broad spectrum of pesticides is measured in a single determination.

Heavy metal content

Heavy metals such as lead, copper, cadmium, arsenic and mercury in herbal drugs can be attributes many causes, including environmental pollution, and can pose clinically relevant dangers to health of the user and should therefore be limited. Plants may accumulate heavy metals from the environment during growth, heavy metals may be intentionally added to products within specific traditional health paradigms such as Ayurveda, and contamination may occur through inadequate quality control. Even though traditional preparation methods for the addition of metals to herbs are intended to render these metals non-toxic, poisonings related to metal exposure have been reported. Instrumental analysis has to be employed when the heavy metals are present in trace amount, in admixture, or when the analyses have to be quantitative. Generally, the main methods are commonly used for atomic absorption spectrophotometry (AAS), inductively coupled plasma (IPC) and neutron activation analysis (NAA).
Microbial contamination
Herbal drugs may be associated with a broad variety of microbial contaminants, represent by bacteria, fungi and viruses. Microbial contamination can occur during the collection and the processing of ingredients or finished products. Some microbial species are common in the environment, while others can be introduced due to poor quality control or hygiene practices. For ensuring of microorganism safety of intake as oral route, determination of microorganism is to be carried out. The World Health Organization (WHO) guideline limits for aerobic bacteria and *Escherichia coli* should not be more than 10 per 10 g of the sample. The limits for yeast, mould, *Pseudomonas aeruginosa* and *Staphylococcus aureus* should not be more than $10^3$ per 10 g of the sample. *Salmonella* should be nil in crude drug samples.

Radioactive contamination
There are many source of ionization radiation occurring in the environment. Hence certain degree of exposure is inevitable. Microbial growth in herbals is usually avoided by irradiation. This process may sterilize the plant material but the radioactivity hazard should be taken into account. The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic Energy (IAE) in Vienna and that of World Health Organization (WHO). Therefore, at present, no limits are proposed for radioactive contamination.

CONCLUSION
The field of the herbal drugs is very vast and there is still lot to explore on the subject of standardization of these. So, while developing an herbal drug it is must to have all the related knowledge of that particular drug including all its macroscopic characters to phytoconstituents to pharmacological action to its standardization in respect to various parameters via various techniques. 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. Monographs as compiled in the standard books like Indian Pharmacopoeia, Ayurvedic Pharmacopoeia of India, Wealth of India and Ayurvedic formulary, provide all the details for the various tests to be performed in order to determine the conformity of the herbal drug with the standards lay. It is recommended that various government agencies should follow a more universal approach to herbal drug quality by adopting the WHO guidelines and also developing monographs using the various quality parameters outlined above. Hence standardization plays an important role to quality control of herbal drugs and meet standard of quality, safety and efficacy.
REFERENCES


