Faculty of Pharmacy

Introduction – part 3

Pharmacognosy & Phytochemistry

Dr. Yousef Abusamra
Quality of drugs

- Quality refers to the **intrinsic value** of the drug.
  - Example: the amount of medicinal principles or active constituents present.
- The constituents include carbohydrate, glycosides, tannins, v. oils, resins, steroids, alkaloids, peptides, hormones, enzymes and other proteins, vitamins, antibiotics.

Name and origin
- Characters
- Identity
- Purity tests which comprise presence of foreign material such as soil, sand, stones, mould, insects and animal excretions.

Quality of drugs
Quality of drugs

- The evaluation of a drug involves a number of methods which may be classified as follows:
  1. Organoleptic
  2. Microscopic
  3. Biological
  4. Chemical
  5. Physical
  6. Chromatographic methods

Evaluation

1. Organoleptic Evaluation

- **Organoleptic evaluation**: is evaluation 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
**Evaluation**

- **Microscopic Evaluation:**
  - The microscope has been employed in the examination of drugs since 1847.
  - 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.
3. Chemical Evaluation:

- Chemical methods of evaluating crude drugs are increasingly important.

- Chemical tests are employed to identify certain drugs:
  - **Example 1**: the characteristic red color developed in *Cascara sagrada* when treated with ammonia TS.
  - **Example 2**: The blue color of iodine with starch.

**Examples of chemical tests**

- Determination of the saponification value, the iodine value and the acid value of fixed oils.

- The reducing effect of sugars with:
  1. Molish’s test.
  2. Barfoed’s test.
Chemical Evaluation

• Other examples:
  ❖ The color reaction of **alkaloids** with alkaloidal reagents.
  ❖ The reducing effect of sugars with Molish’s and Barfoed’s tests.
  ❖ Determination of the saponification value, the iodine value and the acid value of fixed oils.

Physical Evaluation

➢ The application of typical physical constants is **very rare**:
  • 1. **Solubility**: usually expressed as the number of mL of solvent required to dissolve 1 g of the drug.
  • 2. **Specific gravity**: particularly used for fats and volatile oils. **(Specific Gravity (SG))** is a dimensionless unit defined as the ratio of the density of a substance to the density of water - at a specified temperature.
  • 3. **Optical rotation**: of many compounds like **sugar**.
    ➢ {The degree of rotation of polarized light as it passes through an optically active material}.
    ➢ {Optical activity is a property unique to chiral substances, for example 2-butanol, which possess a chiral center (one carbon bound to four different ligands)}.  

Physical Evaluation

4. **Refractive index**: for part of fixed and v. oils.

*The refractive index or index of refraction of a material is a dimensionless number that describes how light propagates through that medium.*
Physical Evaluation

- 6. Water content: as determined by drying to constant weight in an oven.
- Example: loss on drying-dry homatropine hydrobromide (Anti-cholinergic at the muscarinic receptors. It is used in form of eye drops as mydriatic and cycloplegic) at 105°C for 2 hrs., it loses not more than 1.5% of its weight.

Biologic Evaluation

- 5. 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.
Chromatographic Evaluation

- 6. Analysis and separation of organic and inorganic materials, quantitatively or qualitatively.
  - It is proved more effective than other means of separation and identification.
  - Sample components are separated due to the differential migration or partitioning between 2 phases, the mobile and the stationary phase.

Microchemistry

- Microchemistry includes the study of active constituents of crude drugs by the application of chemical and physical methods to milligram quantities of drug in powdered form or to histologic sections of the drug.
- It offers a means by which constituents of many drugs may be isolated and purified.
- The following outline denotes the principle techniques:
Isolation

• I. Isolation of Constituents
  • A. By chemical solvents: which includes:
    • 1. Micro extraction
    • 2. Micro filtration
    • 3. Micro crystallization
  • B. By micro-sublimation

Example: Isolation of caffeine by sublimation by decreasing pressure (green line).

Identification

• II. Identification of Constituents:
  • A. By crystallography:
    (Identifying substances by the typical arrangement of the atoms in their crystals – there are 32 modes of these arrangements)
  • B. By melting point determination (for pure compounds)
  • C. By confirmative tests
    • 1. Chemical tests.
    • 2. Physical tests.
Confirmatory Tests

• PHYSICAL:
  ➢ Example: menthol is isolated from peppermint oil.
  ➢ It occurs as colorless, hexagonal crystals, usually needle-like.
  ➢ The m.p. of L. menthol from natural sources is between 41-43.
  ➢ When L. menthol is triturated (crushed) with an equal weight of camphor, chloral hydrate or phenol, the mixture liquefies, thus confirming the identity.

• CHEMICAL:
  ➢ Many reagents yield characteristic colored-reactions with certain compounds.

  ➢ Example: anthraquinone substances present in Cascara sagrada turn red when treated with alkalies).
Plant Biotechnology

- Plant tissue and cell culture:
  - **Cultivation of plants on an artificial medium** was first described in 1939.
  - Is an important technique in several areas, such as the
    1. Commercial production of ornamental plant.
    2. Plant breeding.
  - **The basis** for plant tissue and cell culture is that *each* cell contains all the genetic information relating to the whole plant. It is thus possible to start with a single cell which is allowed to multiply by division and form tissue of loosely attached cells called a callus

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Plant tissue and cell culture

- **Callus culture**:
  - Callus culture can be started from a **single cell** or **small piece of plant tissue**:
    1. Tips of stems.
    2. Roots.
    3. Leaves.
    4. Cotyledon from very young plants.
    5. Tissues from the ovary or stamens, and parts of the cambial zone of root or stems.
  - The plant part taken to establish a culture is termed the **explant**.
Plant tissue and cell culture

- The surface of the explant must be sterilized using sodium hypochlorite.
- It is left for 5 to 30 minutes.
- The explant is rinsed with sterile water and transferred to the sterile growth medium:
  a) Agar gel in a Petri dish.
  b) Flask.
Nutrient of tissue culture

- The medium must contain salts, nitrogen, organic carbon source, vitamins which cannot be synthesized by the callus tissue.
- Ammonium nitrate (NH₄NO₃), arginine, L-aspartic acid, L-asparagine, glycine, L-glutamic acid and L-glutamine are the source of nitrogen.
- Some growth regulators should be used such as auxins and cytokinins.
- Auxins stimulate lengthwise growth of stems and have the opposite effect on the root (swelling).
- Cytokinins promote cell division.
- Callus are usually stored in the dark.

<table>
<thead>
<tr>
<th>Indole-3-acetic acid [IAA] = Natural auxin</th>
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<tr>
<td>2,4-dichlorophenoxy acetic acid [2,4-D] = Artificial auxin</td>
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<tr>
<td>Kinetin = Natural cytokinin</td>
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- α-naphthaleneacetic acid [NAA] is another natural auxin.
- 2iP and zeatin are another two natural cytokinins.
Natural auxins:

- Indole-3-acetic acid (IAA).
- α-naphthaleneacetic acid (NAA).

Artificial auxins:

- 2, 4-dichlorophenoxyacetic acid (2,4-D).

Natural cytokinins:

- Kinetin.
- 2iP.
- Zeatin.

Organ culture and regeneration of plants

- Plant organs are developed depending on the balance between the concentrations of auxin and cytokinin.
- The salt concentration in the medium and the light conditions are also important.
- Shoots are regenerated in presence of light and a medium of high osmolarity containing chelating agents e.g. EDTA. {compounds that are capable of binding metals; cations particularly, Ca^{+2}. ROLE: binding to calcium preventing clumping of cells grown in liquid suspension, or detaching adherent cells}.
- The auxin should be lower than the cytokinin.
SUSPENSION CULTURES

Suspension cultures:

- In callus cultures, where cells are in contact with one another, they form an unorganized tissue.
- In suspension culture the cells are free in the medium or form small aggregate.
- The same medium can be used as for a callus culture, the only difference being that the agar is omitted.
- Suspension cultures grow much faster than callus cultures, because of the ease with which nutrients access the growing cells.
Environmental factors of importance for plant tissue and cell culture

- **Temperature**: the optimum temperature for in vitro growth of plant cells is in the range 25-30.
- **Aeration**: plant cells in a culture are aerobic and must therefore have access to oxygen in order to prevent infection by microorganisms.
- Also, exchange of gases with the outside environment must be permitted.
- For flask culture, the flask is usually closed with a plug of cotton or wool, which permits exchange of gases but prevents microorganisms from entering the flask.
- The flask is placed on a shaker, which keeps the uptake of oxygen.

- **Light**: Plant cells in tissue culture normally do not carry out photosynthesis, it is therefore necessary to control the light conditions with respect to intensity.
- The irradiation needed to allow plant tissue or cell culture to develop is much lower than that need by normal living plants.
- The best light source for tissue culture is the fluorescent lamp.
- The influence of light on plant culture has been studied at levels of cellular tissues and organs.
Environmental factors of importance for plant tissue and cell culture

- Thus, callus growth and shoot initiation can be either enhanced or inhibited depending on the wave length and irradiance (amount of energy/unit area).
- High near UV {visible to birds, insects and fish} and blue light have been found to inhibit the growth of callus culture.
- Red light can cause both enhancement or inhibition of callus growth depending on the species.
- Example: *Digitalis lanata* when exposed to light for 2-3 periods of 15 minutes, showed that the cardiac glycosides content of the exposed culture was about 10 times higher with each period.