

Phytochemical Studies on Medicinal Plants

Purpose of the study

- ❖ After this lecture you will be able to know.
 - What is phytochemical investigation?
 - Steps in phytochemical investigation
 - Extraction methods
 - Bioactivity/bioassay-guided fractionation
 - Different isolation techniques:-
 - » Crystallization/precipitation
 - » Fractional distillation
 - » Derivatization
 - » Chromatography

Case study

- Imagine while you are on vacation in your village you have found one medicinal plant which is used for the treatment of rabies virus, snake bite, and various tumours by a traditional herbal medicine practitioners. So, you wandered by the healing power of this plant as pharmacists (discoverer of drugs) you want to carryout phytochemical study on this plant so that this plant could be a potential source of new pharmaceutical drug. How can you start your investigation?

Introduction

- Natural products are sources
 - For more than 25% of modern medicine
 - For lead compounds for another 20% modern medicine
- Only 15% of higher plants have been investigated for biological activity.

Phytochemical screening

❖ **Phyto** = plant

❖ **Phytochemical**:- different chemical constituents of a plant

❖ **Phytochemical screening**:- study related to isolation, identification, and characterization of all or individual chemical constituents of a plant in a pure form

❖ It follows the **biological investigation** of each constituent in pure form **to find their biological /therapeutic activities**

❖ It is an **interdisciplinary work** of botany, pharmacognosist, pharmacologist, chemist, and toxicologist.

Steps of phytochemical screening

1. Selection
2. Collection
3. Botanical identification
4. Literature review on identified plant
5. Preparation of the plant for extraction
6. Extraction and preliminary phytochemical screening
7. Biological/pharmacological test of the crude extract
8. Bioactivity-guided fraction and isolation of active constituents
9. Structural elucidation of each active constituent isolated.

Steps of phytochemical screening...

1. Selection of Plant Material

Depends on

- Its medicinal use in traditional medicine
- Trial and error/blind screening
- Chemotaxonomic data on related families, genus or species.

Steps of phytochemical screening...

2. Collection Should consider the:-

- » Time/season
- » Distribution
- » Part of plant used
- » Age of the plant.

Affect the qualitative & quantitative accumulation of the active constituents

Steps of phytochemical screening...

- Collection should be done at **optimal state of development of the plant/its part**.
 1. **Roots and rhizomes** are collected at the end of the vegetation period, i.e. usually in the autumn.
 - In most cases they must be washed free of adhering soil and sand.
 1. **Bark** is collected in the spring.
 2. **Leaves** and **herbs** are collected at the flowering stage.
 3. **Flowers** are usually gathered when fully developed.
 4. **Fruits** and **seeds** are collected when fully ripe.

Steps of phytochemical screening...

3. Botanical identification of the plant

- Identification of the plant can be done by:-
 - ▶ Consulting a **taxonomist or a pharmacognosist**
 - ▶ Comparing the characteristic features of the sample plant with the **reference authentic plant/published monograph**
- The **whole plant at flowering stages** should be provided to the taxonomist or pharmacognosist.
- The authenticated sample should be kept in the **herbarium**



Steps of phytochemical screening...

The voucher letter should contain:

- **Site of collection:** Bahir Dar, West Gojjam, Ethiopia
- **Local name:** *Feto* (Amharic), Garden cress (English)
- **Scientific name:** *Lepidium sativum* L. (seed),
- **Habitat:** a herb found in Bahir Dar zuria woreda.
- **Collector(s) Name:** Yenesew Wudu
- **Date of collection:** 07/11/2018
- **Specimen number:** YW01/2018
- **Identified by:** Getnet Chekole (Botanist, UoG)

Steps of phytochemical screening...

4. Literature review on the identified plant

- Searching any work done on your study plant in published literatures such as scientific journals, books, internet, etc.
- **Literature review helps:-**
 - ◆ Identification of proper plant to work on
 - ◆ Proper preparation, extraction method, and appropriate solvent to be used can be obtained.
 - ◆ Identification of different constituents of the plant

Steps of phytochemical screening...

5. Preparation of the study plant for extraction

Involves:-

- ▶ Removal of foreign extraneous matters,
- ▶ Drying,
- ▶ Size reduction/powdering

◆ Drying

- Artificial drying E.g. Oven drying
- Natural drying
 - ✓ Shade drying
 - ✓ Light drying

Steps of phytochemical screening...

- **Drying:-**
 - ▶ Prevents enzymatic hydrolysis of active ingredients (<10% moisture content, enzymes are in active)
 - ▶ Prevent attack by molds and bacteria
 - ▶ Facilitate size reduction or powdering, etc.
- **Grinding:** improve extraction by
 - Making the sample more homogenous,
 - Increasing the surface area of contact b/n solvent & powder,
 - Decrease the amount of solvent required for extraction by allowing the powder to be packed densely, and
 - Facilitating the penetration of solvent into the cells.

Steps of phytochemical screening...

6. Designing appropriate extraction methods

- **Involve -**
 - ▶ Selection of appropriate solvent
 - ▶ Selection of appropriate extraction methods
- If the plant comes from traditional medicine, use the same extraction solvent and extraction method.
- Choice of extraction method depends on **Nature of**
 - **The plant/part used:** dry/fresh, root, leaf, stem, bark, ...
 - **Active ingredients:** thermolabile/stable, polar/nonpolar...

Steps of phytochemical screening...

- Water and alcohols of aliphatic carbons are usually solvents used to prepare the traditional medicine.
- Choice of extraction solvent follows the principle of “like dissolves like”
 - Non polar/lipophilic solvents are used for extraction of non polar constituents.
 - Polar/ Hydrophilic solvents are used for extraction of polar compounds.

Steps of phytochemical screening...

Extraction: separation of components soluble in the solvent

- Transfer of mass of soluble material from a solid to a fluid.
- Both the active and inactive impurities can be extracted.
- **Extraction involves:**
 - Diffusion of the solvent into cells
 - Dissolution of the metabolites in the solvent
 - Diffusion of the solution out of the cells
 - Continued till equilibrium is set up between the solution in the cells and external env't.

Steps of phytochemical screening...

- **Menstruum** :- the solvent used for extraction
- **Crude extract**: - plant constituents left after removal of the dissolving media by **evaporation**.
- **Residue or mark**: - the **undissolved** constituents of the plant left in the filter paper.
- **Filtrate**: - **Solvent + dissolved plant constituent**

Steps of phytochemical screening...

Methods of extraction

Cold extraction methods



- ☐ Maceration
- ☐ Percolation
- ☐ Expression/cold press
- ☐ SCF extraction

Hot extraction methods



- ☐ Soxhlet extraction
- ☐ Infusion
- ☐ Digestion
- ☐ Decoction

Steps of phytochemical screening...

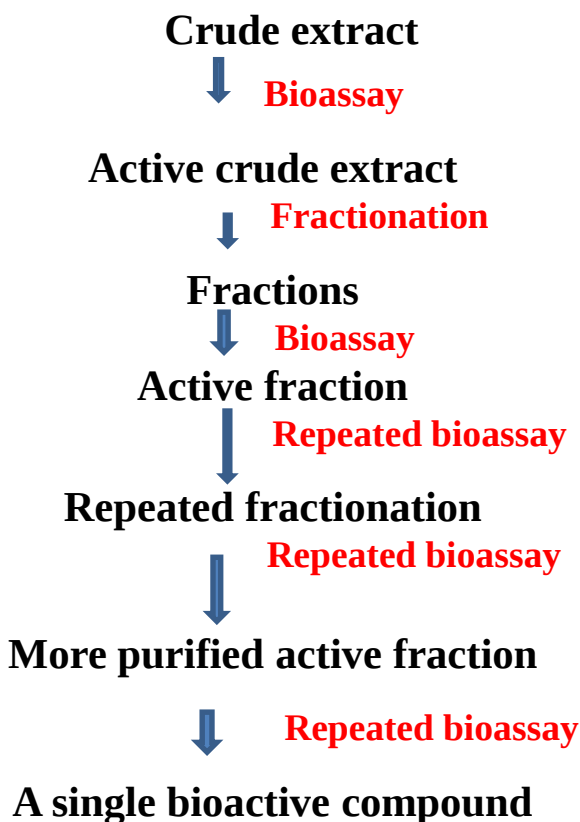
7. Checking extracts/ fractions for pharmacological activity

- *In-vivo, in-vitro, and ex-vivo* evaluation methods
- Can be evaluated for:
 - Anti-bacterial
 - Anti-fungal
 - **Antimalarial**
 - Anti-inflammatory and analgesic
 - Immunomodulatory activity etc...

Steps of phytochemical screening...

8. Bioassay guided fractionation

Testing of **non polar to polar fraction** for biological activity followed by the isolation of individual pure components from biologically active fraction



Steps of phytochemical screening...

Ideal bioassay should be

- ⊕ Inexpensive
- ⊕ Sensitive to small amount of active material
- ⊕ Selective for specific type of bioactivity
- ⊕ Simple to run and maintain

Steps of phytochemical screening...

9. Chemical analysis of the crude extract/fractions/ isolates/Phytochemical screening

- Identification of classes of compounds presents (alkaloids, glycosides, tannins, steroids, etc.).
- Conducted by **chemical tests** (using **specific reagent**)

Steps of phytochemical screening...

10. Isolation and purification of active ingredients

Classical methods



- ☐ Filtration
- ☐ Centrifugation
- ☐ Crystallization
- ☐ Solvent extraction
- ☐ Distillation.
- ☐ Fractional distillation
- ☐ Sublimation

Modern methods



- ☐ various chromatographic methods

Loading....