

### 3.2.4. Physical Evaluation

Physical evaluation of crude drugs is a necessity. The physical parameters remain constant in rare cases, but they help in evaluating the following in different solvents:

- 1) **Moisture Content:** The active chemical constituents in crude drugs are expressed in percentage on air-dried basis. Thus, a drug's moisture content should be determined by heating it at 105°C in an oven up to a constant weight (**table 3.1**).

**Table 3.1: Crude Drugs with Limit for Moisture Content**

Drugs	Moisture Content (%)
Aloes	Not more than 10
Digitalis	Not more than 5
Ergot	Not more than 8
Acacia	Not more than 15
Starch	Not more than 15

- 2) **Viscosity:** A liquid drug's viscosity at a given temperature is constant and is an index of its composition; thus, is used for its standardisation. **Table 3.2** shows some examples:

**Table 3.2: Crude Drugs with Limit for Viscosity**

Drugs	Viscosity Limit
Liquid Paraffin	Kinematic viscosity not less than 64 centistokes at 37.8°C.
Pyroxylin	Kinematic viscosity, 1100 - 2450 centistokes.

- 3) **Melting Point:** A crude drug's purity can be determined by its melting point. Pure chemicals or phytochemicals have very sharp and constant melting points. **Table 3.3** mentions the range of melting points which is determined for establishing the purity of the given crude drugs:

**Table 3.3: Melting Point Range for few Crude Drugs**

Drugs	Melting Point (°C)
Colophony	75 - 85
Kokum butter	39 - 42
Cocoa butter	30 - 33
Bees wax	62 - 65
Wool fat	34 - 44

- 4) **Solubility:** An adulterant can be detected in a crude drug by solubility studies. Alkaloidal bases are soluble in chloroform, while alkaloidal salts are soluble in polar solvents. Glycosides can be extracted with alcohol and water, while their aglycone moieties are soluble in non-polar solvents (e.g., benzene or solvent ether).
- 5) **Optical Rotation:** Some optically active substances either in pure form or in solution form can rotate the plane of polarised light; this property is called **optical rotation**. The plane of polarised light can be rotated either towards right (dextrorotatory) or towards left (levorotatory). Optical rotation is determined using sodium lamp (light source) at 25°C. Optical rotations of some crude drugs are mentioned in **table 3.4**:

**Table 3.4: Optical Rotation Values for few Crude Drugs**

Drugs	Angles of Optical Rotation
Caraway oil	+75° to +80°
Castor oil	3.5° to +6.0°
Clove oil	0° to -1.5°
Honey	+3° to -15°
Eucalyptus oil	0° to +10°
Chenopodium oil	-30° to -8°

- 6) **Refractive Index:** A light ray bends from its original path on passing from one medium to another of different density. Thus, the ratio of light velocity in vacuum to its velocity in substance is called **refractive index of the second medium**. This property is constant for a liquid based on purity, and can be used for its standardisation. A substance's refractive index changes with the incident light wavelength, temperature, and pressure. Refractive indices of some substances determined using sodium light at 25°C are given in **table 3.5**:

**Table 3.5: Refractive Indices of Some Phytoconstituents**

Drugs	Refractive Index
Arachis oil	1.4678 - 1.470
Caraway oil	1.4838 - 1.4858
Castor oil	1.4758 - 1.527
Clove oil	1.527 - 1.535

## 7) Ash Values/Content and Extractives

- i) **Ash Content:** It is the residue left behind after incineration of the drug. Ash content of drug represents inorganic salts occurring naturally in it or adhering to it or added to it purposely, as an adulterant. Total ash of the drug includes both **physiological** and **non-physiological ash**. The former is obtained from plant tissues, while latter is the residue of extraneous matter (sand, soil, etc.) found adhered to the herb. Ash content of a drug can be determined by incinerating it in powdered form so that its organic matter burns completely. Ash value of a crude drug is a standard for identifying it or determining its purity. Total ash comprises of carbonates, oxides, phosphates, silicates, and silica. **Table 3.6** enlists the total ash values of some crude drugs:

**Table 3.6: Crude Drugs with their Ash Contents**

Drugs	Total Ash (% w/w)	Acid Insoluble Ash (% w/w)
Agar	–	1.00
Aloe	05.0	–
Ashoka	11.0	–
Bael	03.5	–
Belladonna	–	3.00
Black catechu	06.0	–
Cannabis	15.0	5.00
Cardamom	06.0	3.50
Clove	07.0	0.75
Gelatine	03.6	–
Ginger	06.0	1.7(Water soluble ash)
Valerian	12.0	–

- ii) **Extractives:** When crude drugs are exhausted they leave behind extracts which represent the estimated measures of their chemical constituents. The following types of extractives can be determined by using various solvents selected by considering the variability in chemical nature and properties of contents of drugs:

- a) **Water-Soluble Extractives:** These extractives contain water-soluble active constituents of crude drugs (e.g., tannins, sugars, plant acids, mucilage, glycosides, etc.), given in **table 3.7**:

**Table 3.7: Water-Soluble Extractive Values of Some Crude Drugs**

Drugs	Water-Soluble Extractive Value (% w/w)
Aloe	Not less than 25.0
Glycyrrhiza	Not less than 20.0
Linseed	Not less than 15.0
Senna leaves	Not less than 30.0
Senna pods	Not less than 28.0
Ginger	Not less than 10.0

- b) **Alcohol-Soluble Extractives:** Various chemicals like tannins, resins, etc. are extracted using alcohol as an ideal solvent. Thus, an estimate of the resin content of drug can be determined by this method. For determining alcohol-soluble extractive, around 95% ethyl alcohol is used.

Diluted alcohol is also used in some cases; however it depends on the solubility of constituents of crude drugs. **Table 3.8** mentions the limits for alcohol-soluble extractive values for some drugs.

**Table 3.8: Alcohol-Soluble Extractive Values of Some Crude Drugs**

Alcohol-Soluble Extractive Values		
Drugs	(% w/w)	
Aloe	Not less than	10.0
Benzoin	Not less than	90.0 (Siam benzoin) 75.0 (Sumatra benzoin)
Asafoetida (90% alcohol)	Not less than	50.0
Ginger (90% alcohol)	Not less than	04.5
Valerian (60% alcohol)	Not less than	30.0
Alcohol-Insoluble Extractive Values		
Myrrh	Not less than	70.0
Benzoin	Not less than	24.0

- c) **Ether-Soluble Extractives:** Volatile and non-volatile types of ether-soluble extractives are determined for crude drug evaluation. **Table 3.9** mentions the limits for non-volatile ether-soluble extractive values for some drugs:

**Table 3.9: Non-Volatile Ether-Soluble Extractive Values of Some Drugs**

Drugs	Limits for Non-Volatile Ether Soluble Extractives (% w/w)
Capsicum	Not less than 12.0
Male fern	Not less than 01.5
Linseed	Not less than 25.0
Nutmeg	Not less than 25.0

- 8) **Volatile Oil Content:** The aromatic crude drugs are pharmaceutically vital owing to their volatile oil content. Therefore, such drugs are standardised based on their volatile oil content. The **examples** of some drugs are given in **table 3.10**:

**Table 3.10: Volatile Oil Content of Some Crude Drugs**

Drugs	Volatile Oil Content (% w/w)
Caraway	Not less than 2.5
Fresh lemon peel	Not less than 2.5
Clove	Note less than 15.0
Fennel	Not less than 1.4
Dill	Not less than 2.5
Cardamom seed	Not less than 4.0

- 9) **Foreign Organic Matter:** The monograph of crude drugs has the maximum limit for foreign organic matter. If the matter exceeds the mentioned limits, the drug quality declines. The limit for foreign organic matter is mentioned in the respective monographs particularly for natural drugs of vegetable origin. The physical as well as physicochemical parameters are used for determining the quality profile of a crude drug, thus form an important part of qualitative evaluation.

### 3.2.5. Chemical Evaluation

Chemical evaluation of crude drugs involves different chemical tests and assays. It also involves isolation, purification, and identification of active constituents of drugs. Preliminary phytochemical screening forms a part of chemical evaluation. The qualitative type of chemical tests is used for detecting the adulteration type.

Chemical assay comprises of the conventional titrimetric techniques as used for determining alkaloids from crude drugs, ester and aldehyde contents of volatile oils, gravimetric methods, etc. Chemical evaluation is defined as the **chemical process of determining the active constituents in a drug**.

Acid value, iodine value, saponification value, ester value, etc. are some quantitative physicochemical constants used for evaluation of fixed oils and fats.

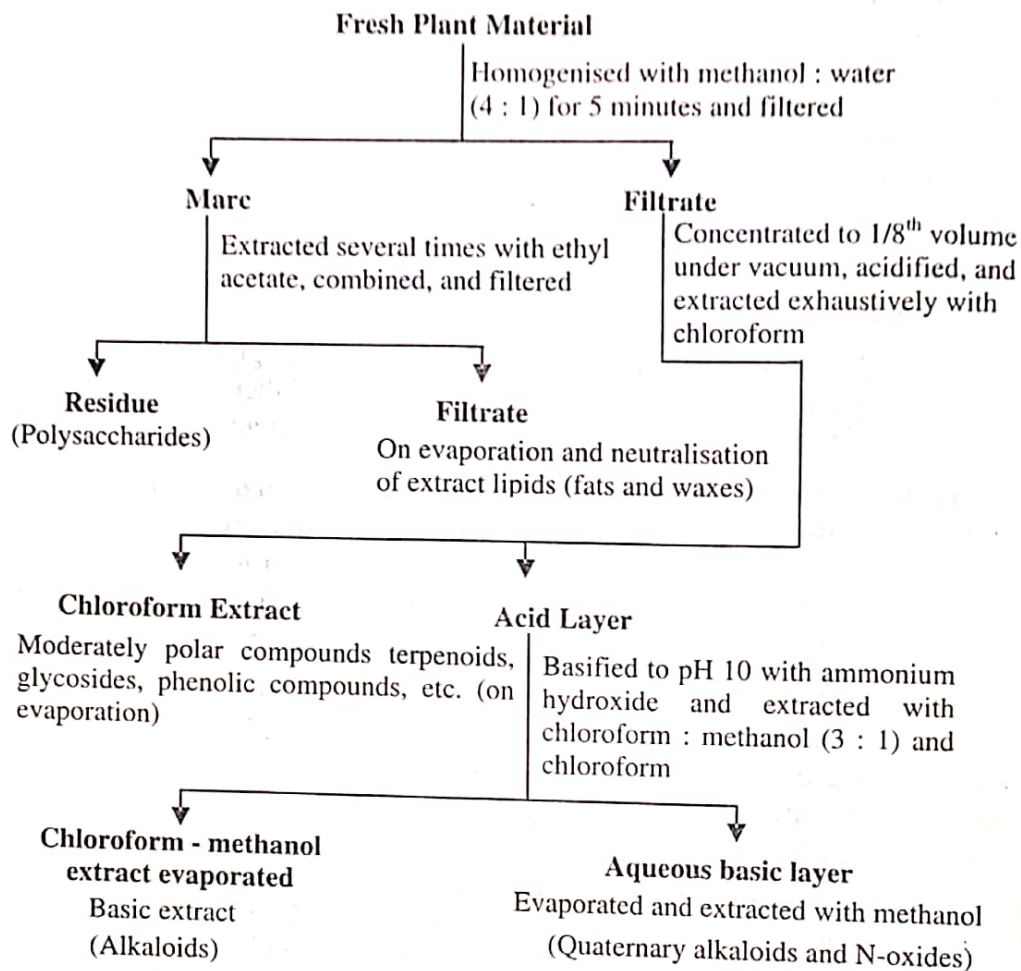


Figure 3.9: Extraction of Constituents from Plant Material

### 3.2.5.1. Phytochemical Investigations

Plant materials' investigation for their phytochemical behaviour involves the following stages:

- 1) Procurement of raw material and quality control,
- 2) Extraction, purification, and characterisation of the pharmaceutically active constituents,
- 3) In process quality control,
- 4) Investigations of biosynthetic pathways to particular compounds, and
- 5) Quantitative evaluation.

**Extraction** is a technique widely used for separating active substances from crude drugs using different solvents. The **basic method** of extracting different constituents from fresh plant is illustrated in figure 3.9.

Fractional crystallisation process makes use of the differential solubility of components of a mixture in a particular solvent. Very often derivatives of particular components, e.g., picrates of alkaloids, osazones of sugars, etc. are used.

### 3.2.5.2. Qualitative Chemical Examination

The obtained extracts are subjected to qualitative tests for identifying the following plant constituents:

- 1) **Detection of Alkaloids:** Separate and small amounts of solvent-free chloroform, alcoholic and water extracts are stirred with a few drops of dilute hydrochloric acid

and filtered. The filtrate obtained is tested with Mayer's reagent, Dragendorff's reagent, Hager's reagent, and Wagner's reagent; and the presence of alkaloids is confirmed if the filtrate gives a precipitate of cream, orange brown, yellow, and reddish-brown colour on treating with respective reagents.

- 2) **Detection of Carbohydrates and Glycosides:** These constituents can be detected by the following methods:
  - i) 200mg of separate alcoholic and aqueous extracts are dissolved in 5ml distilled water and filtered. The obtained filtrate is then treated with Molisch's test for detecting carbohydrates.
  - ii) One more small quantity of extract is hydrolysed for few hours using dilute hydrochloric acid in water bath and then treated with Liebermann-Burchard's, Legal's, and Borntrager's tests for detecting different glycosides.
  - iii) A small quantity of extract is dissolved in water and then treated with Fehling's, Barfoed's, and Benedict's reagents for detecting different sugars.
- 3) **Detection of Phytosterols:** Petroleum ether, acetone, and alcoholic extracts are separately refluxed using alcoholic solution of potassium hydroxide until they are completely saponified. The obtained mixture is subjected to dilution with distilled water and extraction with ether. The obtained ethereal extract is evaporated and the residue (unsaponifiable matter) is treated with Liebermann's and Burchard's tests.
- 4) **Detection of Fixed Oils and Fats:** Petroleum ether and benzene extracts in small quantities are separately pressed between two filter papers. The presence of fixed oil is confirmed if oil stains are left on the paper. To the petroleum ether or benzene extract, a few drops of 0.5N alcoholic potassium hydroxide and one drop of phenolphthalein are added and mixed. The presence of fixed oils and fats is confirmed if on heating this mixture for 1-2 hours on water bath, soap is formed or the alkali undergoes partial neutralisation. The presence of volatile oil can also be confirmed by hydro-distillation of 50gm air-dried or fresh plant material.
- 5) **Detection of Saponins:** 1ml of alcoholic and aqueous extracts are separately diluted with distilled water up to 20ml and shaken for 15 minutes in a graduated cylinder. The presence of saponins is confirmed if 1cm layer of foam is formed. The test solution may also be subjected to test for haemolysis.
- 6) **Detection of Phenolic Compounds and Tannins:** Presence of these compounds can be confirmed by treating small quantities of alcoholic and aqueous extracts in water with 5% dilute ferric chloride solution, 1% gelatin solution containing 10% sodium chloride, 10% lead acetate, and aqueous bromine solutions.
- 7) **Detection of Proteins and Free Amino Acids:** Presence of these compounds can be confirmed by dissolving small quantities of alcoholic and aqueous extracts in a few ml of water and subjecting the resultant solution to millon's, biuret, and ninhydrin tests.
- 8) **Detection of Gums and Mucilages:** 10ml of aqueous extract is mixed with 25ml absolute alcohol with constant stirring. The precipitate obtained is air-dried and examined for its swelling properties and for the presence of carbohydrates.
- 9) **Detection of Volatile Oils:** These can be detected by hydro-distillation of 50gm powdered material in a volatile oil estimation apparatus. In the distillate (collected in the graduated tube of the assembly), the aqueous portion automatically separates out from the volatile oil portion (if present in the drug), after which it is returned back to the distillation flask.

### 3.2.6. Biological Evaluation

Some drugs need to be evaluated by biological means if not successfully assayed by chemical or physical methods of evaluation. Biological evaluation is performed using whole animals, animal preparations, isolated living tissues, or microorganisms. This assay method is termed **biological assay** or **bioassay** as living organisms are used. The methods of biological evaluation are comparatively less precise, more time-taking, and expensive.

With the help of a biological assay, the biological activity of a given sample can be determined. In a complete single test, animals of one strain only should be used. Bioassay is the measure of the capability of sample under test to produce biological effect in comparison to the standard preparation. Such activity is represented in units known as **International Unit (I.U.)**.

#### 3.2.6.1. Biological Testing of Herbal Drugs

Herbal drugs are standardised by the methods evaluating their biological efficacy. These methods utilise an animal model suitable for testing and control, experimental methods, and result assessment.

The **protocols** for the evaluation of the following biological activities are discussed below:

- 1) **Hepatoprotective Activity:** Male/female albino rats are used for evaluating this activity. Hepatitis is caused by the hepatitis virus, and also by certain chemicals, drugs, industrial pollutants, ethyl alcohol, and immunogenic reactions. Carbon tetrachloride is most commonly used for liver damage by acute toxicity; alcohol, paracetamol, and rifampicin can also be used for the same purpose as they cause sub-acute or chronic toxicity.

**Liver toxicity** can be evaluated by the following **parameters**:

- i) Physiological (hexobarbital hypnosis),
  - ii) Biochemical serum estimation of enzymes like SGPT, SGOT, Superoxide dismutase, and other factors like blood proteins, cholesterol, triglyceride levels, etc., and
  - iii) Histopathological (liver tissue necrosis and fatty degeneration).
- 2) **Hypoglycaemic Activity:** Several plant extracts, e.g., karela (*Momordica charantia*), jambul (*Syzgium cumini*), fenugreek (*Trigonella foenum-graecum*), and gudmar (*Gymnema sylvestre*) have been used for evaluation of their hypoglycaemic activity. These extracts are used for treating diabetes mellitus. During the test, diabetes is experimentally induced in animals by a suitable dose of alloxan (derived from urea), which causes selective necrosis of pancreatic  $\beta$  cells, thus inducing moderate diabetes (having fasting blood sugar level of 180-250mg/ml). Herbal drug extracts are used in such cases for checking their efficacy.

Glucose oxidase and *ortho*-toluidine are used for measuring the **blood glucose levels** in all the cases.

Radio-Immunoassay (RIA) or Enzyme Linked Immuno Sorbant Assay (ELISA) is carried out for measuring blood insulin levels.

- 3) **Anti-Fertility Testing:** There are many herbal drugs which have been used for testing their abortifacient or antifertility activity; however only a few have shown scientific evidences, e.g., embelin from *Embelia ribes* have abortifacient activity or gossypol from *Gossypium* species is a male anti-fertility agent. These activities occur in the following ways:

- i) **In females**
  - a) By destroying zygotes, thus leading to early abortion, and
  - b) By preventing ovulation, fertilisation, or implantation.

ii) **In males**

- a) By acting as a spermicidal, and
- b) By acting as anti-androgenic.

Abortifacients and contraceptives are anti-fertility drugs. The anti-fertility activity in females is determined by measuring the pregnancy rate, thus including anti-ovulation and anti-implantation drugs. The anti-fertility activity in males is determined by measuring the inhibition rate of spermatogenesis or sperm motility.

- 4) **Anti-Inflammatory Activity:** Certain plant-derived drugs due to their anti-inflammatory activity are used for treating rheumatoid arthritis, gout, dysmenorrhoea, etc. These drugs can produce their action on many sites in body. Inflammation can result due to mechanical causes, infections, or autoimmune diseases.

The evaluation of anti-inflammatory activity is based on the principle of reducing local oedema induced by injecting irritant, inflammatory substances in rat paw. Carrageenan (a mucopolysaccharide isolated from Irish Sea moss, *Chondrus crispus*) in the dose of 0.1ml, 1%w/v in saline is most commonly used as an inducer. The antagonist (herbal drug extract) is given either 60 minutes or 30 minutes before the test via oral or intraperitoneal route, respectively; and the agonist (carrageenan) is injected into plantar surface of the right hind-paw. The volume of paw is measured just after the injection and after every hour atleast for 5 times by volume displacement method, e.g., using plethysmometer.

- 5) **Neuropharmacological Activity:** There are a large number of plants having neurological activity. Such plants, e.g., opium, pilocarpus, brahmi, etc. are used for medicinal or narcotic purposes. The pharmacological spectrum and biological efficacy of these herbal drugs can be established due to the development of various neuropharmacological testing protocols. (5)