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Practical Medicinal Plants
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المرحلة الثالثة - الدراستين الصباحية والمسائية

الفصل الدراسي الثاني

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Lab.1 Medicinal plants collection and drying

All cultures from ancient times to the present day have used plants as a source of medicines. Today, according to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary health care needs. The greater part of traditional therapy involves the use of plant extracts or their active principles.

Botanists identify plants using herbarium specimens, which are dried, pressed plants in folders or on paper or card. The advantage of herbarium specimens is that they take up little space and that they last for hundreds of years. Using them, a botanist can compare a specimen collected with hundreds of other specimens. Therefore, samples of medicinal plants should be collected and prepared as herbarium specimens.

Collection of medicinal plants

Drugs may be extracted from wild or cultivated plants. The good quality of active constituents of medicinal plants are affected by many factors.

The Factors and points should be considered before the collection

1- Decide which part (s) of the plant involved in study need (s) to collect.

2- Specimens of the collective plants should be labeled . The **labeling** includes (1) the collector's name and a sequential number, e.g. Ahmed 2568. (2) Material from the same collective specimens, such as microscope slides. (3) The label should also include full details of the locality, habitat, height of trees and the color of flowers. (4) Its scientific (Latin) name , and its vernacular name. (5) Whether these plants are cultivated or not.

3- Time of the year:

The plant may contain a substance in winter that is not present in summer, or its amount varies markedly e.g ***Colchicum corm*** is free from bitterness cause by the alkaloid **colchicine** in autumn, hence is used in Austria as a food, instead of potatoes. **Bitterness** starts to appear in spring and early summer when it is which is used pharmaceutically to treat **gout and mediterranean fever**..

4- Time of the day:

Every plant' part has a perfect time to be collected:

Flowers and inflorescences should be collected in early morning or before sun rise to prevent decreasing of volatile oils which are the main active components in flowers.

Leaves or aerial parts should be collected around midday because leaf is the main factory of primary and secondary metabolic compounds produced by photosynthesis which is occurred at its highest rates during midday.

Seeds and fruits should be collected afternoon to approach the maximum nourishment values within these parts because once the metabolic compounds produced inside aerial parts, thus, it takes some time to transport proportions from these compounds to the seeds and fruits.

For example: ***Digitalis*** is a plant contains several active chemical constituents such as **cardiac glycosides (digitoxin)** which is used for the treatment of heart conditions (i.e. **congestive heart failure**). The digitoxin is produced in different times of the day. Being highest in the **afternoon.**

5- Stage of maturity and age:

For instance: ***Conium*** called **hemlock** produces **coniine**, it is a very toxic **alkaloid** causing paralysis of the central and peripheral nervous system, this plant was a cause for **Socrates' death** by the hands of his enemies, the highest levels of coniine recorded when the fruits are **mature and unripe**.

Drying of medicinal plants

Reasons for drying:

1- To reduce their size and weight.

2- To help in their preservation.

3- To fix their constituents, by preventing reactions that may occur in presence of water such as enzymatic destructions of these active constituents.

4- To prevent the growth of micro-organisms such as bacteria and fungi.

5- To facilitate their grinding.

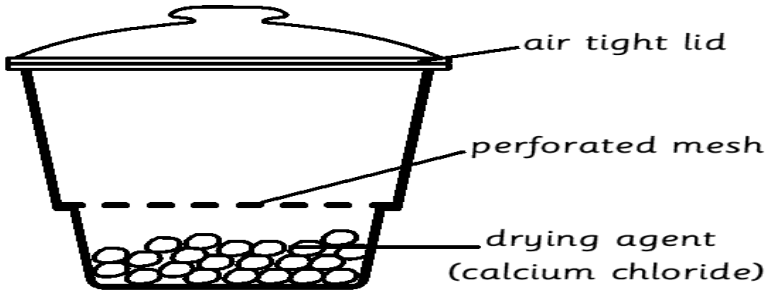
Methods of drying

Drying is carried out either by natural or artificial methods.

1- Natural drying: this is accomplished by natural air in sun or shade.

2- Artificial drying: this is a rapid method of drying done at well-controlled temperature and its accomplished by: (1) direct fire. 2) Use of heated stones. 3) stoves. 4) Lyophilization (Freeze drying). 5) Chemical drying using desiccators. 6) Electrical oven consist of closed space with several movable trays, arranged to allow the circulation of heated air.

The Desiccator



Notes:

1- Electrical ovens used to dry stems, roots, leaves and barks and even some flowers and fruits Such as *Ficus religiosa* fruits (except the aromatic one and lipids constituents). (12-48 h.) and (40-55 C°) are the directions of this procedure..

2- Sunshine use to dry fruits and seeds with lipids constituents in aeriated places and specific plants as *Citrullus colocynthis* fruits.

3- Shadows or shades use to dry the aromatic or fragrant parts such as *Coriandrum sativum* fruits and *Mentha spicata* leaves.

Several changes may occur after drying:

1. **Size and weight**: remarkably decreases due to loss of 80-90% of water.

2. **Shape and appearance**: some shrivel (flowers) on drying, others have their surface wrinkled (fruit's peel).

3- Texture: plants on drying become brittle (leaves), harder (roots), while those containing starch become horny.

4- Color: Tea leaves change from green to brown finally turn almost into black.

5- Odor: ***Hyoscyamus*** lose its bad odor on drying. While ***Orris*** acquires a pleasant odor on drying.

6- Taste: this may be altered. Fresh ***Gentian*** is very bitter becoming pleasant on drying.

There are two principal reasons for deterioration of medicinal plants:

A- Physical factors

1. **Moisture:** moisture sometimes affects drugs adversely through activating the enzymes (as in cardiac glycosides).

2. **Heat:** rise of temperature up to 45 C° activates the enzymes causing decomposition of active constituents. Volatile oil decreases by higher temperature.

3. **Air:** oxygen oxidizes certain constituents of crude drugs, e.g. lemon oil, it causes **rancidity** of fixed oils and **resinification** of volatile oils.

Rancidity: condition produced by aerial oxidation of unsaturated fat (fixed oil) present in foods and other products, marked by unpleasant odor or flavor. When a fatty substance is exposed to air, its unsaturated components are converted into hydroperoxides. Saturated fats such as beef tallow are resistant to oxidation and seldom become rancid at ordinary temperatures.

Volatile oils do not become rancid as do the fixed oils, but instead, on exposure to light and air, they oxidize and resinify in a process called **resinification**.

4- **Light:** it affects drugs, especially those having marked colors. e.g. yellow color of ***Rhubarb*** changes to reddish.

B- Biological factors

1. **Bacteria:** cotton fibers are became brittle by bacterial attack which makes the cotton and wool dusty.

2. **Molds**: the mycelium produces an unpleasant mass in powdered drugs.

3. **Insects**: they seem to attack all drugs but have preferences to certain plants as ginger, belladonna.

4- **Rodents**: they cause most spoilage of crude drugs during storage, especially if wrapped in paper, cloth or wooden containers .

Lab.2 Identification of medicinal plants

The importance of medicinal plants classification is to select the right plant to use for drug preparation experiments.

There are different ways for classification which are very scientific as follows:

1- Morphological taxonomy:

This depend on plant external morphology for all parts. This way of classification depended on identification keys until the genus and species name approached.

2- Anatomical studies:

In this way we suppose to know the storage tissue and the storage compounds which they could be used as drug for diseases treatment. However, the description of drying and extraction of plant parts are on the important steps for drug preparation.

3- Chemical constituents in each plant part used for drug preparation:

This is important in plant identification and differentiation from each other such as alkaloids, terpens and volatile oils.

4- Advance procedure to identify the plants:

Such as DNA by PCR and protein electrophoresis.

Examples for the plant identification importance to obtain safe and success drugs:

1- *Matricaria chammomella* and *Anthemis noblis*

In the local herbal stores you can buy these plants, but you have to know that there is a common confusion could be occurred between both plants due to the similarities

between them specially for those how are not experienced in this field. The differences between those two genera could be summarized :

M. chammomella	*A. noblis*
1- Inflouresence head smaller	1- Larger
2- Redial flowers are sloped when they are mature.	2- Redial flowers are less sloped
3- Inflouresence head is convex	3- Inflouresence head is flat
4- Peduncle is cylindrical	4- Peduncle is angular
5- With smell	5- Without smell
6- No bitter	6- bitter
7- Contain matricharin	7- contain anthemol
8- Uses: anti-inflammatory, anti- vomiting, anti-ulcer.	8- uses: antibacteria.

A. *noblis* contain anthemol, this compound is responsible for the damage of liver cells thus, it is not recommended to be given to children under six years, while *M. chammomella* is safe and it could be used for new borne babies.

2- *Mentha spicata* and *Mentha longifolia* leaves:

See the differences between them in morphology, In addition to their smell. *M. spicata* is useful as sedative for pains, carminative, diuretic, some sort of respiratory inflammation could be treated by this plant, and heart stimulate. Therefore, it is not useful for people who have some heart problems.

M. longifolia has the same use, in addition to their use as anti-anthelmentic and accelerate women birth, therefore is not recommended for women during their first months of pregnancy.

3- Leaves of some Umbelliferae family (Apiaceae) plants:

These leaves are similar to each other in morphology and it is difficult to differentiate between them easy such as: *Apium graveolence, Petroselinum hortense,* and *Coriandrum sativum*

The differentiate between them by smelling, tasting, leaves morphology, length of their petioles and their margins.

Petroselinum hortense is important for menstrual disturbances and pains, rich in minerals such as **Fe, CO, Mg**,….etc., *P. hortense* contain **glycosides (appin)**, this compound is hydrolysed into **apigenin, glucose, and apiose**, in addition to that its **greenish volatile oil** contain **apiol** responsible for the special smell of this plant.

Apium graveolence is diuretic, frittering stones in bladder….etc, this plant contain volatile oil (**yellowish in color**), this v. oil consist of **limonene 60%, selenine 10%, sedanolide 2.5-3%.** Also this plant contain **appin and apiol**.

Coriandrum sativum is diuretic, carminative, and frittering stones in bladder, this plant contain **colorless oil** consist of **45-70% linalool**.

***4-Melilotus indicus* and *Trigonella foenum- graceum* leaves:**

T. foenum- graceum is nutritional plant and used as diuretic, the morphology of this leaves are similar to leaves of *M. indicus*.

M. indicus some information indicated that toxic coumarin could be detected immediately after collection of the plant and its toxicity will be lost later, therefore it could be collected during the morning and given to cows after that during evening

while in *T. foenum- graceum*: leaves don't have these toxic substance, therefore it could be given to animals any time during the day.

5- Ocimum basilicum and *Lallemantia iberica:*

Notice the differences between seeds of *Ocimum basilicum* and *L. iberica*, The seeds of both plants used as anti-anthelmentic and useful for constipation, diuretic and ease renal system pains, coughing….etc .*L. iberica* seeds are harmful to digestive canal in high doses. Typical dose is one tea spoon of seeds in a glass of boiled water.

The seeds of *L. iberica* produce mucilage layer surround these seeds in case they are immersed in water, while *O. basilicum* don't produce the mucilage layer.

Lab 3

Glands and Excretory tissues and their roles in storage and excrete the active constitutes in medicinal plants.

The active chemical compounds which have medicinal effects in plants considered as secondary metabolites in these plants. These compounds accumulated in special secretory structures which could be classified as follows:-

1.External or Dermal glands.

2.Internal or Globular glands.

3.Secretory canals or ducts (tubular type).

4.Hydathodes.

The first 3 structure will be discussed as follows:-

1.External or Dermal glands:-

These structure produced from multiple divisions of protoderm cells. In certain places of this layer. However, these structure called Dermal glands…..

These types of glands included the following types of glands:-

1.Glandular hairs
2-Nectaries

3.Digestive glands in plants which can use insect as food and have the ability to digest them.

The external or dermal glands could excrete nectar, terpenes ,gums

or sugar solutions.

A -Glandular hairs:-

Initiated from multiple divisions of the protoderm cells and produce elongated structure similar to the root hairs, that is why they called glandular hairs. these glandular hairs consist of stalk and head, the stalk consist of one or two cells and the head represented by the apical cells. The head considered as the secretor part. ***Urtica urene***s is one of the

plant which contain the glandular hairs in addition to ***Rosmarinus officinalis*** أكليل الجبل these compounds which produced by these glands important to act as (Anti-inflammatory ,help in injuries healing, stimulate the arteries…..etc).

B-Nectar glands or Nectaries:-

Nectar (this word derived from latin nectar which is mean favored drink of Gods) and this is a sugar-rich liquid produced by gathering of special cells called nectaries or nectar glands, this type of glands is located either within the flowers then called **floral nectaries** with which attract pollinating animals, common nectar-consuming pollinators include mosquitoes, hoverflies, wasps, bees, butterflies and moths, hummingbirds. Or located on stem, leaves or any parts of a plant except floral parts such glands called **extrafloral nectaries**, which provide a nutrient source to animal mutualists (provide anti herbivore protection). The main ingredients in nectar are sugars in varying proportions of **sucrose, glucose, and fructose**. In addition, nectars have diverse other phytochemicals serving to both (1) attract pollinators and (2) discourage predators, such as: {**Carbohydrates, amino acids, and volatile oils** }function **to attract some species**, whereas {**alkaloids and polyphenols**} appear **to discourage predators**.

2-Internal or globular glands:-

These glands initiated from the ground meristem, however they are mostly present in cortex and their morphology is coccoidal. In the transverse section could be seen by naked eyes and consist from one layer or multiple layers from cells surrounded the central cavity.

The cavity produced by the following ways:

a) Gradual separation of secretarial cells.
b) Lysis of secretarial cells often the secretion of their compounds.
c) Or by both ways mentioned above and then called separative cavities or degenerated cavities or schizolys genous.

The central cavity contain the oil secretion which the smelling type attributed to it.

Example: ***Eugenie caryophyllata*** nectar glands are located on flowering buds, or in fruit peels such as those of the oranges and lemons(***Citrus*** *spp*.)

3-Secretory canals or ducts(tubular type)

The initiation of these ducts are different in different plants such as :-

a)Resisn ducts

-These ducts which are tubular structure consist of empty place surrounded by cells the which have thin cell walls(without lignin) and called Epithelial cells.

present as: normal primary or secondary tissues' such as those present in **pins** and **larex** . however they could be present due to the injuries in the plant such as **cedrus**.

b) Gum canals or ducts:

these initiated from the modification of compounds in cell walls which converted from cell wall to un crystallized compounds which have the gum in morphology . this secretion called Gummosis and this could be common to happen in Barks of ***Acacia senegal*** السفط السنغالي .

Main differences between Resin and gum:

Resin	Gum
1- It is initially produced by oxidation of volatile oil (responsible for the essence of resin) in a process called resinification.	1- It is produced by a process called Gummosis(التعريف ذكر سابقا
2- It is a secreted by certain trees such as *cedrus* when they are incised.	2- Secreted by certain trees such as *Acacia senegal* when they are incised.
3- insoluble in water	3- insoluble in alcohol (with few exceptions)
4- soluble in alcohol	4- soluble in water

c)Laticifers or laticiferow canals:

Laticifers are highly specialized cells which can produce a wide variety of secondary metabolites, this type of ducts produce a type of secretion called latex (is a milky fluid consisting of many compounds such as proteins, starches, oils and gums that coagulate on exposure to air. The latex have a role in wound healing and as defense against herbivorous. Laticifers found in the leaves and/or stems of certain plants such as: Moraceae, Euphorbiaceae, Apocyanaceae.

Some of active canal non active compounds present in medicinal plants:-

Active compounds	**Non- active compound**
1- Volatile oils	1- cellulose
2- Saponins	2- legnin
3- Tannins	3 -suberins
4- Resins	
5- Alkaloids	
6- Sterols	
7- Lipids	
8- Carbohydrates	
9- Glycosides	

Lab.4 Methods of Preparing Herbal Remedies

In traditional herbal medicine systems, herbal remedies are prepared in several ways which usually vary based on the plant utilized, and what condition is being treated. Some of these methods include: infusions (hot teas), decoctions (boiled teas), tinctures (alcohol and water extracts), and macerations (cold-soaking). Others include preparing plants in hot baths (in which the patient is soaked in it or bathed with it), inhalation of powdered plants, or steam inhalation of various aromatic plants boiled in hot water.

Active Plant Chemicals Equal Active Remedies
The therapeutical activity of a medicinal plant is closely related to the plant chemicals within it. These chemicals can be classified into major groups such as essential oils, alkaloids, acids, steroids, tannins, saponins and so forth. Each one of these classes may have a preferred method of extraction which facilitates getting the chemicals out of the plant. For example, some active chemicals found in plants are not soluble or dissolved in water, therefore just preparing a hot tea with the plant, or even boiling the plant in hot water won't extract these chemicals into the resulting water, these chemicals may be more soluble in alcohol.

Preparing of Remedies or herbal drugs
When a plant is finely ground, it usually makes a stronger remedy as more surface area of the plant is available to extract in the solvent. Extra time filtering is normally required when working with plant powders, but many herbalists prefer working with powders instead of bulky cut herbs since they make stronger remedy extracts. It is also recommended to use distilled or purified water when extracting medicinal plants. Regular tap water can contain chlorine and other chemicals which might have an interaction or chain reaction with one or more of the many chemicals found in plants.

Instructions for the main extraction methods are as follows.

Macerations
This method of extraction is certainly the easiest. The fresh or dried plant material is simply soaked in cool water overnight. The herb is strained

out and the liquid is taken. Normally this is used for very tender plants, or those with delicate chemicals that might be disfigured by heating or which might be degraded in strong alcohol.

Infusions

Infusions are typically used for fresh /dried leaves and tender herbs or plants. Preparing an infusion is much like making a cup of tea. Water is boiled and then poured over an herb (or combination of herbs), cover the cup and allow to sit/steep for 10-15 minutes or more (get the liquid and leave the sediment in the bottom of the cup). The ratio of herb to water can vary depending on the remedy and the plant. Generally using 30-60 g of herb in 180-240 ml water is sufficient, while the proper dosages are (2-3) cup daily.

Decoctions

Decoctions are usually used with tougher and more fibrous plants, barks and roots (and which have water soluble chemicals). Instead of just steeping it in hot water, **Use 500 ml. of water for every 30 gm of dried herb. Cover the container and boil** the plant material for a longer period of time 15-20 min. to soften the harder woody material and release its active constituents.

Tinctures

A tincture is an alcohol and water extract which is used when plants have active chemicals that are not very soluble in water, and to prepare a concentrate plant extract (in its chemical component) for longer term storage. Properly prepared plant tinctures can last several years or more without losing therapeutical potency (shelf life). To prepare a tincture with a shelf-life of at least one year, by using a minimum of 40% alcohol, in addition to a clean, dark and glass bottle with tight fitting lid. A standard (1:4) tincture usually means 1 part herb to 4 parts liquid (or as above, 1 ounce herb to 4 ounces of liquid). Seal the container and store at room temperature away from direct sunlight. Shake the bottle/jar at least once daily for at least two weeks. If using a powdered plant for the tincture, three days will be sufficient and the powder will settle to the bottom. Dosages needed for tinctures are usually much less than infusions and decoctions. Average dosages for tinctures are about 1-2 milliliters (about 30 to 60 drops) two to three times daily. The tincture can be placed

directly in the mouth for immediate absorption, or placed in a small amount of water or juice.

Compresses

Compresses are simply soaking a cloth in a prepared infusion, tincture or decoction and laying the cloth onto the affected part of the body/skin. Or even chewing up fresh leaves or roots and spitting them out onto the skin.

Bathing Remedies

Medicinal plants are added to a bath water and the patient is soaked in it. The skin is a wonderful organ capable of absorbing plant chemicals (and even synthetic chemicals) directly thru the skin, and into the underlying fat tissue, then into the bloodstream.

Pills (Honey Pills)

Pills can be made by mixing thoroughly the powdered drug with equal quantity of honey cooked to bright red syrup. The moment the mixture starts to cool off, it can be rolled to desired tubular strands and cut into small pieces.

Ointment:

Herbal creams and ointments are natural remedies that are mainly used for skin conditions (wounds and burns) or muscle and joint aches.

1. Start by preparing the herbs. Chop any fresh herbs and then weigh them.

2. Melt 600ml of emulsifying oil (as olive oil) in the glass dish over low heat.

3. Add 270ml of glycerol and 300ml of water.

4. Then add 120g of herbs and simmer for 3 hours, stirring occasionally. If the water runs low, top it up again.

Choice of proper solvent for extraction

1- Not expensive.
2- Safety (have no negative impacts against mankind and environments).
3- Inert or does not have the ability to interact with the active compounds within the plant materials.
4- Highly selective for the compounds need to be extracted.
5- Viscosity, Vapour Pressure, Freezing Point : These should be low for ease in handling and storage, for example, a high viscosity leads to difficulties with dispersion and mass-transfer rate.

Note: the reasons for mix alcohol and water are:

1- Induce soften and enlargement of plant part (s) compose from cellulose by imbibitions.
2- Increase the porosity of the plant cells walls which facilitates diffusion of chemical compounds outward the cells in aid of the aqueous phase (solvent).

Lab.5 Methods of extraction the active compounds

Extraction could be started by the following steps:

1-The chemical constituents: Which need to be extracted and isolated such as: alkaloids, tannins, saponins…….etc.

2-The plant parts: need to be extracted should be known such as leaves, roots, flowers, or barks.

3-Choose the right organic solvent to extract the plant parts:

There are 3 types of organic solvents: high polar organic solvents such as ethanol and methanol, low polar solvents such as hexane, diethyl ether and ether, and intermediate polarity solvents such as chloroform, butanol, and ethyl acetate.

Some organic solvents such as hexane and diethyl ether used to extract oils, in addition to water could be used to extract some chemical constituents as polar solvents.

Types of extraction:

1-Water extracts:

In this method the water could be used for extraction and the resulted extract called water extract and it could be called crude due to water which is high polar solvent similar to ethanol and methanol which extract all chemical compounds in the plant part. Water could be used as cold or hot and called hot or cold water extract. In this type of extraction glycosides could be extracted.

2- Alcoholic extraction:

There are 2 types of alcoholic exraction:

a- Alcoholic extraction by shaking:

In this type of extraction 250 ml of solvent put in beaker and 50 g of plant powder put over it and mix them well, then covered it and leave it for 24h, then filtrate by using muslin cloth then by using filter papers and the supernatant put in petri dishes and put it in oven to evaporate the solvent, and

the extract kept in refrigerator until use. The extract called according to the organic solvent used when we used ethanol called crude ethanolic extract….etc.

b- **Alcoholic extraction by using soxhlet apparatus:**

In this type of extraction 200 ml of solvent and 10 g of plant powder put in soxhlet apparatus and extracted for 8h, then the extract put in rotary evaporator at 40°C to concentrate the extract. The resulted extract called crude alcoholic extract.

3-Steam water extraction:

This type of extraction could be used to extract volatile oils by using clavenger apparatus.

Oil extraction:

different plant parts. Volatile oil found in aromatic plant parts such as flowers and leaves, and it is volatile in room temperature, and extracted by steam water in clavenger apparatus. While fixed oil are found in seeds, bark, and root and them are not volatile in room temperature and extracted by using soxhlet apparatus with hexane or diethyl ether.

Oils have important function such as insects deterrent, and have therapeutically properties due to their antibacterial, antifungal activity and recently many topics have been elucidated their effects against harmful algae.

Lab: 6 Plant chemical metabolic compounds

Plant chemical metabolic compounds are divided to primary and secondary metabolic compounds.

Primary metabolic compounds: are produced by plants and play an important role in growth and reproduction of plants.

Secondary metabolic compounds (S.M.C.): are chemical compounds that produced through primary metabolic process and don't have any role in plant growth and reproduction, but they have played a role in plant persist under special ecological conditions.

Function of secondary metabolic compounds:

1- **Defense:** such as phenols (pesticides).
2- **Attractive:** such as anthocyanin (for pollination).
3- **Allelopathy.**
4- **Structural:** such as lignin.
5- **Therapeutical effects:** (for treated ulcer,and blood pressure).

The S.M.C.are divided into 3 basic groups: Terpens, Alkaloids, and Phenols.

1- Terpens:

Terpens are S.M.C., non nitrogenous, non toxic, composed from isoperine units. Most of it have cyclic structure combined with 1 or more of active groups (hydroxyle). Terpens are chemically classified according to isoperinestructure(C5H8) into the following:

1- Isoperine (5c)*2=(10c) called monoterpens such as volatile oils.
2- Isoperine (5c)*3=(15c) called sesequiterpens such as ABA hormone also volatile oil.

3- Isoperine (5c)*4=(20c) called diterpens such as GA3 hormone.
4- Isoperine (5c)*6=(30c) called triterpens such as steroids (hormones in the flowers ovary) and wax.
5- Isoperine (5c)*8=(40c) called tetraterpens such as xanthophylls and carotins.

Examples of Terpens compounds:

1- Saponins
2- Glycosides.
3- Resins.
4- Volatile oils.

Terpens have some properties such as food preservative, antimicrobial, and facilitate the digestion.

Terpens extraction:

10g of dried plant part such as leaves of *Dodonaea viscosa* successively extracted in soxhlet extractor for 8h with 200ml chloroform. The solvent was removed by rotary evaporator at 40°C. Then the extract kept until used.

Indicators of some terpens compounds:

1- **Detaction of saponins:**

This test was used for the detection of saponins, bottle containing aqueous or alcoholic extract was shaken, the appearance of a foam for a long time refer to a positive result.

2- **Detection of glycosides:**

Some drops of HCL were added to the plant extract and mixed well, then placed in a water bath for 2 min. then 2 ml of benedict reagent were

added and placed in water bath for 5 min., the appearance of red precipitate refer to positive result.

3- Detection of resins:

About 50 ml of (95%) ethyl alcohol was added to 5g of plant extract powder, put in water bath and boiled for 2 min. after cooling the mixture filtrate, 10ml of distilled water containing 4% HCL were added to the filtrate solution, turbidity appearance refer to resins existence.

4- Detection of volatile oils:

10ml of plant extract filtrate with filter paper, then the filter paper was exposed to UV. transilluminator. The appearance of pink color refer to the positive result.

2- Alkaloids:

Alkaloids are one of the largest secondary chemical compound groups found in more than 300 families. They are nitrogenic compounds, heterocyclic, and found as crystals forms as a salts for some plant acids like citric acid, lactic acid, acetic acid, oxalic acid, malic acid, tartaric acid, fumaric acid, and benzoic acid. They contain one or more of nitrogen atom, carbon, hydrogen, and oxygen. Alkaloids are colourless compounds, they are crystals at room temperature if O2 included and liquid such as nicotine if O2 is absent from their structures.

The plant: *Hemelock* is contain many poisoning alkaloids, these alkaloids are extracted and used for poising Socrat.

Example of alkaloids: Coccaine, Caffeine, and Morphine.

Alkaloids have many effects on insects, some alkaloids have different effects against some parasites, and used for treatment the increase of sugar in blood and for treatment the lesion of stomach and colon.

Alkaloid indicators:

1- **Mayer reagent:**

This reagent was used for the detection of alkaloids. The stock solution (1) was prepared by dissolving 13.5g HgCL2 in 60ml H2O, stock solution (2) was prepared by dissolving 5g KI in 10ml H2O, then combined with stock (1) and (2) and diluted with H2O up to 100ml, then 1-2 ml of mayer reagent were added to 5 ml of aqueous or alcoholic extract. A creamy or white precipitate indicated the presence of alkaloids.

2- **Wagner reagent:**

This reagent use for detection of alkaloids, this reagent prepared by: (dissolved 2g of KI in 5ml of D.W. then add 1.27g of Iodine and mix well then add D.W. until the volume reach to 100ml), then 1 ml from reagent added to 1 ml of plant extract. The brown precipitate indicated the presence of alkaloids.

Terpenes	**Alkaloids**
1- Non nitrogenous and (volatile with fragrant)	1- Nitrogenous and non-volatile
2- isoprene unit is the building unit	2- Amino acids are the building units
3- FDA recognized terpene as (safe)	3- very toxic
4- are colorless, mainly insoluble in water	4- Alkaloids are heterocyclic, colorless, insoluble in water they

	are crystals at room temperature if O2 included and liquid such as nicotine if O2 is absent

Lab : 7 **Phenols Extraction**

Phenols are secondary product in plant, which contain benzen cycle (aromatic) 1 or more combined with hydroxyle group (C-OH) 1 or more . phenollic compounds are soluble in water , sometime combined with sugar compound of phenols were found .

Phenolic compound were divided in to following compounds:

1-Tannins

2-Flavonoids

3-coumarins

Preparation of phenolic extracts

200 g of dried materials (leaves) of Euphorbia peplus were divided into two equal quantities, one was mixed with 300 ml of D.W. and the another one was mixed with 300 ml of 1 % HCL.

Then samples were homogenized in electrical shaker for 5 min then filtered by using muslin cloth and then by using centerfuge . the supernatants were mixed with equal volume of N – propanol and concentrated by using rotary evaporator . the upper layer was dried by rotary evaporator at 40 c . Dry material for both layers was kept until use.

The isolated compounds indicators:

1-Ferric chloride reagent for detection of phenolic compounds (in general)

Plant powder 10 g was added to 50 ml of D.W. , and then heated till boiling , the solution left to cool . and filtered . then 1 % of Ferric chloride (Fecl3) was added to the filtered solution . the appearance of blue green color refer to the phenolic compounds existence .

2-Detection of Flavonoids components :

Solution (A) : the plant extract .

Solution (B) : was prepared by adding 10 ml of (50%) ethanol to 10 ml of (50%) KOH two equal volumes of solution (A) and (B) were mixed .appearances of yellow color refer to flavones existence.

3-Detection of coumarins :

About 1-2 ml of alcoholic extract in a test tube added and covered with filter paper (exposed with (NAOH) and placed in water bath ,heated until boiling , then the filter paper exposed to UV transilluminator , a bright yellow green color refer to the coumarins existence .

Lab. 8 COLUMN CHROMATOGRAPHY :

What is Column Chromatography?

In chemistry, Column chromatography is a technique which is used to separate a single chemical compound from a mixture dissolved in a fluid. It separates substances based on differential adsorption of compounds to the adsorbent as the compounds move through the column at different rates which allow them to get separated in fractions. This technique can be used on small scale as well as large scale to purify materials that can be used in future experiments. This method is a type of adsorption chromatography technique. The applications of this technique are wide reaching and cross many fields including biology, biochemistry, microbiology and medicine.

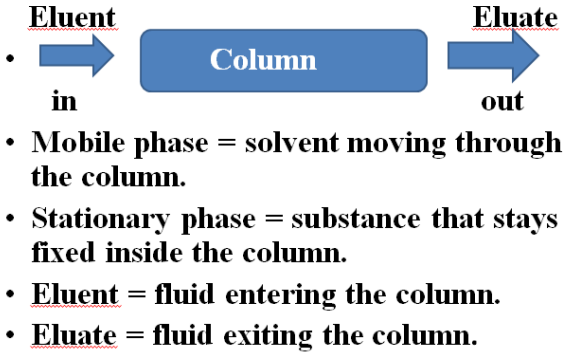
Column Chromatography Components:

The column mostly consists of a glass tube packed with a suitable stationary phase. A glass wool/cotton wool or an asbestos pad is placed at the bottom of the column before packing the stationary phase to prevent it from descending from the bottom of the column.

Stationary phase: It is a solid material that should have good adsorption properties, fixed inside the column.

Mobile phase: This phase is made up of solvents to help the sample mixture can be introduced in the column, some examples of solvents used as mobile phase based on their polarity are ethanol, acetone, water, acetic acid, pyridine, etc.

Chromatography



Eluent: fluid (mobile phase + sample) entering the column, helps in the separation of components in the sample to forming bands. **Eluate:** fluid exiting the column, the components that are separated during the experiment are removed from the column.

Types of packing

Dry Packing: In this required quantity of adsorbent is poured as a fine dry powder into the column and the solvent is allowed to flow through the column till equilibrium is reached.

Wet packing: This is the ideal technique. The slurry of adsorbent with the mobile phase is prepared and poured into the column. The stationary phase settles uniformly. There is no entrapment of air bubbles. The bands eluted from the column will be uniform and ideal for separation.

The column must be packed as uniformly as possible to minimize the distortion of the chromatographic boundaries Channeling is usually caused by the inclusion of air bubbles during packing.

Choosing a Stationary Phase

Alumina (slightly basic) and silica (slightly acidic) are the two most popular stationary phases in column chromatography which possess **surface hydroxyl groups** and **Lewis acid-type interactions determine their adsorption characteristics.** The

elution order of compounds from these adsorptive stationary phases can often be predicted on the basis of their relative polarities. The more polar compounds with the most polar functional groups will be retained on the stationary phase longer. Thus the least polar compound will elute from the column first, followed by each compound in order of increasing polarity. While nonpolar solutes are eluted first.

Choosing Solvents (Mobile Phase)

A separation will begin by using **a nonpolar or low polarity solvent**, allowing the compounds to adsorb to the stationary phase, then **SLOWLY** switching the polarity of the solvent **(choosing a low polarity solvent)** to desorb the compounds and allow them to travel with the mobile phase. Some typical solvent combinations are ligroin dichloromethane, hexane-ethyl acetate and hexane-toluene. Often an experimentally determined ratio of these solvents can sufficiently separate most compounds. Solvents such as methanol and water are normally not used because they can destroy the integrity of the stationary phase by dissolving some of the silica gel.

Principle of Column Chromatography?

Principle of adsorption chromatography involves competition of components of sample mixture for active site on adsorbent. Separation occurs because of the fact that an equilibrium is established between molecules adsorbed on stationary phase and those which are flowing freely in mobile phase. The separation of compounds from one another by passing a mixture through a column that retains some compounds longer than others. When the mobile phase along with the mixture that needs to be separated is introduced from the top of the column, the movement of the individual components of the mixture is at different rates. The components with lower adsorption and affinity to the stationary phase travel faster when compared to the greater adsorption and affinity with the stationary phase. The components that move fast are removed first whereas the components that move slow are eluted out last.

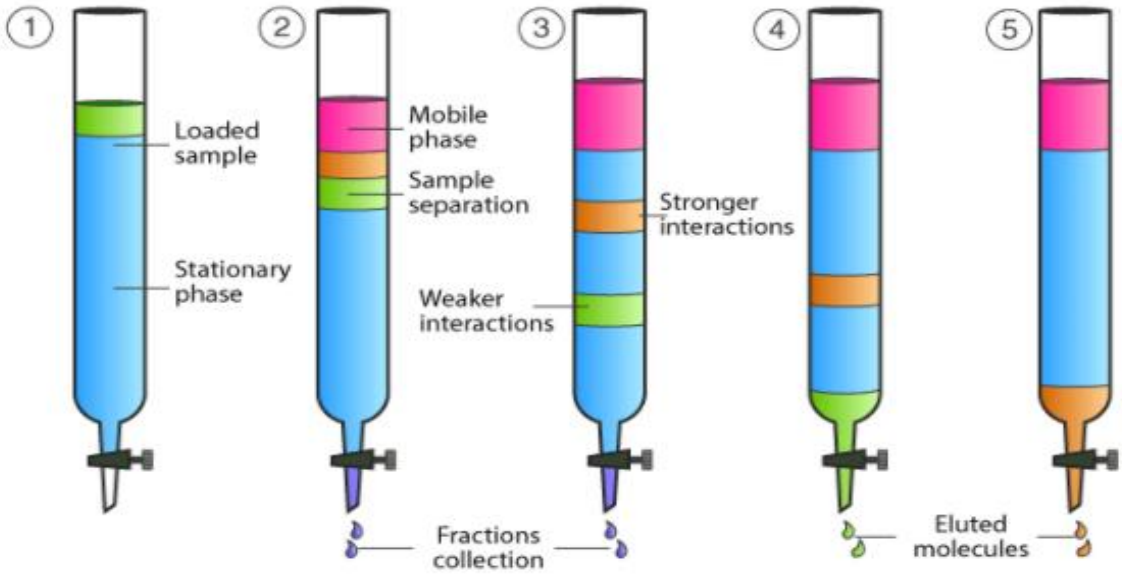
Adding the Sample

The sample is loaded directly to the top of the column, normally, a minimum amount of a polar solvent 5-10 drops, is used to dissolve the mixture then the solution is carefully added to the top of the column using a pipet without **disrupting the flat top surface of the column**. A thin horizontal band of sample is best for an optimal separation. Once the mixture is added and then, continuously adds the solvent eluent while collecting small fractions at the bottom of the column. Using a pipet to add the first bit of solvent on top of the packing and sample, will minimize disturbance of the column and diluting the sample. Collecting small fractions (1-3 ml) is important to the success of your column separation.

Monitoring the Column

If the mixture to be separated contains colored compounds, then monitoring the column is very simple. The **colored bands will move down the column along with the solvent** and as they approach the end of the column, collect the colors in

individual containers. Most organic molecules are colorless, in this case, the reaction must be monitored by **TLC** or measure by **spectrophotometer**.



Lab. 9 Poisonous plants

ıous plants were grouped based on the major organ system affected by consumption plant. The toxicity of these plants came from plenty of various chemical compounds s: Ricin, nicotine, caffeine, crystals of calcium oxalates and many others. Several of poisonous plants gathered depending on their health hazards will be mentioned , Such as:

nts That Cause Irritation of the Oral Cavity

plants belonging to the Araceae family contain needle-shaped calcium oxalate s in their leaves. When the plant leaves are chewed, the oxalate crystals are expelled, g an immediate burning sensation in the oral cavity tissues.

ıts That Affect the Gastrointestinal Tract

s seeds contain ricin which is comprised of two subunits: (Unit B) for binding in r membranes, whereas (unit A) (for activity) is an enzyme capable of inactivating mes in eukaryotic cells.

nts That Affect the Coagulation of Blood Coumarins are the causative factors t in *Melilotus* spp.

diotoxic Plants Cardiac glycosides are a specific type of toxic glycosides that affect diac muscle, increase the contraction force of the heart by inhibiting the myocardial ATP-ase, which can lead to cardiac arrest, cardiac glycosides are present in *Digitalis ea* and *Nerium oleander.*

ıatotoxic Plants : **Pyrrolizidine alkaloids (PAs)** are a large group of hepatotoxins. ıunds in plants known to cause intrahepatic cholestasis are **sporidesmin** a mycotoxin

l by a fungus on grasses and **saponins** present in several grasses.

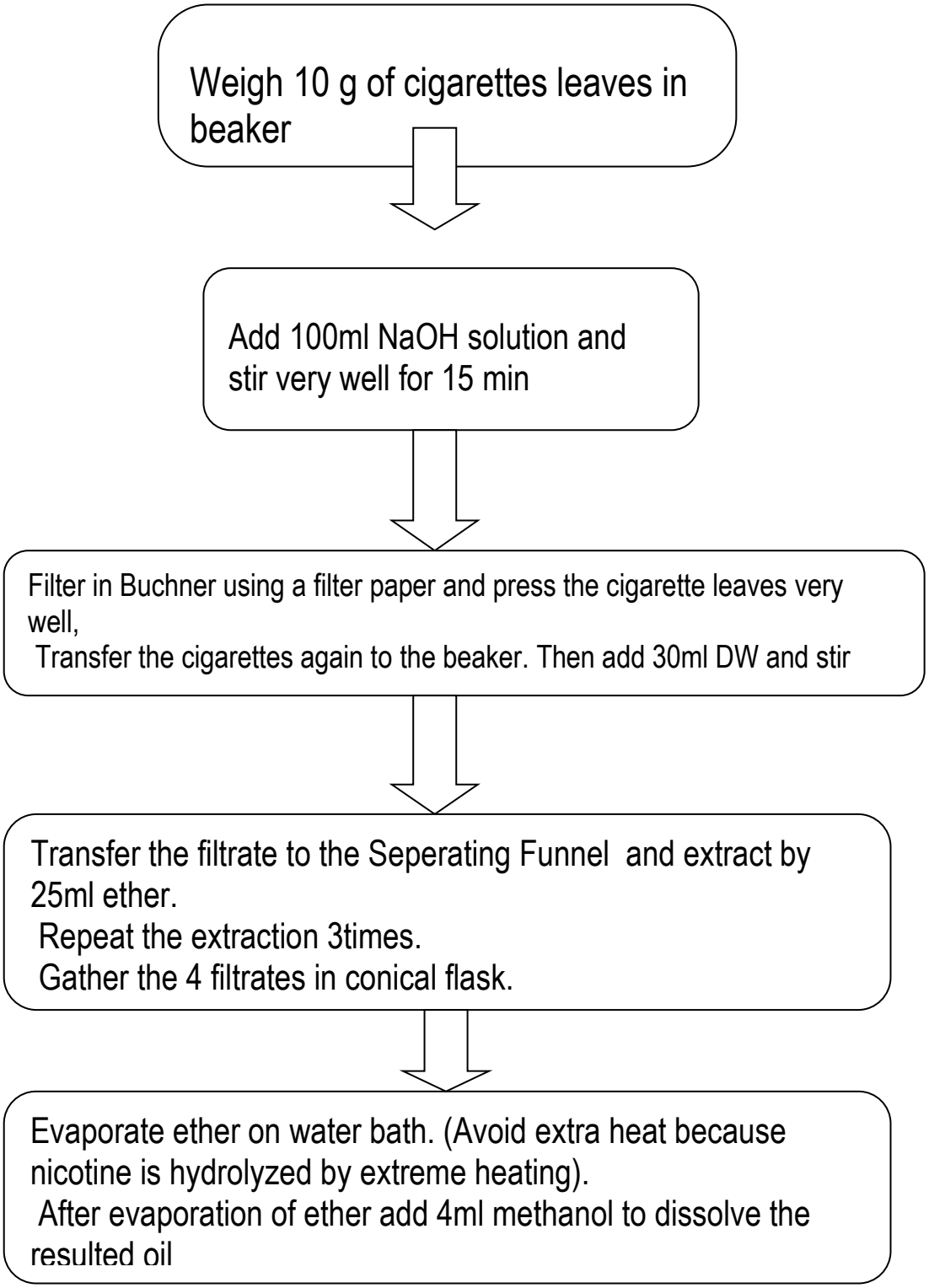
ypes of toxic (in a certain dose) will be elaborated here: Nicotine and ne.

ne is an alkaloid occurs in the leaves of *Nicotiana tubacum* Nicotine is copic, oily liquid that is miscible with water in its base form, this d is easily penetrates and irritates the skin (Be aware while you in touch or extract it) . Tobacco smoke consists of a heterogeneous mixture of which contains 4000 compounds such as **(1) nicotine(2), carbon ide (3), polycyclic aromatic hydrocarbons and (4)heavy metals.** ng is a major risk factor for

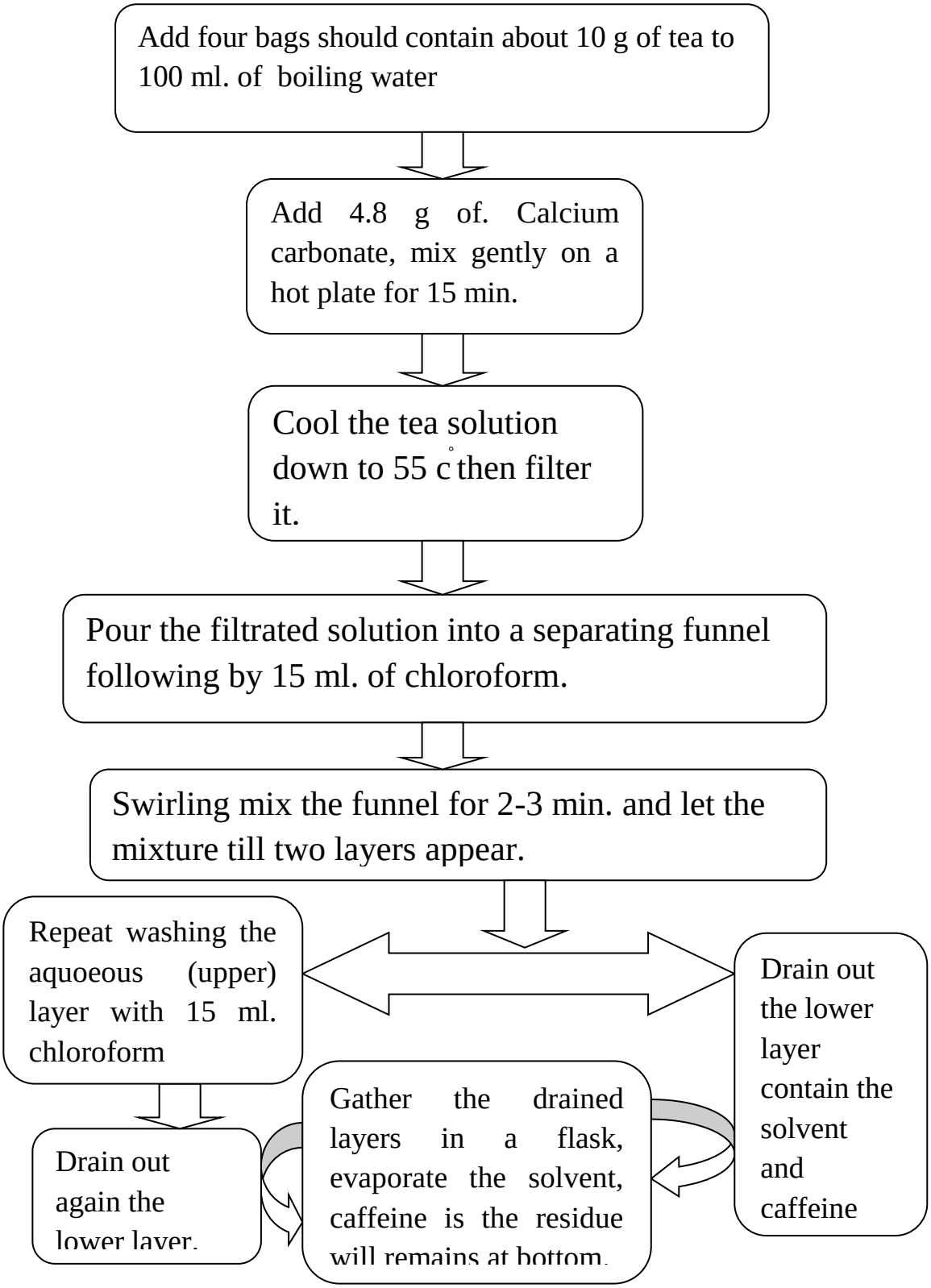
periodontal disease, causing bone and tooth loss. Cigarette smoking increases the risk of heart diseases, cancer of lung and digestive tract.

Caffeine is another alkaloid widely use around the world siping while drinking beverages such as coffee, tea, soft drinks (pepsi) and energy drinks. In humans, caffeine acts as a central nervous system stimulant, temporarily warding off drowsiness and restoring alertness. It is the world's most widely consumed psychoactive drug. Part of the reason caffeine is classified by the Food and Drug Administration as GRAS (generally recognized as safe) is that toxic doses (over 10 grams) are much higher than typically used doses (less than 500 milligrams).

Nicotine extraction procedure:



Caffeine extraction procedure :



Lab.10

Essential oil extraction

Essential oil it is a concentrated hydrophobic liquid containing volatile aroma compounds from plants, essential oils are also known as volatile oils, ethereal oils or aetherolea, or the plant from which they were extracted an oil is "essential" in the sense that it carries a distinctive scent, or essence, of the plant. And it is composed of monoterpens e.g. of monoterpens (α-pinene, β-pinene, limonene, myrecene,frontaline,ect…), which have important functions :

1- Insects deterrent.

2- Have allelopathic effects which provide dominance for their producers over other competitor plants in vicinity.
3- Common in their therapeutically properties due to their antibacterial, antifungal activity which includes also their significant effect on eggs and larvae of many insects, those medical characteristics result in using the essential oils to cure many skin lesions, respiratory tract infections and rheumatoid arthritis and many other diseases. Recently many topics have been elucidated their effects against harmful algae.

4- Sexual attraction in some insects (Pheromones interactions). In addition to the role of carotenoids (tetraterpens) colors in the pollination.

5- In the stomach, the effect is carminative, relaxing the gastric sphincter and encouraging eructation.

Notes: 1- All the essential oils are extracted using steam distillation method which carried out in aid of cleavenger apparatus.

2- Taken by mouth, many essential oils can be dangerous in high concentrations. Typical effects begin with a burning feeling, followed by salivation.

Eucalyptus oil

A part from essential oils used mainly in foods, the best-known essential oil world-wide might be eucalyptus oil, produced from the leaves of *Eucalyptus* sp.. Steam-distilled eucalyptus oil is used as a primary cleaning/disinfecting agent added to soap and cleaning solutions, it also possesses insect and limited vermin control properties. The oils are used as an inhalant with steam and other preparations for relief of colds and influenza symptoms. Because of its refreshing odour and its efficiency in killing bacteria, the oil is also used as an antiseptic.

Method of essential oil extraction