Pharmacognosy and Phytochemistry

Introduction-Part 3

B. Pharm. Semester-1

Course Code: 0510221; Session: 2022-2023

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Learning Outcomes

At the end of this lesson, students will be able to explain

- **Drug Adulteration and its types**
- **Crude Drugs Evaluation:-**
  - **Organoleptic Evaluation**
  - **Microscopic Evaluation**
  - **Physical Evaluation**
  - **Chemical Evaluation**
  - **Analytical Evaluation**
  - **Biological Evaluation**
- **Quality Control of Crude Drugs**

Objective

The objective of this course is to give to the students of pharmacy the basic knowledge about the Medicinal Plants and discussion of Medicinal Plants according to their uses and their effects upon the different organs of the body.

Drug Adulteration

- Drugs Adulteration, in the broad and legal sense is the debasement (cheating) of any article.
- It is the practice of substituting a crude drug partially or whole with other similarly-looking substances which are either free from therapeutic properties or inferior in terms of chemical and therapeutic properties.
- Inferior, spoiled or deteriorated drugs represent the greatest percentage of cases of drug adulteration.
- Adulteration may be deliberate or accidental. It is the addition of one article to another through accident, ignorance, or carelessness.
- Adulteration involves different conditions: Inferiority, Spoilage deterioration, admixture, sophistication and substitution.

Drug Adulteration types

- **Inferiority**: Any substandard drug mixing to main drug.
- **Spoilage**: Due to attack of microorganism.
- **Deterioration**: Impairment of the quality of the drug
- **Admixture**: It is the addition of one article to another through accident, ignorance, or carelessness. Admixture is an unintentional (indeliberate) adulteration.

Drug Adulteration types

- **Sophistication**: It is the intentional or deliberate type of adulteration.

  Example of Sophistication: addition of **wheat flour** to powdered **ginger**, with enough **capsicum** to restore or enhance the pungency (taste).

- **Substitution**: It occurs when entirely different article is sold or used in place of the required one.

  Example of substitution: cotton seed oil sold as olive oil.

  However, all types of substitution are considered legally as adulteration.

Drug Evaluation

Drug Evaluation: It is the confirmation of its identity, and determination of its quality and purity.

(1) Organoleptic Evaluation

(2) Microscopic Evaluation

(3) Physical Evaluation

(4) Chemical Evaluation

(5) Analytical Evaluation

(6) Biological Evaluation

(1) Organoleptic Evaluation

It is the confirmation of its identity by means of the organs of sense, and includes the macroscopic (observable by naked eye) appearance of the drug, its odor and taste.

If necessary, a magnifying lens (6x to 10x) may be used.

1. Shape and size.
2. Color and external markings.
3. Fracture (the appearance of the fracture plane — whether it is fibrous, smooth, rough, granular, etc.) and internal color.
4. Odor and taste

(1) Organoleptic Evaluation

It includes the study of morphology and sensory characters.

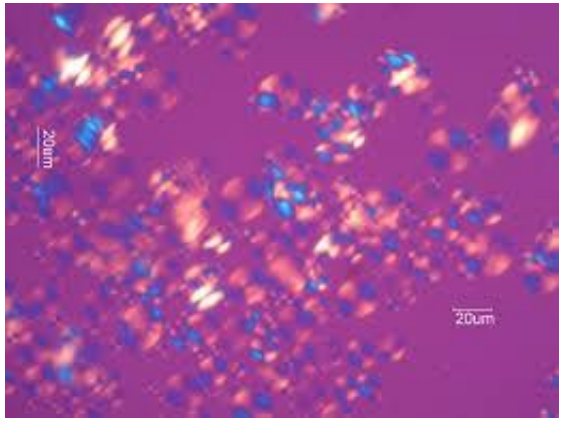
S.NO:	CHARACHTER	DRUG EXAMPLE
1	Brown **colour**	Cinnamon
2	Aromatic **odour**	Umbelliferous fruits
3	Sweet **taste**	Liquorice
4	Fractured **surface**	Cinchona
5	Wavy **shape**	Rauwolifia
6	7 to 8mm width 25 to 60 mm length (**size**)	Senna leaf

(2) Microscopic Evaluation

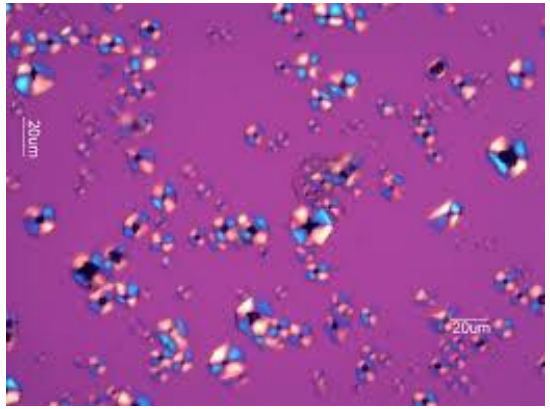
The microscope is not only essential to the study of adulteration in powdered plant and animal drugs, but it is also indispensable in the identification of pure powdered drugs.

Those sections of the official monograph headed histology and powder deal with the microscopic appearance of the drug in sectional view and powdered form.

Wheat Starch



Corn Starch



(2) Microscopic Evaluation

The microscope is not only essential to the study of adulteration in powdered plant and animal drugs, but it is also indispensable in the identification of pure powdered drugs.

Before microscopic analysis, the material must be prepared suitably.

1. Palisade Ratio
2. Stomatal Number
3. Stomatal Index
4. Stomata
5. Vein-islet Number
6. Vein-termination Number
7. Trichomes or plant hairs
8. Calcium oxalate crystals

Quantitative Microscopy

1.Lycopodium spore method

(3) Physical Evaluation

Physical constants are extensively applied to the crude drugs.

Physical constants:-

I. Moisture Content
II. Viscosity
III. Melting point
IV. Solubility
V. Optical Rotation
VI. Refractive Index
VII. Ash values
VIII. Extractive values
IX. Volatile oil Content
X. Foreign organic matter
XI. swelling factor

Examples of loss on drying: Dry homatropine hydrobromide [Anti-cholinergic drug, used in form of eye drops]. Heating at 105 C° in the oven for 2 hrs, it loses **not** more than 1.5% of its weight.

(4) Chemical Evaluation

Chemical tests are extensively used to the crude drugs.

1.Instrumental methods: They make use of various instruments for evaluation like colorimetry, flourimetry spectrophotometry etc.

2.Chemical constants tests: These are like acid value, iodine value and ester value etc are used for the identification of fixed oils and fats.

3.Individual chemical tests: These are the tests which are used for identifying particular drugs.

4.Microchemical tests: These are the tests which are carried on slides.

Example: Euginol in clove oil is precipitated as potassium euginate crystals.

(5) Analytical Evaluation

Chromatography-separation technique used to the crude drugs to isolate the chemical constituents

Chromatographic techniques:

a)TLC-Thin layer chromatography

b)HPTLC-High performance thin layer chromatography

c)HPLC-High performance/pressure liquid chromatography

d)GLC-Gas chromatography

e)CC-column chromatography

f)Gel permeation chromatography

g)Affinity chromatography

(5) Analytical Evaluation

Spectroscopic technique used to the crude drugs to elucidate the structure of the isolate the chemical constituents

Spectrophptometric methods:

i) UV- Ultra violet /visible spectroscopy

ii)IR-Infra Red spectroscopy

iii) Fluorescence analysis

iv) NMR-nuclear magnetic resonance spectroscopy

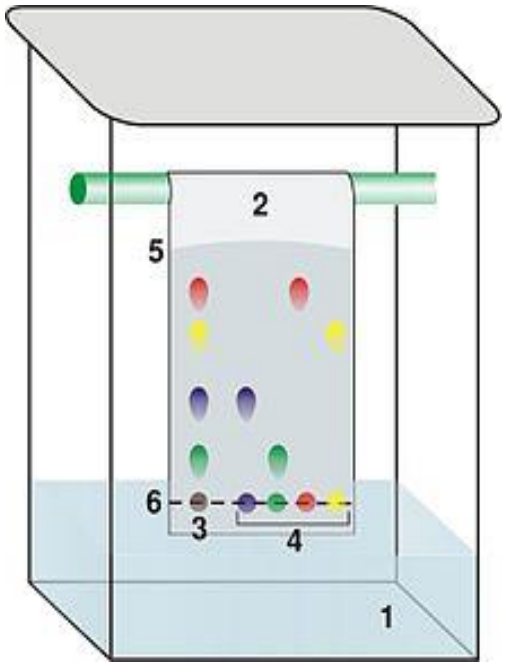
v) MS-Mass spectroscopy

vi) X-ray diffraction

vii) RIA-radio immuno assay

(5) Analytical Evaluation

Thin Layer Chromatography: Sample components are separated due to the differential migration or partitioning between 2 phases, the mobile and the stationary phase.



(6) Biological Evaluation

The pharmacological activity of certain drugs has been applied to their evaluation and standardization.

Assays on living animals or excised organs often indicate the strength of the drug or its preparation.

Since living organisms are used, the assays are called biologic assays or bioassays although the determination of pharmacologic activity is not strictly within the duties of pharmacognosy.

Quality Control of Crude Drugs

The Sampling

Before a consignment of a drug can be evaluated, a sample must be drawn for analysis; considerable care must be taken to ensure that this sample is truly representative.

Preliminary examination

In the case of whole drugs the macroscopical and sensory characters are usually sufficient to enable the drug to be identified.

Foreign matter

The difficulty of obtaining vegetable drugs in an entirely pure condition is fully recognized, and pharmacopoeias contain statements as to the percentage of other parts of the plant or of other organic matter which may be permitted.

Quality Control of Crude Drugs

The extract should always be prepared from a plant material of well known quality according to a pharmacopoeia or any other reliable monograph.

Weight ratio between the crude drug and the extract should be stated.

Example: 1:8 ratio of senna leaves ; 1 g dry extract corresponds to 8 g crude drug.

Control of dry extract should include:

IDENTITY: determined by Organoleptic qualities, Thin-layer chromatography and high performance liquid chromatography. Compare these of the tested drug to those of a standard.

MOISTURE: 5% is permitted.

Quality Control of Crude Drugs

Loss on drying: at 105 °C is suitable for large number of samples.

Karl Fischer titration to determine moisture/water content:

Used for expensive drugs and samples containing small quantities of moisture.

Composition of reagent: a solution of iodine, sulphur dioxide and pyridine in dry methanol.

Basis: the reagent is titrated with sample containing water which causes the loss of the dark brown color. At the end point, when no more water exists, the color persists.

Water is equivalent to the reduced iodine (in terms of moles).

SOLVENT RESIDUE:

For ethanol max. 0.5%. For noxious solvents such as methanol and methylethyl ketone: 0.05 % and 0.002% are the limits.

Gas-liquid chromatography, GLC: the technique preferred.

Karl-Fischer Titration

The elementary reaction responsible for **water quantification** in the Karl Fischer titration is oxidation of sulfur dioxide with iodine.

$$\mathbf{H_2O} + SO_2 + I_2 \rightarrow SO_3 + 2HI.$$

This elementary reaction consumes exactly one molar **equivalent of water Vs iodine**.

Iodine is added to the solution until it is present in excess, marking the end point of the titration, which can be detected by potentiometry.
The reaction is run in an alcohol solution containing a base, which consume the sulfur trioxide and hydroiodic acid produced.

Karl-Fischer Titration

The end point: the detector circuit maintains a constant current between the two detector electrodes during titration.

Prior to the equivalence point, the solution contains I^- but little I_2.

At the equivalence point, excess I_2 appears and an **abrupt** voltage drop marks the end point.

The amount of charge needed to generate I_2 and reach the end point can then be used to calculate the amount of water in the original sample.

Quality Control of Crude Drugs

HEAVY METALS (HM):

Some heavy metals, such as **copper**, **zinc** and **molybdenum** appear as micro-components in diet.

However, these metals shouldn't exceed the stated limits.

Also, metals such as **lead**, **mercury** and **cadmium** should not exceed the allowed limits.

Atomic absorption spectrophotometry: It is the technique of HM determination. The sample is either combusted or digested by a mixture of conc. nitric, sulphuric and hydrochloric acids, and then absorption of metals is measured at certain wavelength, e.g. Cd at 228.8 nm, Cu 328.8 nm.

For example, accepted limits: **Cd < 0.02 ppm** (parts per million), **Pb < 0.05 ppm** and **Hg < 0.0005 ppm**.

Quality Control of Crude Drugs

MICROBIAL CONTAMINATION:
The pharmacopoeia states the accepted levels and limits of bacteria and fungi existing in samples (per unit weight or volume).
Absence of bacteria such as. Salmonella, Escherichia coli, Enterobacteria.

Tests For Aflatoxins, Pesticides And Radioactive Contamination.

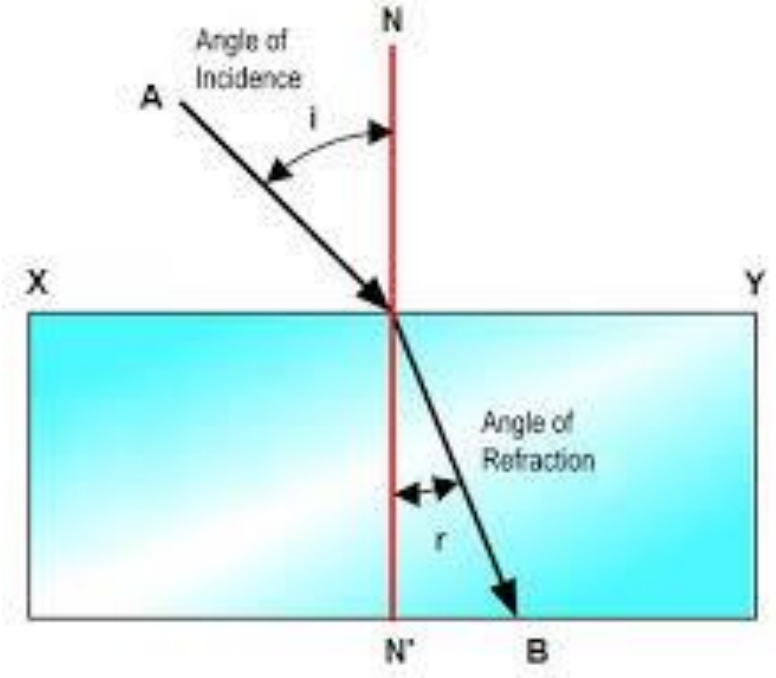
Content Of Pharmacologically Active Compound(s):
To give a reliable dose, these compounds should be determined (such as pyrrolizidine alkaloids), quantitatively.

HPLC methods: A complex mixture of substances; GLC methods: volatile oils.

Quality Control standards for Volatile and Fixed oils

Refractive Index:

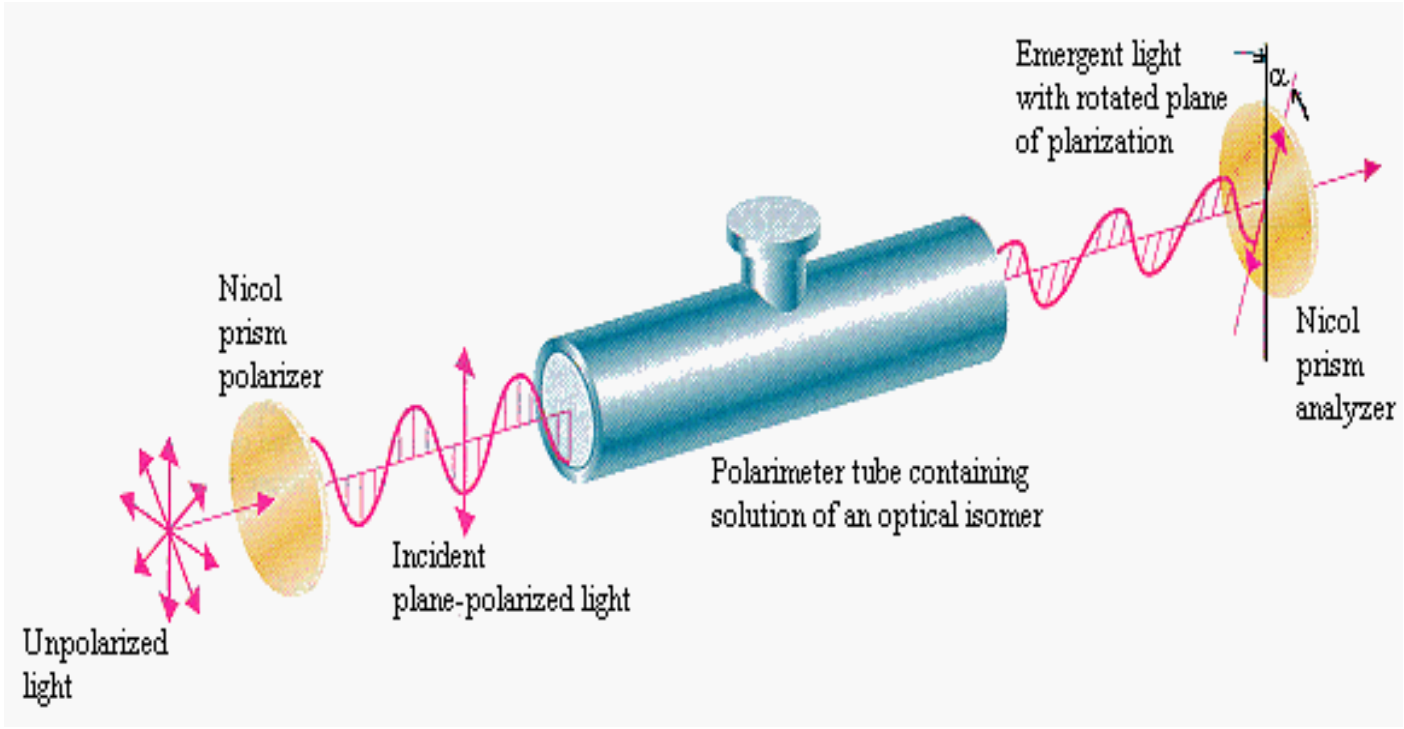
The refractive index of a substance is the ratio between the velocity of light in air and the velocity in the substance under test.



Quality Control standards for Volatile and Fixed oils

Optical rotation:

The optical rotation of a liquid is the angle through which the plane of polarization of light is rotated when the polarized light is passed through a sample of the liquid; this rotation may be either clockwise or anticlockwise.



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2. **Textbook of Pharmacognosy and Phytochemistry 2nd Edition, 2019, Authors: B. Shah, A. N. Kalia, Publisher: Elsevier, ISBN: 978-978-9386217738.**
3. **Medicinal Natural Products: A Biosynthetic Approach, 2nd Edition, 2002, Author: Paul M Dewick, Publisher: John Wiley and Sons Ltd, ISBN: 0471496405.**

Supplementary book:

Fundamentals of Pharmacognosy and Phytotherapy. A Guide for Health Care Professionals by Carol A. Newal, Linda A. Anderson and J. David Phillipson. (2010). the Pharmaceutical Press, London, UK; ISBN: 0 85369-474-5.