بسم الله الرحمن الرحيم

434 PHG

Recent Approaches in Medicinal Plant Analyses

الطرق الحديثة لتحليل النباتات الطبية

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Reference books

1. Quality Control of Herbal Drugs By Dr. Pulok K. Mukherjee.

2. Quality control methods for medicinal plant materials.
(World Health Organization Geneva)

Questions to be answered after completion of the course

1.What are major evaluation parameters for herbal products?

2.Describe the methods of detection for evaluation of herbal products?

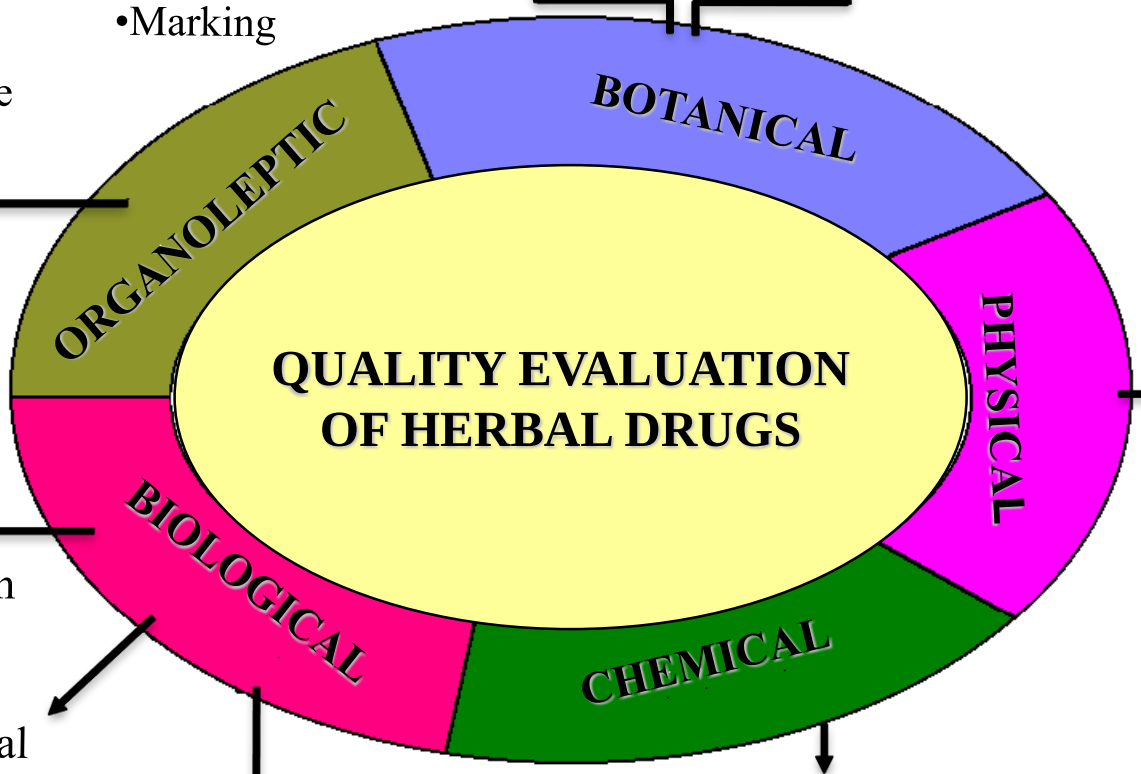
3. How you will define standardization of herbal products?

4. What is marker compound?

5. What are the recent requirements which need to be focused during standardization of herbal drugs and formulations?

Evaluation of crude drugs

Standardization & Quality Evaluation of Herbal drugs



- Colour
- Odour
- Taste
- Texture

- Shape
- External
- Marking

Macroscopic

Microscopic

- Qualitative
- Quantitative
- Scanning Electron Microscopy (SEM) Studies
- Powder Studies

- Moisture Content
- Extractive values
- Ash values
- Fluorescence analysis

Microbial Contamination

- Toxicological
- Pharmacological
- Other specific activities

Antagonistic

- Bacterial
- Fungal

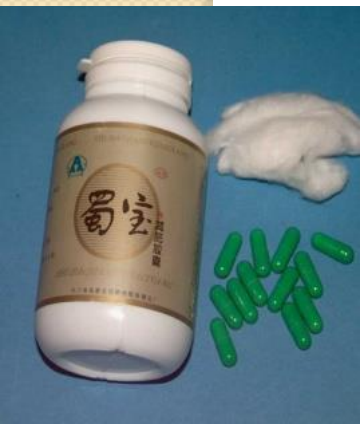
- Qualitative (Chemical tests)
- Quantitative
- Chromatography
- Heavy metal
- Pesticide residue
- Mycotoxin

HPTLC
GLC
HPLC

- HPTLC Finger printing (Secondary Metabolites , DNA Finger printing)

Evaluation of Herbal Products

1. Methods of Identifications.
2. Detection of foreign matters.
3. Assay methods for active constituents.
4. Safety Measures.



- Quality control for efficacy and safety of herbal products is of paramount importance. **Quality** can be **defined** as the status of a drug that is determined by identity, purity, content, and other chemical, physical, or biological properties, or by the manufacturing processes.
- **Quality control** is a term that refers **to processes** involved in maintaining the **quality** and **validity** of a manufactured product.
- **In general**, all medicines, whether they are of synthetic or of plant origin, should fulfill the basic requirements of being efficacious and safe, and this can be achieved by suitable clinical trials.

- **Several problems not applicable to synthetic drugs influence the quality of herbal drugs:**

1. Herbal drugs are usually mixtures of many constituents.
2. The active principle(s) is (are), in most cases unknown.
3. Selective analytical methods or reference compounds may not be available commercially.
4. Plant materials are chemically and naturally variable.
5. The source and quality of the raw material are variable.
6. The methods of harvesting, drying, storage, transportation, and processing (for example, mode of extraction and polarity of the extracting solvent, instability of constituents, etc.) have an effect.

- The term **“herbal drugs”** denotes plants or plant parts that have been converted into phytopharmaceuticals by means of simple processes involving harvesting, drying, and storage.
- **A practical** addition to the definition is also to include other crude products derived from plants, which no longer show any organic structure, such as **essential oils, fatty oils, resins, and gums.**
- **Derived or isolated compounds in the processed state such as extracts or even isolated purified compounds (e.g. strychnine from *Strychnos nux-vomica*) or mixtures of compounds (e.g. abrin from *Abrus precatorius*) are, as a rule, <u>not included in the definition</u>.**

- **In general, quality control is based on three important pharmacopoeial definitions:**

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

- **Identity** can be achieved by macro- and microscopical examinations.
- Voucher specimens are reliable reference sources. Outbreaks of diseases among plants may result in changes to the physical appearance of the plant and lead to incorrect identification.
- At times an incorrect botanical quality with respect to the labeling can be a problem.
- For example, in the 1994s, a South American product labeled as “Paraguay Tea” was associated with an outbreak of anticholinergic poisoning (tachychardia, dry skin and mouth, agitation and flushed skin) in New York. Subsequent chemical analysis revealed the presence of a class of constituents that was different from the metabolites normally found in the plant from which Paraguay tea is made **(belladonna alkaloid).**

- **Purity** is closely linked with the safe use of drugs and deals with factors such as ash values, contaminants (e.g. foreign matter in the form of other herbs), and heavy metals. However, due to the application of improved analytical methods, modern purity evaluation also includes microbial contamination, aflatoxins, radioactivity, and pesticide residues.
- Analytical methods such as photometric analysis, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC) can be employed in order to establish the constant composition of herbal preparations.

- **Content or assay** is the most difficult area of quality control to perform, since in most herbal drugs the active constituents are not known. Sometimes markers can be used. In all other cases, where no active constituent or marker can be defined for the herbal drug, **the percentage extractable matter with a solvent may be used as a form of assay, an approach often seen in pharmacopeias.**
- The choice of the extracting solvent depends on the nature of the compounds involved, and might be deduced from the traditional uses. For example, when a herbal drug is used to make a tea, the hot water extractable matter, expressed as milligrams per gram of air-dried material, may serve this purpose.

- Special form of assay is the determination of essential oils by steam distillation.
- When the active constituents (e.g. **sennosides** in *Senna*) or markers (e.g. **alkylamides** in *Echinacea*) are known, a vast array of modern chemical analytical methods such as ultraviolet/visible spectroscopy (UV/VIS), TLC, HPLC, GC, mass spectrometry (MS), or a combination of GC and MS (GC/MS), can be employed.

- **Standardization** involves adjusting the herbal drug preparation to a defined content of a constituent or a group of substances with known therapeutic activity by adding excipients or by mixing herbal drugs or herbal drug preparations.
- **Botanical extracts** made directly from crude plant material show substantial variation in composition, quality, and therapeutic effects.
- **Standardized extracts** are high-quality extracts containing consistent levels of specified compounds, and they are subjected to rigorous quality controls during all phases of the growing, harvesting, and manufacturing processes.

- No regulatory definition exists for standardization of dietary supplements. As a result, the term “standardization” may mean many different things. Some manufacturers use the term standardization **incorrectly** to refer to uniform manufacturing practices; following a recipe is not sufficient for a product to be called standardized. Therefore, **the presence of the word “standardized” on a supplement label does not necessarily indicate product quality.** When the active principles are unknown, marker substance(s) should be established for analytical purposes and standardization.
- **Marker** substances are chemically defined constituents of a herbal drug that are important for the quality of the finished product. **Ideally**, the chemical markers chosen would also be the compounds that are responsible for the botanical’s effects in the body.

- **There are two types of standardization**

1- In the first category, "**true**" standardization, a definite phytochemical or group of constituents is known to have activity. **Ginkgo** with its **26% ginkgo flavones and 6% terpenes is a classic example.** These products are highly concentrated and no longer represent the whole herb, and are now considered as phytopharmaceuticals. In many cases they are vastly more effective than the whole herb.

2- The other type of standardization is based on manufacturers guaranteeing the presence of a certain percentage of marker compounds; these are not indicators of therapeutic activity or quality of the herb.

Standardization of Herbal drugs- (Raw Drugs)

- Passport data of Raw Plant Drugs (Crude drugs)
- Correct taxonomic identification & authentication.
- Study on the medicinal part: root, stem, bark, leaves, flowers, fruits, nuts, gum, resins etc.
- Collection details: Location, stage & development/ growth of the plants, time, pre-processing storage etc.
- Organoleptic examination of raw drug:
  - Evaluation by means of sensory organs: touch, odour, taste.
- Microscopic & molecular examination.
- Chemical composition (TLC, GLC, HPLC, DNA fingerprinting).
- Biological activity of the whole plant.
- Shelf life of raw drugs.

Standardization of Herbal drugs- (Herbal Formulation)

- Follow defined Good Manufacturing Practices (GMP).
- Scientific Verification.
  - Toxicity evaluation.
  - Chemical profiling.
  - Pharmacodynamics – effect of drug in the body.
  - Pharmacokinetics – absorption, distribution, metabolism, mechanism of action and execution.
  - Dosage.
  - Stability and shelf life.
  - Presentation and Packing.
  - Therapeutic merits – Compared with other drugs.

Good Practices/Techniques in Herbal Products

- Good Survey of literature (Ancient & Modern).
- Develop and Observe Norms of:
  - Good Agricultural Practices (GAP).
  - Good Collection/Harvesting and Post Harvest Handling Practices (GCP/ GHP & GPHP).
  - Good Laboratory Practices (GLP).
  - Good Clinical Practices (GCP).
  - Good Manufacturing Practices (GMP).
  - Good Marketing Techniques (GMT).

A. Organoleptic evaluation

Definition: Organoleptic evaluation of drugs refers to the evaluation of a drug by colour, odour, size, shape, taste and special features including touch, texture etc.

= Organs of sense = Macroscopic appearance of drug.

- The majority of information on the identity, purity and quality of the material can be drawn from these observations, they are of primary importance before any further testing can be carried out.
- Authentic specimen of the material under study and samples of pharmacopoeial quality should be available to serve as a reference.
- This evaluation procedure provides the simplest and quickest means to establish the identity and purity and thereby ensure quality of a particular sample.

- If it is found to be devoid of or significantly different from the specified sensory characters like colour, consistency, odour, etc., it is considered as not fulfilling the requirements.
- ***However judgment based on the sensory characteristics like odour, taste etc., may vary from person to person and time to time based on individual's nature.*** So the description of this features are very difficult so that often the characteristic like odour and taste can only described as 'characteristic' and reference made to the analyst's memory.
- No preliminary treatment is necessary for evaluating the sample in this manner excepting the softening and stretching of the wrinkled and contracted leaves and flowers etc.

B - Microscopical evaluation

Dealing with microscopic appearance of the herb in sectional view and in powdered form.

Microscopical evaluation is useful in the study of:

1- Histologic elements of herbs.
2- Detection of adulterant.
3- Quantitative microanalysis.
4- Study of the constituents by application of chemical method to small quantities of powdered drug (called chemo microscopy).

❖**Histology:**

refers to the **character** and **arrangement** of these tissues as they are present in the herb.

Histologic studies include:

1- Very thin transverse (radial) section.
2- Longitudinal (tangential) sections (entire organ).

Powdered herbs posses **very few** macroscopic features of identification outside of color, odor and taste.

Keys for the identification of powdered crude drugs:

1- Leaves:

a- Trichomes (glandular and nonglandular).

b- Crystals of calcium oxalate.

c- Stomata.

d- Epidermis cells, palisade.

e- Vessels.

2- Flower:

a- Trichomes (glandular and nonglandular).

b- Epidermis, stigma, anther.

c- Pollen grain.

d- Volatile oil.

3- Fruits and seeds:

a- Starch.

b- Aleurone.

c- Sclerenchyma.

d- Vitta.

e- Endocarp.

4- Bark & wood:

a- Phloem, xylem.

b- Tracheides, Parenchyma, wood Parenchyma.

c- Fibers, medullary rays, cork, cambium.

In some cases, the drug may have the **same diagnostic** element, **they are known as closely related species**. So, microscope is not the method of choice.

Microscopical numerical value:

- It is used to identify **closely related species**.

a-Microscopical linear measurement

Used **only** in **Root, Rhizomes, Bark.**

E.g1., distinguished between Cinnamon القرفه السيلانيه (as quill) and Cassia القرفه الصينيه (as flat)

[Both have same diagnostic element].

So differentiate between both by:

Microscopical linear measurement	Cinnamon	Cassia
1- Diameter of starch granules	< 8 µm	> 10µm
2- Width of phloem fiber	30 µm	30-45 µm

Active constituent	Cinnamon	Cassia
Volatile oil	0.7-1% v/w	1-2%v/w
Cinnamic aldehyde	60-75%	Not less than 85%
Phenolics	4-10%	-
Eugenol	10%	-
Tast	Astringent	More astringent
Cost	Cheap	Cheaper

E.g2., Rio Ipecacuanha and Cartagena Ipecacuanha

Starch granule

Rio Ipecacuanha — **15 µm**

Cartagena Ipecacuanha — **17-20 µm**

Why we need to differentiate between Rio and Cartagena Ipeca?

Because Rio Ipeca contains more emetine alkaloid.

Microscopical numerical value (cont.):

If closely related species are **leaves**, we need to determine

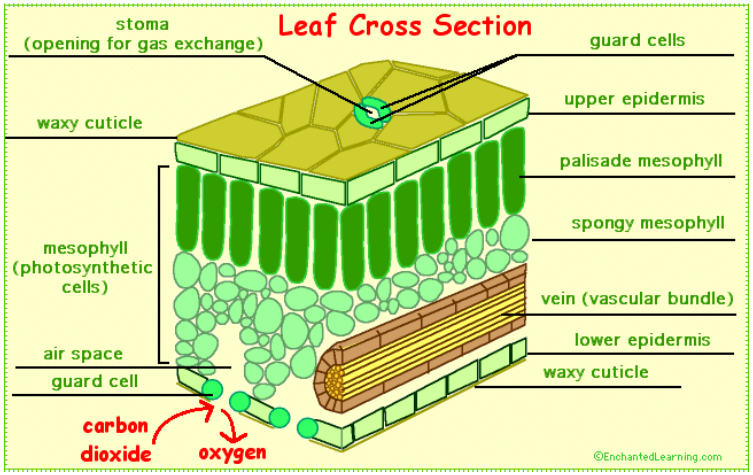
b- Ratio value (leaf contents) :
(Microscopical quantitative value)

- Palisade ratio.
- Stomata index.
- Vein islet number.
- Veinlet terminate number.

1- Palisade ratio:

Def: Average numbers of palisade cell under one epidermal cell using four continuous epidermal cells for the count.

To do the ratio value is determined by "camera lucida".



2- Stomatal index (%):

Def: it is the percentage of the number of stomata to the total number of epidermal cells including the stomata, each stomata being counted as one cell.

Stomatal index= S/E+S x 100

(S) Number of stomata per unit area

(E) Number of epidermal cells in the same unit area.

Cassia angustifolia (both surface) 17.1-18.7-20

Cassia acutifolia (both surface) 11.4- 12.2-13

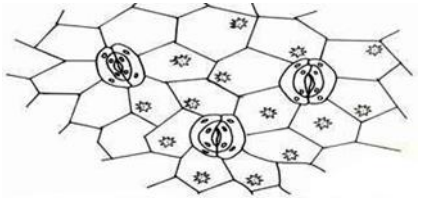


Fig. 2a: Lower leaf epidermis of X. aethiopica (x 400).

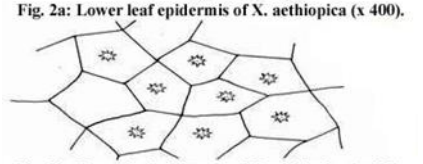


Fig. 2b: Upper leaf epidermis of X. aethiopica (x 400).

Determination of stomatal numbers:

It is an average number of stomata per mm^2 of epidermis.

	Stomatal number	
Plant	**Upper surface**	**Lower surface**
Atropa belladonna	**7.5-10-17.5**	**77.5-113-176.5**
Atropa acuminate	**6-14-37.5**	**62.5-93-174**

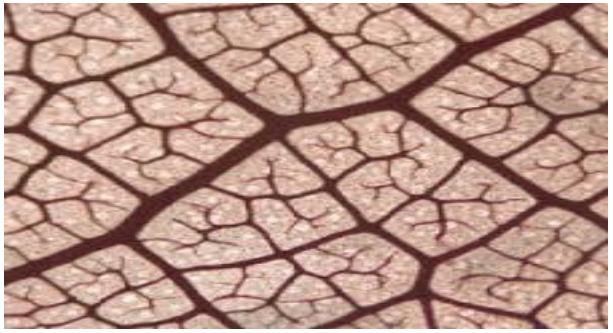
3- Vein islet number:

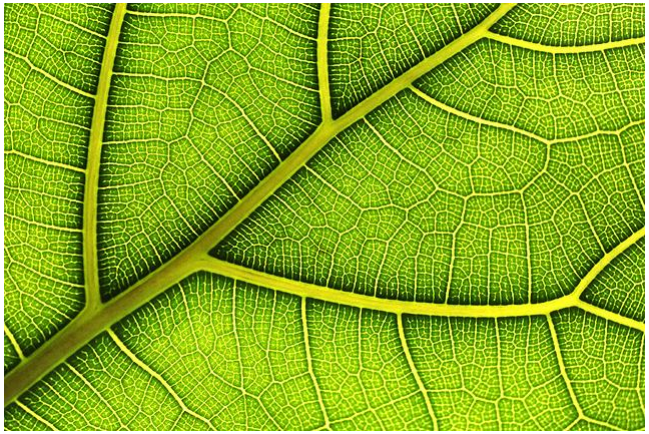
Def of vein islet: The small areas of green tissue outlined by the veinlets are termed vein islet.

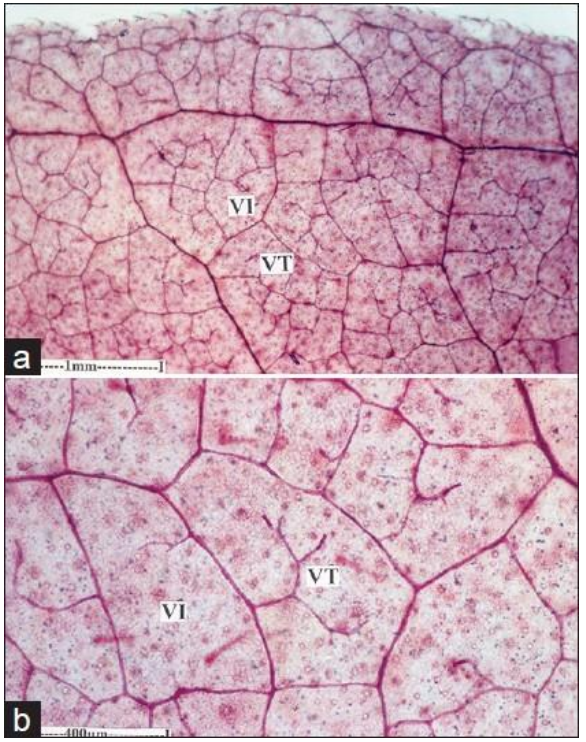
Def of vein islet number: is the number of vein islet per mm^2.

Cassia angustifolia: 25-30

Cassia acutifolia: 19-23







VI= Vein islet

VT= Veinlet terminate

4- Veinlet terminate number:

Def: it is the number of veinlet termination per mm^2 of leaf surface.

- A veinlet termination:Is the ultimate free termination of a veinlet or branch of a vienlet.

- It can be used to distinguish between leaves of closely related species.

Atropa belladonna **6.3-10.3**
Digitalis purpurea **2.5-4.2**
Hyoscymus niger **12.4-19.0**

- This value has been shown to be constant for any species and unaffected by the age of the plant or the size of the leaves.

How to determined the mm^2 ?

By using Eye-peice micrometer and stage micrometer

C- Chemical Evaluation-:

Chemical evaluation is include:

1-Isolation

2-Purification and identification of active constituents

3- Chemical test

4- Assay

5- It also includes phytochemical evaluation

1- Isolation of active constituents:

A- By chemical solvents:

1- Micro-extraction

2- Micro-filtration

3- Micro-crystallization

B- By micro-sublimation

A- By chemical solvents:

1- Micro-extraction:

Def:

It is a separation of the constituents from a small quantity of the drug and depends on the solubility of the constituents in a solvent.

The factors must be considered during micro-extraction:

- State of division of the drug
- Type of solvent used
- Temperature
- Nature of impurities
- Nature of substances

1-If soluble in polar solvent means it is a polar compounds.

2- If soluble in non-polar solvent means it is a non-polar compounds.

3-All substances soluble in 90-95% alcohol.

2-Micro-filtration methods:

To secure small quantities of the extracted substances in a clear solution.

3-Micro-crystallization:

To obtain the extracted constituent in a pure form necessitates crystallization and re-crystallization.

B- Micro-sublimation:

1- It is refer to a method of obtaining a **constituent of a drug** by heating the drug to **vaporize** its chief constituent to a **gaseous state** and then **condensing** the vapor back into **a solid form**.

2- This method is employed only when the drug or its constituents are not decomposed by heat.

3- When the constituent condenses on a cool place, the resulting crystals develop in a pure form.

4- Caffeine is sublimed from powdered Kola or from powdered coffee.

2- Identification of constituents:

A- By crystallography
B- By melting point determination
C- By confirmative test
    1- Chemical test.
    2- Physical test

A- By crystallography: It is a science dealing with:

i- Classification of crystals

ii- Form of crystals

iii- Structure of crystals

iv- Properties of crystals e.g., crystal is:

- Isotropic
- Anisotropic
- Uniaxial
- Biaxial

B- By melting point determination:

It is very important as a means of identifying pure substances.

C- By confirmative test:

1- Chemical test.

2- Physical test.

The use of the **petrographic microscope** is very important in the determination of the **optic constants** of crystalline substances. It is a rapid method for identification of **very small amounts of chemical compounds.**

3- Chemical test

The active constituents should be extracted and purified before applying the chemical test.

Preliminary chemical test for active constituents: (Qualitative test)

1- Test for glycosides and carbohydrate

2- Test for cardiac glycosides

3- Test for anthraquinone glycosides

4- Test for flavonoids

5- Test for saponin

6- Test for tannins

7- Test for alkaloids