

School of Medical and Allied Sciences

Course Code : PCY 410

Course Name: Standardization of Herbal Drug

Module 1

Herbal Drug Technology

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Disclaimer

All the content material provided here is only for teaching purpose.

The logo of Galgotias University is a stylized circular emblem. It features a central white swirl that transitions into a light blue swirl, which then transitions into a yellow swirl, and finally into a light pink swirl. The overall effect is a sense of motion and energy.

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The logo of Galgotias University is a circular emblem with a stylized, multi-colored swirl in the center. The swirl consists of several curved segments in shades of yellow, orange, and light blue, set against a light pinkish-red background. The entire logo is semi-transparent and centered on the slide.

Herbs as raw Material

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HERBS

- ARE - crude plant material such as leaves, flowers, fruit, seed, stems, wood, bark, roots, rhizomes or other plant parts, which may be entire, fragmented or powdered.

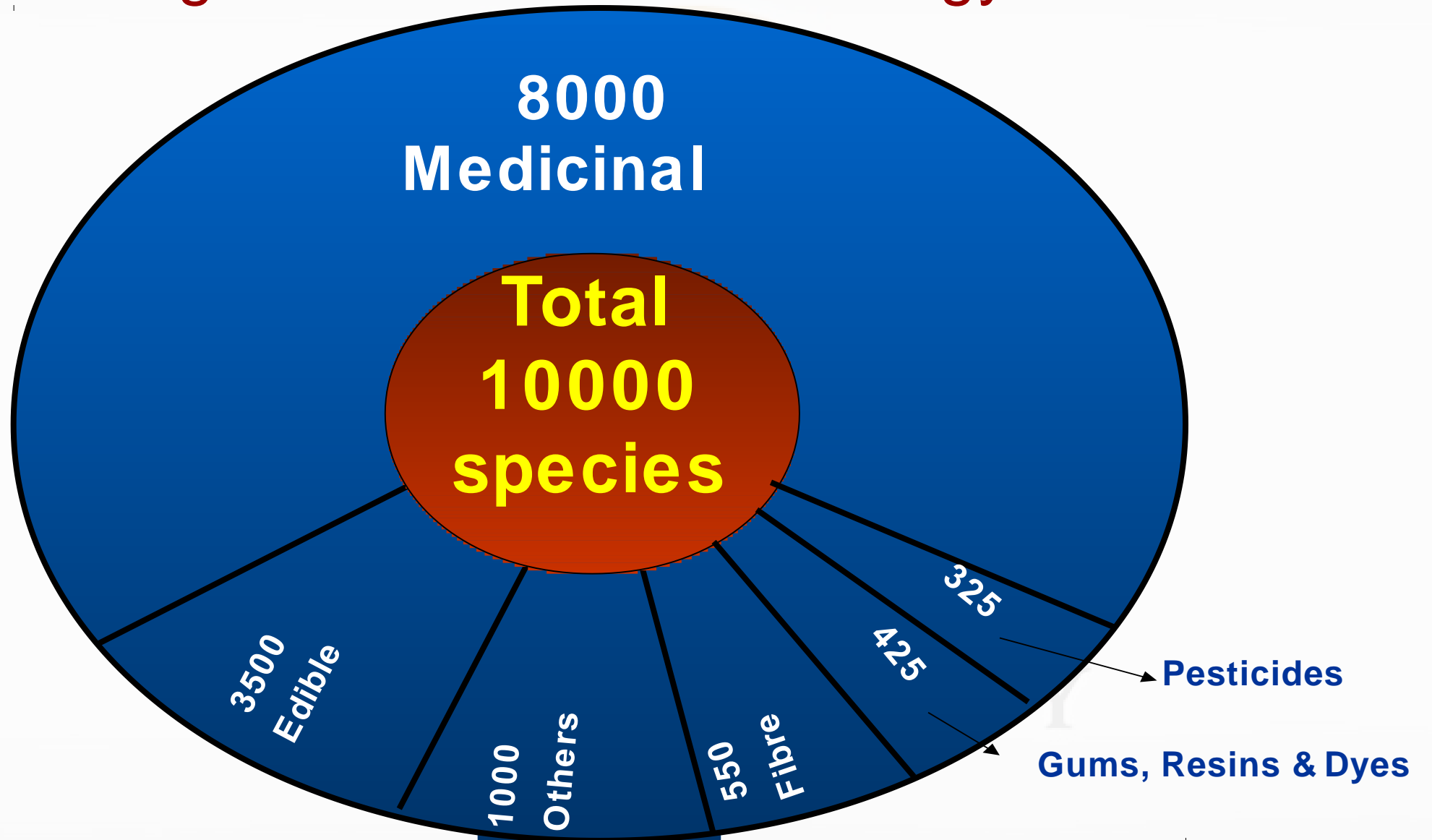
HERBAL DRUGS/ HERBAL FORMULATION

- ARE – Finished labelled products that contain active ingredients such as aerial or underground parts of plant or other plant material or combinations thereof, whether in the crude state or as plant preparations.

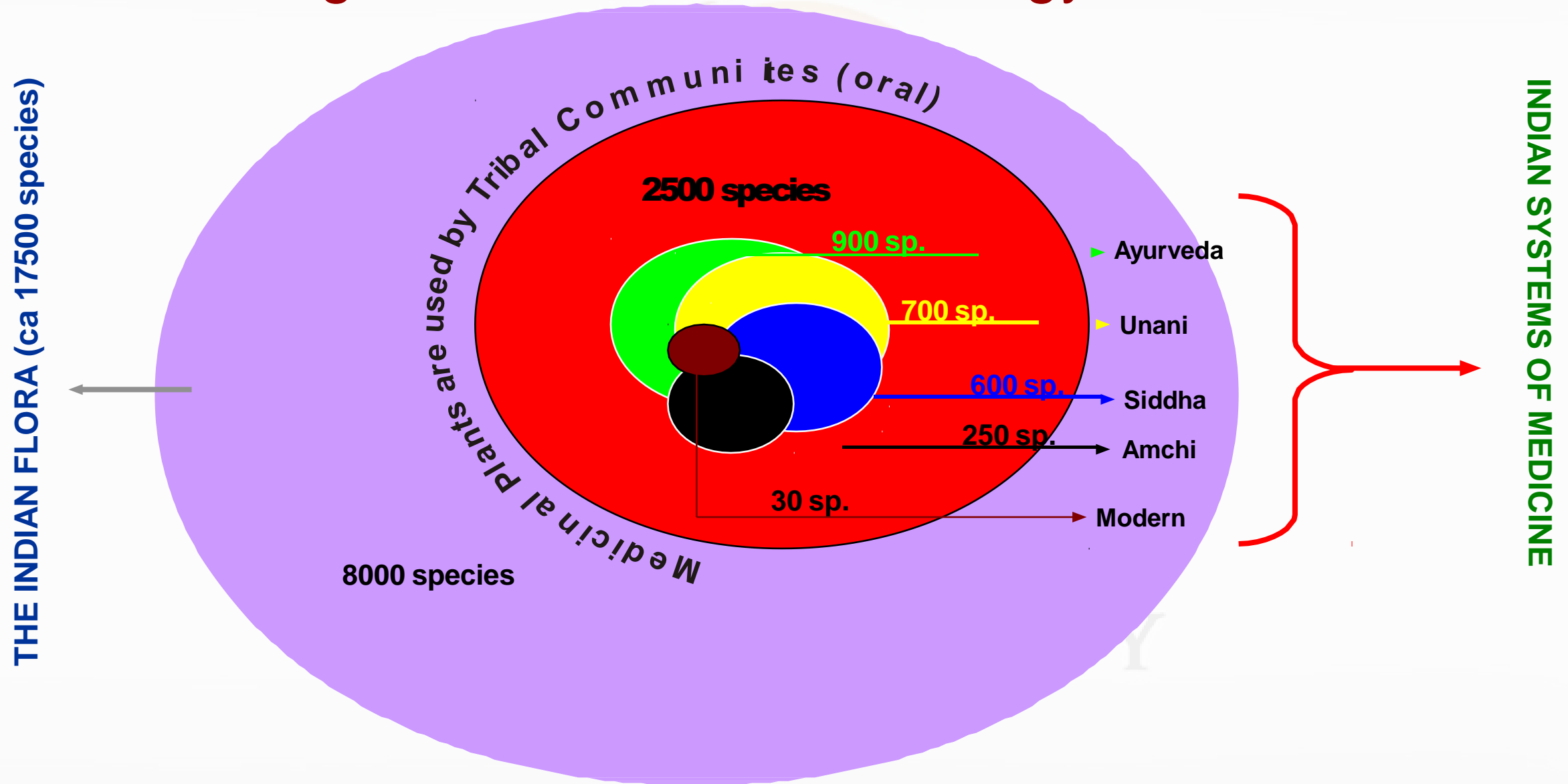
HERBAL DRUGS

- **Phytomedicines or Phytopharmaceuticals sold as Over The Counter (OTC) products in modern dosage forms such as Tablets, Capsules & Liquids for oral use.**
- **Dietary Supplements containing Herbal Products, also called Nutraceuticals available in modern dosage forms.**
- **Herbal Medicines consisting of either Crude, Semi Processed or Processed Medicinal Plants.**

India's strength in Herbal Technology



India's strength in Herbal Technology



Selection of herbs

- **Random selection**
- **Using Ethnopharmacological report**
- **Chemotaxonomic**
- **Geographical**
- **Compound Structure**



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WELCOME!

The new NAPRALERT web platform accommodates two query levels.

1. Free searching of NAPRALERT

Once registered, selective queries of Organism, Pharmacology Chemistry and Author are supported through our free searching platform.

Free reports will be limited to a total of 50 citations per week, up to a total of 800 citations per 12-month period for registered Academic users and 400 citations per 12-month period for non-Academic users.

If your query or queries of interest exceed annual limits for free searching, you will receive your report in installments. Data can be made available immediately using the ANYTIME REFERENCES fee-based service option, when citation limits on free searches have been reached.

Reports are available on your My Reports page. Detailed instructions on query strategies and report generation are available in the help.

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ANYTIME REFERENCES

- Selective queries of the database, including Organism, Pharmacology, Chemistry and Author are available.
- Academic users will be charged \$0.50 per citation, Non-academic users will be charged \$1.00 per citation.
- Once a distinct citation has been purchased, users will not be charged for the same citation generated in any additional NAPRALERT queries.
- Once registered, using NAPRALERT secure server (transmitted via 128-bit encryption for your security) via the University of Illinois payment system, pre-purchase citation credits, available in \$100 increments.
- Reports are available on your My Reports page. Detailed instructions on query strategies and report generation are detailed in the help.

SUPPORTING MEMBERSHIP

- \$5000 minimum purchase required
- 10% discount per citation will be applied to \$5000 membership level, 20% discount per citation will be applied to \$10,000 or more.
- Data can be output in either html-based text or in sortable (csv) format



Organism

Organism information

Family: Genus: Species: Sub-taxon:

Country:

Information type

Reviews can only be selected alone.

Ethnomedical Information Compound List Review only

Experimental Biological Testing Type

All In Vivo In Vitro In Situ

Human Clinical Plants

Pharmacologies

Citation parameters

From year: To year: Authors:

Identification of plants

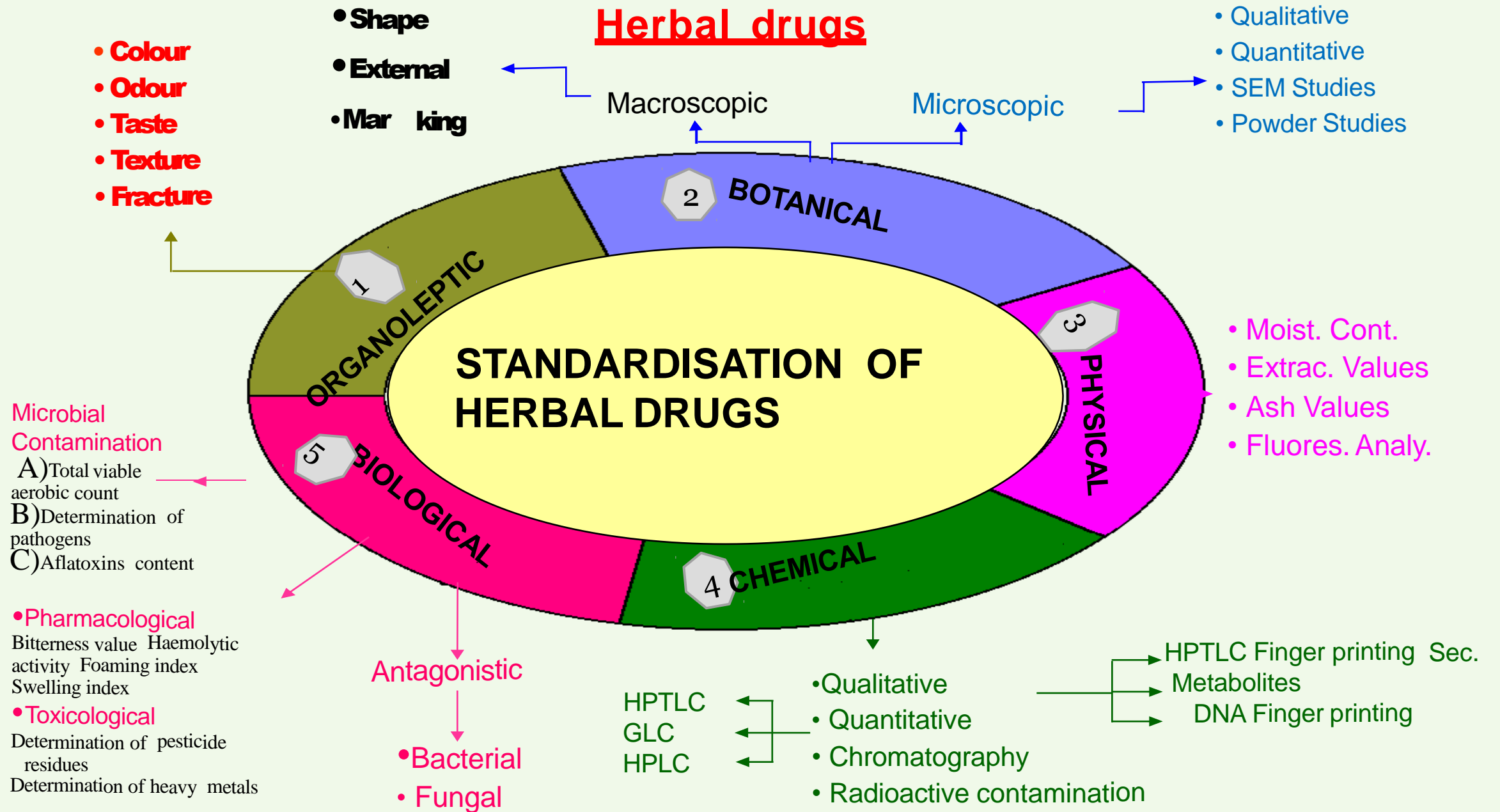
- **Homonyms : Sharing of a popular name by 2 or more species**
- **Systematic botanist can solve the problem**
- **Plant material should be identified in a local national herbarium by taxonomist**
- **A card with details of place, altitude, environment and characteristics along with sample**

Identification of plants (comparison)

- 1. Current scientific literature**
- 2. Monograph from National Pharmacopoeia**
- 3. Botanical Authentication**
- 4. DNA based technique using marker compound**

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Herbal drugs



Guidelines for Quality Control of Herbal formulation

- Quality control of crude drugs material, plant preparations and finished products
- Stability assessment and shelf life
- Safety assessment; documentation of safety based on experience or toxicological studies
- Assessment of efficacy by pharmacological informations and biological activity evaluations

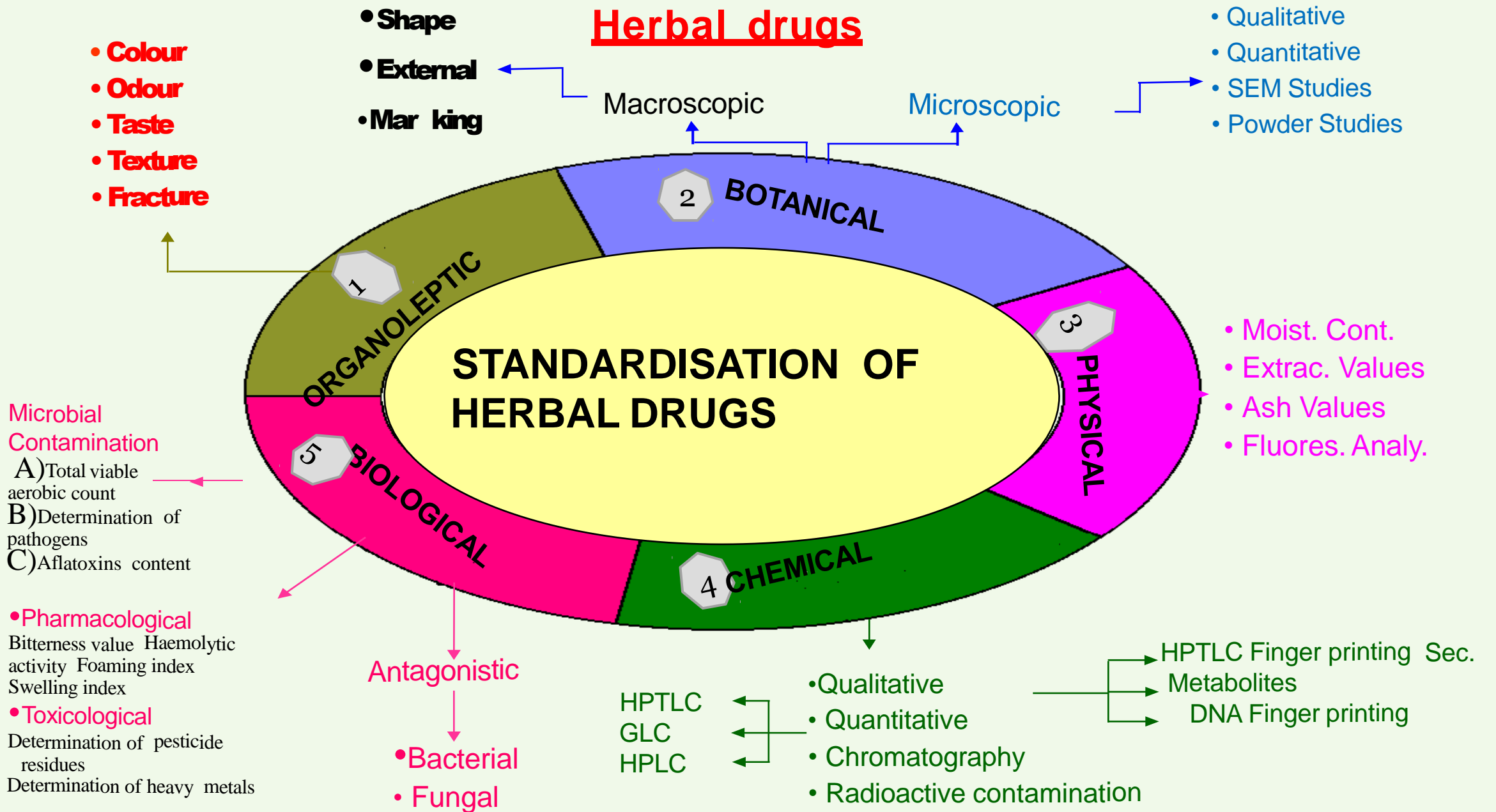
STANDARDISATION

- Standardization of drug means
“confirmation of its identity and determination of its quality and purity and detection of nature of adulterant by various parameters like morphological, microscopical, physical, chemical and biological observations.”



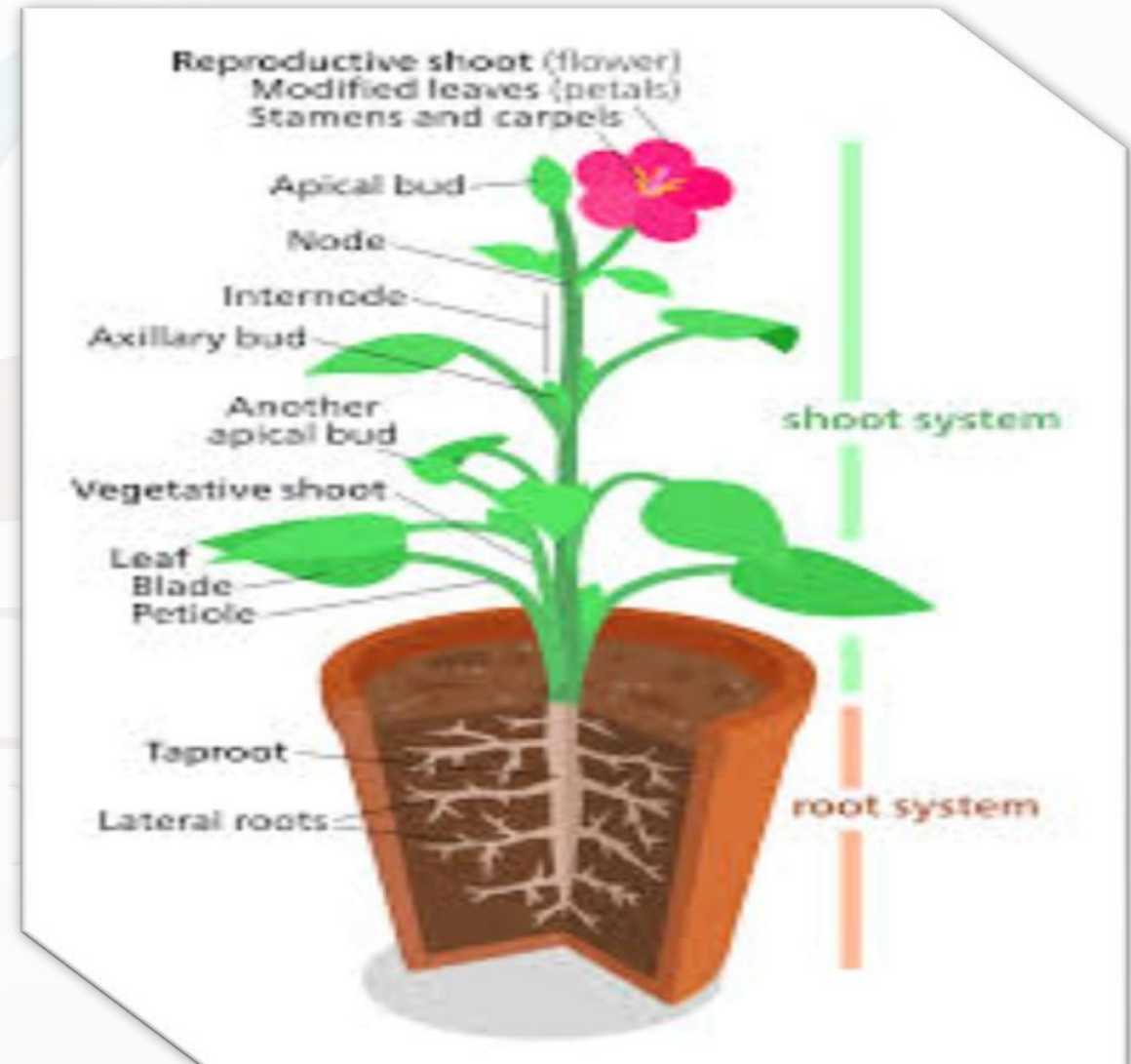
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Herbal drugs



Macroscopic study

- Visual inspection provides the simplest and quickest means by which to establish identity, purity and quality.
- Macroscopic identity of medicinal plant materials is based on shape, size, colour, surface characteristics, texture, fracture characteristics and appearance of the cut surface.



Microscopic study

- Detail of cell structure and arrangement of the cells useful for differentiating similar species.
- Select a representative sample of the material & If it is dried parts of a plant than it may require softening before preparation for microscopy, preferably by being placed in a moist atmosphere, or by soaking in water.
- Any water-soluble contents can be removed from the cells by soaking in water. Starch grains can be gelatinized by heating in water.



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- Histochemical detection

- Starch grains
- Aleurone grains
- Fats, fatty oils, volatile oils and resins
- Calcium oxalate/carbonate crystals
- Lignified cell wall
- Cellulose cell wall
- Mucilage
- Tannin

- Measurement of specimen

- Stomatal number
- Stomatal index
- Palisade ratio
- Vein-islet number
- Vein termination number
- Lycopodium spore method

Foreign organic matter

- Parts of the medicinal plant material or materials other than those named with the limits specified for the plant material concerned;
- Any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned;
- Mineral admixtures that is adhering to the medicinal plant materials, such as soil, stones, sand, and dust.

Foreign matter: NMT 2% w/w



Ash value

- It involves non-volatile inorganic components.
- High ash value is the indicative of contamination, substitution, adulteration or carelessness in preparing the crude drugs.



Total ash

- Total ash is designed to measure the total amount of material produced after complete incineration of the drug material at as low temperature as possible (about 450°C) to remove all the carbons.
- Total ash usually consists of carbonates, phosphates, silicates and silica.
- IP and USP: 675±25°C
- BP : 600±25°C
- WHO: 500-600°C

Acid insoluble ash

- Ash insoluble in HCl is the residue obtained after extracting the total ash with HCl. It gives idea about the earthy matter
- IP method: 25mL 2M HCL solution USP method: 25mL
- 3N HCL solution BP method: 15mL water and 10mL
- HCL
- WHO method: 25 ml of hydrochloric acid (~70g/l)

Water soluble ash

- Total ash content which is soluble in water. It's good indicator of presence of previous extraction of water soluble salts in the drug or incorrect preparation or amount of inorg. matter
- Carbonated ash: Ash is treated with ammonium carbonate.
- Nitrated ash: Ash is treated with dilute nitric acid.

Extractive value

- Amount of the active constituents present in crude drug material when extracted with specific solvent.
- There are following Methods for determination of Extractive value.

- a) Cold method
- b) Hot method
- c) Soxhlet method



COLD EXTRACTION METHOD :



Cold extraction :



Cold extracts on water bath

- Water soluble extractive value
- Alcohol soluble extractive value: Solvent strength: 20- 95% v/v
- Solvent Hexane soluble extractive value: Continuous extraction for 20 hours *eg. Phyllanthus amarus*: NLT 3%
- Volatile ether soluble extractive value:
Anhydrous ether- continuous extraction for 20hours
- Nonvolatile ether soluble extractive value:
Drugs having lipid content, fixed oils *eg. Colocynth fruits* : NMT 3% (Pulp- medicinal value)



Insoluble matter:

- Presence of woody matter or vegetable debris or pieces of bark materials.
- Eg. In catechu
Water insoluble matter: NMT 33% Alcohol insoluble matter: NMT 30%

Total solid content

- The residue obtained when prescribed amount of preparation is dried to constant weight under the specified condition (Residue on evaporation)
- Powdered extract: NLT 95%
- Semisolid extract: NLT 70%



Water Content

- Loss on drying (Gravimetric determination)
- Volumetric Azeotropic distillation (toluene distillation) method
- Titrimetric Karl fisher method
- Gas chromatographic method

Volatile oil content

- Volatile oils are the liquid components of the plant cells, immiscible with water, volatile at ordinary temperature and can be steam distilled at ordinary pressure
- Many herbal drugs contain volatile oil which is used as flavouring agent.
- E.g. Clove: NLT 15% v/w



Bitterness value



- Medicinal plant materials that have a strong bitter taste ("bitters") are employed therapeutically, mostly as appetizing agents. Their bitterness stimulates secretions in the gastrointestinal tract, especially of gastric juice.
- The bitter properties of plant material are determined by comparing the threshold bitter concentration of an extract of the materials with that of a dilute solution of quinine hydrochloride.
- The bitterness value is expressed in units equivalent to the bitterness of a solution containing 1g of quinine hydrochloride R in 2000 ml.

- Bitterness value calculated in units per g using the following formula:

$$\frac{2000 \times c}{a \times b}$$

Where,

a= the concentration of the stock test solution (S_T) (mg/ml),

b = the volume of test solution S_T (in ml) in the tube with the threshold bitter concentration,

c = the volume of quinine hydrochloride R (in mg) in the tube with the threshold bitter concentration.

Haemolytic activity

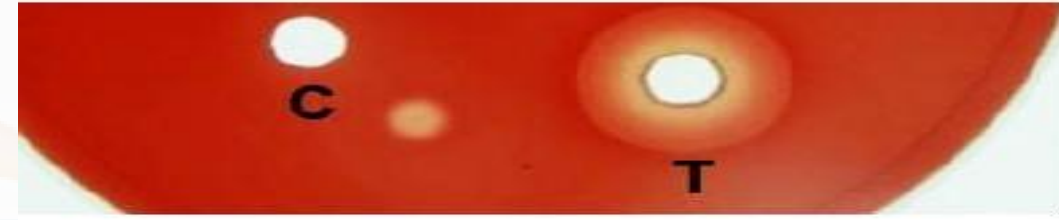


Fig. Hemolytic activity of the crude extract on blood agar plate. (PBS buffer, pH 7.4), T: crude extract.

- Many medicinal plant materials, of the families Caryophyllaceae, Araliaceae, Sapindaceae, Primulaceae, and Dioscoreaceae contain saponins.
- The most characteristic property of saponins is their ability to cause haemolysis; when added to a suspension of blood, saponins produce changes in erythrocyte membranes, causing haemoglobin to diffuse into the surrounding medium.
- The haemolytic activity of plant materials, or a preparation containing saponins, is determined by comparison with that of a reference material, saponin R, which has a haemolytic activity of 1000 units per g.

Serial dilution for the preliminary test

	Tube no.			
	1	2	3	4
Plant material extract (ml)	0.10	0.20	0.50	1.00
Phosphate buffer pH 7.4 TS (ml)	0.90	0.80	0.50	-
Blood suspension (2%) (ml)	1.00	1.00	1.00	1.00

- Calculate the haemolytic activity of the medicinal plant material using the following formula:

$$1000 \times a/b$$

Where,

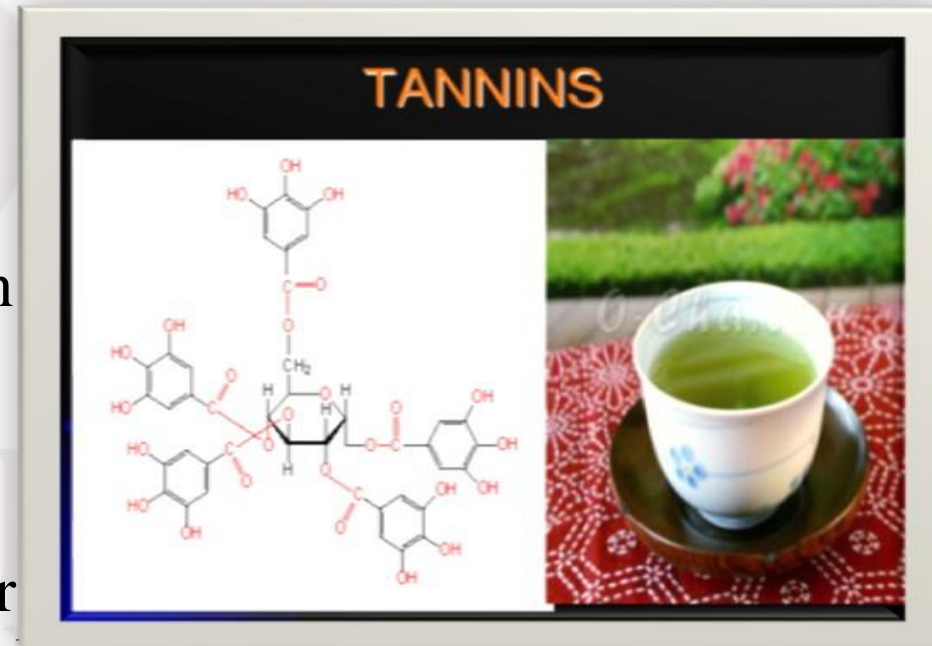
1000 = the defined haemolytic activity of saponin R in relation to ox blood,

***a* = quantity of saponin R that produces total haemolysis (g)**

***b* = quantity of plant material that produces total haemolysis (g)**

Determination of tannins

- Tannins (or tanning substances) are substances capable of turning animal hides into leather by binding proteins to form water-insoluble substances that are resistant to proteolytic enzymes.
- This process, when applied to living tissue, is known as an "astringent" action and is the reason for the therapeutic application of tannins.
- Chemically, tannins are complex substances; usually occur as mixtures of polyphenols that are difficult to separate and



- Calculate the quantity of tannins as a percentage using the following formula:

$$\frac{[T_1 - (T_2 - T_0)] \times 500}{w}$$

where w = the weight of the plant material in grams T1= Weight of material extracted in water
T2= Weight of material not bound to hide powder T0= Weight of hide powder material soluble in water

Determination of swelling index

- The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under specified conditions.
- Its determination is based on the addition of water or a swelling agent as specified in the test procedure for each individual plant material (either whole, cut or pulverized).



Determination of foaming index

- Many medicinal plant materials contain saponins that can cause a persistent foam when an aqueous decoction is shaken.
- The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index. Calculate the foaming index using the following formula:



$$\text{foaming index} = \frac{1000}{a}$$

where a = the volume in ml of the decoction used for preparing the dilution in the tube where foaming to a height of 1 cm is observed.

Determination of pesticide residues

- Not more than 1%
- An ARL (in mg of pesticide per kg of plant material) can be calculated on the basis of the maximum acceptable daily intake of the pesticide for humans (ADI), as recommended by WHO, and the mean daily intake (MDI) of the medicinal plant material.

$$ARL = \frac{ADI \times E \times 60}{MDI \times 100}$$

ADI = **maximum acceptable daily intake** of pesticide (mg/kg of body weight); E = extraction factor, which determines the transition rate of the pesticide from the plant material into the dosage form;

MDI = **mean daily intake** of medicinal plant product.

ARL= Acceptable residual level

Some example of Pesticides :



- Chlorinated hydrocarbons and related pesticides: BHC, DDT
- Chlorinated phenoxyalkanoic acid herbicides: 2,4-D; 2,4,5-T
- Organophosphorus pesticides: malathion, methyl parathion, parathion
- Carbamate insecticides: carbaryl (carbaril)
- Dithiocarbamate fungicides: ferbam, maneb, nabam, thiram, zineb
- Inorganic pesticides: calcium arsenate, lead arsenate
- Miscellaneous: ethylene dibromide, ethylene oxide, methyl bromide
- Pesticides of plant origin: tobacco leaf and nicotine; pyrethrum flower, pyrethrum extract and pyrethroids; derris root and rotenoids

Determination of arsenic and heavy metals

- Contamination of medicinal plant materials with arsenic and heavy metals can be attributed to many causes including environmental pollution and traces of pesticides.
- Limit test for arsenic
- Limit test for cadmium and lead
- The contents of lead and cadmium may be determined by inverse voltametry or by atomic emission spectrophotometry.
- The following maximum amounts in dried plant materials, which are based on the ADI values, are proposed:
 - lead, 10 mg/kg;
 - cadmium, 0.3 mg/kg.



Determination of microorganisms

Test strains and culture media for use in validating the tests for specific microorganisms

Microorganism	Strain number ^a	Medium
<i>Escherichia coli</i>	e.g. NCIMB 8545 (ATCC 8739, CIP 53.126)	lactose broth
<i>Pseudomonas aeruginosa</i>	e.g. NCIMB 8626 (ATCC 9027, CIP 82.118)	soybean-casein digest medium
<i>Salmonella typhimurium</i>	No strain number is recommended. Species not pathogenic for humans, such as <i>Salmonella abony</i> (NCTC 6017, CIP 80.39), may be used	lactose broth
<i>Staphylococcus aureus</i>	e.g. NCIMB 8625 (ATCC 6538 P, CIP 53.156) or NCIMB 9518 (ATCC 6538, CIP 4.83)	soybean-casein digest medium

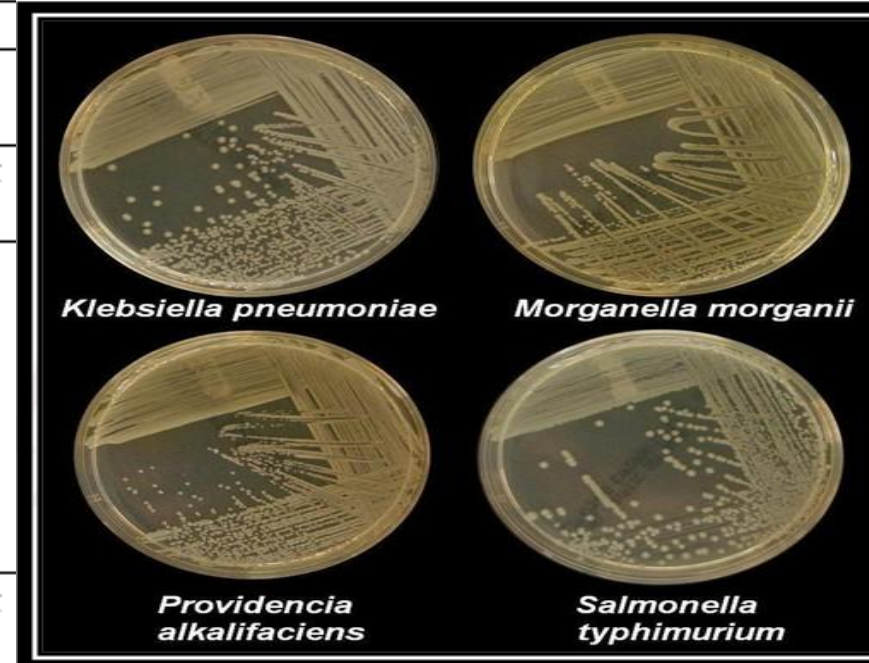
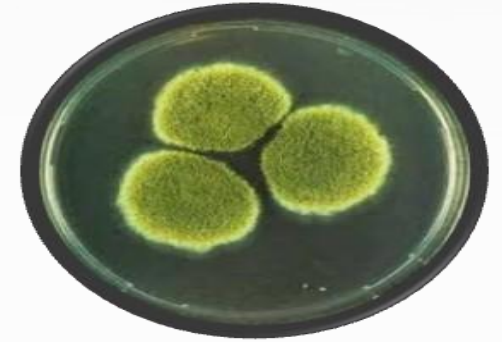


Table 1. Limits for microbial contaminants in finished products & Raw materials

Microorganism	Finished product	Raw materials
<i>E. coli</i>	10^1	10^4
Salmonella	-	-
Total aerobic bacteria	10^5	-
Enterobacteria	10^3	-

U N I V E R S I T Y

Aflatoxins Content

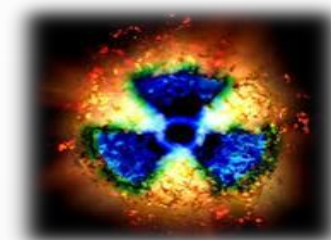


- Aflatoxins are naturally occurring mycotoxins produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*.
- The presence of aflatoxins can be determined by chromatographic methods using standard aflatoxins B1, B2, G1, G2 mixtures.
- IP method: NMT 2 $\mu\text{g}/\text{kg}$ of aflatoxins B1 & Total aflatoxins 4 $\mu\text{g}/\text{kg}$
- USP method: NMT 5ppb of aflatoxins B1 & Total aflatoxins 20ppb



Radioactive contamination

- The range of radionuclides that may be released into the environment as the result of a nuclear accident might include long-lived and short-lived fission products, actinides, and activation products.
- Microbial growth in herbals is usually avoided by irradiation. This process may sterilize the plant material but the radioactivity hazard should be taken into account.
- The nature and the intensity of radionuclides released may differ markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc.).
- The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic



CHROMATOGRAPHY OF HERBAL DRUG

- Separation, identification, impurity detection and assay of herbal drug in the formulation or in the extract are carried out by following methods :-

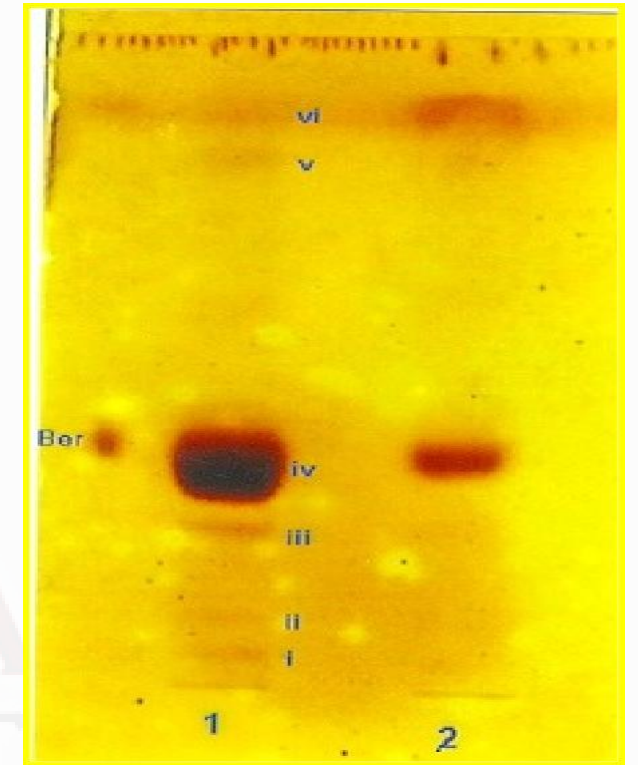
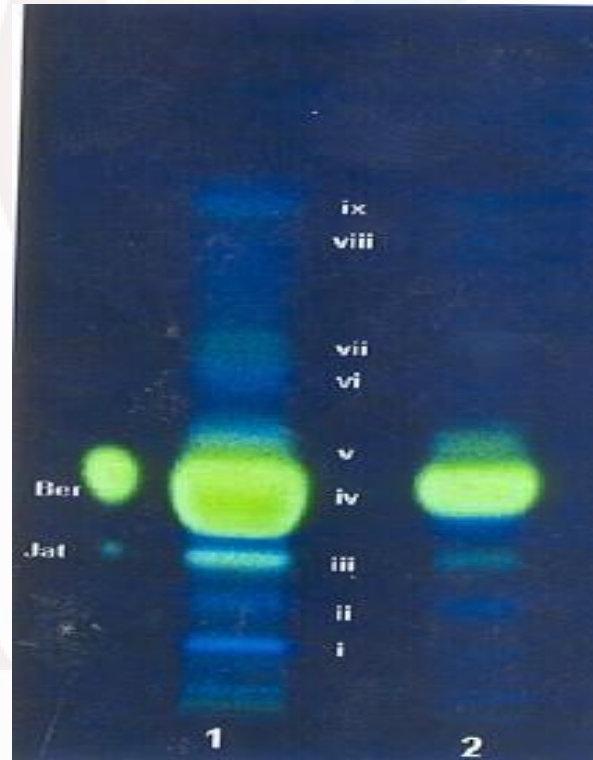
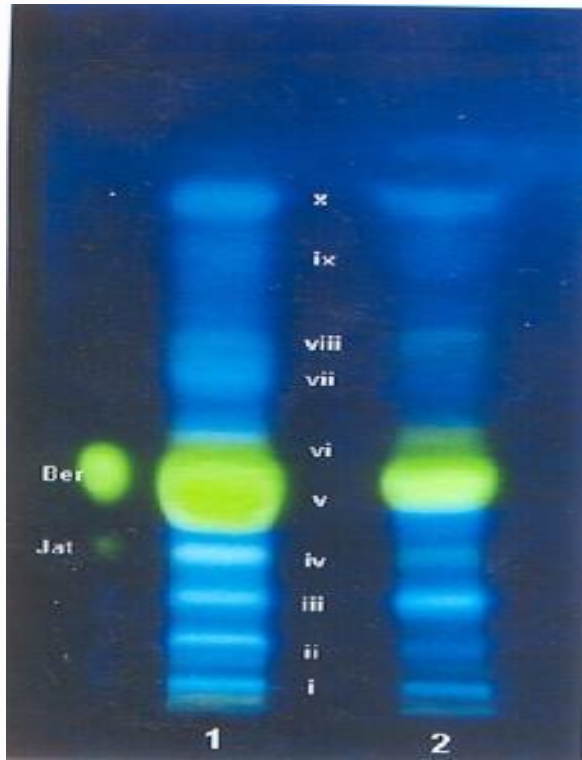
a) TLC b) HPTLC

c) HPLC/Densitometric chromatography

d) GLC

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a) T.L.C. Finger-print profile of Methanolic extract of *Coscinium fenestratum*

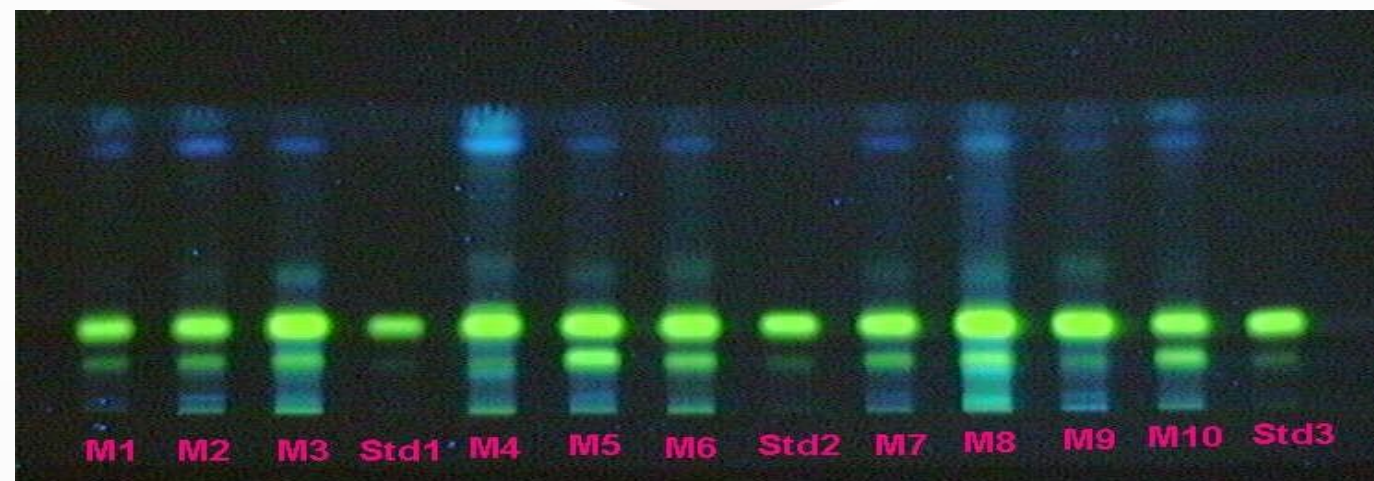
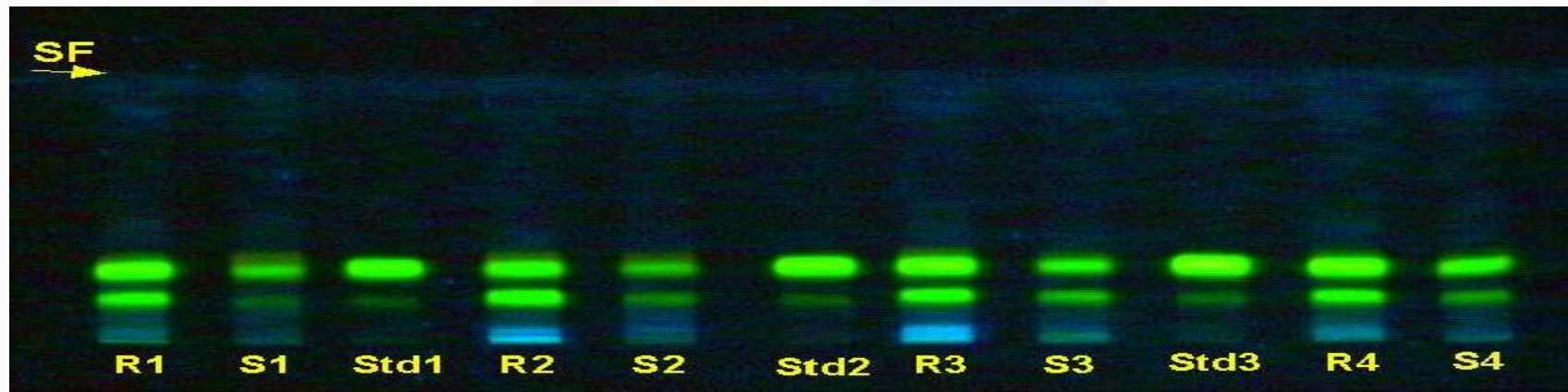


Detection :

(a) Under UV 366nm

(b) Under UV 254 nm. (c) Under visible light after

b) Comparative HPTLC profile of Berberine in different market samples



c) Densitometric scan of different samples of

