EVALUATION OF CRUDE DRUGS

EVALUATION OF CRUDE DRUGS

It ensures the identity of a drug and determines the quality and purity of drugs

Reasons behind the evaluation of crude drugs

- Biochemical variation in the drug
- Effect of treatment and storage of drugs
- Adulteration and substitution

ORGANOLEPTIC EVALUATION

- Qualitative evaluation based on study of morphologial or sensory characters
- Study of form of drugs – Morphology
- Study of description of form - Morphography

- Colour : brown colour of Cinnamon

- Odour : aromatic odour of volatile oil drugs
- Taste : pungent taste of Capsiucm, sweet taste of Liquorice
- Shape : wavy shape of Rauwolfia, Ovoid tears of Acacia, disc shape of Nuxvomica

- Size : small size of umbelliferous fruits
- Special features like touch : silky texture of Nux vomica
- Fractured surface: in cinchona and quassia
- General appearance: percentage of seeds in colocynth, stalks in clove

MICROSCOPIC EVALUATION

- To confirm structural details of drugs of plant origin.
- More detailed evaluation
- Qualitative evaluation
- In entire form or powder form

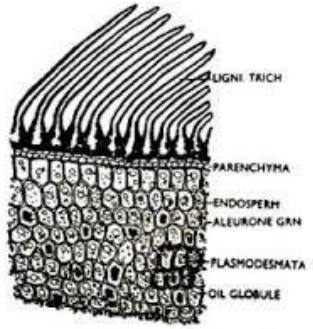


Fig. 17: T. S. of Nux vomica seed

- **Transverse section/Longitudenal section**
  - Lignified trichomes of Nuxvomica
  - Wavy medullary of cascara bark

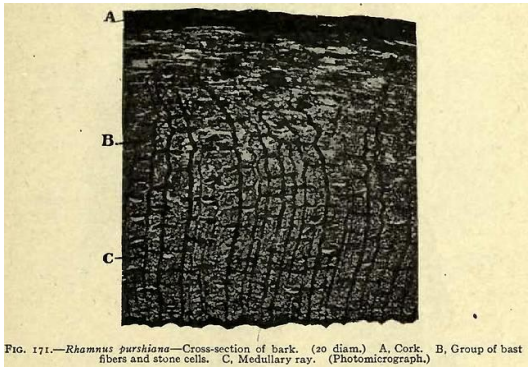


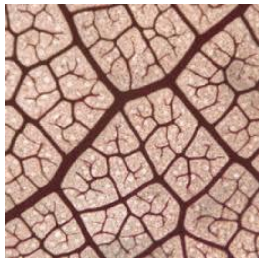
FIG. 171.—*Rhamnus purshiana*—Cross-section of bark. (20 diam.) A, Cork. B, Group of bast fibers and stone cells. C, Medullary ray. (Photomicrograph.)

- **Chemomicroscopy:** Section/powder + chemical reagents
  - Study of constituents by application of chemical methods in histology or powder form
  - eg. Phloroglucinol+conc. HCl --- gives pink colour --- lignified cells.
  - Mucilage + ruthenium red --- pink colour
  - Starch + iodine --- blue colour

MICROSCOPIC EVALUATION

- **Leaf constants**

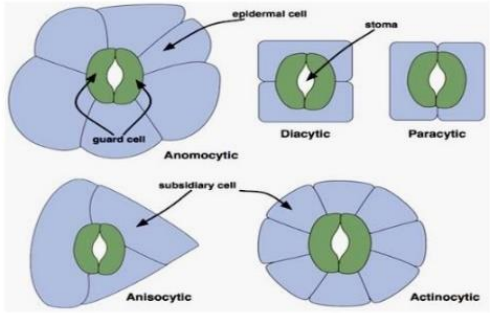
- Stomatal number: Average number of stomata per sq. mm of epidermis of leaf
- Stomatal Index: Percentage which the number of stomata form to the total number of epidermal cells in an unit area
- Vein islet number: Number of vein islets per square mm of the leaf surface midway between midrib and margin.
- Vein termination number: Number of vein terminations per sq. mm of the leaf surface midway between midrib and margin.
- Palisade ratio: Average number of palisade cells beneath each epidermal cell.



MICROSCOPIC EVALUATION

❑ Stomata

- A stoma is a minute epidermal opening present on aerial parts of the plants with a central pore, two kidney shaped guard cells and varying number of subsidiary or epidermal cells covering the guard cells.
- The primary and most important function of stomata is gaseous exchange and the secondary function is transpiration.



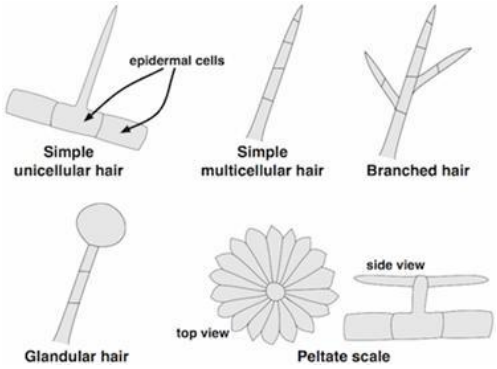
- Paracytic or rubiaceous stomata
- Diacytic or caryophyllaceous stomata
- Anisocytic or cruciferous stomata
- Anomocytic or ranunculaceous stomata
- Actinocytic or radiate celled stomata

MICROSCOPIC EVALUATION

- **Trichomes or plant hairs**

- These are tubular elongated or glandular outgrowth of the epidermal cell.
- Trichomes as such have no function, but sometimes, perform secretory function.
- Depending on the structure and the number of cells present in trichomes, they are classified as

  - Covering trichomes or non-glandular trichomes or clothing trichomes
  - Glandular trichomes
  - Hydathodes or special type of trichomes



MICROSCOPIC EVALUATION

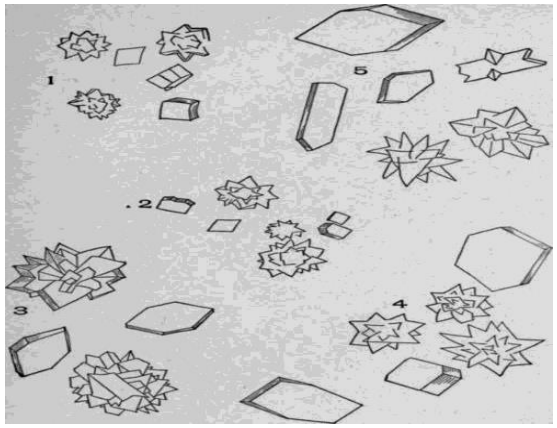
- **Cell contents**

- Aleurone grains, mucilage, tannin, starch grains, fixed and volatile oil globules and other inorganic components like calcium carbonate, calcium oxalate and silica are diagnostic characters of the plants.

- **Calcium oxalate crystals**

- Clacium oxalate is a dimorphic salt of which both the types occur in the plant body.

  - Cubical or prisms
  - Rhombic or diamond
  - Tetragonal
  - Monoclinic
  - Acicular
  - Rosettes or clusters



MICROSCOPIC EVALUATION

- **Microscopic measurements**
- This is used to differentiate two species of the same genus.
- **Quantitative microscopy or Lycopodium spore method**
- It is an important technique for powdered drugs
- It is inexpensive technique with official status.
- Lycopodium spores are very characteristic in shape and appearance and exceptionally unifrom in size (25 µ)
- On an average, 94,000 spores per mg of powdered lycopodium are present.
- A powdered drug is evaluated by this technique, if it contains
  - Well defined particles which may be counted
  - Single layered cells or tissues, the area of which may be traced under suitable magnification and actual area calculated or
  - The objects of uniform thickness, the length of which can be measured under suitable magnification and actual are calculated.
- The percentage purity of an authentic powdered ginger is calculated using the following equation –

$$\frac{NxWx94,000x100}{SxMxP}$$

CHEMICAL EVALUATION

- It covers isolation, purification and identification of active constituents.
- It may be chemical tests or chemical assays.

- **Qualitivative tests/identification tests**

a. Regular chemical tests or specific tests

- Van urk test – ergot alkaloids
- Tropane alkaloids – vitali morin test
- Murexide test – purine alkaloids

b. Detection of adulteration

- Halphens test – cotton seed oil
- Fiehes test for honey
- Colophony is identified by copper acetate
- Baudouin test – sesame oil

- **Quantitative tests/chemical assays**

- Useful in determining single active constituent or group of related constituents present in the same drug.
- To know the purity of drug
- These may be titrimetric methods or spectroscopic methods
  - Alkaloidal content of a drug eg. Morphine in Opium, Reserpine in Rauwolfia
  - Digoxin in Digitalis
  - Eugenol in clove
  - Aldehydes in lemon oil
  - Cinnamic acid and benzoic acid in resins
  - Acid value, saponification value, ester value, iodine value – estimation of gums, resins, balsams, volatile oils, fixed oils, etc.

- **Phytochemical investigation**

- It is a Systematic investigation
- Involves 4 steps

- Procurement and authentication of raw material
- Extraction, purification and characterization of the constituents and in process quality control
- Investigations of biosynthetic pathways to particular compounds
- Quantitative evaluation

- **Extraction**

- Separation of active substances from pure drug involves use of different solvents.
- Dried plant material is used for extraction.
- Choice of solvent depends on active constituents.
- Petroleum ether: fixed oils, volatile oils, steroids, aglycones
- Chloroform and ether: alkaloids, quinones
- Alkaloids – basification is necessary
- Glycosides – water soluble and alcohol soluble
- Tannins – soluble in water, alcohol, ethyl acetate
- Process is either maceration with agitation, percolation or continuous hot extraction

- **Successive solvent extraction**: based on polarity – from non polar solvent to polar solvent

- Air dried plant material is extracted in soxhlet assembly with petroleum ether, benzene, solvent ether, chloroform, acetone, ethanol, methanol and water.
- Extraction with these solvents can also be prepared by maceration or cold extraction.
- Each time before extraction with next solvent, plant material is dried at $\leq 50^0$C in an oven
- Extract is concentrated by distilling off the solvent
- Evaporating to dryness on water bath
- Extract is weighed and percentage is calculated on air dried basis
- Colour and consistency are noted

- **Purification**

- To remove impurities like chlorophyll, pigments, inorganic and organic acids, resins, fatty substances, etc.
- Commonly by partitioning between two immiscible solvents or precipitation of desired compound or impurity by certain reagent.
- finally by fractional crystallization, fractional liberation, fractional distillation, sublimation, steam distillation.

- **Characterization or qualitative chemical examination**

- Detection of alkaloids – dragendroffs test
- Detection of glycosides – hydrolysis followed by molisch test, benedicts test, fehlings test
- Detection of phytosterols – Liebermann burchard test, salkoswki test
- Detection of fixed oils and fats – solubility in petroleum ether, oil stain test, soap formation
- Detection of saponins – foam test
- Detection of phenolic compounds and tannins – ferric chloride test
- Detection of proteins and amino acids – ninhydrin test, biuret test, xanthoproteic test
- Detection of gums and mucilages – ruthenium red test, lead acetate test
- Detection of volatile oils

PHYSICAL EVALUATION

- Physical standards are used.
- These are rarely constant.
- Mostly done for unorganized drugs.
  - Moisture content
  - Viscosity
  - Melting point
  - Solubility
  - Optical rotation
  - Refractive index
  - Ash values
  - Extractive values
  - Volatile oil content
  - Foreign organic matter
  - Chromatography
  - spectroscopy

PHYSICAL EVALUATION

- Spectroscopy
  - UV-Visible
  - IR
  - NMR
  - Mass
- Chromatography – Separation technique
  - Column chromatography
  - Paper chromatography
  - Thin layer chromatography
  - HPLC
  - GC
  - HPTLC

BIOLOGICAL EVALUATION

- Conformity of therapeutic activity of raw material and finished product
- When the estimation of potency of crude drug or its preparations is done by means of its effect on living organisms like bacteria, fungal growth, entire animal or animal tissues, it is known as Bioassay.
- Bioassay is a measure of sample being tested capable of producing biological effect as that of standard preparation.
- Activity is represented in International Units (IU)
- Eg: Digitalis – 1 IU contained in 76 mg of standard preparation
- Biological assays are 3 types
    - Toxic method: whole animal is used
    - Symptomatic method: whole animal is used
    - Tissue method: done on isolated organ or tissue

BIOLOGICAL EVALUATION

- Toxicity studies are performed in suitable animal models to decide the lethal dose and effective dose of crude drugs. Generally mice are used.
- Pigeons are used to assay digitalis glycosides by transfusing the drug through alar vein to the blood stream and observing the lethal effects.
- The drugs that have an effect in eyes are assayed on rabbits eye.
- Effect of ergot are carried out on cocks comb or rabbits intestine or its uterus.
- Next to the animals, the studies are carried out in human beings also.
- Microbiological assays are carried out to determine the effects of drug in various microorganisms, and this is employed in the identification of antimicrobial drugs.
- In other microbiological methods, the living bacteria yeast moulds are used for assaying vitamins.