STANDARDISATION OF HERBAL DRUGS

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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 Suppliments containing Herbal Products, also called Neutraceuticals available in modern dosage forms.

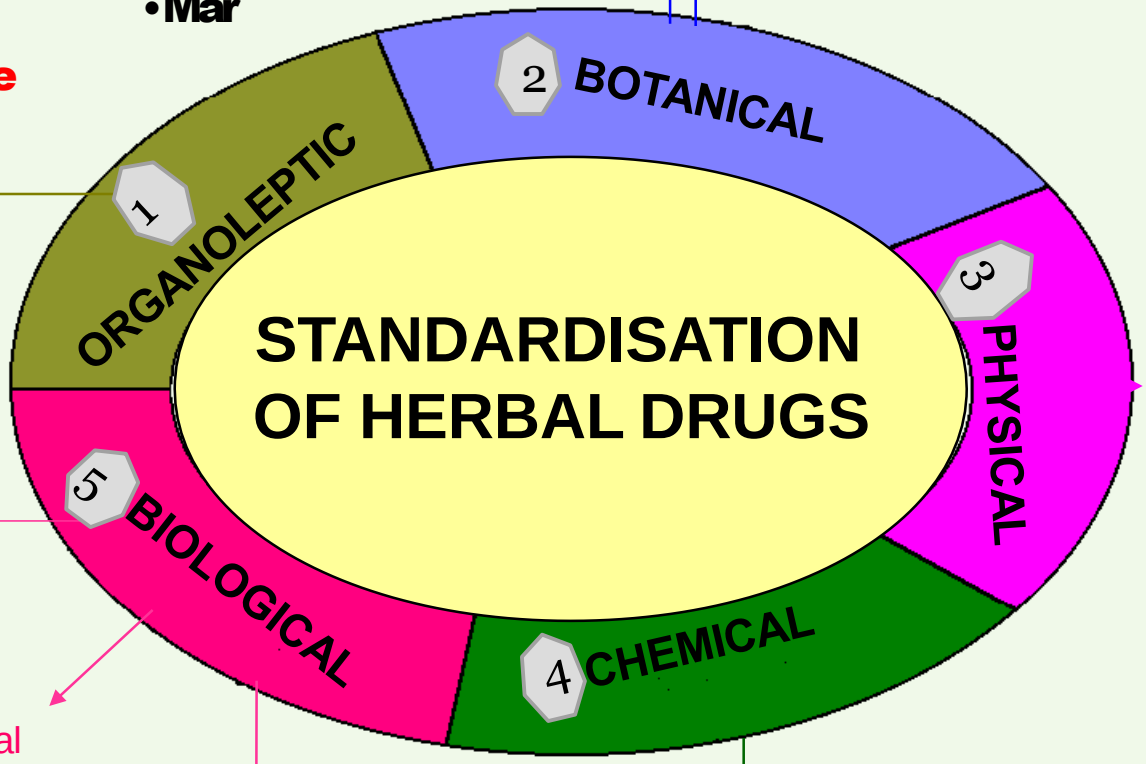
¬Herbal Medicines consisting of either Crude, Semi Processed or Processed Medicinal Plants.

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."

Standardization & Quality Evaluation of Herbal drugs



- Colour
- Odour
- Taste
- Texture
- Fracture

- Shape
- Extern al king
- Mar

Macroscopic

Microscopic

- Qualitative
- Quantitative
- SEM Studies
- Powder Studies

- Moist. Cont.
- Extrac. Values
- Ash Values
- Fluores. Analy.

Microbial Contamination

A) Total viable aerobic count

B) Determination of pathogens

C) Aflatoxins content

- Pharmacological

Bitterness value
Haemolytic activity
Foaming index
Swelling index

- Toxicological

Determination of pesticide residues
Determination of heavy metals

Antagonistic

- Bacteria l
- Fungal

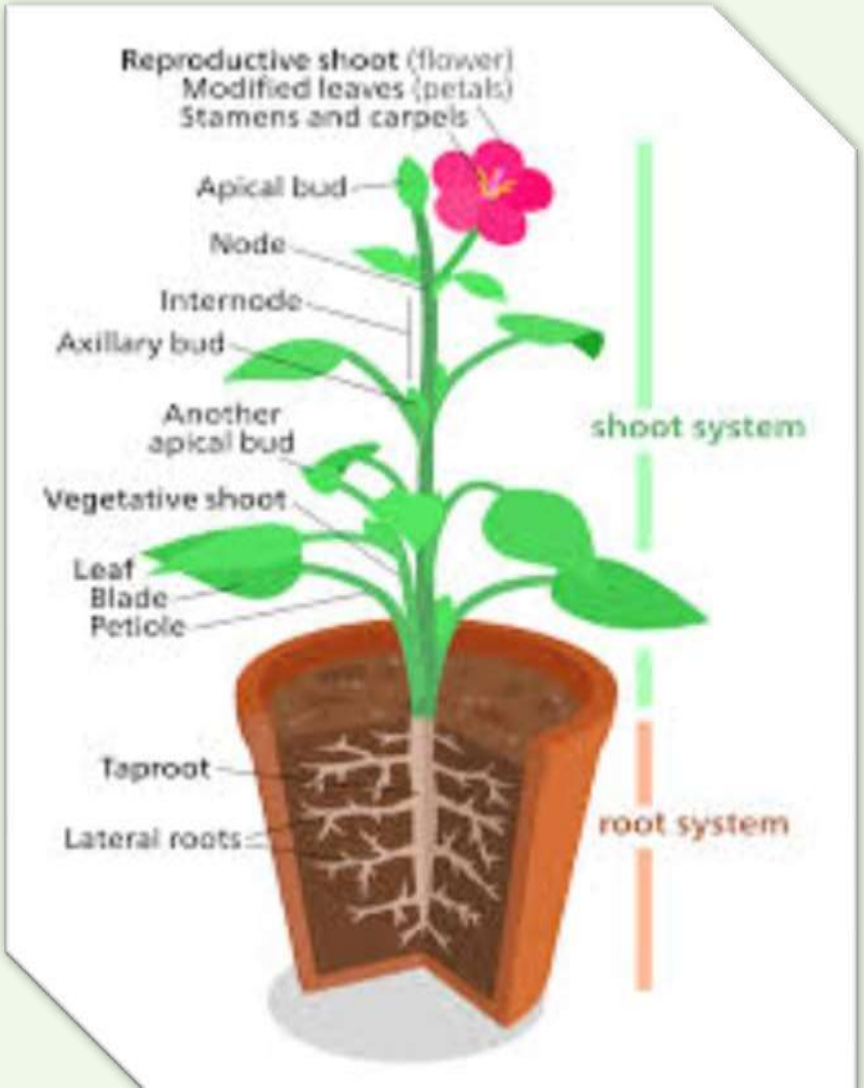
HPTLC
GLC
HPLC

- Qualitative
- Quantitative
- Chromatography
- Radioactive contamination

HPTLC Finger printing
Sec. Metabolites
DNA Finger printing

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.

- 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: 15mLwater 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 determin--nation 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 flavourig 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

. 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:

$$\frac{[T_1 - (T_2 - T_0)]x500}{w}$$

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

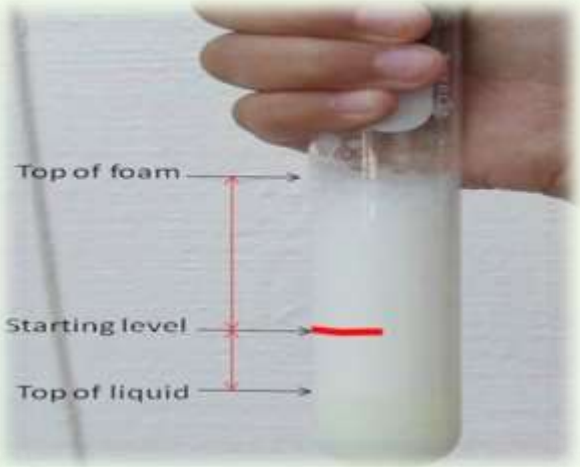
Determination of swelling index

- 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 WHO, and the mean daily intake (MDI) of the medicinal plant material.

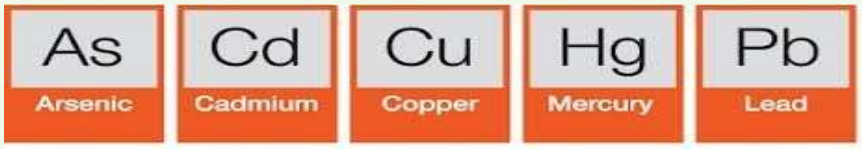
$$ARL = \frac{ADI \times E \times 60}{MDI \times 100}$$

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.

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

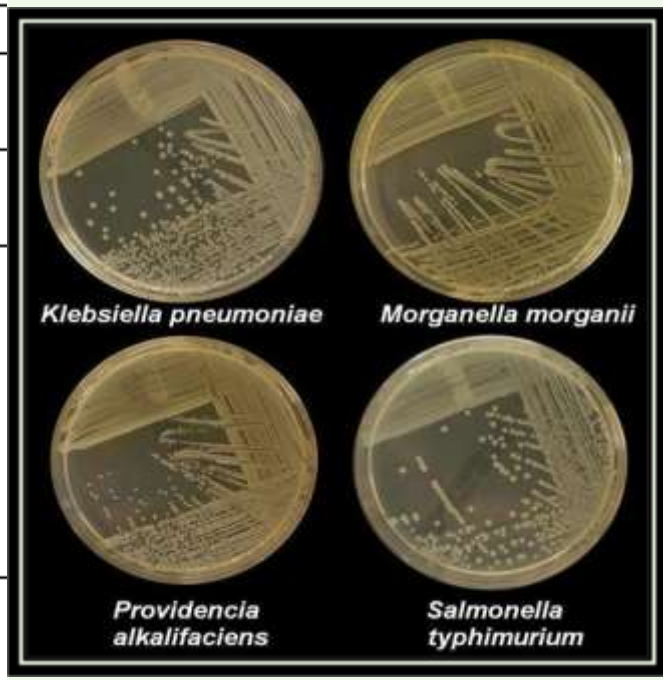


Table 1. Limits for microbial contaminants in finished products & Raw materials

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

Aflatoxins Content

- Aflatoxins are naturally occuring 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 µg/kg of aflatoxins B1& Total aflatoxins 4 µg/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.

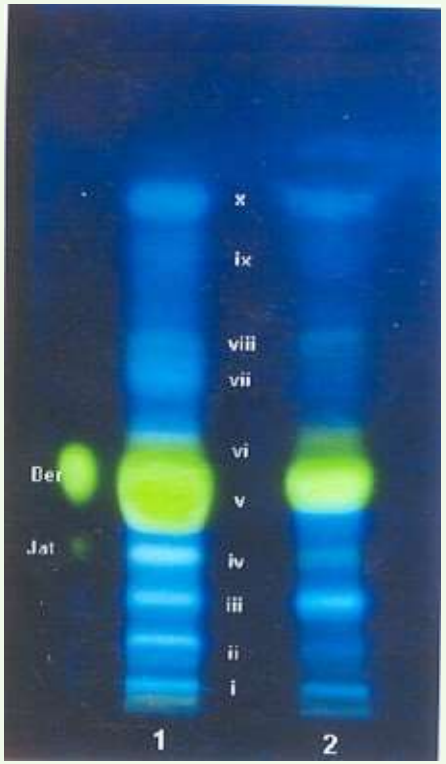
- The nature and the intensity of radionuclides released may differ markedly and depend on the source (reactor, reprocessing plant, fuel fabrication plant, isotope production unit, etc.).
- The radioactivity of the plant samples should be checked accordingly to the guidelines of International Atomic research centre

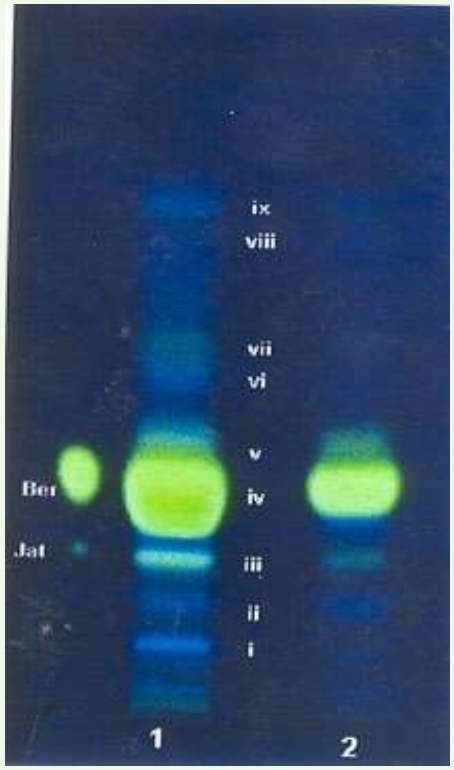
CHROMATOGRAPHY OF HERBAL DRUG

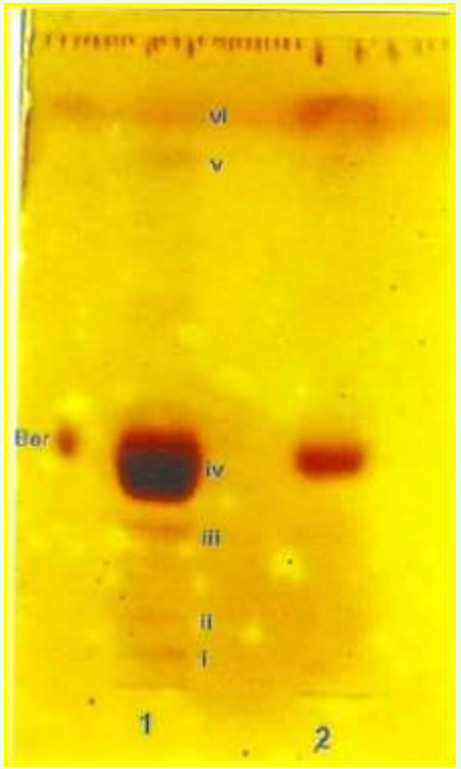
- Seperation, identification, impurity detection and assay of herbal drug in the formulation or in the extract are carried out by following methods :-

a) TLC
b) HPTLC
c) HPLC/Densitometric chromatography
d) GLC

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



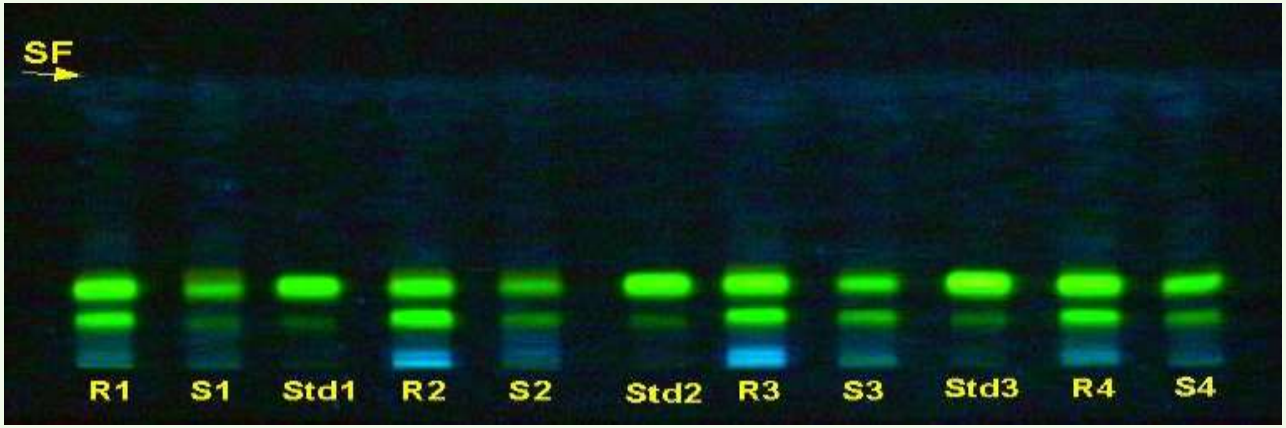


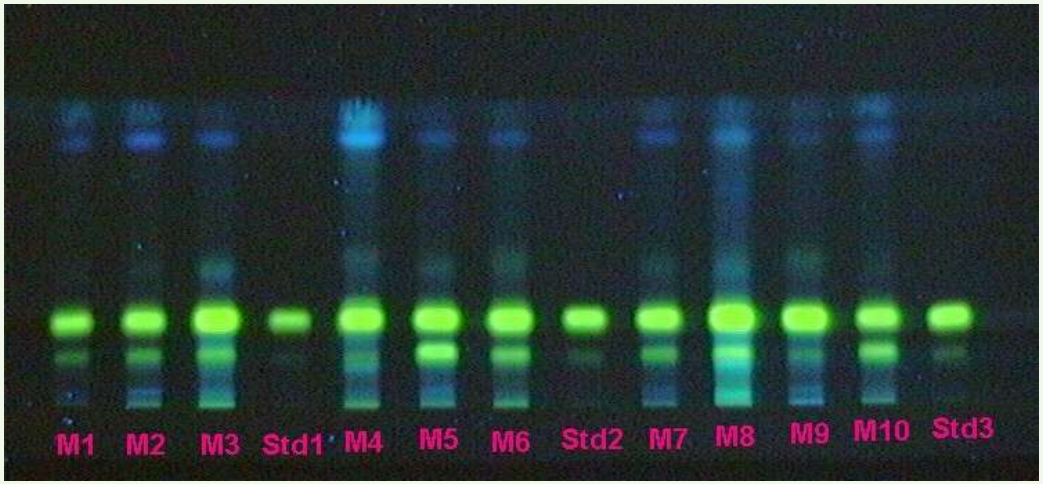


Dectetion :

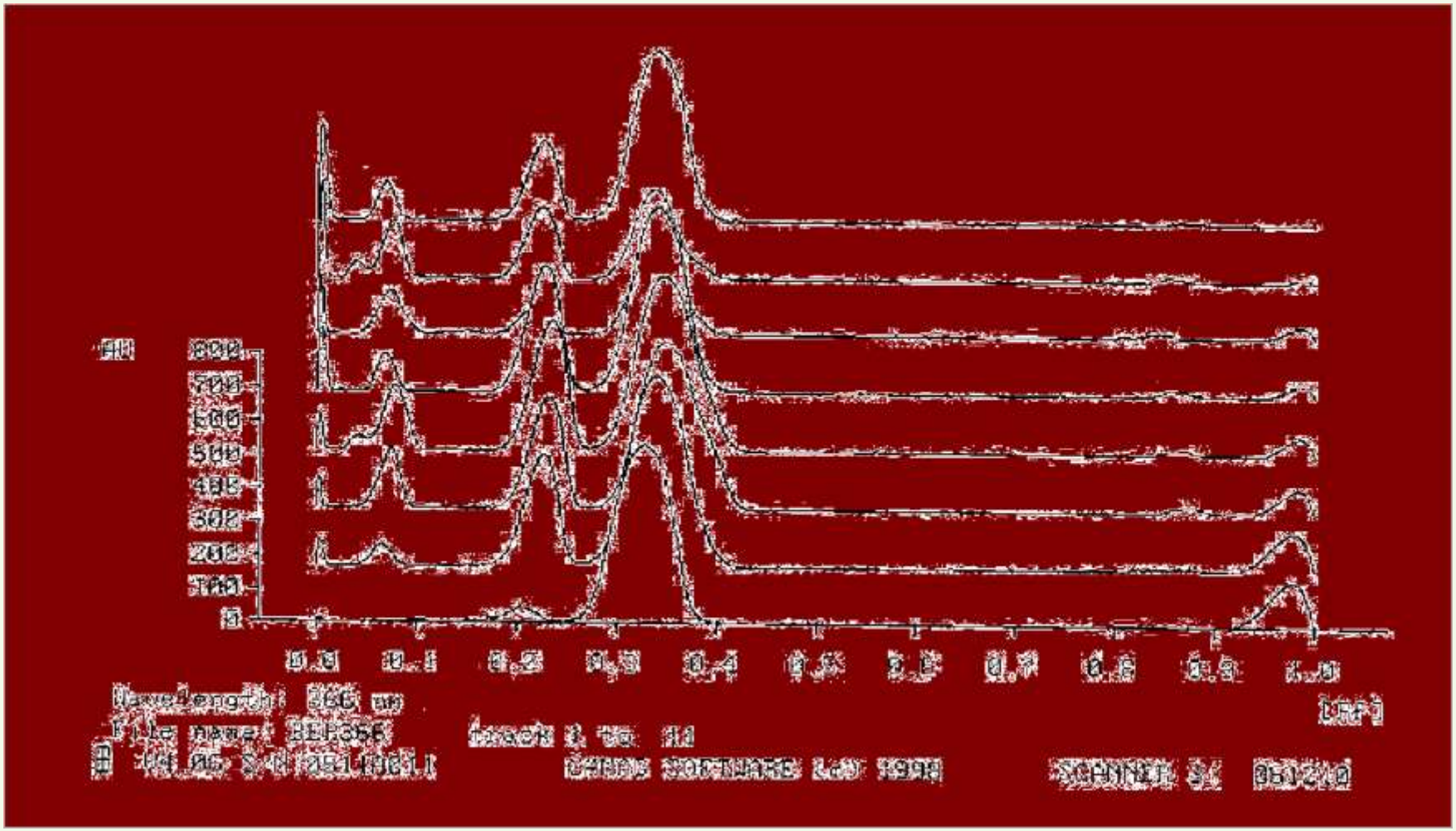
(a) Under UV 366nm **(b) Under UV 254 nm. (c) Under visible light after**

b) Comparative HPTLC profile of Berberine in different market samples





c) Densitometric scan of different samples of Berberis spp. at UV 266 nm



ANALYTICAL SPECIFICATIONS OF VATI/GUTIKA (TABLET/PILLS)

1. Description Colour
   Odour
2. Weight variation
3. Disintegration time -Not more than 15 min
4. Identification TLC/HPTLC/GLC
5. Assay
6. Test for heavy/Toxic metals
   Lead
   Cadmium
   Mercury
   Arsenic
7. Microbial contamination
   Total bacterial count
   Total fungal count
8. Test for specific Pathogen
   E. coli
   Salmonella spp.
   S.aureus
   Pseudomonas aeruginosa
9. Pesticide residue
   Organochlorine pesticides
   Organophosphorus pesticides
   Pyrethroids

11 Test for Aflatoxins (B1,B2,G1,G2)

ANALYTICAL SPECIFICATIONS OF SYRUP (LIQUID ORAL)

1. Description, Colour
2. Odour
3. Total – ash
4. Acid – insoluble ash
5. Water-soluble extractive
6. Alcohol – soluble extractive
7. PH
8. Total sugar content
9. Viscosity
10. Identification TLC/HPTLC/HPLC
11. Test for heavy metals
    - Lead
    - Cadmiu
    - m
    - Mercury
12. Microbial contamination
    - Total bacterial count
    - Total fungal count
13. Test for specific Pathogen
    - E. coli
    - Salmonella spp.
    - S.aureus
    - Pseudomonas aeruginosa
14. Pesticide residue
    - Organochlorine pesticides
    - Organophosphorus pesticides
    - Pyrethroids

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