

## Standard Operating Procedures for the Quality Control Studies of Herbal Drugs

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### ABSTRACT

Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Eighty of world population is dependent on herbal drugs and to enter into the global market it is vital to maintain its quality. Because of commercial gains, adulteration and substitution is quite normal. As a result the quality of herbal drugs and products is highly variable and necessitates the need for quality control studies of raw material. Standardisation is a relatively new concept in pharmaceutical manufacturing to ensure total quality management and to assure products of best quality. 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. So standardization is important as it helps in giving correct dosage to patient, in detecting adulteration in commercial samples and also in authentication of other species. Various parameters and their methods of determination used to conduct standardisation of plant drugs are discussed in this article.

**Keywords:** Standardisation parameters, Pesticide Residue, Loss on Drying, Importance.

### INTRODUCTION

Plant materials are used throughout developed and developing countries as home remedies, over-the-counter drug products and raw materials for the pharmaceutical industry, and represent a substantial proportion of the global drug market. Herbal medicines are the synthesis of therapeutic experiences of generations of practicing physicians of indigenous systems of medicine for over hundreds of years. Eighty of world population is dependent on herbal drugs and to enter into the global market it is vital to maintain its quality. [Sagar Bhanu P.S. et al, 2007]

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. [Patel P.M. et al, 2006]

However, the last few years have seen a major increase in their use in the developed world. Herbs are defined in several ways depending on the context, in which the word is used. In the field of medicine, they are most accurately defined as crude drugs of vegetable origin utilized for the treatment of diseases stated, often of a chronic nature or to attain or maintain a condition of improved health. Pharmaceutical preparations made by extracting herbs with various solvents to yield tinctures, fluid extracts, are known as phytomedicinals (plant medicines) [Stafford 1994]. Herbal medicines are plant derived materials or products with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants. In some traditions, material of inorganic or animal origin may also be present. Herbal medicines are also in great demand in the developed world for primary health care

because of their efficacy, safety and lesser side effects. They also offer therapeutics for age-related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available.[Clarke ECG, 1967]

According to the definition, there are three kinds of herbal medicines: Raw plant materials, processed plant materials, and medicinal herbal products. The definition does not apply where the active component has been identified, and either isolated or synthesized as a chemical component of a drug.[WHO International pharmacopoeia, 2001]

- **Ingredient:** The substance in a herbal formulation which may not be a purified chemical component.
- **Medicinal herbal products:** Finished, labelled pharmaceutical products in dosage forms that contain one or more of the following: Powdered plant materials, extracts, purified extracts or partially purified active substances isolated from a plant material. Medicines containing plant material combined with chemically defined active substances, including chemically defined isolated constituents of plants, are not considered to be herbal medicines.
- **Medicinal plant:** A plant, which has been used for medicinal purposes at one time or another and which although not necessarily a product or available for marketing is the original material of herbal medicine.
- **Processed plant materials:** Plant material treated according to traditional procedure to improve their safety and efficacy to facilitate their clinical use or to make medicinal preparation.
- **Raw plant materials:** Fresh or dry plant materials, which are, marketed whole or simply cut into small pieces

### **How herbs are different from chemical entities?**

Herbs are more dilute than the concentrated chemicals.

--Herbs often contain additional active principles other than major active principles and many physiologically inert substances such as cellulose and starch; unlike the chemical entities, which contain one active ingredient plus a number of inert substances, which make up the dosage form (such as tablet, capsule, and lozenge) [Stafford 1994].

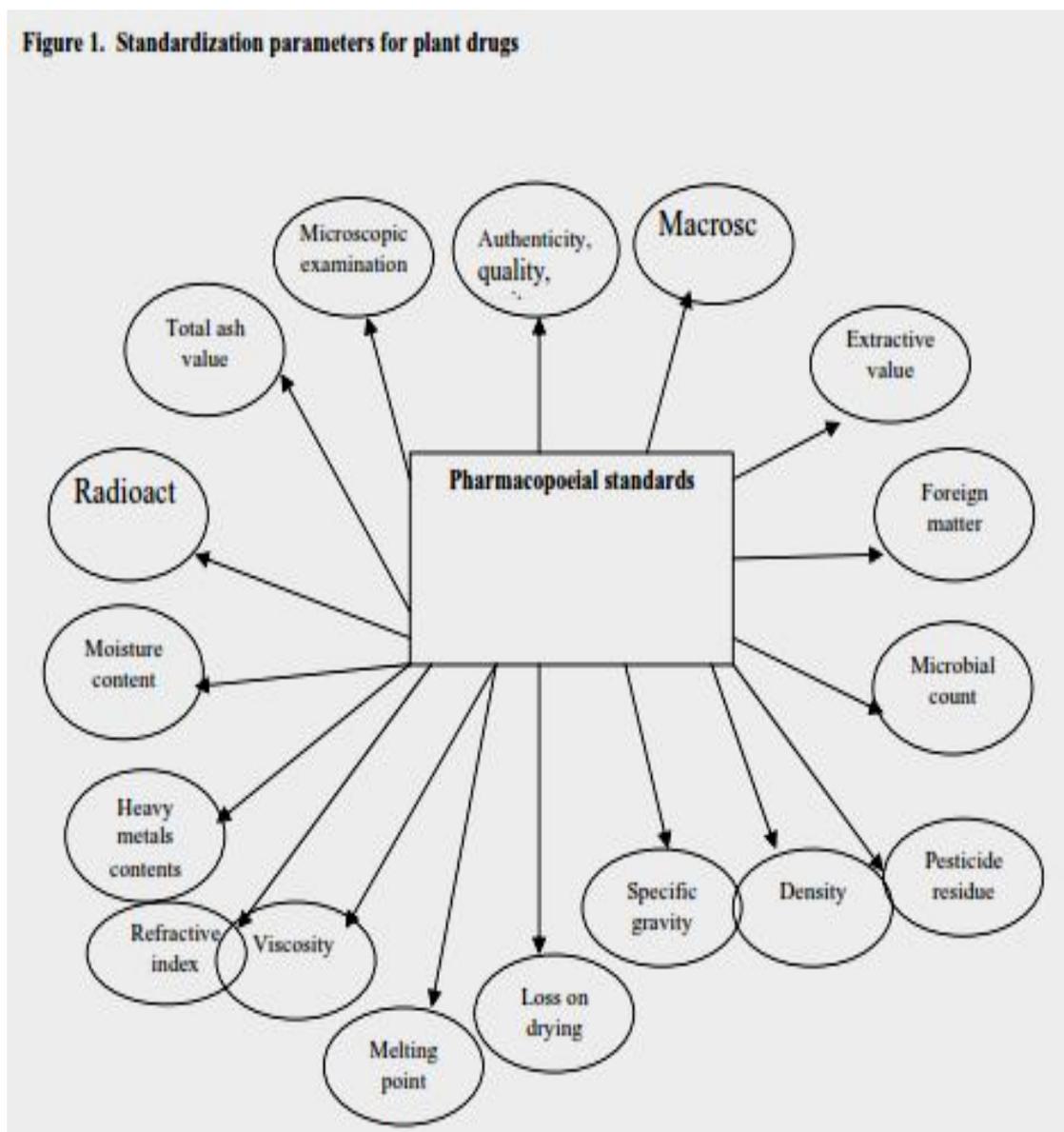
India despite its rich traditional knowledge, heritage of herbal medicines and large biodiversity has a dismal share of the world market due to export of crude extracts and drugs. Pharmaceutical research is aimed at meeting the medical needs of the population for whom appropriate therapeutic remedies are not available or at those that are available are unsafe for prophylactic use for various disorders. While meeting medical needs, research also has to ensure that market needs for such exist and that the product will command sales and profits proportionate to investments. In cases where there are mismatches between these two, the products suffer the status of orphan drugs. The selection of an appropriate R&D portfolio is a strategic management exercise for a company, which should take into account a system to ensure that every packet of medicine that is being sold has the correct substances in the correct amount and will induce its therapeutic effect this is known as standardization. It is very important that a system of standardization is established for every plant medicine in the market because the scope for variation in different batches of medicine is enormous. Plant material may vary in its photochemical content and therefore in its therapeutic effect according to different places of collection, with different times in a year for collection, with collection at the same time and places but in different years and with different environmental factors surrounding the cultivation of a particular medicinal

plant. Adding to this variability is the fact that in herbal medicine several plants may be used together in the same preparation. This means that there should be a quality control test for the entire preparation to ensure quality of the product. World Health Organization (WHO) encourages, recommends and promotes traditional /herbal remedies in national health care programs because these drugs are easily available at low cost, safe and people have faith in them. The WHO assembly in

number of resolutions has emphasized the need to ensure quality control of medicinal plant products by using modern techniques and applying suitable standards count apart from medical needs, innovative potential for success and available resources.

Therefore, standardization is needed as it helps in giving correct dosage to the patient, in detecting adulteration in commercial samples and authentication and differentiation from similar plants of other species.

Figure 1. Standardization parameters for plant drugs



### Procedure for analyzing certain Important Parameters of Herbal Drugs

#### 1. Determination of Ash Value

The ash value includes 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. "Physiological ash" is the residue after ignition of the extraneous matter (e.g. sand and soil) adhering, determines both kinds of ashes and is

referred as the "Total ash" test. 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 be lost.

Total ash usually consists of carbonates, phosphates, silicates and silica to get more consistent ash, the EP and BP use sulphated ash, which involves treatment of the drug with dilute sulphuric acid before ignition. In this all oxides and carbonates are converted to sulphates and the ignition

is carried out at high temperature.(Chaudhari RD,1996).

About 1 to 4 gm of the graduated material was accurately weighed in to previously ignited and tarred crucible(platinum or silica) the material was spread in an even layer in the crucible and ignited by gradually increasing the heat to 500-600 degree centigrade until free from carbon. It was cooled in desiccators and weighed. If carbon free ash could not be obtained in this manual, the crucible was cooled and the residue was moistened with about 2ml of water or saturated solution of ammonium nitrate. It was dried on a waterbath. The content of total ash was calculated as percentage of ash with reference to the air dried plant 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.

25ml of hydrochloric acid was added to the crucible containing the total ash and covered with a watch glass and boiled gently for 5minutes.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 to constant weight. The content of the acid insoluble ash was calculated in percentage of ash and reference to the air-dried plant material.

#### **Water soluble ash:**

Water soluble ashes 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 the total ash and boiled gently for 5minutes.The insoluble matter was

collected in a sintered glass crucible. or an ash less filter paper. It was washed with hot water and ignited for 15 minutes at a temperature not exceeding 450 degree centigrade. The weight of the residue in mg was subtracted from the weight of total ash. The contents of water soluble ash were calculated in percentage of ash with reference to the air-dried plant material.[ Ansari S.H, 2005]

#### **Sulphated ash:**

Heated a silica or platinum crucible to redness for 30 minutes, allowed to cool in a desiccators and weighed. 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 degree centigrade. 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.

#### **2. Determination of Extractable Matter:**

The determination of water soluble or ethanol soluble extractive matter is used as a means of evaluating drugs, The constituents of which are not readily estimated by other means. But a suitable assay became available(e.g. with anthraquinone containing drugs)the extractive tests are no longer required as pharmacological standards(Ayurvedic pharmacopoeia,1999).

#### **Cold maceration method:**

About 4gm of coarsely powdered air-dried material was accurately weighed in to glass stopper conical flask. It was macerated with 100ml of specified solvents for the given plant material for 6hours ,shaking frequently

with then allowed to stand for 18hours.It was rapidly filtered in order to avoid loss of solvent.25ml of the filtrate was transferred to a tarred flat bottom disk and evaporated to dryness on a water bath. It was dried at 105 degree centigrade for 6 hours, cooled in a desiccator for 30 minutes and weight was calculated in percentage of extract with reference to the air-dried materials.

### 3. Loss on Drying:

It determines the amount of volatile matter of any kind including material. Dried petridishes were weighed. The samples are kept in the petridishes and accurately weighed the petridish and their contents. The sample was distributed as evenly to a depth not exceeding 10mm. The petridishes were placed in the drying chamber. The samples were dried in an oven at a temperature of 105 degree centigrade, until content weight of sample was obtained. After drying was completed the drying chamber was opened, the petridishes were allowed to cool to room temperature in a dessicator and weighed for LOD calculation (Ayurvedic pharmacopoeia, 1999 ).

### 4. Fluorescence analysis:

Many herbs undergo fluorescence wet surface and powder is exposed to UV light .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 day light and UV light.

### 5. Thin layer chromatographic studies:

Preparation of the alcoholic extraction:10gm of the powdered drug sample was added to 100ml of absolute alcohol and refluxed for 5minutes on a water bath. The extract was filtered through whatman filter no.1and concentrated to 1/4<sup>th</sup> of its volume and was used for the TLC(Joseph Sharma etal,1999).Similarly the chloroform,

petroleum ether and aques extracts were prepared.

### Preparation of the plates:

Silica Gel G was used as a stationary phase for spreading on TLC chromatographic plates.Silicagel G(35gm) was triturated with little amount of distilled water in a mortar with a lass pestle. The silics gel G was allowed to swell for 10-15 minutes and then sufficient amount of water was added so as to make 80ml with vigorous stirring to et slurry. It was then spread on a clean glass plates. Thickness of silica gel was about 0.5mm the prepared plates were air-dried at room temperature.

### Activation of plates:

The air-dried plates were kept in an oven at 110degree centigrade for an hour and were stored in a desecrator subsequently.

### Saturation of the chromatographic chamber:

About 1cm height of the solvent taken in a clean dry chamber and then the walls of the chamber were lined with a strip of filter paper pre ignited with the solvent system.The chamber was closed to saturate with vapors of solvent.

### Application of spot:

The base line was marked at about 1.5cm above from the cover edge. The dissolved fractions were spotted on the plates with a fine capillary tube and then allowed to air dry.

### Development of chromatogram:

The spotted plates were kept for 30minutes in the saturated chromatographic chamber containing the pure solvent or solvent mixture with filter paper lining. The chamber were covered with greased glass plates. The solvent system was allowed to ascend up to 3/4<sup>th</sup> of the plate.

### Optimization of the mobile phase:

Pure solvents from different selective areas were tested. Diethyl ether, ethanol, methanol, ethyl acetate, acetonitrile, toluene, chloroform, benzene and cyclohexane were used as pure solvents.

The polarity of the solvent system in which the R<sub>f</sub> value was too high or low was adjusted by using a combination of solvents.

#### Detection of the spots:

The air-dried plates were viewed in UV light chamber to look for the coloured fluorescent spots, if any. The plates were then viewed in iodine vapor chamber for 5-10 minutes and spots were noted. Iodine is the universal detection reagent, the detection is usually non-destructive and reversible. Substrates, which chemically react with iodine, give colourless spots, otherwise brown spots on yellow background were observed. Since iodine has fluorescence quenching property, iodine containing spots appear as dark spots when viewed under short wavelength UV (254nm).

The plates were also sprayed with spray reagent 5-10%v/v ethanolic sulphuric acid and then kept in oven at temperature of 105 degree centigrade for 10-15 minutes and the spots were noted. It is a universal reagent and all classes of compounds can be detected by charring at elevated temperature. Usually dark brown spots appear as results of charring (Setri PD et al, 1997). The R<sub>f</sub> value (Retention factor) was calculated by using formulae,

**R<sub>f</sub> = Distance travelled by the solute front / Distance travelled by the solvent front.**

#### 6. Foreign Matter

Weigh a sample of plant material, taking the quantity indicated above unless otherwise specified in the test procedures for the plant material concerned. Spread it in a thin layer and sort the foreign matter into groups either by visual inspection, using a magnifying lens (6x or 10x), or with the help

of a suitable sieve, according to the requirements for the specific plant material. Sift the remainder of the sample through a No. 250 sieve; dust is regarded as mineral admixture. Weigh the portions of this sorted foreign matter to within 0.05g. Calculate the content of each group in grams per 100g of air-dried sample.

For some medicinal plant materials where the foreign matter may closely resemble the material itself, it may be necessary to take a pooled sample of the plant material and apply a critical test, either chemical, physical or by microscopy. The proportion of foreign matter is calculated from the sum of the portions that fail to respond to the test.

#### 7. Tannins

To prepare the plant material extract, introduce the quantity specified in the test procedure for the plant material concerned, previously powdered to a known fineness and weighed accurately, into a conical flask. Add 150ml of water and heat over a boiling water-bath for 30 minutes. Cool, transfer the mixture to a 250-ml volumetric flask and dilute to volume with water. Allow the solid material to settle and filter the liquid through a filter-paper, diameter 12cm, discarding the first 50ml of the filtrate.

To determine the total amount of material that is extractable into water, evaporate 50.0ml of the plant material extract to dryness, dry the residue in an oven at 105°C for 4 hours and weigh (T<sub>1</sub>).

To determine the amount of plant material not bound to hide powder that is extractable into water, take 80.0ml of the plant material extract, add 6.0g of hide powder R and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T<sub>2</sub>).

To determine the solubility of hide powder, take 6.0g of hide powder R, add 80.0 ml of water and shake well for 60 minutes. Filter and evaporate 50.0 ml of the clear filtrate to dryness. Dry the residue in an oven at 105°C and weigh (T<sub>3</sub>). Calculate the

quantity of tannins as a percentage using the following formula:

$$\frac{[T1-(T2-T0)]}{\text{-----} \times 500} \times 100$$

W

Where  $w$  = the weight of the plant material in grams.

#### 8. Volatile Oils:

Place the volume of distillation liquid specified in the test procedure for the plant material concerned in the flask, add a few pieces of porous porcelain and join the condenser to the apparatus. Introduce water by tube N until it reaches level B. Remove stopper (K') and introduce the appropriate volume of xylene R or the solvent specified for the given plant material, using a graduated pipette and placing its tip at the bottom of tube K. Replace stopper, heat the

liquid in the flask until it begins to boil and adjust the distillation rate to 2-3 ml per minute, unless otherwise specified in the test procedure.

To determine the distillation rate, lower the level of water while distilling by means of the three-way tap until the meniscus is at the level of the lower mark (see Fig. 1). Close the tap and simultaneously start a stop-watch. As soon as the level in the bulb reaches the upper mark, stop the watch and note the time taken. Open the tap and continue the distillation. Stop the heating after 30 minutes, turn off the heater, wait at least 10 minutes and then record the volume of solvent (xylene) collected in the graduated tube.

Introduce the specified quantity of the plant material being examined into the flask and continue the distillation as described above for the time and at the rate described above for the time and at the rate



**9. Pesticide Residue:**

Chromatography (mostly column and gas) is recommended as the principal method for the determination of pesticide residues. 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. However, these techniques are not universally applicable. Some pesticides are satisfactorily carried through the extraction and clean-up procedures, others are recovered with a poor yield, and some are lost entirely. In chromatography, the separations may not always be complete, pesticides may decompose or metabolize, and many of the metabolic products are still unknown. Consequently, as a result of limitations in the analytical technique and incomplete knowledge of pesticide interaction with the environment, it is not yet possible to apply an integrated set of methods that will be satisfactory in all situations.

It is therefore desirable to test plant materials of unknown history for broad groups of compounds rather than for individual pesticides. A variety of methods meet these requirements. Chlorinated hydrocarbons and other pesticides containing chlorine in the molecule, for example, can be detected by the measurement of total organic chlorine; insecticides containing phosphate can be measured by analysis for total organic phosphorus, while pesticides containing arsenic and lead can be detected by measurement of total arsenic or total lead, respectively. Similarly, the measurement of total bound carbon disulfide in a sample will provide information on whether residues of the dithiocarbamate family of fungicides are present.

If the pesticide to which the plant material has been exposed is known or can be identified by suitable means, an established method for the determination of that

particular pesticide residue should be employed.

**10. Arsenic:**

The amount of arsenic in the medicinal plant material is estimated by matching the depth of colour with that of a standard stain.

**Preparation of the sample by acid digestion**

Place 35-70g of coarsely ground material, accurately weighed, in a Kjeldahl flask, capacity 800-1000 ml. Add 10-25 ml of water and 25-50 ml of nitric acid (~1000 g/l) TS and then carefully add 20 ml of sulfuric acid (~1760g/l) TS. Heat cautiously so that no excessive foaming takes place. Gradually add nitric acid (~1000g/l) TS, drop by drop, until all the organic matter is destroyed. This is achieved when no further darkening of the solution is observed with continued heating, and a clear solution with copious vapours of sulfur trioxide is obtained. Cool, and add 75 ml of water and 25 ml of ammonium oxalate (25 g/l) TS. Heat again until sulfur trioxide vapours develop. Cool, transfer with the help of water to a 250-ml volumetric flask, and dilute to volume with water.

**Apparatus**

A suitable type of apparatus is constructed as follows. A wide-mouthed bottle of about 120-ml capacity is fitted with a rubber bung through which passes a glass tube. The latter, made from ordinary glass tubing, has a total length of about 200mm and an internal diameter of exactly 6.5 mm (external diameter about 8 mm). The lower end of the tube is drawn out to an internal diameter of about 1 mm, and there is a hole not less than 2 mm in diameter blown in the side of the tube, near the constricted part. The tube is positioned so that when the bottle contains 70ml of liquid the constricted end is above the surface of the liquid and the hole in the side is below the bottom of the bung. The upper end of the tube has a flat, ground surface at right-angles to the

axis of the tube, with slightly rounded-off edges.

One of two rubber bungs (about 25 mm x 25 mm), each with a central hole of exactly 6.5 mm diameter, is fitted at the upper end of the tube. The other bung is fitted with a piece of glass tube about 3 mm long and with an internal diameter of exactly 6.5 mm and with a similar ground surface. One end of each of the tubes is flush with the larger end of the bungs, so that when these ends are held tightly together with a rubber band or a spring clip, the openings of the two tubes meet to form a true tube. Alternatively, the two bungs may be replaced by any suitable construction satisfying the conditions described in the test.

#### Method

Moisten some cotton-wool with lead acetate (80g/l) TS, allow to dry, and lightly pack into the tube which fits into the wide-mouthed bottle to not less than 25 mm from the top. Between the flat surfaces of the tubes, place a piece of mercuric bromide paper AsR that is large enough to cover their openings (15 mm x 15 mm). The mercuric bromide paper AsR can be fitted by any other means provided that:

- the whole of the evolved gas passes through the paper;
- the portion of the paper in contact with the gas is a circle 6.5 mm in diameter; and
- the paper is protected from sunlight during the test.

Place an aliquot (25-50ml) of the solution being tested, prepared as described above, in the wide-mouthed bottle, add 1 g of potassium iodide AsR and 10g of granulated zinc AsR, and place the prepared glass tube assembly quickly in position. Allow the reaction to proceed for 40 minutes. Compare any yellow stain that is produced on the mercuric bromide paper AsR with a standard stain produced in a similar manner with a known quantity of dilute arsenic

AsTS. Examine the test and standard stains without delay in daylight; the stains fade with time.

The most suitable temperature for carrying out the test is generally about 40°C but, as the rate of evolution of the gas varies somewhat with different batches of granulated zinc AsR, the temperature may have to be adjusted to obtain an even evolution of gas. The reaction may be accelerated by placing the apparatus on a warm surface, care being taken to ensure that the mercuric bromide paper AsR remains dry throughout.

Between successive tests, the tube must be washed with hydrochloric acid (~250g/l) AsTS, rinsed with water and dried.

#### 11. Microorganisms: Enterobacteriaceae and certain other Gram-negative bacteria

##### *Detection of bacteria*

Homogenize the pretreated material appropriately and incubate at 30-37°C for a length of time sufficient for revivification of the bacteria but not sufficient for multiplication of the organisms (usually 2-5 hours). Shake the container, transfer 1g or 1ml of the homogenized material to 100ml of Enterobacteriaceae enrichment broth-Mossel and incubate at 35-37°C for 18-48 hours. Prepare a subculture on a plate with violet-red bile agar with glucose and lactose. Incubate at 35-37°C for 18-48 hours. The material passes the test if no growth of colonies of Gram-negative bacteria is detected on the plate. [Anon et al, 1989]

##### *Quantitative evaluation*

Inoculate a suitable amount of Enterobacteriaceae enrichment broth-Mossel with quantities of homogenized material prepared as described under "Detection of bacteria" above, appropriately diluted as necessary, containing 1.0g, 0.1g and 10µg, or 1.0ml, 0.1 ml and 10µl, of the material being examined. [Wyllie T.D. et al, 1978] Incubate at 35-37°C for 24-48 hours. Prepare a subculture of each of the cultures

on a plate with violet-red bile agar with glucose and lactose in order to obtain selective isolation. Incubate at 35-37°C for 18-24 hours. The growth of well-developed colonies, generally red or reddish in colour, of Gram-negative bacteria constitutes a positive result. Note the smallest quantity of material that gives a positive result. Determine the probable number of bacteria. [Eaton D.L et al, 1994]

### ***Pseudomonas aeruginosa***

Pretreat the material being examined but using buffered sodium chloride-peptone solution, pH 7.0, or another suitable medium shown not to have antimicrobial activity under the conditions of the test, in place of lactose broth. Inoculate 100 ml of soybean-casein digest medium with a quantity of the solution, suspension or emulsion thus obtained containing 1g or 1 ml of the material being examined. Mix and incubate at 35-37°C for 24-48 hours. [Heathcote, J.G et al, 1978] Prepare a subculture on a plate of cetrimide agar and incubate at 35-37 °C for 24-48 hours. If no growth of microorganisms is detected, the material passes the test. If growth of colonies of Gram-negative rods occurs, usually with a greenish fluorescence, apply an oxidase test and test the growth in soybean-casein digest medium at 42°C. The following method may be used. Place 2 or 3 drops of a freshly prepared 0.01 g/ml solution of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride R on filter-paper and apply a smear of the suspected colony; the test is positive if a purple colour is produced within 5-10 seconds. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative. [Finley J.W. et al, 1992]

### ***Staphylococcus aureus***

Prepare an enrichment culture as described for *Pseudomonas aeruginosa*. Prepare a subculture on a suitable medium such as Baird-Parker agar. Incubate at 35-37°C for 24-48 hours. The material passes the test if no growth of microorganisms is detected.

Black colonies of Gram-positive cocci often surrounded by clear zones may indicate the presence of *Staphylococcus aureus*. For catalase-positive cocci, confirmation may be obtained, for example, by coagulase and deoxyribonuclease tests. The material passes the test if cultures of the type described do not appear or if the confirmatory biochemical test is negative. Determine the probable number of bacteria. [Goldbatt, L.A et al, 1969]

### Limits for Microbial Contamination

Microorganism	Finished product	Raw materials
<i>E. coli</i>	101	104
Salmonella	-	-
Total aerobic bacteria	105	-
Enterobacteria	103	-

### 12. Bitterness Value

After rinsing the mouth with safe drinking-water, taste 10ml 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.

In order to save time in the second test, it is advisable to ascertain first whether the solution in tube no. 5 (containing 5 ml of S<sub>7</sub> in 10 ml) gives a bitter sensation. If so, find the threshold bitter concentration of the material by tasting the dilutions in tubes 1-4. If the solution in tube no. 5 does not give a bitter sensation, find the threshold bitter

concentration by tasting the dilutions in tubes 6-10.

All solutions and the safe drinking-water for mouthwashing should be at 20-25 °C.

Calculate the bitterness value in units per g using the following formula:

$$\frac{c \times 2000}{a \times b}$$

where a = the concentration of the stock solution ( $S_a$ ) (mg/ml),

b = the volume of  $S_T$  (in ml) in the tube with the threshold bitter concentration,

c = the quantity of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.\

Based on the above studies, specifications can be drawn for a particular herb. In commercial practice, several problems are encountered when larger consignments are received from different traders. They are adulteration/substitution, mixture of different species, wrongly labeled materials based on local names, substantial quantities of foreign matters, contamination with fungus.

To eliminate such problems and to have better control on raw materials, it is desirable to monitor the following [Chakravarthy BK, 1993].

1. Herbs should be collected when their active principles are maximum.
2. Trained taxonomist/botanist should be entrusted to distinguish between related species of a given plant.
3. Unwanted and foreign material should be removed from the herb.
4. Herbs should be sorted properly into different grades according to the quality, and the best grade be used.
5. Drying of the herbs should be controlled keeping in view the type of active compounds, eg, sun drying/vacuum drying. Moisture should be controlled below 9-10%.

If it exceeds, the herb becomes prone to fungal contamination.

6. Dried herbs should be stored in closed containers. Storage place should be rodent free, cool, dark, and well ventilated and moisture free.
7. Storage period of any crude drug should be studied carefully.
8. Truly representative sample must be drawn for analysis according to prescribed USP/ISI method of sampling.

### PHARMACOPOEIAL STANDARDS

Several pharmacopoeias such as Indian pharmacopoeia, British pharmacopoeia, Japanese pharmacopoeia and USP do cover monographs and quality control tests for a few medicinal plants used in these countries [Chaudhari RD, 1996].

Internationally, several pharmacopoeias have provided monographs stating quality parameters and standards of many herbs and herb products. Brief review of WHO Guidelines concerning the quality control of the herbal material and herbal remedies is narrated below.

### WHO GUIDELINES' MONOGRAPH TITLE

Botanical
Sensory evaluation: Visual Macroscopy/Touch/Odour/Taste
Foreign matter, foreign plants, foreign animals, foreign minerals, etc
Microscopy: Histological observation, histological detection, measurements
Physiochemical
TLC
Ash: Total, acid insoluble, water soluble
Extractable matter: In hot water, cold water and ethanol
Water content and volatile matter, LOD, azeotropic
Volatile oil by steam distillation
Pharmacological
Bitterness value: Units eq. to bitterness of std. soln. of quinine hydrochloride
Hemolytic activity: On ox blood by comparison with std. ref. saponin
Astringency: fraction (tannins) that bind to std. Hide powder
Swelling index: In water
Foaming index: Foam height produced by 1gm material under specified conditions
Toxicological
Arsenic: Stain produced on HgBr <sub>2</sub> paper in comparison to std. stain
Heavy metals, pesticide residues; total organic chloride and total organic phosphorus

Microbial contamination
Total viable aerobic count
Pathogens: <i>Enterobacteriaceae, E. Coli, Salmonella, P. aeruginosa, S aureous</i>
Aflatoxins: By TLC using std. aflatoxins (B1, B2, G1, and G2) mixture.
Radioactive contamination

## CONCLUSION

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.

For the purpose of research work on standardization of herbal formulations and nutraceuticals, a profound knowledge of the important herbs found in India and widely used in Ayurvedic formulation is of utmost importance.

India can emerge as the major country and play the lead role in 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. So standardization is important as it helps in giving correct dosage to patient, in detecting adulteration in commercial samples and also in authentication of other species.

## REFERENCES

1. Sagar Bhanu PS, Zafar R, Panwar R. Herbal drug standardization. *The Indian Pharmacist*; vol. 4(35):May 2005. p.19-22.
2. Patel PM, Patel NM, Goyal RK. Evaluation of marketed polyherbal antidiabetic formulations uses biomarker charantin. *The Pharma Review*; vol.4 (22): June 2006. p.113.
3. Clarke ECG. Isolation and identification of drugs. *The*

Pharmaceutical press; London: 1967.

4. World Health Organization (WHO), *International pharmacopoeia*, Geneva Switzerland, 2001.
5. Sarin YK, Dhor KL, Thappa RK. *Aromatic flora of Himalayan Region Prospects of commercial utilization: In newer trends in essential oil* Flacor .Tata McgrawHill; Newdelhi: 1993.p.194-210.
6. Trotter RT, Logan MK, Rocha JM, Boneta JI. *Ethanography and Bioassay: Combined methods for a preliminary screen of Home remedies for potential pharmacological activities.* *J of Ethanopharmacology*; 1983.p.113-4.
7. Malone MH. *Pharmacological approaches to Natural products screening and Evaluation in new Natural products and plant drugs with Pharmacological, Biological and Therapeutic activity*; 1997.p.23.
8. Valverde JL. *The problem of Herbal Medicinal Legal Status: Pharmaceuticals policy and law.* IOS press; Netherlands:(2).2000.
9. Fransworth NR, Bingal AS. *Problems and prospects of discovering new drugs from higher plants by Pharmacological screening: New natural products and plant drugs with pharmacological, biological and therapeutic activity.*
10. Tempesta E. *Evaluation of local sources in traditional medicine.* *J of Ethanopharmacology*; (2) 1980:p.163.
11. Sandelberg F, Cronund A. *An Ethanopharmacological Inventory of medicinal and toxic plants from Equatorial Africa.* *J of Ethanopharmacology*; (5) 1982:p.187.
12. Ravi V, Agarwal M, Agnihotri DK, Khatoon S, Rawat AKS, Melhrotra S. *Pharmacological evaluation of*

- Leucas aspera Lin. Natural Product Sciences; (2) 2005: p.109-14.
13. Chaudhari R. Herbal Drug Industry: A practical approach to Industrial Pharmacognosy. Eastern publishers, Newdelhi.2006.
  14. Ansari SH. Standardization of crude drugs. Essentials of Pharmacognosy. 1<sup>st</sup> ed; 2005: p.14, 581.
  15. Anon. Mycotoxins, Economic and Health Risks. Council for Agricultural science and Technology. 1989. Report No.116. p.91.
  16. Wyllie TD, Morchause LG. Mycotoxin Fungi, Mycotoxins, Mycotoxicoses-An Encyclopedic Handbook. Vols.1,2, 3. Marcel Dekker, Inc. New york.
  17. Eaton DL Groomman JD. The Toxicology of Aflatoxins. Academic Press; New York: 1994. P.383-426.
  18. Heathcote JG, Hibbert JR. Aflatoxins : Chemical and biological aspect. Elsevier; New York: 1978. p.173-86.
  19. Finley JW, Robinson SF, Armstrong DJ. Food Safety Assessment. American Chemical Society; Washington D.C: 1992. p.261-75.
  20. Wyllie TD, Morchause, LG. Mycotoxin Fungi, Mycotoxins, Mycotoxicoses-An Encyclopedic Handbook. Vols.1,2, 3. Marcel Dekker Inc; New York:1978.
  21. Shaw PC, Pui-Hat Butt P. Authentication of Panax species and their adulterants by random primed polymerase chain reaction. Planta Medica; vol. 61: 1995. p.466-69.
  22. Lazarowych NJ, Pekos P. Use of fingerprinting and marker compounds for identification and standardization of botanical drugs: Strategies for applying pharmaceutical HPLC analysis to herbal products. Drug Information Journal; Vol.32: 1998. p.497-512.
  23. Apers S, Naessens ., Pieters L, Vlietinck A. Densitometric thin-layer chromatographic determination of aescin in an herbal medicinal product containing Aesculus and Vitis dry extract. Jr. of Chromatographic Analysis; vol.1112(1-2): April 2006. p.165-70.
  24. Nair VD, Kanfer I, Hoogmartens J. Determination of stigmasterol, beta-sitosterol and stigmastanol in oral dosage forms using HPLC with evaporative light scattering detection. Journal of pharmaceutical and biomedical analysis; vol. 41(3): June 2006. p. 731-7.
  25. Sarmani SB, Abugassa I, Hamzah A, Yahya MD. Elemental analysis of herbal preparations for traditional medicines by neutron activation analysis with the kO standardization method. Biological trace element research; 1999: p. 365-76.
  26. Careri M, Elviri L, Mangia A. Liquid chromatography-UV determination and liquid chromatography-atmospheric pressure chemical ionization mass spectrometric characterization of sitosterol and stigmasterol in soyabean oil. Jr. of Chromatographic Analysis; vol. 935(1-2): Nov 2001. p.249-57.
  27. Herbone JB. Phytochemical methods Chapman and Hall. 2<sup>nd</sup> ed. London, New York; 1928:
  28. Saraswathy A. Standardization of siddha drugs. Ancient Science of life1, 2; 1994.:p. 53-60.
  29. Ahirwal B, Ahirwal D, Ram A. Evaluation of standards and quality control parameters of herbal drugs. Souvenir, Recent trends in herbal therapy. 2006;p. 25-29.
  30. Santosh MK, Shaila D, Sanjeeva Rao I. Standardization study of dadimastaka and pushyannga

churnas. Asian Jr. of Chemistry. vol.  
16(34); 2004: p. 1735-41.