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بنية المقرر ٠ التقنيات الأحيائية النباتية/ العملي	
الاسبوع	**المادة العلمية**
الاول	التعرف على فوائد النباتات الطبية والصناعية وغيرها من المجالات Significance of medicinal plants to human being
الثاني	التعرف على التقنيات المتعددة في كيفية استخلاص المواد الفعالةExtraction techniques of medicinal plants
الثالث	وفوائده الطبيةTerpenoidsالتعرف على مستخلص مادة Some of Plant Secondary Metabolites (**Terpenoids)**
الرابع	وفوائده الطبيةEssential Oilsالتعرف على مستخلص مادة Some of Plant Secondary Metabolites **(Essential Oils**)
الخامس	**الاختبارالاول**
السادس	التعرف على مستخلص مادة Alkaloids وفوائده الطبية (الجزء الاول) Some of Plant Secondary Metabolites (**Alkaloids**)
السابع	التعرف على مستخلص مادة Alkaloids وفوائدها الطبية (الجزء الثاني) Some of Plant Secondary Metabolites (**Drug application of Alkaloids**)
الثامن	التعرف على مستخلصات Phenolic compounds وفوائدها الطبية Some of Plant Secondary Metabolites **Phenolic compounds** (phenols)
التاسع	التعرف على مستخلصات التانينات **(Tannins copounds)** وفوائدها الطبية Some of Plant Secondary Metabolites **Tannins copounds**
العاشر	التعرف على مستخلصات **Flavonoids** وفوائدها الطبية Some of Plant secondary metabolites **(Type of Flavonoids in plants)**
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الثاني عشر	**الاختبار الثاني**

Significance Of Medicinal Plants To Human Being

Healing with medicinal plants is an old treatment method as old as mankind itself. The connection between human and their search for drugs in nature dates from the far past. Awareness of medicinal plants' usage is a result of the many years of struggles against diseases and man learned to pursue drugs in barks, seeds, fruits, and other parts of the plants. Contemporary science has acknowledged and considered in modern pharmacotherapy the active actions of plant origin drugs, known by ancient civilizations and used throughout the millennia. The knowledge of the development of ideas related to the usage of medicinal plants with awareness has increased the ability of pharmacists and physicians to respond to the challenges that have emerged with the spreading of professional services in facilitation of man's life.

Plant Secondary Metabolites:

These secondary metabolites or products exert in general a profound physiological effect on the mammalian system and thus are known as active principles of plants secondary plan. They are more limited in the plant kingdom and mostly accumulated by plant cells in smaller quantities than primary metabolites, which are essential for plant growth, development, stress adaptation, and defense.

The table below appears the differences between primary and secondary plant metabolites:

Plant Primary Metabolites	Plant Secondary Metabolites
1- Have metabolic functions essential for plant growth and development. 2- Produce in every plants. 3- Include: Carbohydrates, Organic acids , Amino acids, Fatty acids, Steroids and Vitamins.	1- Don't have apparent functions involved in plant growth development. 2- Produce in different plant families, in specific groups of plant families or in specific tissues, cells or developmental stages throughout plant development. 3- Include: Terpenoids, special nitrogen metabolite including: (non-protein amino acids, amines glycosides, glucosinolates, alkaloids and phenols).

The plant material

- The freeing of the plant tissue under study from contamination:
  a- Free from disease (microbial products, infection alter plant metabolism).
  b- Free from other plants.
- Plants may be dried before extraction.
- Period of plant material storage e.g.: Myristicin content nutmeg, *Myristica fragrance* fruits increase slowly on storage, while the more volatile β-pinene content decrease with time.

Methods of extraction:

The precise mode of extraction naturally depends on texture and water content of the plant material being extracted and on the type of substance that is being isolated. In general it is desirable to "kill" the plant tissue, i.e. prevent enzymatic oxidative or hydrolysis occurring.

The flowing general steps followed for optioned plant secondary metabolites:

EXTRACTION:

Extraction is the separation of medicinally mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, terpenoids, and flavonoids using selective solvents through standard procedures. The aim of all solvent extraction methods is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc. The following are the widely used extraction techniques.

1- Maceration, percolation, and decoction.
2- Soxhlet apparatus with a range of solvent such as, ether, petroleum and chloroform). This apparatus used for non-polar compound, lipids and sometimes starting with above solvent while using alcohol and ethyl acetate for more polar compounds.
3- Clavenger for oil extraction.

CONCENTRATION:

Extract can be concentrate by rotary evaporator. Otherwise as a standard precaution against loos of material. Concentrated extract should be stored in a refrigerator and trace of toluene added prevent fungal growth. (Mention other methods of concentrating the plant extract).

NOTE:

All of extract and concentrate techniques tend to produce an extract with an aroma that differs from the aroma of the raw materials. Heat, chemical solvents, or exposure to oxygen in the extraction process may denature some aromatic compounds, either changing their odor character or rendering them odorless, and the proportion of each aromatic component that is extracted can differ.

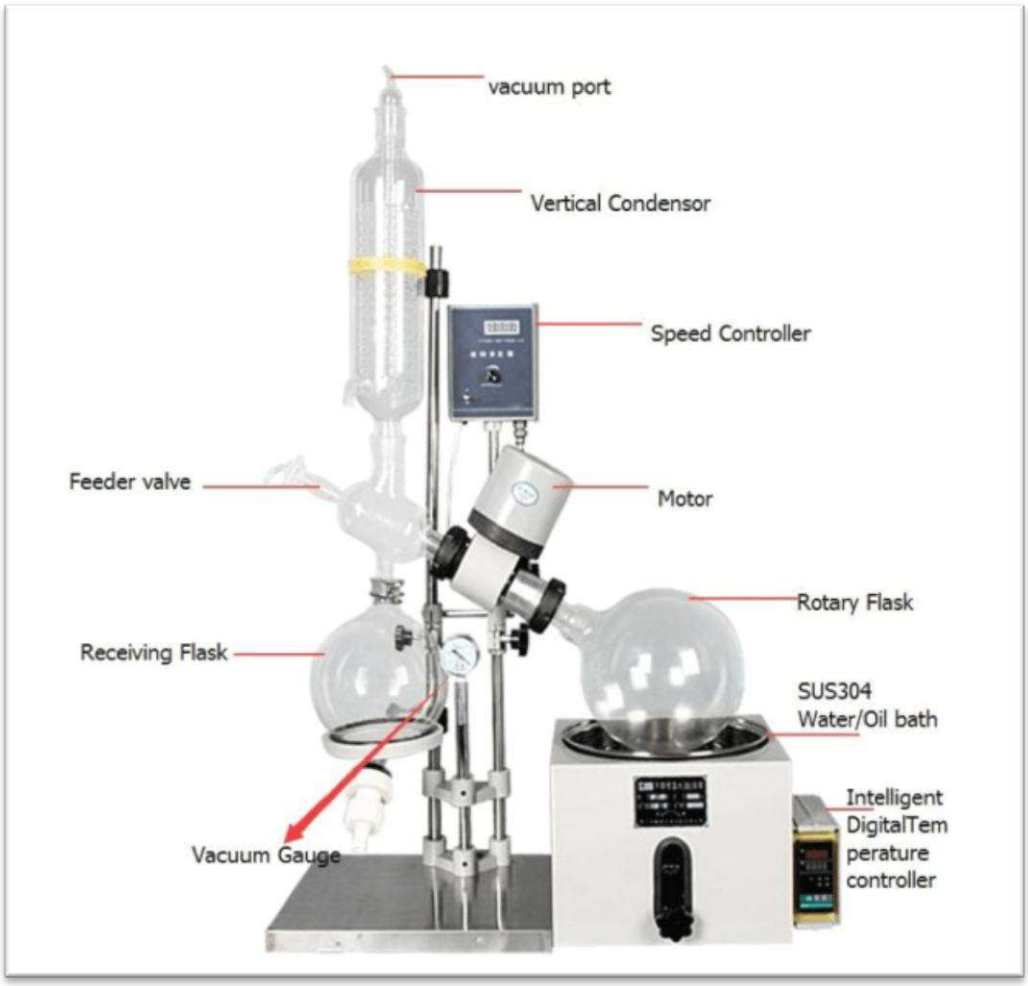


Figure 1: Rotary Evaporator

Extraction Techniques of Medicinal Plants

Extraction as the term is used pharmaceutically, involves the separation of medicinally active portions of plant or animal tissues from the inactive or inert components using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquid, semisolids or powder intended only for oral or external use.

Methods of Plant Extraction

1. Soaking

Plant part powder (fine or coarse particles) was weighed using an electronic weighing balance and differently soaked in suitable solvent, at a ratio of 1:3, 1:4 or 1:5 (plant powder/ solvent). The mixture was agitated using an electric blender (to enhance proper mixing of the solvent with the powder), and then poured into air-tight container. Then the containers with the mixtures were kept in the refrigerator at 4℃ for 48 hours.

2. Maceration

Maceration (for fluid extraction): whole or coarsely powdered plant-drug is kept in contact with the solvent at a ratio of 1:3, 1:4 or 1:5 in a stoppered container (to prevent the solvent evaporates and changed the extraction ratio) for a defined period with frequent agitation until soluble material is dissolved. This method is best suitable for use in case of the thermo labile drugs.

3. Decoction

In this process, the crude drug is boiled in a specific volume of water for a defined time; then it is cooled and strained or filtered. This procedure is suitable for extracted water soluble, heat stable constituents. This process is typically used in ethnopharmacology. The starting ratio of crud drug to water is fixed, e.g. 1:3, 1:4 or 1:5, the volume is boiling during the extraction procedure. Then the concentrated extract is filtered and used as such or processed further.

4. Percolation

This procedure is used must frequently to extract active ingredients in the preparation of tinctures and fluid extracts. A percolator (a narrow, cone-shaped vessel open at both ends) is generally used. The solid ingredients are moistened with an

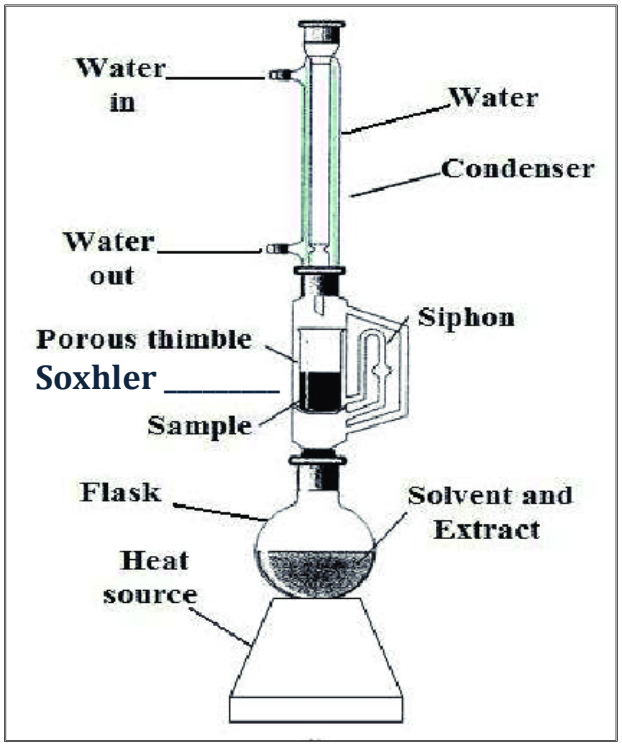
appropriate amount of the specified solvent and allowed to stand for approximately 4 hours in a well closed container.

5. Sonication

The procedure involves the use of ultrasound with frequencies ranging from 20 kHz to 2000 kHz; this increases the permeability of cell walls and produces cavitation. Although the process is useful in some cases, like extraction of rauwolfi a root, its large-scale application is limited due to the higher costs.

6. Serial Exhaustive Extraction

It is another common method of extraction which involves successive extraction with solvents of increasing polarity from a non-polar solvent such as (hexane) to more polar solvent such as (methanol) to ensure that a wide polarity range of compounds could be extracted. Some researchers employ soxhlet extraction of dried plant material using organic solvent. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds.



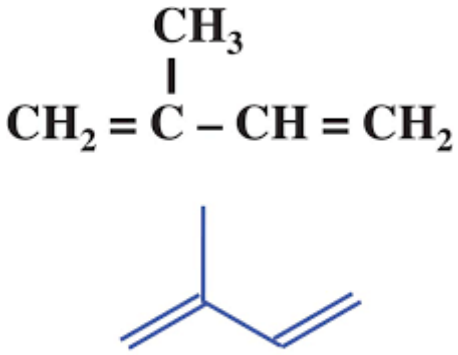
Soxhlet Apparatus

Some of Plant Secondary Metabolites

Plant Secondary Metabolites divided in to three groups (depending on differences in their structure, syntheses pathway and solubility):

1- Terpenoids
2- Polyphenols
3- Nitrogen containing compounds.

* **Terpenoids** are consists of isoprene molecule, they are classified according to number of these units.



Isoprene

The main classes of terpenoids according to number of isoprene are:

1- C_5 Isoprene
2- C_{10} Monoterpenoids, for example: menthol from mint (volatile)
3- C_{15} sesquiterpenoids, for example: abscisins (abscisic acid) (volatile).
4- C_{20} diterpenoids, for example: gibberellins (less volatile)
5- C_{30} triterpenoids, for example, sterols, saponins (nonvolatile).
6- C_{40} tetraterpenoids, for example, carotenoids (nonvolatile).
7- C_{50} polyisoperene, for example, rubber (nonvolatile).

On the other wise, several studies have shown that essential oils of many species have been related to the presence of monoterpenes in their composition.

Extraction:

Terpenoids are normally extracted from plant tissues with organic solvents, such as light petroleum ether or chloroform.

Separation:

Terpenoids separated by **Thin-Layer Chromatography** (TLC) on silica gel or alumina using same solvent of extraction and **Gas Liquid Chromatography** (GLC) for volatile compounds.

Identification:

Volatile terpenes can be identified by GLC, where as in critical cases, both **Fourier Transform Infrared** (FTIR) and mass spectra should be determined.

Procedure of Terpenes extraction

1- Crush a few seeds of Lantana plant to a fine powder using mortar. Cover the powder with ether and leave to extract for at least one hour.

2- Filtered then concentrate the ether extract.

3- Detect Terpenoids by chemical detection: 4 ml of acetic acid anhydride and 1 ml of concentrated sulfuric acid added to 2 ml of chloroform then mixed with 1 ml of crude extract. The appearance of pink color indicated the presence of Terpenoids, while formation of blue color after leaving the sample for one minute indicated the presence of Steroids.

4- Five milliliter of ethanolic crude extract is added to 3 ml of mercuric chloride (1%) solution, formation of white precipitate indicating of saponin. Otherwise, formation of foam after shaking the extract indicate the presence of saponin.

Lecturers: *Sumayah Sami and Haifaa Nori*

Some of Plant Secondary Metabolites

∗ ∗ Essential Oils

Definition and localization:

Essential Oils are odorous and volatile compounds found only in 10% of the plant kingdom and are stored in plants in special brittle secretory structure, such as glands, secretory hairs, secretory ducts, secretory cavities or resin ducts. The essential oils also called volatile or ethereal oils, because they evaporate when exposed to heat in contrast to fixed oils.

Extraction of Essential Oils

Oils contained within plant cells are liberated through heat and pressure from various parts of the plant matter, such as leaves, flowers, fruit, grass. roots and bark. The extraction of the essential oils from plant material can be achieved by various methods, of which solvent extraction, steam and steam/water distillation (using Clavenger apparatus) are the most common methods of extraction. It is also believed that this method produce the best quality essential oil. This technique was invented in the 11^{th} century by Iben Al-Baitar. In fact, the process of the steam distillation was so perfect; it was almost 200 years before improvements were made. The steam distillation method involves placing the flower, plant or herb (which can be either fresh or dried) in the specific flask. Then, pressurized steam is concentrated in the area where the parts of plant are placed. The plant becomes saturated. Once it is saturated, the cell walls of the plant burst, allowing the molecules that contain the essence of the plant out. This occurs until the essence of the plant is sweated out. The sweat or vapor that is left (a combination of essence and water) passes through a spiral tube and is then shocked with cold water. What it left is a mixture of essence and water. This essence oil, is just that, an oil, it is a naturally hydrophobic liquid meaning it does not blend with water. Once this has occurred, the steam and the highly scented molecules are then circulated into another area where it is immediately cooled. Naturally when using cold temperature, water combining essential oil will condensed. However, essential oils are

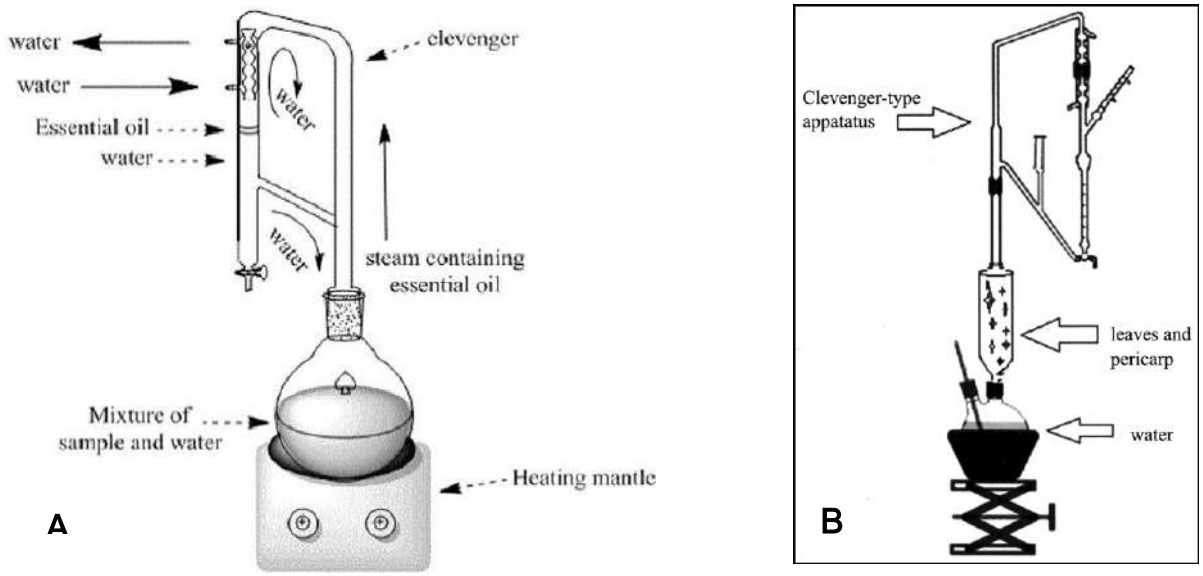
hydrophobic, therefore before essential oil is in its true state (the physicality that we know them in) there is a period of patience needed.

Applications of Essential Oils:

Essential oils are used in perfumes, cosmetics, disinfectants, candles, soaps and body products, and even for flavoring food. In most cases, essential oils can be absorbed from the food matrix or as pure products and cross the blood brain barrier easily. This later property is due to the lipophilic character of volatile compounds and small size.

The action of essential oil begins by entering the human body via three possible different ways including direct absorption through inhalation, ingestion or diffusion through the skin tissue.

Essential oils are used in aromatherapy are readily accepted by human body. This is due to the chemical structure of it. It so closely resembles that of human protein, so that the human body does not notice the difference.



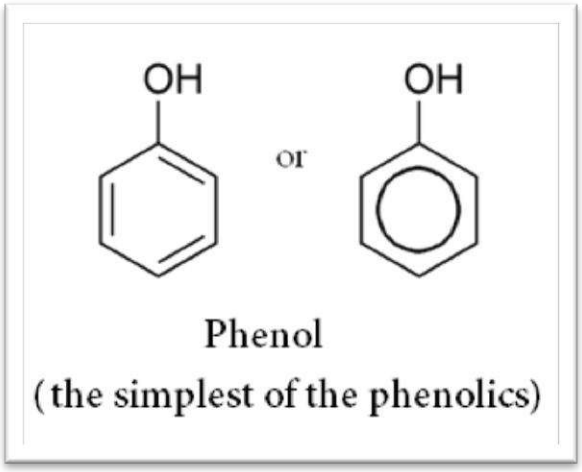
A-Clevenger-type apparatus B- Modified Clevenger

Lecturers: *Sumayah Sami and Haifaa Nori*

Some of Plant Secondary Metabolites

****PHENOLIC COMPOUNDS (PHENOLICS)

The phenolic Compounds (sometimes called phenols) embraces a wide range of plant substances, they are class of chemical compounds consisting of hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. The simplest of the class is phenol**,** which is also called carbolic acid C6H5OH. Phenolic compounds are classified as simple phenols or polyphenols based on the number of phenol units in the molecule. Most of phenols are glycosides and also it includes several important groups are flavonoids, lignins and tannins.



Phenol
(the simplest of the phenolics)

Role of phenolic compounds in plants

1- In soils, larger amounts of phenols are released from decomposing plant waste against other natural plant community as competitive manner (allelopathic interactions).

2- Phenolic compounds can act as protective agents, inhibitors, natural animal toxicants and pesticides against invading organisms, i.e. herbivores, nematodes, phytophagous insects, and fungal and bacterial pathogens. The scent and pigmentation conferred by other phenols can attract symbiotic microbes, pollinators and animals that disperse fruits.

3- Volatile phenolic compounds are found in plant resin where they may attract benefactors such as parasitoids or predators of the herbivores that attack the plant.

4- Structural materials of cell wall.

5- Act as plant pigments such as anthocyanins and flavonoids**.**

NOTE:

Plant phenols can be a considerable nuisance because of their ability to complex with protein by hydrogen bonding. When plant cell constitutes come together and the membranes are destroyed during isolation procedure, the phenols rapidly complex with protein and as a result there is often inhibition of enzyme activity in crude plant extract.

EXTRACTION OF PHENOLIC COMPOUNDS FROM PLANTS

The extraction of phenols is affected by the polarity of solvents that used. Therefore, it is very difficult to develop an extraction procedure suitable for the extraction of all plant phenols. The phenolic extracts from plant materials are always a diversified mixture of plant phenolics soluble in the solvent system that used. Additional steps may be required to remove the unwanted phenolics and non-phenolic substances such as waxes, terpenes, fats, and chlorophylls. Likewise, the extraction procedure is sequential and systematically carried out using an aqueous organic solvent to extract phenolic compounds in plant samples (because of phenols tend to be water soluble). This traditional method is called liquid- liquid extraction (LLE).

Phenols are themselves very susceptible to enzymic oxidation and phenolic material may be lost during isolation procedure due to the action of specific (phenolase) enzymes present in all plants. Extraction phenols with boiling alcohol

normally prevent enzymic oxidation occurring, and this procedure should be adopted routinely.

SIMPLE PROCEDURE OF PHENOLIC COMPOUNDS EXTRACTION:

One hundred grams of plant part ground was mixed with 400 ml of hydrochloric acid (2%) and the mixture was placed in water bath for one hour at 90°C. Then the mixture was stirred on magnetic stirrer for two hours. Filtration was achieved using Buchner funnel, the filtrate solution was treated with 200 ml of diethyl ether with the same volume of filtrate. The mixture was put other time in water bath for one hour, then it was evaporated by using rotary evaporator and finally crude phenols were gotten.

TEST FOR PHENOLIC COMPOUNDS:

About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black coloration. A few drops of alcohol and ferric chloride solution were mixed with the plant extract. A blue green or red color indicates the presence of phenol.

APPLICATIONS AND USES OF PHENOLIC COMPOUNDS

Phenols are important raw materials and additives for industrial purposes in:

- Laboratory processes.
- Chemical industry.
- Chemical engineering processes.
- Wood processing.
- Plastics processing.

- Some natural phenols can be used as : biopesticides, Furanoflavonoids like karanjin or rotenoid are used as acaricide or insecticide.
- Some phenols are sold as dietary supplements. Phenols have been investigated as drugs. And its derivatives have been made of phenolic

compound, combretastatin A-4, an anticancer molecule, including nitrogen or halogens atoms to increase the efficacy of the treatment.

- Phenols have potential as a pharmaceutical effect, since it possesses antioxidant, anti-inflammatory, anti-mutagenic and cancer preventive.
- Stomach problems, reduces blood cholesterol.
- Anti-viral, antifungal and antibacterial compounds.
- Blood-sugar lowering and other beneficial cardiovascular effects.

Lecturers: *Sumayah Sami and Haifaa Nori.*

Some of Plant Secondary Metabolites

***** Glycoside in Medicinal Plant:

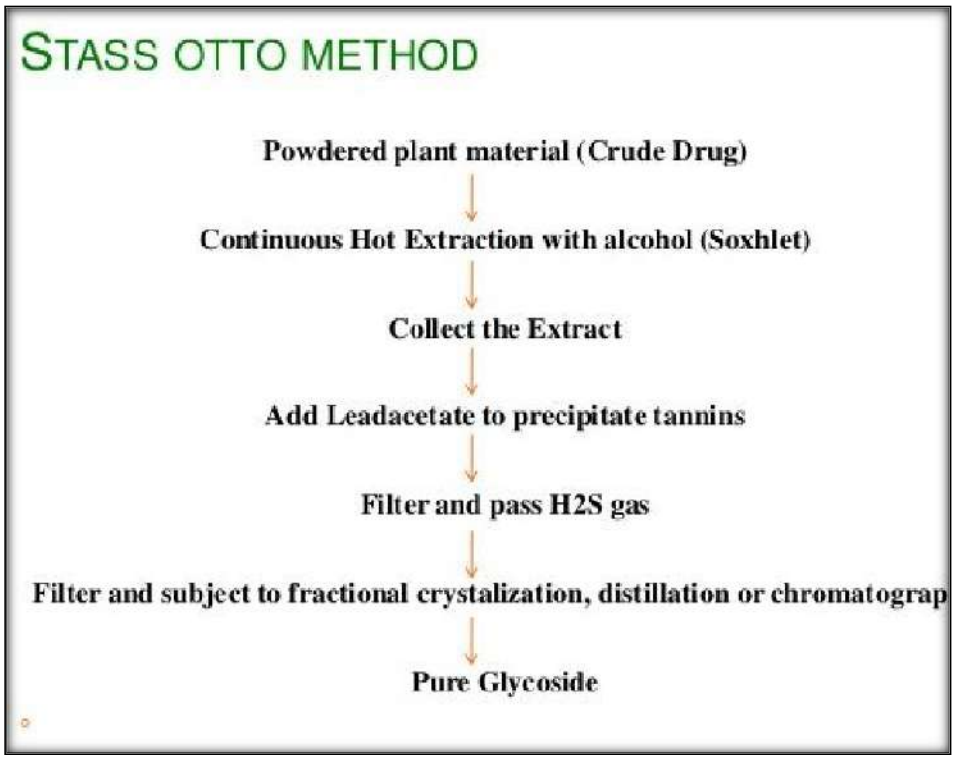
A glycoside: is an organic compound, usually of plant origin (medicinal plant), and comprising a sugar portion is called glycone linked to a non-sugar moiety is called the aglycones or genin in a particular manner. They exert therapeutically significant effect on human and animals. Traditionally used in modern medicine because of their **cardio tonic**, **purgative**, **analgesic**, **anti-arrhythmic**, **demulcent action**.

Glycoside →Aglycone (genin) + Glycone (Sugar)

Extraction and Types of Glycoside in Plant

General method of glycoside Extraction

1. The glycoside isolation method (extraction) is called **stas-otto method**.
2. The dried plant material is rendered into a moderately coarse powder. The powder is then extracted in a Soxhlet apparatus with aqueous ethanol.
3. The non-glycosidal impurities which get extracted along with glycosides are removed by precipitating them with **lead acetate solution**.
4. The excess of lead acetate is then removed by passing **hydrogen sulphide gas** through the extract. Lead gets precipitated as **lead sulphide**, which is filtered out.
5. The filtrate contains the **glycosides**. The glycoside can be obtained by removal of the solvent under **reduced pressure** or any other suitable procedure.
6. Further purification of the isolated glycosides is done by **column chromatography.**
7. The extract molecular structure is determined by the spectrophotometer, Ultra Red assays, Infra-red , NMR and mass spectroscopy etc.



Types of Glycoside in Medicinal Plant

1. **Anthraquinones glycosides (Purgatives).**

i) Senna ii) Cascara iii) Aloe ii) Rhubarb

2. **Cardiac glycosides (Poisons).**

i) Digitalis ii) Squill

3. **Saponin glycosides (Soaps):**

i) Asparagus roots ii) Ginseng

4. **Cyanophore glycosides**

i) Wild cherry ii) Bitter almond

5. **Flavone glycosides (Anti-inflammatory)**

i) Lemon ii) Sweet orange-Hesperidin iii) Bitter orange

6. **Aldehyde glycoside**

i) Vanilla

7. **Phenolic glycoside (analgesic – aspirin)**

Sennoside: anthraquinones glycosides of senna plant (*Cassia senna* and *Cassia angustifolia*) used to make remedies are now mass-cultivated for medicinal purposes – primarily for their laxative properties and moisture activity.

Part of plant: dried leaflets

Extraction OF Anthraquinone glycosides (Senna)

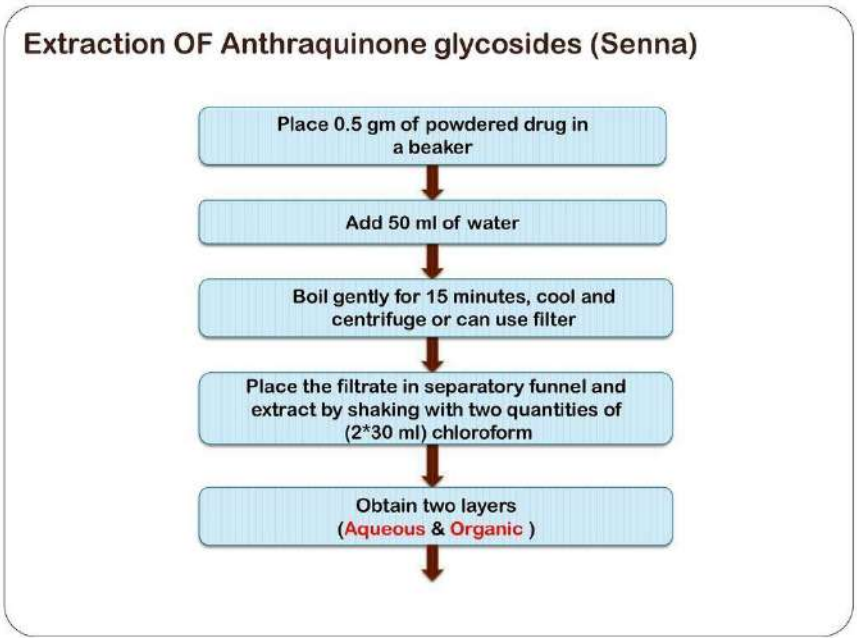
1- Place 0.5 gm of powdered drug in a beaker.

2- Add 50 ml of water.

3- Boil gently for 15 minutes, cool and centrifuge or can use filter.

4- Place the filtrate in separator funnel and extract by shaking with two quantities of (2*30 ml) chloroform.

Will Obtain two layers: (Fraction A) Organic layer contain free Aglycone and (Fraction B) Aqueous layer contain glycoside as a whole



5- Take Fraction B (Aqueous Layer): concentrate to about 1ml. The other part is place in a 250 ml round bottom Flask.

6- Adding to it 3.5ml of ferric chloride solution (60%w/v). Reflux for 20 min.

7- Add 2ml of diluted HCl acid, continue heating for further 20 min, shaking the flask occasionally to dissolve as possible of the precipitate, and allow cooling.

8- Place the hydrolysate in a separatory funnel and extract with two quantities of (2*30ml) chloroform.

9- Placed in small flask and evaporate carefully almost to dryness on a rotary evaporator

Macerate the powdered plant material with 70% ethanol

↓

After filtration

↓

Place the filtrate in separator funnel and shaking with same quantity of chloroform 1 x 2

↓ ↓

Fraction (A) Organic layer — Fraction (A) Aqueous layer

Mix few amount of Fraction B then add $FeCL_3$ and diluted HCL on water Bath for 10 min

↓

Partition with chloroform

↓ ↓

Fraction (C) Organic layer — Fraction (D) Aqueous layer

Identification of Free Anthraquinone by Borntrager's Test

1. Take (Fraction C) Organic layer and boil with 1ml diluted sulphuric acid in a test tube for 5 min (glycoside will hydrolyzed to aglycone and sugar) by boiling with acid
2. Mix with chloroform in separatory funnel which will contain the free aglycone
3. Then mix with diluted ammonia or KOH to form two immiscible layer.
4. Pink or red color produced in the alkaline layer indicate the presence of free anthraquinone

Drug forms:

Lecturer: *Dr. Maysaa Kadhim Al-Malkey*

Some of Plant Secondary Metabolites

****PHENOLIC COMPOUNDS (PHENOLICS)

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3- Volatile phenolic compounds are found in plant resin where they may attract benefactors such as parasitoids or predators of the herbivores that attack the plant.

4-Structural materials of cell wall.

5-Act as plant pigments such as anthocyanins and flavonoids**.**

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- wood processing.
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Some natural phenols can be used as : biopesticides, Furanoflavonoids like karanjin or rotenoid are used as acaricide or insecticide.

Some phenols are sold as dietary supplements. Phenols have been investigated as drugs. For instance, Crofelemer (USAN, trade name Fulyzaq) is a drug under development for the treatment of diarrhea associated with anti-HIV drugs. Additionally, derivatives have been made of phenolic compound, combretastatin A-4, an anticancer molecule, including nitrogen or halogens atoms to increase the efficacy of the treatment.

Lecturers: *Sumayah Sami and Haifaa Nori*

Some of Plant Secondary Metabolites

******* Flavonoid in Medicinal Plant:

Introduction to Flavonoid

1. Flavonoids are a large family of polyphenolic plant compounds.
2. Non nitrogenous universal plant pigment responsible for plant pigmentation (i.e. color of flowers, fruits, and sometime leaves; Flavus in Latin means yellow but could be blue or red color).
3. Occur in free state in plant OR as a glycoside (O and C glycoside).

Hesperidin $\xrightarrow{\text{hydrolysis}}$ Hesperitin + Rhamannose + Glucose

{Citrus fruits} {Flavonoid aglycone} {Sugar glycone}

4. Foods with a high flavonoid content include parsley, Red onion (*Allium cepa*), blueberries (*Cyanococcus Vaccinium*), black tea (*Camellia sinensis*) and green tea, bananas, all citrus fruits (*Rutaceae*), buckwheat, and dark chocolate with a cocoa content of 70% or greater.
5. Flavonoids have been shown to exhibit anti-inflammatory, anti-thrombogenic, anti-diabetic, and anti-cancer.

Types of Glycoside in Medicinal Plant

1) Anthocyanidins - blueberries
2) Flavonoles – citrus fruit
3) Flavans – black tea
4) Flavanones

Flavonoids Properties

1. Flavonoids are crystalline compounds;
2. Flavonoid glycosides are generally soluble in water and alcohol but insoluble in organic solvents; the aglycones (genins) are only sparingly soluble in water but soluble in ether, chloroform.

3. Under the UV light flavonoids show fluorescence of different colors (yellow, orange, brown, red).

4. They are colored:

a. yellow: flavones, flavonoles, and chalcones

b. red: anthocyanidins in acidic media

c. blue: anthocyanidins in alkaline media

d. colorless: catechins, flavans, and flavanones

4. Chemically, being phenolic dissolve in alkalis $\longrightarrow$ yellow solution $\xrightarrow{Hcl}$ colorless

5. Flavanoides + lead aceate $\longrightarrow$ Precipitate

5. Flavanoides solution + $FeCl_3$ $\longrightarrow$ Green , purple to red brwon color

General Method of Flavonoids Extraction

Ethanol and methanol are frequently used to extract flavonoids. The common extraction methods include dipping, percolation, reflux, continuous reflux and so on. The **alcohol of high concentration (90–95%)** is applied to **extract free flavonoids**, and the alcohol at the **concentration of about 60%** is applied to **extract flavonoid glycosides**. For example, reflux method was applied to extract total flavonoids from **leaves of Ginkgo biloba with 70% ethanol**, and the product yield was significantly higher than the water decoction method.

Hot water extraction method is applied to **flavonoid glycosides**. It possesses the advantages of low cost, safety, simple equipment and could be applied in industrial production, but much water- soluble impurities, such as proteins and saccharides might be mixed into the product.

Most of the flavonoids are **acidic because of hydroxyl or carboxyl groups**, so they could be **extracted with alkaline water or alkaline dilute alcohol**. The commonly used solvents include **dilute sodium hydroxide, lime water, 5%**

sodium hydroxide dilute ethanol solution and so on. Water- soluble impurities, such as tannins, pectins and mucilages, could be precipitated because of the formation of calcium salts during the extraction with lime water. It has often showed good results if 5% sodium hydroxide dilute ethanol solution was used. However, the product yield might be reduced because some flavonoids obtained after acidification might be dissolved in dilute ethanol solution.

1. **Ferric Chloride test**: Plant extract was mixed with **Ferric chloride solution**- **Dark green color** formed.

Flavonoids Identification test

2. **Shinoda test**: The plant extract was mixed with few drops of **concentrated HCl** to this mixture, pieces of magnesium were added- Pink , red, yellow, or magenta color developed.

3. **Zinc-HCl reduction test**: Plant extract was mixed with concentrated HCl. To this mixture, zinc dust was added A **magenta color** developed.

4. **Lead acetate test:** Plant extract was mixed with **10% lead acetate solution- Yellow precipitate** obtained.

5. **Sodium hydroxide test:** Plant extract was mixed with **10% NaOH solution- Yellow precipitate** was formed.

6. To small quantity of residue, add **lead acetate solution**. **Yellow colored precipitate** is formed.

7. Alkaline reagent test: addition of increasing amount of **sodium hydroxide** to the residue show **yellow** coloration, **which discoloration after addition of acid.**

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Separation and isolation techniques …….. Lab. 9

Separation and isolation techniques are used to separate complex mixtures or crud extracts into its constituent parts (these compounds which are not chemically combined together).

There are various types of separation processes that are:

- Chromatography
- Filtration
- Centrifugation
- Separating funnel
- Crystallization
- Sublimation
- Evaporation
- Simple and Fractional distillation…etc.

Filtration: Separation of solids or groups of solids from the liquid in a mixture, using a medium through which the liquid can pass such as filter papers or membrane filter

Evaporation: The solvent is boiled off and escape into the air while the solute is left behind in the holding container (this method is not suitable for compounds which can decomposed by heating).

Centrifugation is an important separation technique in biochemistry, in which we divide the sample into a solid residue and a supernatant solution.

Distillation is a method used for the separation of components of a mixture containing two miscible liquids (their boiling points are significantly different). The process involves heating a liquid to its boiling points, and transferring the vapors into the cold portion of the apparatus, then condensing the vapors and collecting the condensed liquid in a container

Separator funnel is usually used for (**liquid–liquid extraction**). After placing the two liquids in the separator funnel, we shake the funnel to increase the surface area between the phases. When the extraction is complete, we allow the liquids to separate. The stopcock at the bottom of the separatory funnel allows us to remove the two phases.

Ashwaq Shanan & Dr. Farah Thamer

Separation and isolation techniques …….. Lab. 9

(Chromatography): the main Separation techniques

Chromatography is an important biophysical technique for separating, identifying, and purification mixture components for a qualitative and quantitative study.

- The mixture is dissolved in a **mobile phase** **(gas, solvent, water**, etc.) that transports it through a system of fixed material called **stationary phase** (**column, capillary tube, plate, or sheet**).
- The different constituents of the mixture have different affinities for the stationary phase. The different molecules stay longer or shorter on the stationary phase, depending on their interactions with their surface sites.
- The factors effective in this separation process include molecular characteristics related to **adsorption** (liquid-solid), **partition** (liquid-liquid), and **affinity** or differences among their molecular weights.

Types of chromatography

1. **Thin-layer chromatography:** is used in food and pharmaceutical laboratories, terpenes.
2. **Gas chromatography**: is utilized in the separation of alcohol, petrochemical, lipid, and amino groups.
3. **High-pressure liquid chromatography (HPLC):** in drugs, food, pesticides, alkaloids and plant phenols.
4. **Column chromatography**: is used for the purification of biomolecules
5. **Ion-exchange chromatography:** in separation of ions and macromolecules as nucleic acids, enzymes, sugars, and proteins. Also in purification of water
6. **Gel filtration chromatography**: is used for the purification of RNA, DNA, viruses, enzymes, and proteins.

7. **Affinity chromatography**: is used for separation of macromolecules as nucleic acids, and proteins

8. **Paper chromatography:** for separating water-soluble and colored compounds

9. **Electrophoresis:** is used for the purification of RNA, DNA and proteins.

Gas chromatography

In gas chromatography (GC), the mobile phase is an inert gas such as helium. The mobile phase carries the sample mixture through what is referred to as a stationary phase. The stationary phase is a usually chemical that can selectively attract components in a sample mixture. The stationary phase is usually contained in a tube of some sort. This tube is referred to as a column. Columns can be glass or stainless steel of various dimensions.

GC has a **long, thin column** containing a thin interior coating of a solid stationary phase (capillary column).

GC is used for **semi-volatile, non-polar organic compounds**.

The capillary column is held in an oven that can be programmed to increase the temperature gradually. As the temperature increases, those compounds that have low boiling points elute from the column sooner than those that have higher boiling points. Therefore, there are actually two distinct separating **forces**, **temperature, and stationary phase interactions** mentioned previously.

Applications of GC

• Petrochemical and hydrocarbons analysis.

• Food, fragrance, beverage, and perfume analysis.

• Forensic (arson, explosives, drugs, unknowns).

• Pesticide analysis, food safety, and quality.

• Pharmaceutical and drug analysis.

• Clinical toxicology.

• GC is increasingly used for detection of illegal narcotics marijuana, cocaine.

High-pressure liquid chromatography (HPLC)

(HPLC) is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture.

It relies on pumps to pass a pressurized liquid solvent containing the sample mixture through a column filled with a solid adsorbent material. Each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for the different components and leading to the separation of the components as they flow out of the column. The important components of this device are:

- **Solvent**
- **High- pressure pump**
- **Column**
- **Detector and recorder**

Duration of separation is controlled with the aid of a computerized system, and material is accrued

Application of HPLC

Isolation and purification of biologically active natural products **(drugs, raw and pharmaceutical products, food, pesticides, and plant phenols).**