

**PRE-SCREENING METHODS OF PLANT MATERIALS: A REVIEW****<sup>1</sup>Smita Verma\*, <sup>1</sup>Mayank Srivastava, <sup>2</sup>Shahjahan, <sup>3</sup>Jayant Kumar Maurya, <sup>3</sup>Snjeev Kumar Singh, <sup>3</sup>Swarup Chattarjee**<sup>1</sup>Department of Pharmacology, Shambhunath Institute of Pharmacy, Jhalwa, Allahabad, U.P, 2110012, India<sup>2</sup>Department of Pharmacology, V.B.S.P, University, Jaunpur<sup>3</sup>Department of Pharmaceutics, Prasad Institute of Technology, Jaunpur

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| <b>*For Correspondence:</b><br>Department of Pharmacology,<br>Shambhunath Institute of<br>Pharmacy, Jhalwa, Allahabad,<br>U.P, 2110012, India. | <b>ABSTRACT</b><br>Remedial plants are used widely for the support of primary health care needs of the people accessible in the rural areas. The traditional medicines were obtained from the medicinal plants, but the herbal drugs are obtained from the medicinal plants alone. Here, increasing awareness and general acceptability of the use of herbal drugs in today's medical practice. Here, near about most of the world population depends on herbal medicines. This ascend in the use of herbal product has also given rise to various forms of abuse and defilement of the products leading to consumers' and manufacturers' dissatisfaction and in some instances fatal penalty. The challenge is countless and vast, making the global herbal market unsafe. This review seeks to enlighten herbal medicine on the need to set up quality parameters for collection, handling, processing of herbal medicine and these parameters are also very helpful to ensure the safety of the global herbal market.<br><b>KEY WORDS:</b> Pharmacognostic evaluation, Ash value, Medicinal Plant. |
| <b>Received: 09.12.2016</b><br><b>Accepted: 22.03.2017</b>   |   |
| <b>Access this article online</b><br><b>Website:</b><br><a href="http://www.drugresearch.in">www.drugresearch.in</a>                           |   |
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**1. INTRODUCTION**

This review is interconnected to the Phytocognostic evaluation of plant materials (especially root part of the plant). Many root of the plants shows various medicinal actions like, use as an expectorant, diaphoretic, as a gentle stimulant and plant materials are also used as some carminatives in dyspepsia, it's useful in leprosy and eczema. For example, *Calotropis gigantea* (L) powdered root bark gives relief in diarrhoea and dysentery. It is also relief in cough and asthma (Havagiray R. Chitme). This review presents a range of methods to pre-screen various Pharmacognostic evaluations of plant materials which are useful in a variety of traditional medications. This brings a set make easy to go through the various methods at same time and make it easy to screen the herbal drugs.

**2. SCREENING METHODS:**

A variety of laboratory methods for testing the Phytochemicals in plans materials are,

**2.1. Determination of Physical contents of drug**

- 2.1.1. Moisture content determination
- 2.1.2. Total ash value
- 2.1.3. Acid insoluble ash value
- 2.1.4. Water soluble ash value
- 2.1.5. Fluorescent studies of powder drugs

## 2.2. Successive solvent extraction Method

### 2.3. Quantitative Chemical evaluation

- 2.3.1. Detection of Alkaloid
- 2.3.2. Detection of Glycoside
- 2.3.3. Detection of Tannins
- 2.3.4. Detection of Saponins
- 2.3.5. Detection of Flavonoids
- 2.3.6. Detection of Terpenoids
- 2.3.7. Test for Steroids and Sterols
- 2.3.8. Detection of Carbohydrates and Sugars

### 2.1. DETERMINATION OF PHYSICAL CONTENTS OF DRUG:

Physical standards are to be determined for drug, wherever possible. These are rarely constant for crude drug, but may help in evaluation, specifically with reference to moisture content, ash values, specific gravity, density, refractive index, viscosity and solubility in different solvents. (Kokate C.K.). A few of them are described below:

#### 2.1.1. Moisture content determination

##### Principle:

The percentage of active chemical constituents in crude drug is maintained on air dried basis. Hence, the moisture content of a drug should be determined and should also be controlled. The moisture content of a drug should be minimized to prevent decomposition of crude drugs either due to chemical change or microbial contamination. Moisture content determined by heating a drug at 105 °C in an oven (Kokate C.K.).

##### Procedure:

Accurately weighed of drug (in gram), was taken and dried at 105 °C for three hour in oven and again weighed. Dried and weighed was continued at 1hour interval until difference between two successive weighed corresponds to not more than 0.25%. The reading was taken after a constant weight was reached and moisture content was determined. (Archana Gautum).

#### 2.1.2. Total ash value

##### Principle:

The residue remaining after incineration was the ash content of drug, which simply represents inorganic salts, naturally occurring in drug or adhering to it or deliberately added to it, as a form of adulteration.



Figure 2.1.1: Hot Air Oven



Figure 2.1.2: Muffle furnace and Crucibles



Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The objective of ashing vegetable drugs is to remove all traces of organic matter, which may otherwise interfere in an analytical determination (Varma Rajeev).

**Procedure:**

Powdered plant material was taken in gram. The pre-weighed clean sintered silica crucibles, they were incinerated by gradual increasing of the temperature 400-500 °C in the muffle furnace till white ash obtained until constant weight of ash obtained. The crucible was cooled at room temperature in a desecrator and weighed the ash and calculated the % of total ash with reference to the air-dried sample of the crude drug using following-

**Formula:**

$$\text{Total ash Value (\%)} = \frac{Z-X}{Y} \times 100$$

Where, X= Weight of the crucible; Z = Weight of the crucible with ash; Y = Weight of the powder taken (g).

**2.1.3. Acid insoluble ash value**

**Principle:**

Acid insoluble ash was defined as a part of total ash insoluble in dilute hydrochloric acid. This was also recommended for certain drugs. Adhering dirt and sand may be determined by acid-insoluble ash content (Kokate C.K.).

**Procedure:**

The total ash content of the plant material obtained was taken and added 25ml of 25 % (v/v) HCl (6.25ml) in to a 100-ml beaker, mixed and boiled for 15min and allowed to cool. It was filtered through a whatman filter paper (no. 44, ash less) and washes the residue two times with hot water. The insoluble ash thus retained on filter paper along with paper was ignited in a preweighed sintered crucible (1000 °C). Then the crucible all along with the filtrate was weighed and calculated the acid insoluble ash substance using the following formula (Pratima H and Pratima Mathad, Khandelwal Dr K.R).

$$\text{Acid insoluble ash Value (\%)} = \frac{X}{Y} \times 100$$

Where, X= weight of the residue; Y= Weight of powder taken (g).

**2.1.4. Water soluble ash value**

**Principle:**

As well as the total ash content, it was some time useful to determine the ratio of water soluble to water insoluble ash as gives a useful indication of the quantity of certain foods. Ash was diluted with distilled water then heated to nearly boiling and the resulting solution was filtered. The amount of soluble ash was determined by drying the filtrate and the insoluble ash was determined by rinsing, drying and ashing the filter paper.

**Procedure:**

The total ash was boiled for 5 minutes with 25 ml of distilled water and the insoluble matter was collected on an ash less filter paper, washed with hot distilled water, and ignited for 15 minutes at a temperature not exceeding 450 °C. Weighed the insoluble matter and it was subtracted from the weight of the total ash, the difference in weight represents the water-soluble ash. The percentage of

the water-soluble ash was calculated with reference to the air-dried powdered plant sample. It was calculated by using following formula (Okhale, Samuel).

**Formula:**

$$\text{Water insoluble ash Value (\%)} = \frac{X}{Y} \times 100$$

Where, X= Weight of the residue; Y= Weight of powder taken (g) Water soluble ash Values (%) = Total ash value – Water insoluble ash value.

### 2.1.5. Fluorescent studies of powder drugs

#### Principle:

The organic molecule absorbs light usually over a specific range of wavelength and many of them re-emit such radiations. This phenomenon was called as luminescence. When re-emission of absorbed light lasts only whilst the substance was receiving the exciting rays the phenomenon was defined as fluorescence.

#### Procedure:

When the powdered drug was treated with the various reagents and observed under UV lamp, then powder drug show fluorescence. When the powder was exposed the UV light, this can be useful in their identification. The fluorescence character of the plant powders was studied both in daylight and UV light (254 nm and 366 nm) (Varma Rajeev).

### 2.2. SUCCESSIVE SOLVENT EXTRACTION METHOD:

The dried coarse powder material was subjected to Soxhlet extraction separately and successively with powdered material was defatted with petroleum ether (60-80°C) and successively extracted for 4 hr using chloroform and methanol. (Murali A. *et al.*, 2010) and distilled water solvents in the increasing order of polarity. The solvent was distilled under reduced pressure, controlled temperature (40-50°C) and the resulting semisolid mass was vacuum dried using rotary evaporator (for solvent recovery) (Hitesh Vashrambhai Patel. *et al.* IJBPR).



**Figure 2.1.5: UV fluorescence Analysis cabinet**



**Figure 2.2.1: Rotary Evaporator**

The yield of the extracts was calculated using the following formula (Pratima H and Pratima Mathad):

Solvent – Petroleum ether, chloroform, Methanol and aqueous.

Method – Soxhlet extraction

Residue obtained

Extractive value % =  $\frac{\text{-----}}{\text{Weight of the plant material taken}} \times 100$

### 2.2.1. DETERMINATION OF EXTRACTIVE VALUES:



Figure 2.2.1: Soxhlet assembly

### 2.2.2. Petroleum ether soluble extractive value

The roots of plant were coarse and air dried drug material were washed and allowed to dry in shade for a week and then grounded into fine powder in mixer grinder. The dried powder of plant was subjected to Soxhlet extraction with solvent for 4 hr starting from petroleum ether (60-80 °C). Extraction was concentrated by distilling off the solvent and then evaporated to dryness on water bath. The extract was weighed. Its percentage was calculated in terms of air-dried weighed of plant material. Then after this process the powdered drug was dried in desiccator or use hot air oven (below 150 °C) (Mukharjee, Pulak K. and Kokate C.K.).

### 2.2.3. Chloroform soluble extractive value:

In the process of successive extraction, each time before extracting with the next solvent, the powdered material (which was collected from previous step) was dried in hot air oven (below 150 °C). After drying, the dried powder materials were subjected to Soxhlet extraction with the solvent using chloroform for 4hr. The residue was evaporated using a rotatory evaporator. Average extractive value in percentage w/w (on the dry basis) was calculated with reference to air dried drug or plant material (Mukharjee, Pulak K. and Kokate C.K.).

### 2.2.4. Methanol soluble extractive value:

Each time before extracting with the next solvent, the powdered material (which was collected from previous step) was dried in hot air oven (below 150 °C). Then the dried powder materials were subjected to Soxhlet extraction with the solvent using methanol for 4 hr. The residue was evaporated using a rotatory evaporator. Average extractive value in percentage w/w (on the dry basis) was calculated with reference to air dried drug or plant material (Mukharjee, Pulak K. and Kokate C.K.).

### 2.2.5. Water soluble extractive value:

In last step of successive extraction, plant material was extracting with the next solvent using distilled water (polar solvent). Then the powdered material (which was collected from previous step) was dried in hot air oven (below 150 °C). Then the dried powder material was subjected to Soxhlet

extraction with solvent using distilled water for 4 hr. The residue was evaporated using a water bath. Average extractive value in percentage w/w (on the dry basis) was calculated with reference to air dried drug or plant material (Mukharjee, Pulak K. and Kokate C.K).

## 2.3. QUANTITATIVE CHEMICAL EVALUATION

### 2.3.1. Detection of Alkaloid

A minute amount of various extracts was taken individually in 4ml of 1.5%v/v HCL and filtered. These filtrates were then subjected to following test.

(Ajay Kumar R. Suranaa and Rajendra D. Wagh):

**2.3.1.1. Dragendorff's Test:** The extract (1 ml) of, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An **orange-red** precipitate indicates the presence of alkaloids.

**2.3.1.2. Mayer's Test:** The extract (1 ml) of, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish **yellow** or **cream** colored precipitate indicates the presence of alkaloids.

**2.3.1.3. Hager's Test:** The extract (1 ml) of, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), **yellow** colored precipitate indicates the presence of alkaloids.

**2.3.1.4. Wagner's Test:** The extract (1 ml) of, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids (Kokate C.K).

### 2.3.2. Detection of Glycoside

**2.3.2.1. Legal Test:** Dissolve the extract in (1ml) pyridine + (add) 1ml sodium nitroprusside solution to make it alkaline. The formation of **pink red** to **red** color shows the presence of steroidal glycosides.

**2.3.2.2. Baljet Test:** The test extracts (1 ml) + adds 1ml of sodium picrate solution and the **yellow** to **orange** color reveals the presence of steroidal glycosides.

**2.3.2.3. Borntrager's Test:** Add a few ml of dilute sulphuric acid + the extract solution (1ml), boiled, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of **red** color of the ammonical layer shows the presence of anthraquinone glycosides (Kokate C.K).

### 2.3.3. Detection of Tannins

Ñ Take the little quantity of test extract + mixed with basic lead acetate solution. Formation of **white precipitates** indicates the presence of tannins.

Ñ Take 1ml of the extract + add ferric chloride solution. Formation of a **dark blue** or **greenish black** colour product shows the presence of tannins.

Ñ Take little quantity of test extract + treated with potassium ferric cyanide and ammonia solution. Give a **deep red** colour indicates the presence of tannins (Kokate C.K).

### 2.3.4. Detection of Saponins

- Take small quantity of extract separately + add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm **layer of foam** indicates the presence of Saponins.

### 2.3.5. Detection of Flavonoids

- **Shinoda Test:** Take the test solution + add few magnesium turnings + add concentrate hydrochloric acid drop wise, pink scarlet **crimson red** or **occasionally green** to **blue** color appears after few minutes (Mukharjee Pulak. K)

### 2.3.6. Detection of Vitamin C

- Dilute 1 ml of (2% w/v) solution of drug with 5 ml of water + add 1 drop of freshly prepared (5% w/v) solution of sodium nitroprusside + 2 ml of dilute NaOH solution then add 0.6 ml of HCl drop wise and stir, then it will show yellow color turns **blue** (Varma Rajeev)

### 2.3.7. Test for Steroids and Sterols

- **Libermann-Burchard Test:** 1gm of the test extract + dissolved in a few drops of chloroform + 3 ml of acetic anhydride + 3 ml of glacial acetic acid was added then heat and cooled under the tap and drops of concentrated sulphuric acid was added along the sides of the test tube. Bluish-green coloured appearance shows, the presence of sterol.
- **Salkowski Test:** Dissolve the extract in chloroform + add equal volume of conc. H<sub>2</sub>SO<sub>4</sub> Formation of **bluish red** to **cherry** colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract (Kokate C.K).

### 2.3.8. Detection of Carbohydrates and Sugars

- **Molisch's Test:** Take 2 ml of the extract + add 1ml of  $\alpha$ -naphthol solution + add concentrated sulphuric acid through the side of the test tube. **Purple** or **reddish violet** colour at the junction of the two liquids reveals the presence of carbohydrates.
- **Fehling's Test:** Take 1 ml of the extract + add equal quantities of Fehling solution A and B, upon heating formation of a **brick red precipitate** indicates the presence of reducing sugars.
- **Benedict's test:** Take 5 ml of Benedict's reagent + add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars. (Kokate C.K.)

## CONCLUSION

This review is helpful for evaluations of many medicinal active constituents of plant and gives the various methods of phytochemical screening of various constituents of the plant materials, which is present in plant parts (plant root, bark, leaves etc). This information's are useful in the variety of traditional medications. This brings a set make easy to go through the various methods at same time and make it easy to screen the herbal drugs.

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