

**STANDARDIZATION OF CRUDE DRUGS: A PRECISE REVIEW****Salman Ahmed\* and Muhammad Mohtasheemul Hasan**

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**ABSTRACT**

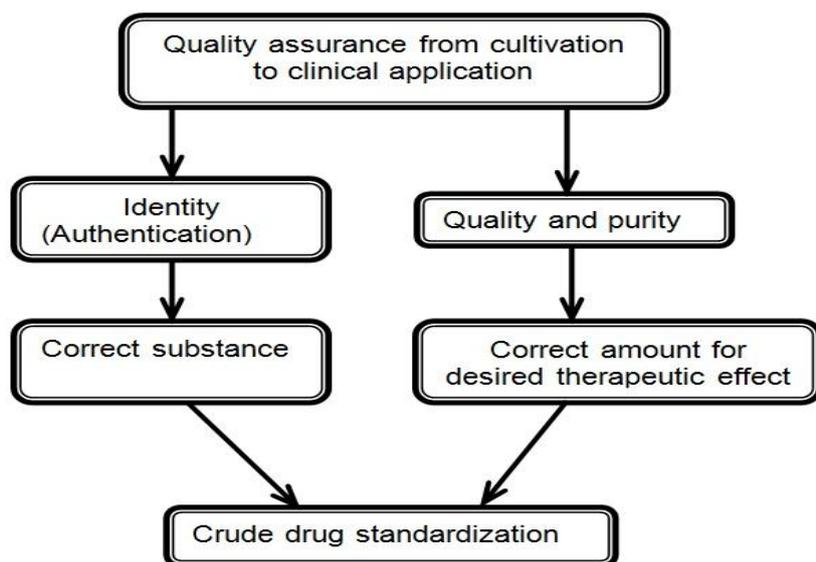
Standardization of crude drugs is a code of conduct and an essential need of a time. Substitution and adulteration are now become a very common practice, which makes global crude drug market unsafe for crude drug depending world population. Moreover, crude drug evaluation is not an easy task because of numerous influential factors affecting bio efficacy and reproducibility of therapeutic effects. The broad spectrum of standardization gives surety of correct substance in correct amount for desired therapeutic effects. It also provides all possible careful measures taken from collection, manufacturing till drug dispensing for clinical application. In a present review, authors have tried to cover precise tools for crude drug standardization.

**KEYWORDS:** Crude drug, evaluation, standardization, qualitycontrol, quality assurance.

**INTRODUCTION**

Standardization is a code of conduct that ensures the correct substance in correct amount for desired therapeutic effect (safety, quality and efficacy) is known as standardization.<sup>[1,2]</sup> It describes all measures taken during manufacturing process and quality control leads to reproducible quality of particular product.<sup>[3]</sup> Standardization confirms drug identity (authentication) and determines the quality and purity.<sup>[4]</sup> The herbal raw material prone to a lot of variation in phyto constituents due to several factors different places of collection (indigenous and naturalized plants), time and season of collection, different environmental conditions, (primary causes like light, moisture, temperature, oxygen etc. secondary causes as involvement of living organisms like bacteria, molds, mites, nematodes, worms, insects etc.), genotypic and chemotypic variation, presence of xenobiotics (foreign chemical substances found within an organism that is naturally not expected to be present within that organism.

So, there is a need of quality control tests for crude drug or medicine to ensure quality.<sup>[1,2]</sup> In order to obtain quality oriented herbal products, care should be taken right from the proper identification of plants, season and area of collection, their extraction and purification process.<sup>[4]</sup> The word standardization should encompass entire field of study from cultivation of medicinal plant to its clinical application.<sup>[5]</sup> and focus all aspects of medicinal plant research from ethno-pharmacology (traditional medicinal use), utilization (how plant use in specific disease), isolation and identification of active constituents to efficacy evaluation, safety, formulation and clinical evaluation.<sup>[6]</sup>



In general, quality control is based on three important pharmacopoeias definitions:

- Identity: Is the herb the one it should be?
- Purity: Are there contaminants, e.g., in the form of other herbs which should not be there?
- Content or assay: Is the content of active constituents within the defined limits.

WHO guidelines for quality standardized herbal formulations are as follows.<sup>[3]</sup>

1. Quality control of crude drug material, plant preparations and herbal products.
2. Stability assessment and shelf life.
3. Safety assessment, documentation of safety based on experience or toxicological studies.
4. Assessment of efficacy by ethno-medicinal information and biological activity evaluation.

## METHODS OF STANDARDIZATION

### AUTHENTICATION OF HERBAL DRUGS<sup>[7]</sup>

Authenticated raw material is the basic starting point in developing a botanical product. In addition, each step of harvest, storage, processing and formulation may dramatically alter the quality and consistency of final product. Therefore methods to ensure quality control in manufacturing and storage are requisite tools to ensure optimal efficacy and safety of these products. Furthermore, such controls are critical for the evaluation of pharmacological, toxicological or clinical studies involving botanical products. Authentication is especially useful in cases of drugs that are frequently substituted or adulterated with other varieties which are morphologically and chemically indistinguishable. Several herbal drugs in the market still cannot be identified or authenticated based on their morphological or histological characteristics. Use of wrong drugs may be ineffective or it may worsen the condition.

#### Taxonomic method<sup>[7]</sup>

The initial step in the identification and authentication of botanical materials. The botanical origin of the drug is identified and its scientific Latin binomial (i.e. genus species) name is determined based on this method. Information such as botanical name, vernacular names, site of collection of plant material, details of collector, habitat, season of collection, altitude and part collected etc. are the essential prerequisites even before authentication.

#### Macroscopic Evaluation, Organoleptic Evaluation, Sensory Evaluation, Conventional Evaluation

Macroscopic identity of botanical materials is based on parameters like shape, size, color, texture, surface characteristics, fracture characteristics, odor, taste and such organoleptic properties that are compared to a standard reference material<sup>[7]</sup> Organoleptic evaluation provides simplest and quickest way to identify crude drug. Macroscopic evaluation<sup>[6]</sup> encompass morphologic description of plant parts by using naked eye. It is the technique of qualitative evaluation based on the study of morphological and sensory profile of whole drugs. eg. The fractured surfaces in *Cinchona*, *Quillia* and *Cascara* barks and *Quassia* wood are important characteristics. Aromatic odor of umbelliferous fruits and sweet taste of liquorice are the examples of this type of evaluation where odor of drug depends upon the type and quality of odorous principles (volatile oils) present. Shape of drug may be cylindrical (*Sarsapilla*), sub cylindrical (*Podophyllum*), conical (*Aconite*), fusiform (*Jalap*) etc., Size represents length, breadth, thickness, diameter etc. color means external color

which varies from white to brownish black are important diagnostic characters. The general appearance of a crude drug often indicates whether it is likely to comply with prescribed standard like furrows (alternate depression or valleys), wrinkles (fine delicate furrows), annulations (transverse rings), fissures (splits), nodules (rounded outgrowth), scars (spot left after fall of leaves, stems or roots). Taste is specific type of sensation felt by epithelial layer of tongue. It may be acidic (sour), saline (salt like), saccharic (sweetish), bitter or tasteless.

### **Microscopic evaluation (presence or absence of type of cells or tissues)<sup>[6]</sup>**

Microscopy is used to determine the structural, cellular and internal tissue features of botanicals. It is usually used to identify and differentiate two herbals that are similar. This is the commonly used technique, convenient, quick and can be applied to proprietary medicines too.<sup>[7]</sup> Microscopic inspection alone can't always provide complete identification but when used in the association with other analytical methods <sup>[4]</sup>.

#### **a. By taking plant section**

Plant part under study usually taken in the form of appropriate (longitudinal or transverse / cross) section to study the presence or absence of type (shape) of cells or tissues. Some of the chemicals like phloroglucinol, chloral hydrate, safranin, methyl orange etc., use for clear visualization of cellular content.<sup>[6]</sup> Using microscope detecting various cellular tissues, trichomes, stomata, starch granules, calcium oxalate crystals and aleuronic grains are some of important parameters which play important role in identification of crude drug (table 2). Crude drug can also be identified microscopically by cutting the thin TS (transverse section), LS (Longitudinal section) especially in case of wood and by staining them with proper staining reagents.

**Table 2: Qualitative microscopic test for detection of cellular contents.**

Chemical test	Constituent	Results
T.S. of crude drug + 1 drop of phloroglucinol+ dilute HCl	lignin	pink color. <sup>[8]</sup>
T.S. of crude drug + 1 drop of iodine solution	starch and hemicellulose	blue color. <sup>[8]</sup>
T.S. of crude drug + ruthenium red	mucilage	pink color. <sup>[6]</sup>

#### **a. Powder drug evaluation**

Dried powder usually taken for studying the presence or absence of cellular contents (type/ shape) by using microscope.<sup>[6]</sup>

Microscopic evaluation also includes study of constituents in the powdered drug by the use of chemical reagents. These reagents used due to abundance of cellular contents, presence of coloring matters, shrinkage or collapse of cell wall which creates hurdles in microscopic evaluation.<sup>[9]</sup>

### **Types of reagents used<sup>[9]</sup>**

#### **1. Aqueous and alcoholic solution of potash (KOH)**

0.3% solution of potash is used to dissolve aleurone grains (specialized dry vacuole where storage proteins accumulate in a stable form in seeds, usually in the endosperm).

5% solution is used to dissolve starch and protein and causes swelling of cell wall.

#### **2. Chloral hydrate solution B.P. (Chloral 80 g; water 20ml)**

It acts as clearing / clarifying agent.

#### **3. Ethanol**

It behaves as clarifying agent by dissolving oils, resins, chlorophyll etc. It does not dissolve gums and mucilage.

#### **4. Ether and ethanol (mixture of equal parts of ether and ethanol 96% + 4% water)**

It is used to remove chlorophyll and fixed oil.

- Removal of fixed oil, fats, resins, volatile oils, tannins or chlorophyll.
- Defatting is necessary in case of oily seed's evaluation.
- Removal of chlorophyll is necessary in order to clarify other cellular contents.

#### **5. Glycerin dilute (50%)**

Its clearing capacity is inferior to chloral hydrate.

#### **6. Iodine solution**

It gives blue color with starch and hemicellulose.

#### **7. Picric acid solution (saturated solution in water)**

It stains aleurone grains and animal fibers.

## 8. Solution of sodium hypochlorite

It behaves as bleaching agent to remove dark color of bark and chlorophyll from leaves. After bleaching, sections should be removed from solution and washed with water to protect contact of solution with cellular contents. If it happens, results the removal of desirable cellular contents.

## 9. Sudan III solution

(Sudan III solution= sudan red solution+ glycerin+ alcohol equal parts)

It stains oils useful in the examination of secretory cells and ducts.

## Qualitative aspects of microscopic studies<sup>[8]</sup>

### • Stomata

There are several types of stomata, distinguished by the forms and arrangement of the surrounding cells, e.g.

(a) Anomocytic (Ranunculaceae) irregular celled: *Digitalis*.

(b) Anisocytic (Cruciferae) unequal – celled: *Datura*.

(c) Diacytic (Caryophyllaceae) cross – celled: *Mentha*.

(d) Paracytic (Rubiaceae) parallel celled: *Senna*.

### • Trichomes

Trichomes are divided and subdivided as follows

#### (i) Covering trichomes

(a) Unicellular trichomes: *Nux vomica*, *Cannabis sativa*.

(b) Uniseriate multicellular unbranched trichomes: *Datura*.

(c) Biseriate multicellular unbranched trichomes: *Calendula officinalis*.

(d) Multiseriate multicellular unbranched trichomes: Male fern.

(e) Multicellular branched trichomes: *Verbascum Thapsus*.

#### (ii) Glandular Trichomes

(a) Unicellular glandular trichomes: *Justicia adhatoda*.

(b) Multicellular glandular trichomes: *Digitalis purpurea*.

#### (iii) Hydathode Trichomes

Piper betle.

**Quantitative aspects of microscopic studies<sup>[8]</sup>****Palisade ratio**

It is defined as average number of palisade cells beneath each epidermal cell. e.g. *Atropa belladonna* (6-10), *Digitalis lanata* (2.5-6.5).

**Stomatal No**

It is defined as average number of stomata per square millimeter area of epidermis. e.g.: *Atropa belladonna*: 6.0 to 14-37.5 (Upper Surface), 62.5 to 93-174 (lower Surface).

**a. Stomatal index**

It is the percentage which the number of stomata forms to the total number of epidermal cells. It is calculated by,

$$S.I. = S \times 100 / (E + S).$$

Where, S.I. = Stomatal Index; S = Number of stomata per unit area;

E = Number of Epidermal cells in the same unit area. e.g.

*Atropa belladonna*: -2.3-3.9 to 10.5 (Upper Surface), 20.2 to 21.7- 23.0 (Lower Surface).

*Digitalis Purpurea*-1.6-2.7 to 4.0 (Upper Surface) to 19.2- 25.2 (lower Surface).

**b. Vein islet number**

It is defined as average number of Vein Islet per square millimeter of the leaf surface midway between midrib and the margin.

i. *Digitalis lanata* — 2.0-8.0.

ii. *Digitalis Purpurea* — 2.0-5.5.

**c. Vein termination number**

It is defined as average number of Vein terminations per square millimeter of the leaf surface midway between midrib and the margin.

iii. *Atropa belladonna* — 6.3-10.3.

iv. *Atropa acuminata* — 1.4-3.5.

**Physicochemical test**

The values of Physicochemical parameters of the individual drugs or the proprietary medicines can be compared with the standard values of pharmacopoeia and standardized.<sup>[7]</sup> These tests are actually the Pharmacopoeial standards for authenticity, quality and purity of herbal drugs and are as follows.<sup>[1]</sup> Mostly used physicochemical methods are as follows.

**Solubility<sup>[10]</sup>**

The presence of adulterant in a drug could be indicated by solubility studies identify by various solvents. e.g., pure *Asafoetida* is soluble in carbon disulphide.

**i. Alcohol**

5 gm of powdered material along with 100 ml of alcohol are shaken well occasionally for the first 6 hours and kept undisturbed for 18 hours. The liquefied extract thus obtained was concentrated in an vacuum oven and the percentage was calculated with the weight of the drug powder taken.

**ii. Water**

The procedure adopted for solubility percentage of alcohol is used with chloroform water instead of alcohol to get the water solubility.

**Viscosity**

Viscosity of a liquid is constant at a given temperature and is an index of its composition. So, it can be used as a means of liquid drugs standardization.

**Determination of moisture /Loss on drying procedure<sup>[11]</sup>**

- Weigh about 1.5g of the powdered drug into a weighed flat and thin Porcelain dish.
- Dry it in the oven at 100°C or 105°C.
- Cool in desiccators and watch the loss in weight is usually recorded as moisture.

**Note**

A very useful form of dish for the determination of moisture and of ash is a thin flat porcelain dish. If a platinum dish available it may be used. The burning of the powder should proceed slowly and the material must not be allowed to catch fire or to give off smoke as dense fumes

**Refractive index<sup>[10]</sup>**

When a ray passes from a one medium to another of different density, it is bent from original path. Thus, the ratio of velocity of light in vacuum to its velocity in a substance is termed as refractive index of the second medium. Depending upon purity, it's constant for a liquid and can be consider as one of its standardization (table 3). Refractive index of a compound varies with the wave length of the incident light, temperature and pressure.

**Table 3: Refractive indices of some crude drugs for sodium light at 25°C.**

Crude drugs (oils)	Refractive indices
<i>Arachis hypogaea</i> (Arachis)	1.467-1.470
<i>Carum carvi</i> (Caraway)	1.4838-1.4858
<i>Ricinus communis</i> (Castor)	1.475-1.527
<i>Syzygium aromaticum</i> (Clove)	1.527-1.535

**Volatile oil content**

Pharmaceutical significance of aromatic drugs is due to their odorous principal that is volatile oils such crude drugs are standardized on the basis of their volatile contain (table 4).

**Table 4: Standardized volatile contents of aromatic drugs<sup>[10]</sup>**

Aromatic drugs	Volatile content ( Not Less Than %w/w )
<i>Carum carvi</i> (Caraway)	2.5
<i>Citrus × limon</i> (lemon peel)	2.5
<i>Syzygium aromaticum</i> (Clove)	15
<i>Foeniculum vulgare</i> (fennel)	1.4
<i>Elletaria cardamomum</i> (cardamom seeds)	4.0

**Melting point**

pure chemicals or phytochemicals possess very sharp and constant melting point. Since the crude drugs from animal or plant origin contain the mixed chemicals, they are described with certain range of melting point.

**Table 5: Melting point range of few crude drugs.**

Crude drug	Description	Melting point (°C)
Colophony	translucent brittle substance produced from pine.	75-85
Kokum butter	oil comes from the seeds of the kokum tree ( <i>Garcinia indica</i> ).	39-42
Cocoa butter	fat obtained from the seeds of <i>Theobroma cacao</i> .	30-33
Bees wax	yellow to grayish brown wax obtained by melting and purifying the honeycomb of the bee, <i>Apis mellifera</i> .	52-65
Wool fat	a fatty substance, extracted from wool.	34-44

**Ash determination**<sup>[4]</sup>

After ignition the remaining material is known as ash. There are two types of ash one is the ash derived from plant tissues (physiological ash) and the other one is residue of extraneous matter adhering to plant surface (non-physiological ash). Ash is determined by following three methods.

**Total ash**

Weight of residue obtained after ignition.<sup>[4]</sup>

About 2gm of powdered drug was weighed accurately into a tarred silica crucible.

- Incinerated at 450°C in a muffle furnace until free from carbon.
- The crucible was cooled and weighed.
- Percentage of total ash was calculated with reference to air-dried substance.<sup>[11]</sup>

Determination of total ash value formula.<sup>[11]</sup>

- Total ash value of the sample =  $100 (z-x)\% / y$ .
- X= weight of empty dish.
- Y= weight of the drug taken.
- Z= weight of the dish + ash (after complete incineration).

**Acid insoluble ash**

Weight of residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter<sup>[4]</sup>. The method of analysis is as follows<sup>[11]</sup>

- Ash obtained from the total ash was boiled with 25ml of 2N HCl for a few minutes. Filtered through an ash less filter paper.
- The filter paper was transferred into a tarred silica crucible.
- Incinerated at 450°C in a muffle furnace until free from carbon.
- The crucible was cooled and weighed.
- Percentage of acid insoluble ash was calculated with reference to air-dried substance.<sup>[11]</sup>

**Water soluble ash**

Difference in the weight between the total ash and the residue after treatment of total ash with water<sup>[4]</sup>. The method is as follows

- Ash obtained from the total ash was boiled with 25 ml of distilled water for a few minutes and filtered through an ash less filter paper.
- The filter paper was transferred into a tarred silica crucible.
- Incinerated at 450°C in a muffle furnace until free from carbon.
- The crucible was cooled and weighed.
- Percentage of water-soluble ash was calculated with reference to air-dried substance.<sup>[11]</sup>

### **Extractive value**

Extractive value determines the amount of active constituents extracted with solvent from a given amount of medicinal plant.<sup>[4]</sup> It gives an idea about the nature of the chemical constituents present.<sup>[11]</sup>

### **Determination of alcohol soluble extractive value**

About 5gms of air dried coarse powdered drug was weighed and macerated with 100ml of 90% alcohol in a closed flask for 24 hours, shaking frequently during the first 6 hrs and these allowed standing for 18 hrs.

- Thereafter it was filtered rapidly taking precautions against loss of the solvent.
- 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed.
- The %age of the alcohol soluble extractive values was calculated with reference to the air-dried drug.

### **Determination of water soluble extractive value**

About 5gm of air-dried powdered drug was taken & macerated with 100 ml of chloroform water in a closed flask for 24 hrs shaking frequently during the first 6 hrs and then allowed to stand for 18 hrs.

- Thereafter, it was filtered rapidly taking precautions against loss of the solvent.
- 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed.
- The percentage of the water soluble extractive value was calculated with reference to the air-dried drug.

**Determination of bitterness value<sup>[4]</sup>**

The bitterness properties of plant material are determined by comparing the threshold bitter concentration of an extract of the material with that of dilute solution of quinine hydrochloride. The bitterness value is expressed in units equivalent to the bitterness of solution containing 1gm of quinine chloride in 2000ml. The bitterness of plant material measures by taste. Bitter taste employed therapeutically. Bitterness stimulates secretions in the gastrointestinal tract especially of gastric juice.

**Determination of swelling index<sup>[4]</sup>**

1gm of plant material dipped in water or swelling agent in glass stoppered measuring cylinder, the material is shaken repeatedly for 1hr and then allowed to stand for a required period of time. The volume of the mixture (ml) taken up by the swelling of plant material is then read. The mixing of whole plant material with the swelling agent is easier than pulverized or reduced plant material as it requires vigorous shaking at specific interval to ensure even distribution of material in swelling agent.

**Determination of foaming index<sup>[4]</sup>**

Many plant materials cause persistent foam (due to the presence of saponins) when an aqueous decoction is shaken. The foaming ability is measured in terms of foaming index.

**Determination of pesticide residues**

The food and agriculture organization of the united nations (FAO) and WHO established limits of pesticide residue for safe consumption of food and animal feed<sup>[4]</sup>. These pesticides are mixed with the herbs during the time of cultivation. Mainly pesticides like DDT, BHC, toxaphene, cause serious side effects in human beings, if the crude drugs are mixed with these agents.<sup>[10]</sup>

**Determination of microorganisms and aflatoxins<sup>[10]</sup>**

Microbial parameters, like total viable content of pathogenic bacteria like enterobacteria (gram negative bacteria like *Escherichia coli*, *Enterobacter*, *Klebsiella*, *Pseudomonas aeruginosa*, *Salmonella*, *Shigella*), other gram positive bacteria and presence of aflatoxins determine for safe herbal drug consumption. Limits given in official books can be utilized as a quantitative or semi quantitative tool to control the amount of purities coming from different steps of preparation, storage and preservation (Small amount or below than limit microorganism will destroy by body immune system).

**Chemical tests<sup>[1]</sup>**

The aims of these tests are as under.

- Preliminary (primary) testing for different chemical (functional) groups
- Quantification (% age) of chemical groups of interest (e.g. total alkaloids, phenolics, triterpenes, tannins) or establishment of finger prints.
- Multiple marker based finger print profiles (use of different marker compounds which indicate the % presence of more than one chemical group).

Qualitative chemical test such as acid value, saponification value etc. Some of these are useful in evaluation of resins (acid value, sulphated ash), balsams (acid value, saponification value and bester values), volatile oils (acetyl and ester values) and gums (methoxy determination and volatile acidity). Preliminary phytochemical screening is a part of chemical evaluation. These qualitative chemical tests are useful in identification of chemical constituents and detection of adulteration.<sup>[1]</sup> Some important preliminary tests are given in table 6.

**Table 6: Some important preliminary tests with their obtaining results and reagents used.**

Chemical test	Reagents used	Results
<b>Alkaloids</b>		
Mayer	Potassium mercuric iodide solution	Creamy precipitate <sup>[10]</sup>
Wagner	Iodine potassium solution	Brown precipitate <sup>[10]</sup>
Hager	Saturated solution of picric acid	Yellow color <sup>[10]</sup>
Dragendorff	Potassium bismuth iodide solution	Reddish brown precipitate <sup>[8]</sup>
<b>Amino acids</b>		
Millon's test	Millon's reagent	White precipitate <sup>[8]</sup>
Ninhydrin	Ninhydrin solution	Violet color <sup>[10]</sup>
Folin	Folin phenol reagent	Blue color <sup>[11]</sup>
Pauly	Sulphanilic acid, sodium nitrite and sodium carbonate	Cherry red color <sup>[11]</sup>
<b>Carbohydrates</b>		
Molisch	Alcoholic $\alpha$ -naphthol + sulphuric acid	Purple to violet color rings <sup>[8]</sup>
Barfoed	Barfoed reagents	Red color(monosaccharide) after 10 min .color form(disaccharide) <sup>[10]</sup>
Selivanoff	Selivanoff's reagents	Rose color(ketone) <sup>[10]</sup>
Tests for pentoses	Hydrochloric acids + phloroglucinol	Red color <sup>[10]</sup>
<b>Anthraquinone Glycosides</b>		
Borntrager	Borntrager reagent	Pink ammonical layer <sup>[8]</sup>

<b>Tannins</b>		
Ferric chloride	Ferric chloride	Blue color <sup>[8]</sup>
<b>Flavonoids</b>		
Alkaline reagent	10% Sodium hydroxide solution	Intense yellow color <sup>[11]</sup>
Ammonium hydroxide	10% ammonium hydroxide	Yellow fluorescence <sup>[11]</sup>
Zinc	Zinc dust and conc. HCl	Red color <sup>[11]</sup>

Quantification of important chemical constituents (extraction / isolation, separation / fractionation, purification, identification of active compounds by using different chromatographic and spectroscopic techniques like.<sup>[1]</sup>

### **Chemometric and Spectral methods<sup>[7]</sup>**

Initially the use of infrared (IR) spectroscopic method is restricted only for structural elucidation of isolated compounds from the herbal matrices. It is also found useful in phytochemical studies as a finger printing device for comparing a natural with synthetic sample. With the advance of computer technology, chemometric method has become a leading tool among the scientific communities towards faster analysis and shorter product development time. Among others, an unsupervised pattern recognition technique such as Principal Component Analysis (PCA) is the most often used method for handling multivariate data without prior knowledge about the study samples.

While the supervised classification procedure using Soft Independent Modeling of Class Analogy (SIMCA) based on making a PCA model to assign unknown samples into the predefined class model has also been applied to the analysis of infrared spectra. A study using FTIR transmission spectroscopy, associated with the appropriate chemometric methods (PCA and SIMCA) was done to classify *Orthosiphon stamineus* Benth (well known as Java tea for treating infection of the urinary tract, kidney and bladder stone disease) based on its geographical origin and varieties from the obtained characteristics infrared spectrum. Chemometric analysis of spectra is rapid and simple since no chemical treatment of samples is required.

### **Chromatographic methods<sup>[7]</sup>**

The most commonly used analytical methods for herbal products are

Capillary Electrophoresis (C.E.).

Gas Chromatography- Nuclear Magnetic Resonance (G.C.-N.M.R.).

High Performance Liquid Chromatography (H.P.L.C.).

High Pressure Thin Layer Chromatography (H.P.T.L.C.).

Liquid Chromatography- Mass Spectroscopy (L.C.-M.S.).

Thin Layer Chromatography (T.L.C.).

**a) Thin Layer Chromatography (TLC)<sup>[7]</sup>**

TLC is frequently used for the analysis of herbal medicines since various pharmacopoeias still use TLC to provide first characteristic fingerprints of herbs. TLC has the advantages of being simple and can be employed for multiple sample analysis.

For each plate, more than 30 spots of samples can be studied simultaneously in one time. In summary, the advantages of using TLC to construct the fingerprint of herbal medicines are its simplicity, versatility, high velocity, specific sensitivity, simple sample preparation and its economy.

**b) High Performance Liquid Chromatography (HPLC)<sup>[7]</sup>**

HPLC is a popular method for the analysis of herbal medicines, because it is easy to learn and use and is not limited by the volatility or stability of the sample compound.

**b) Gas Chromatography (GC)<sup>[7]</sup>**

The GC of the volatile oil gives a reasonable fingerprint and can be used to identify the plant. The analysis of volatile compounds by gas chromatography is very important in chemical analysis of herbal medicines. The extraction of the volatile oil is relatively straight forward and can be standardized and the components can be readily identified using GC-MS analysis. The advantages of GC clearly lie in its high sensitivity of detection for almost all the volatile chemical compounds.

**c) Capillary Electrophoresis (CE)<sup>[7]</sup>**

Capillary electrophoresis (CE) allows an efficient way to document the purity/complexity of a sample and can handle virtually every link of charged sample components ranging from simple inorganic ions to DNA. CE is promising for the separation and analysis of active ingredients in herbal medicines.

**Biological evaluation / Biological assays<sup>[3]</sup>**

Pharmacological activity of crude drugs has been applied to evaluate and standardize them. The assays on living animal and on their intact or isolated organs can indicate the strength of the drug or their preparations. Some drugs have specific biological and pharmacological

activity which is utilized for their evaluation. Actually this activity is due to specific type of constituents present in the plant extract. For evaluation the experiments were carried out on both intact and isolated organs of living animals. With the help of bioassays, strength of drug in its preparation can also be evaluated.

### **Hemolytic activity**<sup>[3]</sup>

The hemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of reference material, saponins.

### **Antibiotic activity**<sup>[7]</sup>

Some bacteria such as *Salmonella typhi*, *Staphylococcus aureus* and *E. coli* are used to determine the antiseptic value (the degree of antiseptic activity e.g. phenol co-efficient of certain drugs). The activity of antibiotics is also determined by using *Klebsiella pneumonia*, *Micrococcus flavus*, *Sarcina lutea* etc. living bacteria, yeast and molds are used to evaluate certain vitamins. Microbiological assays by cylinder plate method and turbidimetric method are used in evaluation.

### **Antifertility activity**<sup>[7]</sup>

Antifertility drugs include contraceptives and abortifacients. Contraceptive drugs are used to prevent pregnancy and abortifacient to terminate pregnancy. Female rats are used for antifertility activity i.e. measure the pregnancy rate (anti ovulation and anti-implantation) and male rats are used for antispermatogenic activity and spermicidal activity of herbal drugs.

### **Hypoglycemic activity**<sup>[7]</sup>

Rabbits, rats or mice are used to test hypoglycemic activity of plant extract. Radio-immunoassay (RIA) or Enzyme linked immunosorbate assay (ELISA) are done for measurement of insulin levels.

### **Neuropharmacological activity**<sup>[7]</sup>

Testing the herbal drugs with effects on central and autonomic nervous system. CNS acting drugs like cocaine (*Erythroxylum coca*), morphine (*Papaver somniferum*), cannabinol (*Cannabis sativa*) are tested using rodents. For testing the herbal drugs for their effects on ANS guinea pig ileum for antispasmodic activity, rabbit jejunum for adrenergic activity, rat phrenic nerve-diaphragm for muscle relaxant activity, frog rectus for skeletal muscles activity.

## AUTHENTICATION OF MINERALS<sup>[7]</sup>

The use of minerals as source of drugs is largely used next to herbals. They are processed with herbal juices and made into their calcified or oxide forms and administered as drugs. Hence their identification is equally important to herbal drugs.

### 1. Physical properties

The physical properties of individual minerals include nature, color, streak, tenacity, transparency, luster, hardness, fracture, cleavage or parting, magnetism and specific gravity. These unique physical characteristics of minerals can be used for the authentication.

### 2. Optical properties

Optically active compounds have the property of rotating the plane polarized light (optical rotation). The optical rotation is determined at 25°C using sodium lamp as light source. There are two types of optical rotations, dextrorotatory (+ve), levorotatory (-ve). This can be determined within limits by means of polarizing microscope. Optical properties include application of optical crystallography in identification of minerals those are crystalline and transparent irrespective of their chemical composition.

### 3. Refractive Index (RI)

It is defined as ratio of velocity of light in media to that in vacuum. It is constant for pure drug and varies with wavelength of incidence light, temperature and pressure. Immersion method is used to determine the RI. For example Alum and Garnet are isotropic, Calcite, Quartz, Sapphire and Ruby are anisotropic – uni axial, and Mica and Gypsum are anisotropic – bi axial. e.g. Castor oil has refractive index 1.475-1.527.

### Chemical properties

The Chemical properties include effect on heat, solubility, reaction with acids like HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, assays which include Volumetric/ Gravimetric / Flame photometry for Na, K, qualitative test for carbonate and sulphate, analysis of heavy metals like Arsenic and other elements.

### 4. Microscopic methods

Usually minerals can be authenticated by light microscopy coupled with polarized microscopy. It is a simple, inexpensive and widely used method for the authentication of minerals.

## 5. Spectroscopic methods

The spectral methods used to authenticate animal include UV-Vis spectrophotometer, Near Infrared and Fourier Transform Infrared spectrometer etc. These methods are specifically employed in the authentication of honey.

### a. Near Infrared Spectroscopy (NIRS)

Near Infrared Spectroscopy has received much attention for chemical quality and process control because of its speed and attribute of requiring little or no sample preparation. NIRS uses the near infrared region of the electromagnetic spectrum (from about 800nm to 2500nm).

### b. Atomic Absorption Spectrometry (AAS)

AAS is based on the light absorption of elements. AAS is used for the quantitative estimation of inorganic minerals in plant drugs/poly herbal formulations, drugs of mineral/ metals and animal origin. The estimation can be made at ppm level and still lower levels by graphite furnace method.

### c. X-ray Diffraction Analysis (XRD)

The X-ray diffraction pattern of a pure substance is like a fingerprint of the substance. The powder diffraction method is ideally suitable for characterization and identification of polycrystalline phases. The main use of powder diffraction is to identify components in a sample by a search/match procedure. Furthermore, the areas under the peak are related to the amount of each phase present in the sample.

### f. X-ray Fluorescence Analysis (XRF)

X-ray fluorescence (XRF) is the emission of characteristic secondary or fluorescent X-rays from a material that has been excited by bombarding with high energy X-rays or gamma rays. The phenomenon is widely used for elemental analysis and chemical analysis particularly in the investigation of metals, and for research in geochemistry, forensic science and archeology.

## AUTHENTICATION OF MARINE AND ANIMAL PRODUCTS<sup>[7]</sup>

### 1. Chromatographic methods

#### a. Gas Chromatography /Mass Spectrometry (GCMS)

GC-MS is the marriage of two analytical methods into a versatile technique for the

identification of complex volatile materials. Gas Chromatography (GC) effectively separates the constituents of the sample for subsequent analysis and identification by Mass spectrometry (MS). The first result obtained is the compiled data of total ion chromatogram (TIC), which is a plot of the total mass eluting from the GC and detected by MS as a function of time.

#### **b. Liquid Chromatography-Mass Spectrometry (LCMS)**

Liquid chromatography mass spectrometry (LC-MS or alternatively HPLC-MS) is an analytical technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals. Raw materials like honey and animal fats can be authenticated by this technique and the spectral fingerprints of them can be generated.

#### **CONCLUSION**

A significant number of methods to authenticate crude drugs have been addressed here. A simple method like organoleptic characteristics may hold good to authenticate certain drugs but some may require highly sophisticated techniques too, based on the adulterants and similarity in the chemical constituents. So, it is in the hands of the researchers to choose the right method suitable for the drug of interest which would match the reference standard. Even before choosing the methods the raw materials should be confirmed with traditional practitioners. In addition, while utilizing chemical methods and other analytical tools it is mandatory to stick on to the latest validated techniques that suit the study. One needs to understand what type of raw material / formulation one is dealing with and the type of preparation to be evaluated. In spite of all these factors, the microbial contamination, pesticide residue and heavy metal analysis should be considered before processing the raw material for drug preparation. This is regarding the safety issue of drugs. This type of analysis is required when evaluating the authenticity of botanicals because these extraneous contaminants can cause undesired physiological effects. To conclude, more basic research should be carried out and many individuals should be trained with these authentication techniques to address this issue prevailing in crude drugs.

**REFERENCES**

1. Nikam PH, Kareparamban J, Jadhav A, Kadam V. Future Trends in Standardization of Herbal Drugs. *J Appl Pharm Sci*, 2012; 2(6): 38-44.
2. Ekka NR, Namdeo KP, Samal PK. Standardization strategies for herbal drugs-an overview. *Res J Pharm & Technol*, 2008; 1(4): 310-312.
3. Mehta SJ, Shah DP, Mehta TJ, Patel PM, Patel NM. Compendial testing method on herbal crude drug- a review. *Asian J Pharm Res*, 2011; 1(2): 49-52.
4. Bele AA, Khale A. Standardization of herbal drugs: an overview. *Int Res J Pharm*, 2011; 2(12): 56-60.
5. Sahil K, Sudeep B, Akanksha M. Standardization of medicinal plant materials. *Int J Res Ayur & Pharm*, 2011; 2(4): 1100-1109.
6. Chanda S. Importance of pharmacognostic study of medicinal plants: An overview. *J Pharmacog & Phytochem*, 2014; 2(5): 69-73.
7. Revathy SS, Rathinamala R, Murugesan M. Authentication methods for drugs used in Ayurveda, Siddha and Unani systems of medicine: an overview. *Int J Pharm Sci & Res*, 2012; 3(8): 2352-2361.
8. Patil SG, Wagh AS, Pawara RC, Ambore SM. Standard tools for evaluation of herbal drugs: an overview. *The Pharma Innovation-Journal*, 2013; 2(9): 60-65.
9. Evans, WT. *Trease and Evans Pharmacognosy*. 2009; Elsevier Limited.
10. Gautam A, Kashyap SJ, Sharma PK, Garg VP, Visht S, Kumar N. Identification, evaluation and standardization of herbal drugs: an overview. *Der Pharmacia Lettre*, 2010; 2(6): 302-315.
11. Ahmad T, Singh SB, Pandey S. Phytochemical screening and physicochemical parameters of crude drugs: a brief review. *Int J Pharm Res & Review*, 2013; 2(12): 53-60.