

Phytochemistry

The phytochemistry investigation of plant may involve the following steps :

- 1 - authentication of the plant
- 2 - extraction of the plant material
- 3 - separation and isolation of the constituents of interest
- 4 - charecterization of the isolated compounds
- 5 - investigation of the biosynthetic pathways to particular compounds
- 6 - quantitatue evaluation

Authentication of the plant : all plant material used should be properly authenticated by specialists . the National Herbarium in Iraq is the main institute for authentication of plants.

Extraction of plant material : The choice of extraction procedure depends on the texture & water content of the plant material being extracted & on the type of substance that is being isolated . Dried materials are usually powdered before extraction where as fresh plants ex: leaves can be homogenized with the solvent ex: alchohol. The methods of extraction are different , they are either cold or hot extraction depending on whether the components to be isolated are heat stable or not.

Cold extraction:

1.Percolation:it is usually one of the most widespread methods employed for plant extraction since it does not require much manipulation or time . The equipment used is a conical glass container with a tap at the base of the apparatus used to set the rate of the solvent elution. Hot or cold solvent may be used. In the former case , a metallic percolator is required .

Very fine powders , resins , & powder that swell or give a viscous eluent cannot be extracted by this method since percolation would be disrupted. The sample should be coarsely fragmented , & particles that pass through a 3-mm sieve would be adequate. Particles of too large a size may produce a high-elution rate precluding the necessary equilibrium for the dissolution of the metabolites , & the menstruum (solvent) would percolate unsaturated . Percolation is more efficient than maceration since it is a continuous process in which the saturated solvent is constantly being displaced by fresh menstruum. Normally , percolation is not used as a contiuous method because the sample is steeped in solvent in the percolator for 24 hour (for up to three times), & then the extracted material are collected & pooled. It has been observed that after a

triple-solvent extraction , the remaining marc does not contain valuable material.

2.Maceration :

In this method the plant is introduced into a suitable container & a sufficient quantity of the required solvent is added & the container is tightly closed & left in away from heat & light for 24 hours after which the solvent can be replaced by a new quantity after filtering the first quantity for another 24 hours & so on until there is exhaustion of the active constituents. Sometimes one time of maceration is enough & this might be left for more than 24 hours. The efficiency of this method may be increased by occasionally shaking the container or by using a mechanical or magnetic stirrer to allow homogenization of the final solution & saturation of the solvent. It is a discontinuous method & the solvent should be renewed until the plant material is exhausted. This requires occasional filtration steps that may produce loss of solvent, metabolites &/or plant material. Such problems may be avoided in part by suspending the ground material in a tied bag in the upper part of the solvent.

Hot extraction (for heat stable material):

There are different methods for hot extraction:

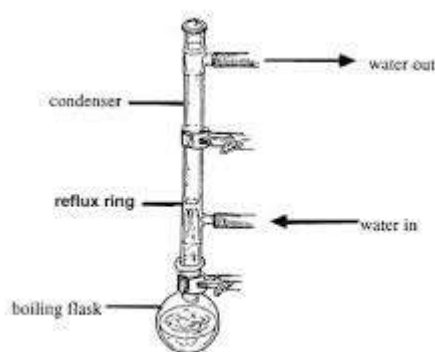
Non continuous hot extraction:

1.Infusion: where by the plant is introduced in a container & a hot solvent is poured on it & the container is covered & left for a certain time then strained.

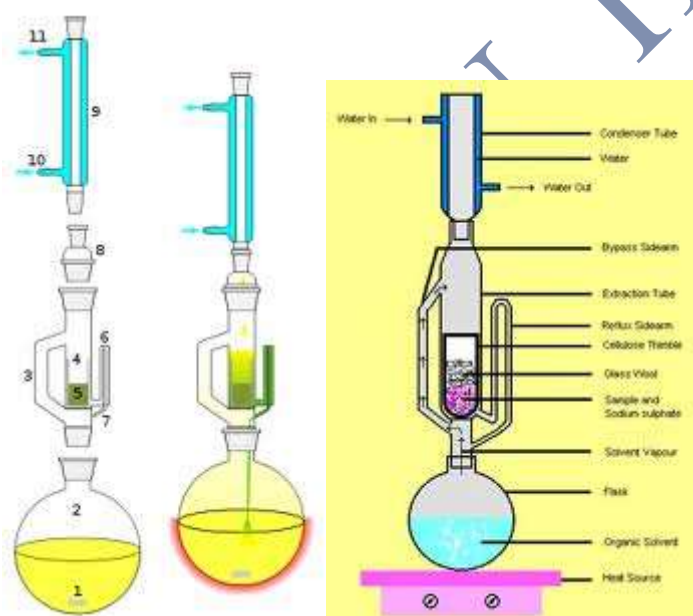
2.Decoction: In this method the plant is boiled with the solvent principally water for certain time taking in consideration the quantity of the solvent so that to avoid dryness and burning of the plant material.

Continuous hot extraction:

1.Reflux extraction: Here the plant is boiled with the solvent in a round flask on which a condenser is placed to insure a complete extraction with out reduction in the quantity of the solvent.



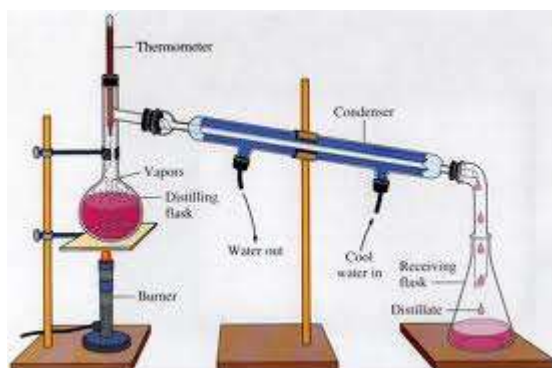
2.Soxhlet extraction: In this method a special apparatus is used called the soxhlet in which the powdered plant is placed in thimble (which is made from cellulose) & the thimble is introduced in the apparatus after plugging it i.e the thimble with cotton wool & the apparatus is placed on a round flask containing the solvent & a cooling condenser is placed on the top of the flask. The solvent is boiled & gets up to the condenser through a side tube , after condensation the solvent will get down & drop on the top of the thimble & extract the plant material inside it. After the trough of the apparatus is filled with the solvent , the solvent will return to the flask by syphoning & so on , the process is repeated until complete extraction. The main advantage of extraction using a Soxhlet apparatus is that it is an automatic , continuous method that does not require further manipulation other than concentration of the extractive & saves solvent by recycling it over the sample. Moreover , this method is not time-consuming , since for a standard-sized sample (500 g), the extraction time is less than 24 h. An obvious disadvantage is that the extractives are heated during the period of extraction at the boiling point of the solvent employed & thermally labile compounds such as carotinoids may hydrolyze , decompose , or produce artifacts.



3. Distillation : which is either :

-steam distillation

-fractional distillation which is used for separation of compounds with different boiling point



Choice of the extracting solvents:

Alcohol is a general solvent for many plant constituents except most of the fixed oils. Water – immiscible solvents are widely used ex:

-light petroleum which is used for extraction of essential & fixed oils, steroids.

-ether & chloroform which might be used for extraction of alkaloids & quinones

-the extraction of organic bases ex: alkaloids usually requires basification of the plant material if a water immiscible solvent is to be used , while for aromatic acids & phenols acidification may be required.

Separation & isolation of the constituents

Different methods may be used in this matter ex:

1. Sublimation : which is some times used on the whole drug , as in the isolation of caffeine from tea , or for the purification of materials present in a crude extract.

2. Distillation : fractional distillation has been traditionally used for the separation of the components of volatile mixtures , mainly components of volatile oils.

3. Fractional liberation : some groups of compounds may be separated by fractional liberation from a mixture ex: when a mixture of alkaloid bases is shaken with NaOH solution the phenolic alkaloids will be separated as salts.

4. Fractional crystallization : the method exploits the differences in solubility of the components of a mixture in a particular solvent .Some times derivatives of the particular components are employed ex: picrates of alkaloids , osazones of sugars.

In closed chromatography mean the stationary phase is packed in closed container & the mobile phase flow through it, while in open column chromatography the stationary phase is found spreaded on a flat surface as an open sheet of paper or a glass plate.

2. classification according to the mechanism of separation (according to the mode of separation). It is perhaps more useful to divide chromatographic forms according to the mode of separation on which each is based. These basic forms of molecular interaction, determine chromatographic behavior. There are four basic mechanisms of chromatography by which separation can occur, & more than one mechanism may be responsible during a given separation:

1. Adsorption chromatography: This involves mobilization of molecule between the surface of a solid stationary phase & liquid mobile phase. The dynamic equilibrium of solutes as they switch between the stationary & mobile phases (processes of sorption & desorption, respectively) is specific for each molecule & is affected by competition that exists between solutes & solvent for sites on the stationary phase. This is a purely physical process involving the formation of no chemical bonds, but only the relatively weak forces of hydrogen bonds, Van der Waal forces, & dipole-dipole interactions. For this reason almost any inert material can in theory be used as an adsorbent, the only proviso being that it does not react either with the sample or the mobile phase & that it is insoluble in the mobile phase. Common examples include silica (as a column or as a TLC stationary phase), cellulose, styrene, alumina etc.

2. Partition chromatography: This type of chromatography employs the separation principle of liquid-liquid extraction. The mechanism involves the relative solubility of the compound between the sorbent (stationary phase) & the solvent (mobile phase). Compounds that are more soluble in the mobile phase will migrate up the plate to a greater extent than components that are more soluble in the stationary phase. When one of the liquids is coated onto a solid support, such as a column of cellulose coated with water, or a silica TLC plates coated with adsorbed water, a stationary phase is created on which separation can be carried out with an immiscible/organic mobile phase, employing the principles of liquid-liquid extraction with the advantages of chromatography. However, this method suffers from the disadvantages that the liquid stationary phase tends to be stripped (leached) from the column as a result of shear forces acting on it from the movement of the mobile phase & by the solubility of the liquid stationary phase in the mobile phase.

Normal phase/reverse phase : If the stationary phase is more polar than the mobile phase, this is normal phase chromatography. An example of this is a

column of silica with its polar silanol groups & a mobile phase of an organic solvent. When the stationary phase is less polar than the mobile phase, this is reverse phase chromatography, exemplified by the hydrocarbons bound to the silica support & a water/acetonitrile mobile phase. Reverse phase chromatography is very widely used as a form of HPLC, & most natural products have a region of hydrophobicity that leads to their retention to some extent on a reverse phase column.

Reverse phase TLC utilizes sorbents that partition natural products between a hydrophobic, fatty (lipid) stationary phase, & an aqueous mobile phase.

Comparison between adsorption & partition chromatography:

Separation in chromatography depends on the type of the **distribution** of the solute (the substance to be separated) between the mobile phase & the stationary phase. In adsorption chromatography the distribution of the solute is between a solid phase which is called adsorbent & a liquid or gas phase which is called the mobile phase. In partition chromatography the distribution of the solute is between a liquid stationary phase & a mobile phase which could be a liquid or a gas.

The ratio of the amount of solute retained by any of these phases is called the distribution ratio. In case of adsorption chromatography the distribution ratio is known as adsorption coefficient & in partition type it is known as the partition coefficient.

In adsorption chromatography where we have a solid stationary phase & a liquid mobile phase, high concentration of the solute will be found at the surface of the solid phase than what is present in the liquid phase. This is known as adsorption & this happens because of the attraction between the surface molecule of the adsorbent or the solid phase & the molecules of the solute. These attractive forces give rise to two types of adsorption:

1. Chemisorption: in this type, the process is irreversible & it is due to a chemical interaction between the solute & adsorbent.

2. Physical adsorption: this type is due to the dipole interaction & physical forces of attraction like Vander Waals forces of attraction. This process is reversible & it plays a big part in the separation of the solute in adsorption chromatography.

Each solute is distributed between the solid & the liquid phase & depending on the nature of the solute, these factors will vary. However, similar solutes having similar functioning groups will behave similarly when both the adsorbent & mobile phase are kept constant.

In chromatography only dilute solutions are used when a weak adsorbent (weak in activity of adsorption) is used & the load (**concentration**) of the solute on the adsorbent affects greatly the separation of the different

components of the solute. When a mixture of solutes is needed to be separated the amount of adsorbent used depends on :

1. the number of these components.
2. The total weight of the solute to be separated.

If more than one solute is present then the solute **competition** for the active site of the surface of the adsorbent is important . This explains why in chromatography the migration of solute might not be predicted from measurement data.

The factors affect the adsorption process will control the process of migration of the solute by chromatography , these factors are the strength of the molecular interaction of the followings:

- 1- the solute- solute
- 2- the solute – solvent
- 3- the solvent – adsorbent
- 4- the solute- adsorbent

In partition chromatography when a solute is separated between two liquid phases & it is soluble in both then we have equation $\alpha = C_A/C_B$

α = the partition coefficient. C_A is the concentration in liquid A , C_B is the concentration in liquid B.

In partition chromatography the solute is distributed between the two liquids A&B according to its solubility in each A&B. This depends on the nature of the solvent & solute. This ratio in which the solute distribute itself is known as the partition coefficient , (α) which is constant at a constant temperature over limited range of concentration. Also in partition chromatography if a mixture of solutes is used **the distribution of each solute is independent on the other** ex: in a mixture of A B & C the distribution of A will be independent on that of B&C as for the others. This is different than the case of adsorption chromatography where by there is a competition of each component for the active site of the adsorbent i.e. in adsorption chromatography the adsorption of A will depend on that of B&C.

As a summary the main differences between adsorption & partition chromatography are:

1. Sample size: In adsorption chromatography the sample is much more larger than in partition.
2. Sample type: Adsorption is generally preferable for non-polar samples while partition for polar.
3. Sample recovery: partition methods generally do not induce chemical alteration or irreversible binding of the sample on the stationary phase, while some adsorbent-sample combination may result in sample loss due to these processes.

4. Speed of separation: Adsorption methods are generally much faster.
5. Convenience: carrying out the separations are less convenient in partition methods.

3.Size-inclusion/-exclusion chromatography: Compounds may be separated by their relative sizes & by their inclusion (or exclusion) into the sorbent.

4.Ion-exchange chromatography: This technique is limited to mixtures containing components that can carry a charge. In this form of chromatography, the sorbent is usually a polymeric resin that contains charged groups & migrating counter-ions, which may exchange with ions of a component as the mobile phase migrates through the sorbent. Separation is achieved by differences in affinity between ionic components & the stationary phase.

Affinity chromatography: This type of chromatography depends on specific interactions of biological molecules such as an antibody-antigen interaction, enzyme-inhibitor interaction, DNA-DNA binding, DNA-protein interaction, or a receptor-agonist/antagonist interaction. The ligand (receptor) is covalently bound to the packing material

Types of chromatography

Adsorption chromatography

- 1- Column chromatography
- 2- Thin layer chromatography TLC

Partition chromatography

- 1- paper chromatography
- 2- Gas liquid chromatography GLC
- 3- High performance liquid chromatography HPLC

Ion exchange

Gel filtration

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