

Investigation of Medicinal Plants by

Scientific Methods

by

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1. Selection of Medicinal Plants: It is advisable to refer the ethnomedical reports and books on medicinal plants in the flora before the selection. The plant selected has to show biological activity. Once it is collected a voucher specimen has to be prepared and this is useful in the identification of plant and for future reference.
2. Extraction of Plant material: Once the plant material is collected it has to be extracted in order to isolate the biologically active compounds. Extraction itself is twofold. You can do either a cold extraction or a hot extraction depending on the solubility or/and stability of the active compound(s) in the selected solvent. In cold extraction you have to dip the material in the solvent and shake it well at room temperature. But the hot extraction needs a special set-up called soxhlet apparatus where extractions are done under reflux conditions. Solvents can be either organic (hexane, CHCl_3 , methanol, etc.) or aqueous (H_2O , dilute acid, etc.). After the extraction is completed the solvent has to be removed by evaporation under reduced pressure.

3. Biological and Phytochemical screening of the extract:

3.1. Biological Screening A large number of biological tests are available. Some examples are described below.

3.1.1. Antitumour: Antitumour active principles are the substances capable of inhibiting the growth of cancer cells. Cytotoxic tests following tumour inhibiting or tumour destroying assays are available for the screening of these extract. In vitro tests are often used for the screening.

3.1.2. Antifertility : Antifertility agents are the substances capable of preventing or terminating pregnancy. Both in-vivo and in-vitro techniques are available for screening of a plant extract. The test can be based on either female antifertility activity or male antifertility activity.

3.1.3. Antimicrobial : This area covers the activity of an extract, against both fungal and bacterial growth. Antimicrobial testing is often done in-vitro due to its efficiency. Thin Layer Chromatographic (TLC) bioassay, spore germination test and agar plate test are used for antifungal assay. The most widely used procedure for antibacterial assay is the disc diffusion method.

3.2. Phytochemical Screening Phytochemical screening is carried out in order to get an idea about the important phytochemical groups present in an extract. Some of the

important phytochemical groups responsible for biological activity are alkaloids, saponins, sterols, terpenes, flavonoids, polyphenols, coumarins etc.

A large number of tests are available for screening plant extracts for different phytochemical groups.

4. Bioactivity directed fractionation of extracts : Fractionation of an extract can be done either by solvent partition or by chromatography. Efficiency of solvent partition method depends upon the distribution constant, volumes of two phases used and on the number of extraction.

Chromatographic separation is the most widely used method and it is based on differences in the distribution of solutes between a mobile and a stationary phase. Chromatographic methods can be classified as column chromatography and open-bed chromatography. In column chromatography fractionation is carried out in a column and both gases and liquids can be used as mobile phase. Thin layer chromatography and paper chromatography are examples for the open-bed chromatography.

However, in activity directed fractionation one has to monitor the biological activity of each of the fractions obtained in each stage of fractionation or separation until the active compound(s) are isolated.

5. Purification of active compounds : The most widely used method of purification is recrystallization. Once purified, it is better for a researcher to record all the physical properties such as colour, melting point (m.p.), optical rotation (L_D).

6. Structure elucidation of active compounds : Two different methods known as chemical methods and physical methods are used in structure elucidation.

6.1. Chemical methods : There are certain chemical tests which give different products or answers depending on the functional groups present in the compound. eg. 2,4-DNP test for carbonyl groups, Tollen's reagent for aldehyde groups etc.

6.2. Physical methods : Due to the availability of modern techniques physical methods predominate over chemical methods in structure elucidation. These techniques are easy to handle, time saving and one can recover the sample after obtaining the required data. The most widely used techniques are Ultra-violet (UV), Infra-red (IR), Nuclear Magnetic Resonance (NMR) and Mass spectroscopy.

6.2.1. UV spectroscopy : Ultra violet radiation is the part of the electromagnetic radiation between x-rays and visible regions which is energetic enough to affect on some electronic energy levels. UV absorption peaks are observed due to absorption of certain amount of energy of the radiation by the different chromophores present in the compound. Chromophore is an isolated functional group capable of absorbing UV or visible radiation. These groups confer colour on substance and most of them have unsaturated bonds such as $C = C$, $C = O$, $N = N$ etc.

- 6.2.2. IR Spectroscopy : IR radiation which extends beyond visible into microwave region is capable of affecting both the vibrational and rotational energy levels of a molecule. IR spectrum gives characteristic absorption for each functional group.
- 6.2.3. NMR Spectroscopy : Compounds also capable of absorbing radiowaves which causes the transition between two orientations of nucleus in the presence of magnetic field, are known as nuclear magnetic resonance (NMR). Most widely used NMR technique is Proton NMR. Here the important point is that the chemical environment of H changes absorbing radio wave also changes for the resonance. So ¹H NMR spectrum shows number and environment of protons present in the molecule. Similarly ¹³C NMR gives number and environment of carbon atoms in the compound.
- 6.2.4. Mass Spectroscopy : In this technique, the molecule is bombarded with energised electrons and molecular ion is formed. The molecular ion is then accelerated under an electric field and let it to travel in a circular path. Depending on the mass of the fragment ions formed, different ions fall in different places and are detected. These ions appear as peaks in the spectrum with molecular weights. High resolution mass spectrum gives molecular formula of the compound.

In summary, UV spectrum gives the information about the chromophore, IR gives an idea about functional groups, ^1H and ^{13}C NMR gives different types and number of H and C atoms in the molecule and MS analysis gives the molecular formula. So combining these information and using the knowledge of chemistry we can get chemical structure of a given compound.