Medicinal Plants Lab. 1

A medicinal plant: Is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes. When a plant is designated as 'medicinal', it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation.

Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes.

Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions, and to defend against attack from predators such as insects, fungi and herbivorous mammals is called herbal medicine. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. At least 12,000 such compounds have been isolated so far. These phytochemicals are divided into:

(1) Primary metabolites : Are kinds of metabolite that are directly involved in normal growth, development, and reproduction. It usually performs a physiological function in the organism (i.e. an intrinsic function).. Some common examples of primary metabolites such as sugars, amino acids and fats, which are found in all plants.

(2) Secondary metabolites : Are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. These includes:

- Alkaloids
- Terpenoids
- Flavonoids
- Glycosides
- Natural phenols

Secondary metabolites aid a plant in important functions such as **protection, competition, and species interactions**, but are not necessary for survival. One important defining quality of secondary metabolites is their specificity. Usually, secondary metabolites are specific to an

individual species. Research also shows that secondary metabolic can affect different species in varying ways. These Compounds are found in a smaller range of plants, serving a more specific function. For example, some secondary metabolites are **toxins** used to deter predation and others are **pheromones** used to attract insects for pollination. It is these secondary metabolites and **pigments** that can have therapeutic actions in humans and which can be refined to produce drugs for examples are **inulin from the roots of dahlias, quinine from the cinchona, morphine and codeine from the poppy, and digoxin from the foxglove.**

Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effect.

Main Steps to study medicinal plants

To start with such project we suppose to follow the following instructions:

A-Collecting: which include the following points:

1- Identify the specific plant needed for study "know the family, Genus and species".
2- Make sure that the plant is not infected with any pathogenic diseases.
3- Decide the part or plant needed to be studied.
4- Decided the time of the day and the season of the year to collect the plant as a whole plants or parts of plants.
5- Plant should be collected without causing any injurie to the epidermis or any order surface.
6- Time of collection should be noted and it could be during early morning when the compounds needed to be extracted are oils. However, the plant should be collected during their flowering time which most possible during spring. In case of flowers and buds are needed to be collected that should be done early in the morning.

B-Cleaning:

Clean the part(which in fact collected)from any dirts. The plant parts should be cleaned by water and brush could be used if necessary to remove any dirts on them. plants supposed to be cleaned from any

stranger matters which some time by using sieves or some mechanical ways.

C-Drying

Dry the plant with specific way to avoid any damage to the chemical constituents in it or some pathogen invasion for the plant parts due to the humidity or any other factors.

There are two types of dryness:

1. **Natural**: like sun and air. Some plants will be dried under the sun shine such as *Citrallus colocynthis* but others will be dried in shadow places such as *Coriandrum sativa*.
2. **Artificial**: by using ovens with temperature control and time control. For example: In roots (10-12h), in leaves (3h.), flowers (6h.) and in seeds (2-7h). Generally plants parts putted in electrical oven but not exceeded the temperatures more than 40-45ºC for about 12 hours.

   After dryness the humidity stay less as possible which is, approximately 3% in leaves , 4-6% in flowers, (3-4%) in fruits, (6-8%) in roots and in seeds (12-14%).

   The fruits or seeds which contains oil or lipids will be dried under sun shine with aeriated place for one day or two or sometimes electrical Oven could be used such as *Ficus religiosa.*

D- Packing and Storage

We should packing and storage the compounds in dry and clean places The dryness should be employed in dry place away from humidity to avoid the fungal contaminations and stop the decomposing enzymes

Roots could be lasted more than 2-5 years it they storage in a suitable ways which keep the chemical compounds active. The same could be happen for the plant Barks. Leaves stay active to about one year then they will lose their activity gradually during the next year. It better to collect the leaves annually to make sure that they have an active constituent. Fruits and seeds which contain volatile oils will be very week within one year after storage. Those which have the starch or proteins will store for 2-2.5 years active.

E-Extraction

Use the specific and write way for extraction using either high polar solvents (water, ethanol and methanol) or low polar solvents (chloroform, hexane and Dimethyl ether).

The identification of medicinal plants.

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

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

1) **Morphological taxonomy**:
   This depended 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 dung for diseases treatment. However the description of drying and extraction of plant parts are one of the important steps for drug preparation.
3) **Chemical constituents:**
   Chemical constituents in each plant part used for drug suppose to be known. (This is important in plant identification and to differentiate them from each other even the close plants). Chemical constituents Such as alkaloids, Essential and volatile oils. ect.) . All these chemical constituent have specific reagents to prove their present or absent in each plant part or extract.
4) **Advanced procedure:**
   There are advanced procedure to identify the plants such as DNA test and some serological experiments in addition to enzymes presence in specific plant parts with in different growth steps stages and their response to be stimulated due to physiological or ecological factors. …ect.

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

1) *Matricaria chammomella* and *Anthemis noblis*

In the popular market you can buy this plant, but you have to know that this is a common name and this could be mixed and contain more than on genus and different species all mixed with each other. However, you will by a mixture of different genus or species of plant with close morphological feature. Those two plants have two different characteristics.

M. Chammomella flower	***A. noblis*** flower
Smaller radial flower	Wide Radial flower
sloped when they are matured	Sloped during maturation
Peduncle mostly are cylindrical	Peduncle mostly are angular

However, the differentiation between those two pants could be by smelling. , *M. chammomella* is smelly and the made drink from it is not bitter, but the other plant *A. noblis* is not smelly and the taste of its drink is more bitter.

These differences prove that the chemical constituents of each plant are completely different. However, *A. noblis* contain Anthemol which is Antibacterial. This compound is not recommended to be given to children because it could affect the liver cells. However, *M. Chammomella* Chemical constituents used to be anti-inflammatory, antiulcer and anti-vomiting, so it is important for new born babies and safe.



Flower of *Anthemis noblis*



Flower of *Matricaria chammomella*

2) *Mentha spicata* and *Mentha longifolia*

Note *the Mentha spicata* leaves and *Mentha longifolia* leaves and see the differences between them in size and morphology in addition to their smell.

Mentha spicata is useful as sedative for pains, carminative and heart stimulant … ect. However, some sort of Respiratory inflammation could be treated by this plant and it is diuretic. Accordingly it is not useful for those people who have some heart problems.

Metha longifolia plant has the same use in addition to their use for women quick birth, Anthelminitic. However, it is not useful for women during thier pregnancy.





3) *Apium graveolens, Petroselinum Hortense* and *coriandrum sativum*

Leaves for some Umbellifereac family planta ,these leaves are similar to each other in morphology and it is difficult to differentiate between them easy such as: *Apium graveolens, Petroselinum hortense, coriandrum sativum.*

Note: under the binocular microscope and magnifies lenses the fruit morphology for each plant and try to differentiate between them by smelling and testing. In addition to the leaves morphology, length of their petioles, and their margins.

Petroselinum Hortense or *P. sativum* it is important for menstrual disturbances and pains, rich in vitamins and minerals especially Fe, Co, Mg, K . *Apium graveslens* is diuretic, used against stones in the ureters and bladder . *Coruandrum sativum*: it is diuretic. Carminative, used against stones in ureters and bladder .. ect.

P. sativum have smooth ovate fruits. It has high commercial benefits due to glycoside (appin) which during its hydrolysis will give (apigenin) with glucose and other sugar (apiose). In addition to that it contain Apiol with special smell for this plant.

Apium graveoleus: with very small seeds, flattened , However some strains of this plant have bigger seeds. This plant contains volatile oils yellowish in color. This V.oils consist of the following**:** Limonene 60%, Selenine 10%, Sedanolide 2.5-3%.. However, the Glycoside appin is the dominant compound in this plant seeds.

C. sativum: contains oils without color usually and contain 45-70% linalool and their fruits are rounded shaped.

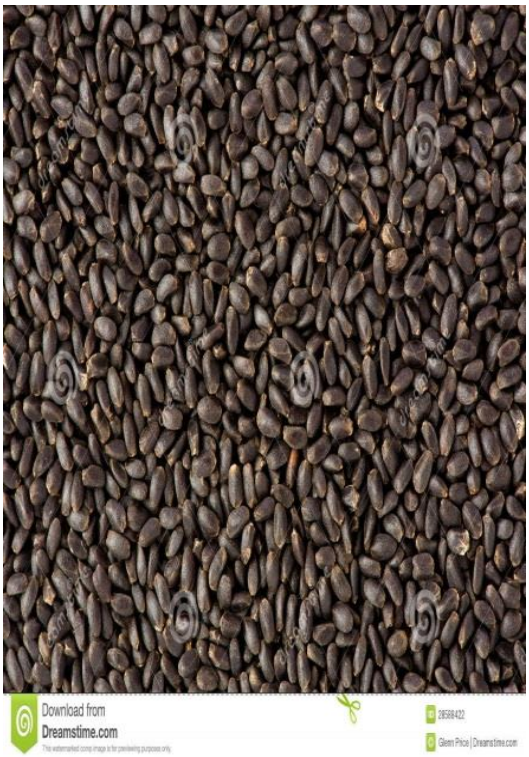


4) *Ocimum basilicum and Lallemantia iberica*

The seeds of both plants are similar to each other; mucilage will be produced around them when they are immersed in water. However, the uses of each plant medicinally are different. *O. basilicum* seeds usually used as anthelmintic and useful for constipation, but *L. iberica* seeds are harmful to digestive channels.

Typical doose is (Tea spoon (small) with glass of boiled water could be taken when the water cool down). In addition to this it is diuretic and stopping for renal system pains, coughing … ect.





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

The active chemical compounds which medicinal effects in plants have considered as secondary metabolites in the 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- Hydrothodes

The three first structures will be discussed as follows:

1- External or Dermal glands:

These structures produced from multiple divisions of protoderm cells. In certain places of this layer. However, these structures called dermal glands. These types of glands include the following types of glands:

1. Glandular hairs.
2. Nectaries.
3. Digestive glands. in plants which can use insects as food and have the ability to digest them.

The external or dermal glands could excrete Nectar, Terpenes, Gums and sugar solutions.

A) Glandular hairs:

Initialed 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 topical cell. The head considered as the secretory part.

Urtica urense is one of the plants which contain the glandular heirs in addition to *fritillaria imperia*. These compounds which produced by these glands important to as (Anti-inflammatory, help in injuries healing, stimulate the arteries … etc.).

B) Nectary glands or Nectaries:

These gland present in those plants which inoculated by flying insects such as the bees. These glands could secrete attractive compounds to these insects which called Nectar. This Nectar secreted from specific

parts in the plant which contain those special cells which covered the flowering parts which called Nectary glands or Nectaries. These cells differs from normal epidermal calls by morphology which is palisaded and from the parenchyma mature cells by their thin cell wall and their secretional morphology.

2- Internal or Globular glands

These glands initiated from the ground meristem, however they are mostly present in the cortex and their morphology is coccoidal. In the transverse section could be seen by nacked eyes and consisted 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 after the secretion of their compounds.
c) Or by both ways mentioned above and then called separated cavities or degenerated cavities or Schizollys genous. The central cavity contains the oily secretion which the smelling type attributed to it.

Example: *Eugenie caryophyllata* which have this on the flowering buds. and the glands which actually present in fruit skins of arranges and lemons (*Citrus* spp.).

3- Secretory canals or ducts (Tubular type)

The initiation of these ducts is different in different plants. Such as:

a) Resin ducts

- These ducts which are tubular structure consist of empty place surrounded by cells which have thin cell walls (without lignin) and called epithelial cells.
- The Resin ducts considered as a character of the conifers plants and these ducts are present as: Normal primary or secondary tissues such as those present in pinus and Larix. 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 specially Arabian Gums which produced in rich amount from barks of *Acacia senegal*.

c) Lactiferous canals:

Secretion structures which secreted gummy compounds consist of plenty of special particles and look like milk in color. This is why these compounds called plant milk (Latex). This compound present in plenty of Angiosperms plant which have more than 80 families most

of them are dicotyledous plants such as: moraceae, euphorbiaceae, Apocynaceae, and papaveraceae.

Lab. (4)

Methods of Extraction

After selection of the plant parts, dried them very carefully by avoiding to exceed the temperature over 45-50ºC. In order to extract medicinal ingredients from plant material, the following **sequential Steps Involved in the Extraction of Medicinal Plants are**: **1.** Size reduction **2.** Extraction **3.** Filtration **4.** Concentration **5.** Drying

Before the extraction we should notice the following:

1) The chemical constituents which need is be extracted and isolated such as alkaloid, Glycosides, saponins or Tannins.. ect.
2) The plant parts need to be extracted should be known such as leaves, Roots, flowers, seeds or Barks.
3) Choose the write organic solvents to extract the plant parts. The soulvent should be:
   - Be highly selective for the compound to be extracted.
   - Have a high capacity for extractions in .
   - Not react with the extracted compound or with other compound in the plant material.
   - Have low price.
   - Be harmless to man and environment.
   - Be completely volatile. Aliphatic alcohol with up to three carbon atoms, or mixtures of the alcohols with water, are the solvent with the greatest extractive power for almost all natural substances of low molecular weight like alkaloids, suaponines and flavonoid.
   - Ethyl alcohol is the solvent of choice for obtaining classic extracts such as: tincture and fluid.
   - There are organic solvents which are polar such as Methanol, ethanol. However there are some organic solvents which are intermediate polarity such as chloroform, Butanol, Acetone, However, the low polar solvents such as Hexane.. ect.
   - Some organic solvents such as Acetone or n-Butanol.. ect. used to extract particular chemical compounds and Benzene, Ether or Hexane used to extract oil. In addition water could be used to extract some chemical constituents as polar solvents.

Types of solvent

1) **Water**: In this extraction the water could be used for extraction and the Resulted extract called water extract and it could be called crude extract due to water which is a polar solvent similar to EOH and

MeOH. Water could be used as cold or hot water and called hot or cold water extract. In this way of extraction Glycosides could be extracted.

2) **Steam water**: could be used to extract to essential and volatile oil.
3) **Alcoholic**: Ethanol or Methanol could be used in this type of extraction because these solvents are polar (high polar) and most of the chemical constituents dissolved in the except essential oil.
4) **Other organic solvents**: Other organic solvents used such as chloroform, Acetone.. ect. All these organic solvents are non-polar solvents and most of the non-polar chemical constituents dissolved in them. The extract called according to the organic solvents used (chloroformic extract … ect.)
5) **Oil extraction**: In this ether could be used or Benzenc or Hexane to extract oil from the plant pants. These organic solvents could dissolve the oil from the plant parts.

Note: more there one organic solvents could be used to extract the chemical constituents from the plant parts. This procedure could be followed gradually and start with first organic solvents until the isolation of all compounds.

Extraction

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 by using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use.

These include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, semisolid extracts and powdered extracts.

The purposes of standardized extraction procedures for crude drugs are to attain the therapeutically desired portion and to eliminate the inert material by treatment with a selective solvent known as *menstruum*.

The extract thus obtained may be ready for use as a medicinal agent in the form of tinctures and fluid extracts, it may be further processed to be incorporated in any dosage form such as tablets or capsules, or it may be fractionated to isolate individual chemical entities such as ajmalicine, hyoscine and vincristine, which are modem drugs. Thus, standardization of extraction procedures contributes significantly to the final quality of the herbal drug.

General Methods of Extraction of Medicinal Plants

Maceration: In this process, the whole or coarsely powdered crude drug is placed in a stoppered container with the solvent and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.

Infusion: Fresh infusions are prepared by macerating the crude drug for a short period of time with cold or boiling water. These are dilute solutions of the readily soluble constituents of crude drugs.

Digestion: This is a form of maceration in which gentle heat is used during the process of extraction. It is used when moderately elevated temperature is not objectionable. The solvent efficiency of the menstruum is thereby increased.

Decoction: In this process, the crude drug is boiled in a specified volume of water for a defined time; it is then cooled and strained or filtered. This procedure is suitable for extracting water- soluble, heat-stable constituents.
This process is typically used in preparation of Ayurvedic extracts called "quath" or "kawath". The starting ratio of crude drug to water is fixed, e.g.1:4 or 1:16; the volume is then brought down to one-fourth its original volume by boiling during the extraction procedure. Then, the concentrated extract is filtered and used as such or processed further.

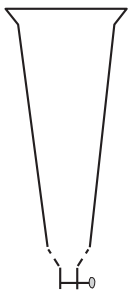


Figure 1: Percolator

Percolation: This is the procedure used most 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 (Figure 1). The solid ingredients are moistened with an appropriate amount of the specified menstruum and allowed to stand for approximately 4 h in a well closed container, after which the mass is packed and the top of the percolator is closed. Additional menstruum is

added to form a shallow layer above the mass, and the mixture is allowed to macerate in the closed percolator for 24 h. The outlet of the percolator then is opened and the liquid contained therein is allowed to drip slowly. Additional menstruum is added as required, until the percolate measures about three-quarters of the required volume of the finished product. The marc is then pressed and the expressed liquid is added to the percolate. Sufficient menstruum is added to produce the required volume, and the mixed liquid is clarified by filtration or by standing followed by decanting.

Hot Continuous Extraction (Soxhlet): In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in chamber E of the Soxhlet apparatus (Figure 2). The extracting solvent in fl ask A is heated, and its vapors condense in condenser D. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in chamber E rises to the top of siphon tube C, the liquid contents of chamber E siphon into fl ask A. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method, compared to previously described methods, is that large amounts of drug can be extracted with a much smaller quantity of solvent. This effects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

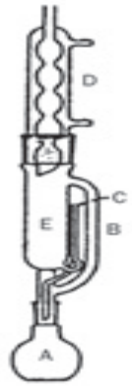


Figure 2: Soxhlet apparatus

Aqueous Alcoholic Extraction by Fermentation: Some medicinal preparations of Ayurveda (like asava and arista) adopt the technique of fermentation for extracting the active principles. The extraction procedure involves soaking the crude drug, in the form of either a powder or a decoction (kasaya), for a specified period of time, during which it undergoes fermentation and generates alcohol in situ; this facilitates the

extraction of the active constituents contained in the plant material. The alcohol thus generated also serves as a preservative. If the fermentation is to be carried out in an earthen vessel, it should not be new: water should first be boiled in the vessel. In large-scale manufacture, wooden vats, porcelain jars or metal vessels are used in place of earthen vessels. Some examples of such preparations are karpurasava, kanakasava, dasmularista. In Ayurveda, this method is not yet standardized but, with the extraordinarily high degree of advancement in fermentation technology, it should not be difficult to standardize this technique of extraction for the production of herbal drug extracts.

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Counter-current Extraction: In counter-current extraction (CCE), wet raw material is pulverized using toothed disc disintegrators to produce a fi ne slurry. In this process, the material to be extracted is moved in one direction (generally in the form of afine slurry) within a cylindrical extractor where it comes in contact with extraction solvent. The further the starting material moves, the more concentrated the extract becomes. Complete extraction is thus possible when the quantities of solvent and material and their fl ow rates are optimized. The process is highly efficient, requiring little time and posing no risk from high temperature. Finally, sufficiently concentrated extract comes out at one end of the extractor while the marc (practically free of visible solvent) falls out from the other end.

This extraction process has significant advantages:

i) A unit quantity of the plant material can be extracted with much smaller volume of solvent as compared to other methods like maceration, decoction, percolation.
ii) CCE is commonly done at room temperature, which spares the thermo labile constituents from exposure to heat which is employed in most other techniques.
iii) As the pulverization of the drug is done under wet conditions, the heat generated during comminution is neutralized by water. This again spares the thermo labile constituents from exposure to heat.
iv) The extraction procedure has been rated to be more efficient and effective than continuous hot extraction.

Ultrasound Extraction (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. One disadvantage of the procedure is the occasional but known deleterious effect of ultrasound energy (more than 20 kHz) on the active constituents of medicinal plants through formation of free radicals and consequently undesirable changes in the drug molecules.

Supercritical Fluid Extraction: Supercritical fl uid extraction (SFE) is an alternative sample preparation method with general goals of reduced use of organic solvents and increased sample throughput. The factors to consider include temperature, pressure, sample volume, analyte collection, modifier (cosolvent) addition, flow and pressure control, and restrictors. Generally, cylindricalextraction vessels are used for SFE and their performance is good beyond any doubt. The collection of the extracted analyte following SFE is another important step: significant analyte loss can occur during this step, leading the analyst to believe that the actual efficiency was poor. There are many advantages to the use of CO2 as the extracting fluid. In addition to its favorable physical properties, carbon dioxide is inexpensive, safe and abundant. But while carbon dioxide is the preferred fluid for SFE, it possesses several polarity limitations. Solvent polarity is important when extracting polar solutes and when strong analyte-matrix interactions are present. Organic solvents are frequently added to the carbon dioxide extracting fluid to alleviate the polarity limitations. Of late, instead of carbon dioxide, argon is being used because it is inexpensive and more inert. The component recovery rates generally increase with increasing pressure or temperature: the highest recovery rates in case of argon are obtained at 500 atm and 150° C. The extraction procedure possesses distinct advantages:

i) The extraction of constituents at low temperature, which strictly avoids damage from heat and some organic solvents.
ii) No solvent residues.
iii) Environmentally friendly extraction procedure.

The largest area of growth in the development of SFE has been the rapid expansion of its applications. SFE finds extensive application in the extraction of pesticides, environmental samples, foods and fragrances, essential oils, polymers and natural products. The major eterrent in the commercial application of the extraction process is its prohibitive capital investment.

Aromatic Plant Extracts

1. **Concrete**
2. **Absolutes**
3. **Resinoids**
4. **Hydrodistillation**
5. **Essential Oil Extraction by Expression**

In order to isolate essential oils by hydrodistillation, the aromatic plant material is packed in a still and a sufficient quantity of water is added and brought to a boil; alternatively, live steam is injected into the plant charge. Due to the influence of hot water and steam, the essential oil is freed from the oil glands in the plant tissue. The vapor mixture of water and oil is condensed by indirect cooling with water. From the condenser, distillate fl ows into a separator, where oil separates automatically from the distillate water.

Types of Hydrodistillation

Three are three types of hydrodistillation for isolating essential oils from plant materials:

A. Water distillation: In this method, the material is completely immersed in water, which is boiled by applying heat by direct fi re, steam jacket, closed steam jacket, closed steam coil or open steam coil. The main characteristic of this process is that there is direct contact between boiling water and plant material. The laboratory apparatus recommended for trial distillations is the Clevenger system.



Figure: Clevenger-type laboratory-scale hydrodistillation apparatus.

B. Water and steam distillation: In water and steam distillation, the steam can be generated either in boiler or within the still. water and steam distillation does not require a great deal more capital expenditure than water distillation. Also, the equipment used is generally similar to that used in water distillation, but the plant material is supported above the boiling water on a perforated grid.

C. Direct steam distillation: As the name suggests, direct steam distillation is the process of distilling plant material with steam generated outside the still in a satellite steam generator generally referred to as a boiler. As in water and steam distillation, the plant material is supported on a perforated grid above the steam inlet. A real advantage of satellite steam generation is that the amount of steam can be readily controlled. Because steam is generated in a satellite boiler, the plant material is heated no higher is the most widely accepted process for the production of essential oils on large scale. Throughout the flavor and fragrance supply business, it is a standard practice.

5- Essential Oil Extraction by Expression: Expression or cold pressing, as it is also known, is only used in the production of citrus oils. The term expression refers to any physical process in which the essential oil glands in the peel are crushed or broken to release the oil. One method that was practiced many years ago, particularly in Sicily (spugna method), commenced with halving the citrus fruit followed by pulp removal with the aid of sharpened spoon-knife (known as arastrello). The oil was removed from the peel either by pressing the peel against a hard object of baked clay (concolina) which was placed under a large natural sponge or by bending the peel into the sponge. The oil emulsion absorbed by the sponge was removed by squeezing it into the concolina or some other container. It is reported that oil produced this way contains more of the fruit odor character than oil produced by any other method.

Experiments (1):-

Preparation of water extracts:

1) 50 g of dried plant parts could be taken such as (*Nerium oleander, Vinca rosa*), and put it in 250ml of Boiled sterilized distilled water with continuums stirring until it cool down.
2) Filtrate the resulted solution with filter paper and replace it in closed sterilized container prior to be stored in the fridge until use.

Experiments (2):-

Preparation of alcoholic extracts:

1. Followed the same method in 1 but replace the boiled water with ethanol. Separating funnel used in this experiment with continuous stirling for 24-48 hr.
2. Saxhlet apparatus used in this method for extraction and the same amount of dried plant parts and alcohol used in this apparatus for

6-8 hr. then filtrate the extract resulted which should be put in sterilized container in the fridge until used.

Experiments (3): Extraction of oil (Essential oils)

1) Take 50 g from Aromatic plant such as: *Linum usitatissimum, Nigella sativa, Sesamum orientale.*
2) 250 ml of Hexane added and replace it in the continuous extractor or steam distillators.
3) Collect the resulted oil in the flask and keep it in the fridge until use.

Experiments (4): Extraction of volatile oils:

(*Eucalyptus* sp., *Myrtus communis*)

1) 15 g of plant part (dried and in powder form) and 50 ml of Boiled distilled water adding to it with continuous stirling until it cool down.
2) Filtrate the extract.
3) Store the extract with sterilized clean container.
4) 1 ml of the extract (water extract) take and one drop of the reagent added to it.
5) Registar the reagents results .

Secondary metabolites

Secondary metabolites are organic compounds that are not directly involved in the normal growth, development, or reproduction of an organism. Unlike primary metabolites, absence of secondary metabolites does not result in immediate death, but rather in long-term impairment of the organism's survivability, fecundity, or aesthetics, or perhaps in no significant change at all. Secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. Secondary metabolites often play an important role in plant and other interspecies defenses. Humans use secondary metabolites as medicines, flavorings, and recreational drugs.

Organic compound is any member of a large class of gaseous, liquid, or solid chemical compounds whose molecules contain carbon. For historical reasons discussed below, a few types of carbon-containing compounds, such as carbides, carbonates, simple oxides of carbon such as CO and CO2, and cyanides are considered inorganic. The distinction between organic and inorganic carbon compounds, while "useful in organizing the vast subject of chemistry is somewhat arbitrary" Organic chemistry is the science concerned with all aspects of organic compounds. Organic synthesis is the methodology of their preparation. Organic compounds as containing carbon-hydrogen bonds, and inorganic compounds are compounds that do not contain such links. Thus, carbonic acid is an organic, while formic organic acid, meaning that the first fatty acid organic, although it can be called "carbonic acid will" also form the "anhydrous" monoxide Carbon is organic.

Alkaloids

Alkaloids are generated by various living organisms, especially by higher plants – about 10 to 25% of those contain alkaloids. Therefore, in the past the term "alkaloid" was associated with plants. The alkaloids content in plants is usually within a few percent and is inhomogeneous over the plant tissues. Depending on the type of plants, the maximum concentration is observed in the leaves (black henbane), fruits or seeds (Strychnine tree), root (*Rauwolfia serpentina*) or bark (cinchona). Furthermore, different tissues of the same plants may contain different alkaloids. Beside plants, alkaloids are found in certain types of fungi, such as psilocybin in the fungus of the genus *Psilocybe*, and in animals,

such as bufotenin in the skin of some toads. Many marine organisms also contain alkaloids. Some amines, such as adrenaline andserotonin, which play an important role in higher animals, are similar to alkaloids in their structure and biosynthesis and are sometimes called alkaloids.

Plant groups containing alkaloids: -

Are nitrogenous organic compounds combine with alkaline acid which is found in all parts of the plant, or in the special parts of the plant (such as bark, roots, fruits, seeds, leaves)

An alkaline effect compounds ranges focus from 1-15% of the dry weight of the plant.

The alkaloids of the most important groups in the world of medicine and treatment is fairly natural compounds that may be chemically or physiologic vary, but they share being organic materials containing nitrogenous bases complicated installation carrier Atoms of C, O, H, N These groups act as if the base which is having an impact in the physiology of the organism, even if found in plants in small quantities and this effect varies depending on the nature and amount of alkaloids have been separated so far more than 2,000 alkaloid.

Families containing alkaloids: -

- Papaveraceae
- Solanaceae
- Liliaceae
- Acanthaceae
- Apocynaeae

Alkaloids uses and benefits: -

- Cocaine and morphine extracted from poppy
- Quinine extracted from cinchona
- There caffeine from coffee, tea cocaine from coca
- ephedrine drug to treat colds, asthma, ephedra, a genus of plants, Rizrbin of Alrolivea Serbntinh.
- Altupuk and Rrarin plant of curare poison extract. Material used toxic alkaloid extracted from the plant extract of curare in some primitive tribesmen to poison arrows.
- It contains plant hemlock (SAM), which is used to execute the Greek philosopher Socrates plant, a lethal amounts of materials and semi-Universalist other alkaline.
- Also, the Alkontin extracted from the plant is highly toxic Aconitum.
- And nicotine extracted from tobacco is toxic to humans and is used to kill insects.

Detection of alkaloids: - To make this disclosure has been using one of two methods 1. Marquis reagent, which gives gray granulated evidence of

the presence of alkali 2. Meyer detector that gives a white sediment is also evidence of the alkali

Glycosides

It is a group of organic compounds in which sugar group linked with other clique by glycosidic bond - oxygen (O-glycosidic bond) Or sulfur glycosidic - bond (S-glycosidic bond), in the latter case can call glycosides (thioglycosides) or sulfur glycoside.
Glycosides play an essential role in biology, used as many plant glycosides medical medicament.
It contrast, many authors are demanding to be associated with the sugar molecule genin part to distinguish the compound that he Glycosides, so the Many (polysaccharide) are excluded from Glycosides. According to this term is called the diabetes :(glycone) The a glycone called (a glycone(
Or the basic part :(genin) of Glycosides
A glycone can consist of a single sugar group (monosaccharide) or several groups of sugar (oligosaccharide).

Plant groups containing glycosides: -

Glycosides are an important part of the active substances in medicinal plants
Are compounds produced when analyzed by one or more sugars as part consists of Diabetes and genin part, which may be a composite (alcoholic or phenolic or Aldehydes or ketones)

Type of Glycosides :-

1- Glycosides Digitoxin Tonic to heart muscles
2- Glycosides Rutine Tonic wall of blood vessels and anti-bleeding
3- Glycosides Salicin user analgesic

Plant families containing glycosides:-

- Apocynaceae الذفلة عائلة
- Liliaceae بَقية الس العائلة
- Rosaceae الوردية العائلة

Glycoside importance for the plant: -

1. help regulate the growth and photosynthesis and metabolism within the plant
2. Protects and keeps the plants from animals or birds or insects that may feed on them

Glycoside significance for humans: -

1- Therapy to stimulate the heart muscle, such as glycosides in oleander plant, Ruta, onions
2- Strengthen the walls of blood vessels, such as plant Ruta, buckwheat
3- Therapy constipation, such as Cucumbers Plant
4- Treatment of skin diseases such as the plant Ruta

5- The treatment of rheumatism and joint pain, such as willow plant and the poplars plant,

Detection glycosides: -
Add 2 ml of Benedict's reagent to 1ml of the plant extract the subject in a test tube and then shake the solution well and put in a boiling water bath for 5 minute
The tube then left to cool ... Red precipitate shows the presence of glycosides

Volatile oils : - are oils that have a propensity to evaporation or volatilization when exposed to the atmosphere in the degree normal temperature without degrade or change installed, these oils consist of terpenes materials and oxygen.
These oils are found in more than one plant, as are found in about 60 plant species, attributed the medical impact of basic oils because they contain ISO carbonaceous materials and oxygen are used different types of the oils to treat various ailments such as flu, cold and expelling gases and diuretic and stimuli for the digestive system and anti microorganisms, particularly bacteria Bacillus.
Plant families containing volatile oils: -

- Pinaceae
- Ranunculaceae
- Myrtaceaea
- Labiatae
- Umbellferae .

Their therapeutic benefits : -

- anise oil :- Eliminates head lice
- Chamomile oil :- Strengthens the blood and reduce the heat
- Coriander oil :- It regulates the menstrual cycle
- basil oil :- Feeds the hair and strengthens
- Oregano oil :- Expels worms and kill it
- juniper oil :- Much benefit in the treatment of paralysis
- Clove oil :- Antiseptic oral analgesic for the pain of the teeth and gums hardener

• Almond oil :- Treats hemorrhoids, eczema, itching, urinary stones crumbling, and the inhabited of the middle ear pain and strengthens the membrane of the eardrum, and treat freckles and burns.

Extracting volatile oils Method : -

1. extraction distillation.
2. organic solvent extraction.

Detection of volatile oils: -

Were taken (10) ml of the extract plant was nominated and satiated their nomination papers well, were exposed to a source of UV,
Indicate appearance of bright pink color on the presence of volatile oils.

'Tannins

Tannins called Cementing materials and be of a complex chemical structure in which phenolic compounds amorphous crystals are interference a difficult separation or compounds,
A **tannin** (or **tannoid**) is an astringent, polyphenolic biomolecule that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids.

Properties of tannins :-

• holding materials (diarrhea)
• materials to stop the bleeding
• antiseptic
• treatment (for poisoning by heavy metals, alkaloids, glycosides)

Types of tannins :-

1. Hydrolyzable:- tannins that soluble in water
2. Proanthocyanidin:- tannins is not soluble in water
3. Pseudo Tannins :- false tannins.

Families plant containing tannin: -

• Theaceae
• Fagaceae
• Hamumelidiaceae .

Uses of tannin: -

1. leather tanning: - the ability of these materials to the deposition of proteins and alkaloids from their solutions, and this process is carried out

at the tanning of leather, which is characterized by this group of botanical ingredients when precipitate the proteins that are the skin becomes non-biodegradable. (To prevent the decomposition of proteins in the skin, it remains for a long time without decomposition)
2. Treatment of intestinal distress clutch effect on the intestine and its impact cleanser
3. It causes constriction of blood vessels, and is used to stop bleeding and heal wounds and sores and burns.

Detection of tannin: -

Boiling (10) grams of plant powder in 50 ml of distilled water and then filtered solution and leave to cool, then the filtrate is divided into two parts:-
1. Add a solution of 1% Lead acetate to deduce the presence of tannins sediment the emergence of a white gelatinous textures.
2. Add 1% solution of Ferric Chloride as it indicates the emergence of a bluish green color and the presence of tannins.

Chromatography Techniques (part 1)

Chromatography techniques are used to separate mixture of various chemicals into their individual forms. The process involves separation of the mixture with the help of different mobile and stationary phases...

Chromatography is the science which involves the study of separation of molecules based on differences in their composition and/or structure. It is the collective term for a set of laboratory methods used for the separation of mixtures. It is a wonderful technique to separate mixtures and involves the preparation of a test solution, which will interact differently with the stationary support, thereby leading to separation of similar molecules. Now how does this happen? Well, the molecules in the test solution which have tighter interactions with the stationary support will move slower than those molecules with weaker interactions. In this manner, different types of molecules in a solution can be separated.

The basic principle of chromatography is that different chemicals have different degrees of dissolving power in a liquid and different powers of sticking to a solid surface. Thus, chromatography can identify chemical components in a mixture and separate them by making them visible on a surface. Such separations using chromatography techniques can be done by using various supports like glass plates (thin layer chromatography), insoluble molecules (liquid chromatography), volatile gases (gas chromatography) etc. Let s have a closer look at the different types of chromatography techniques.

Chromatography techniques are one of the most widely used chemical techniques for separation of contaminants from chemical plants. For example, chemical plants use the techniques for removal of pesticides from ground water. Moreover, pharmaceutical companies also use these techniques to prepare large quantities of pure compounds required to prepare medicines. Manufacturers also use these chromatography techniques to check the presence of any contaminants in their manufactured compounds.

Different Types of Chromatography Techniques

The different chromatographic techniques by which similar molecules can be separated are as follows:

1- **Paper Chromatography:** This technique involves the presence of a solvent that moves along blotting or filter paper. In this technique, the cellulose of the filter paper acts as an inert support. When a few drops of the mixture is applied to this filter paper and dipped into the container with water, the water begins to move upwards by capillary action. The water then spreads out the final color of the mixture into their constituent colors. Thus, interactions between the sample or mixture, water or solvent and the filter paper brings about separation of the components. Paper chromatography is used to separate most colored compounds and is widely used in artificial and natural pigment research.

2- **Thin Layer Chromatography** (TLC): This technique involves the use of an inert piece of glass, plastic or metal. It is a simple and inexpensive technique used to judge the purity of a synthesized compound. It is also used to detect the extent of progress of a particular chemical reaction. A drop of solution to be analyzed is added to the TLC glass plate, which has already been coated with a thin layer of

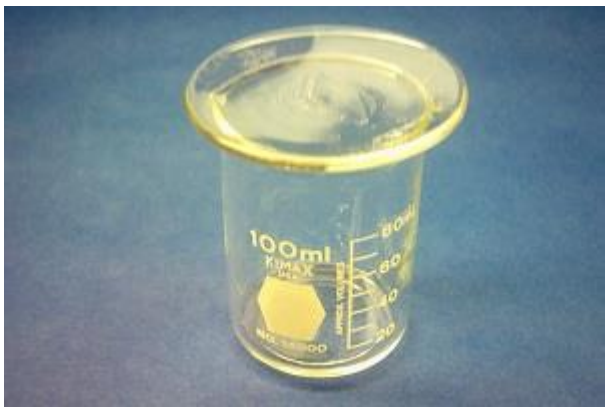
silica gel. This silica coated glass plate acts as the stationary phase. The sheet is then placed in a glass chamber containing a solvent, which forms the mobile phase. Via capillary action, the solvent moves upwards and carries the molecules of the solution to be detected at different rates. The components of the solution will appear in the form of a series of spots at various locations on the plate. Calculations are made based on ratio of the distance that the substance travels to the distance that the solvent travels up the plate. Read more on thin layer chromatography.

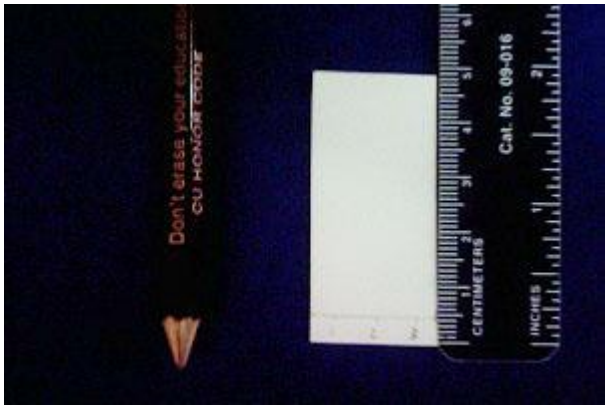
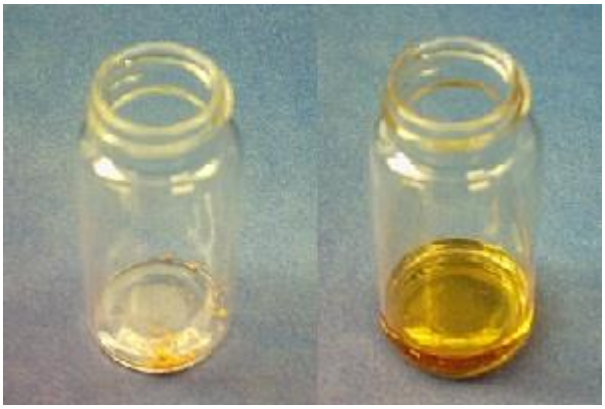
TLC is a simple, quick, and inexpensive procedure that gives the chemist a quick answer as to how many components are in a mixture. TLC is also used to support the identity of a compound in a mixture when the R_f of a compound is compared with the R_f of a known compound (preferably both run on the same TLC plate).

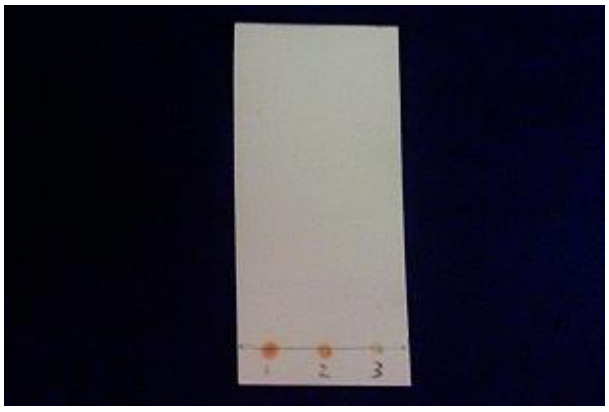
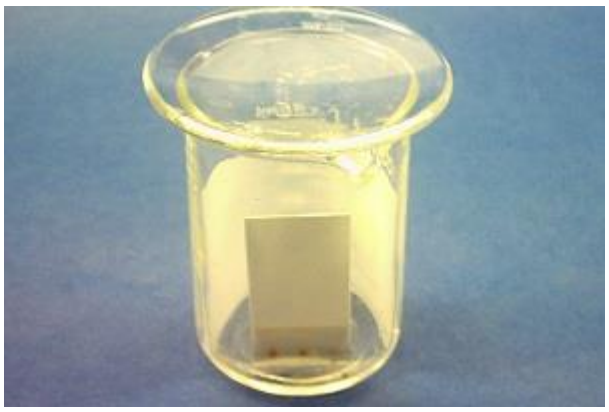
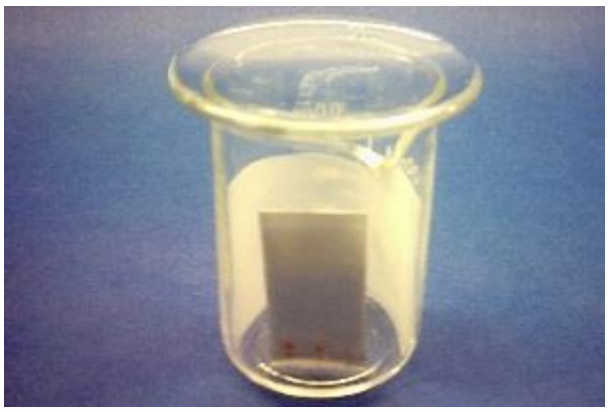
A TLC plate is a sheet of glass, metal, or plastic which is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only the very bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action.

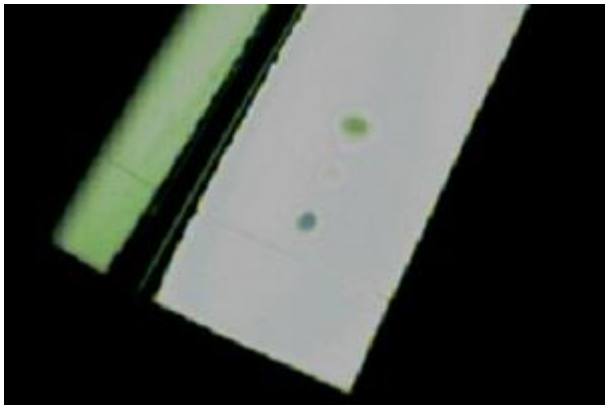
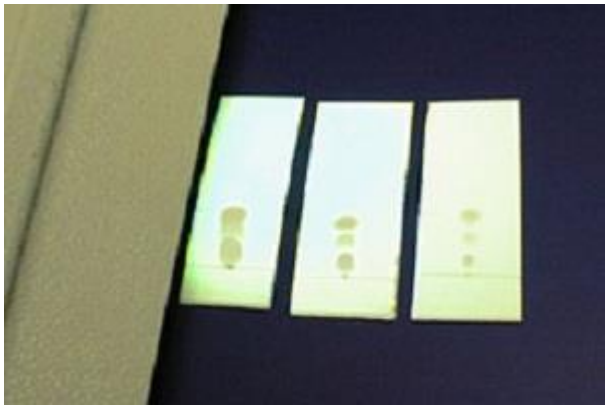
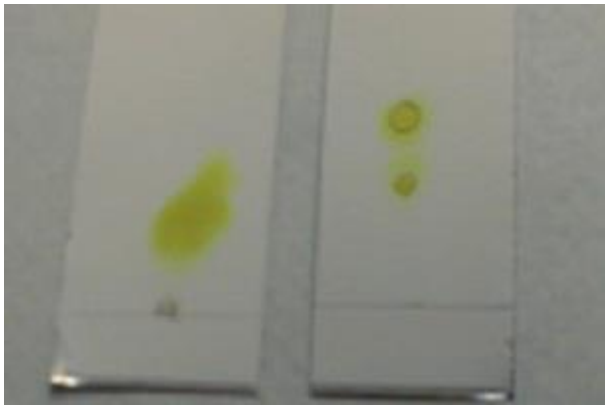
As the solvent moves past the spot that was applied, an equilibrium is established for each component of the mixture between the molecules of that component which are adsorbed on the solid and the molecules which are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized. If the compounds are colored, visualization is straightforward. Usually the compounds are not colored, so a UV lamp is used to visualize the plates. (The plate itself contains a fluorescent dye which glows everywhere *except* where an organic compound is on the plate.)

Experiments:

	**Step 1: Prepare the developing container** The developing container for TLC can be a specially designed chamber, a jar with a lid, or a beaker with a watch glass on the top (the latter is used in the undergrad labs at CU). Pour solvent into the chamber to a depth of just less than 0.5 cm. To aid in the saturation of the TLC chamber with solvent vapors, you can line part of the inside of the beaker with filter paper. Cover the beaker with a watch glass, swirl it gently, and allow it to stand while you prepare your TLC plate.

<table>
<tr>
<td></td>
<td>Step 2: Prepare the TLC plate
TLC plates used in the organic chem teaching labs are purchased as 5 cm x 20 cm sheets. Each large sheet is cut horizontally into plates which are 5 cm tall by various widths; the more samples you plan to run on a plate, the wider it needs to be. Shown in the photo to the left is a box of TLC plates, a large un-cut TLC sheet, and a small TLC plate which has been cut to a convenient size. Handle the plates carefully so that you do not disturb the coating of adsorbent or get them dirty.</td>
</tr>
<tr>
<td>
</td>
<td>Measure 0.5 cm from the bottom of the plate. Using a pencil, draw a line across the plate at the 0.5 cm mark. This is the origin: the line on which you will spot the plate. Take care not to press so hard with the pencil that you disturb the adsorbent. Under the line, mark lightly the name of the samples you will spot on the plate, or mark numbers for time points. Leave enough space between the samples so that they do not run together; about 4 samples on a 5 cm wide plate is advised.</td>
</tr>
<tr>
<td></td>
<td>Step 3: Spot the TLC plate
If the sample is not already in solution, dissolve about 1 mg in 1 mL of a volatile solvent such as hexanes, ethyl acetate, or methylene chloride. As a rule of thumb, a concentration of 1% usually works well for TLC analysis. If the sample is too concentrated, it will run as a smear or streak (see troubleshooting section below); if it is not concentrated enough, you will see nothing on the plate. Sometimes you will need to use trial and error to get well-sized, easy to read spots.</td>
</tr>
</table>

	Obtain a a microcapillary. In the organic teaching labs, we use 10µL microcaps - they are easier to handle than the smaller ones used in research labs. Dip the microcap into the solution and then **gently** touch the end of it onto the proper location on the TLC plate. Don't allow the spot to become too large - if necessary, you can touch it to the plate, lift it off and blow on the spot. If you repeat these steps, the wet area on the plate will stay small.
	This example plate has been spotted with three different quantities of the same solution and is ready to develop. If you are unsure of how much sample to spot, you can always spot multiple quantities and see which looks best.
	**Step 4: Develop the plate** Place the prepared TLC plate in the developing beaker, cover the beaker with the watch glass, and leave it undisturbed on your bench top. The solvent will rise up the TLC plate by capillary action. Make sure the solvent does not cover the spot.
	Allow the plate to develop until the solvent is about half a centimeter below the top of the plate. Remove the plate from the beaker and immediately mark the solvent front with a pencil. Allow the plate to dry.

	**Step 5: Visualize the spots** If there are any colored spots, circle them lightly with a pencil. Most samples are not colored and need to be visualized with a UV lamp. Hold a UV lamp over the plate and circle any spots you see. Beware! UV light is damaging both to your eyes and to your skin! Make sure you are wearing your goggles and do not look directly into the lamp. Protect your skin by wearing gloves.
	If the TLC plate runs samples which are too concentrated, the spots will be streaked and/or run together. If this happens, you will have to start over with a more dilute sample to spot and run on a TLC plate.
	Here's what overloaded plates look like compared to well-spotted plates. The plate on the left has a large yellow smear; this smear contains the same two compounds which are nicely resolved on the plate next to it.

TLC Solvents Choice

When you need to determine the best solvent or mixture of solvents (a "solvent system") to develop a TLC plate or chromatography column loaded with an unknown mixture, vary the polarity of the solvent in several trial runs: a process of trial and error. Carefully observe and record the results of the chromatography in each solvent system. You will find that as you increase the polarity of the solvent system, all the components of the mixture move faster (and vice versa with lowering the polarity). The ideal solvent system is simply the system that gives the best separation.

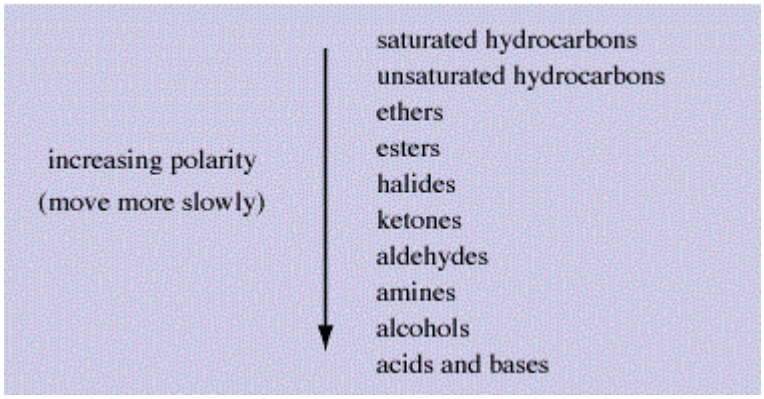
TLC elution patterns usually carry over to column chromatography elution patterns. Since TLC is a much faster procedure than column chromatography, TLC is often used to determine the best solvent system for column chromatography. For instance, in determining the solvent system for a flash chromatography procedure, the ideal

system is the one that moves the desired component of the mixture to a TLC R_f of 0.25-0.35 and will separate this component from its nearest neighbor by difference in TLC R_f values of at least 0.20. Therefore a mixture is analyzed by TLC to determine the ideal solvent(s) for a flash chromatography procedure.

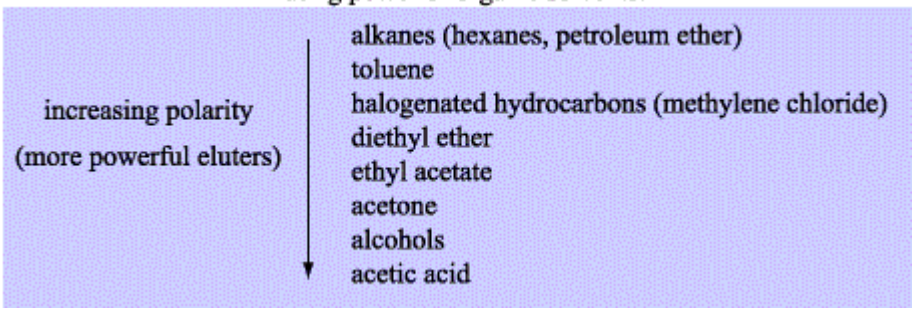
Beginners often do not know where to start: What solvents should they pull off the shelf to use to elute a TLC plate? Because of toxicity, cost, and flammability concerns, the common solvents are hexanes (or petroleum ethers/ligroin) and ethyl acetate (an ester). Diethyl ether can be used, but it is very flammable and volatile. Alcohols (methanol, ethanol) can be used. Acetic acid (a carboxylic acid) can be used, usually as a small percentage component of the system, since it is corrosive, non-volatile, very polar, and has irritating vapors. Acetone (a ketone) can be used. Methylene chloride or and chloroform (halogenated hydrocarbons) are good solvents, but are toxic and should be avoided whenever possible. If two solvents are equal in performance and toxicity, the more volatile solvent is preferred in chromatography because it will be easier to remove from the desired compound after isolation from a column chromatography procedure.

Ask the lab instructor what solvents are available and advisable. Then, mix a non-polar solvent (hexanes, a mixture of 6-carbon alkanes) with a polar solvent (ethyl acetate or acetone) in varying percent combinations to make solvent systems of greater and lesser polarity. The charts below should help you in your solvent selection. You can also download

The expected elution order of organic classes.



Eluting power of organic solvents.



Interactions Between the Compound and the Adsorbent

The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces. With silica gel, the dominant interactive forces between the adsorbent and the materials to be separated are of the dipole-dipole type. Highly polar molecules interact fairly strongly with the polar SiOH groups at the surface of these adsorbents, and will tend to stick or adsorb

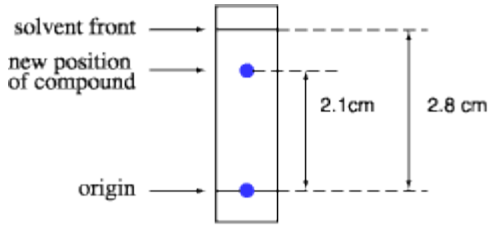
onto the fine particles of the adsorbent while weakly polar molecules are held less tightly. Weakly polar molecules generally tend to move through the adsorbent more rapidly than the polar species. Roughly, the compounds follow the elution order given above.

The R_f value

The retention factor, or R_f, is defined as the distance traveled by the compound divided by the distance traveled by the solvent.

$$R_f = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

For example, if a compound travels 2.1 cm and the solvent front travels 2.8 cm, the R_f is 0.75:



$$R_f = \frac{2.1}{2.8} = 0.75$$

The R_f for a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant:

- solvent system
- adsorbent
- thickness of the adsorbent
- amount of material spotted
- temperature

Since these factors are difficult to keep constant from experiment to experiment, relative R_f values are generally considered. "Relative R_f" means that the values are reported relative to a standard, or it means that you compare the R_f values of compounds run on the same plate at the same time.

The larger an R_f of a compound, the larger the distance it travels on the TLC plate. When comparing two different compounds run under identical chromatography conditions, the compound with the larger R_f is less polar because it interacts less strongly with the polar adsorbent on the TLC plate. Conversely, if you know the structures of the compounds in a mixture, you can predict that a compound of low polarity will have a larger R_f value than a polar compound run on the same plate.

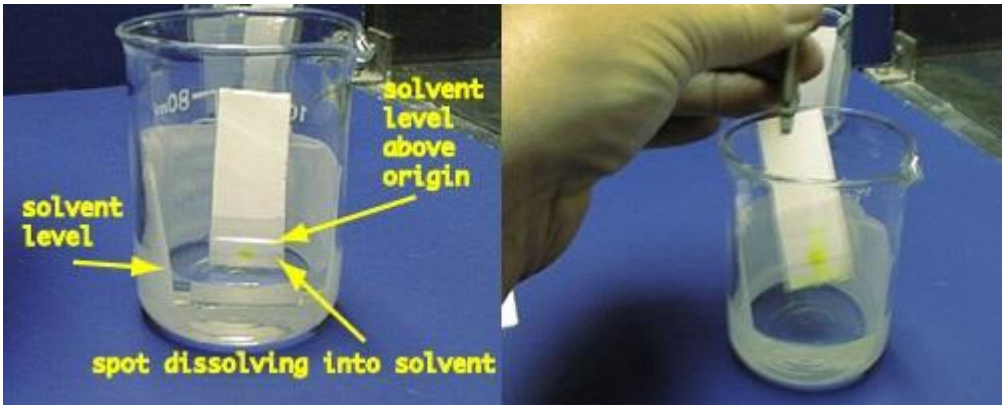
The R_f can provide corroborative evidence as to the identity of a compound. If the identity of a compound is suspected but not yet proven, an authentic sample of the compound, or standard, is spotted and run on a TLC plate side by side (or on top of each other) with the compound in question. If two substances have the same R_f value, they are likely (but not necessarily) the same compound. If they have different R_f values, they are definitely different compounds. Note that this identity check must be performed on a single plate, because it is difficult to duplicate all the factors which influence R_f exactly from experiment to experiment.

Troubleshooting TLC

All of the above (including the procedure page) might sound like TLC is quite an easy procedure. But what about the first time you run a TLC, and see spots

everywhere and blurred, streaked spots? As with any technique, with practice you get better. Examples of common problems encountered in TLC:

- **The compound runs as a streak rather than a spot:** The sample was overloaded. Run the TLC again after diluting your sample. Or, your sample might just contain many components, creating many spots which run together and appear as a streak. Perhaps, the experiment did not go as well as expected.
- **The sample runs as a smear or a upward crescent:** Compounds which possess strongly acidic or basic groups (amines or carboxylic acids) sometimes show up on a TLC plate with this behavior. Add a few drops of ammonium hydroxide (amines) or acetic acid (carboxylic acids) to the eluting solvent to obtain clearer plates.
- **The sample runs as a downward crescent:** Likely, the adsorbent was disturbed during the spotting, causing the crescent shape.
- **The plate solvent front runs crookedly:** Either the adsorbent has flaked off the sides of the plate or the sides of the plate are touching the sides of the container (or the paper used to saturate the container) as the plate develops. Crooked plates make it harder to measure R_f values accurately.
- **Many random spots are seen on the plate:** Make sure that you do not accidentally drop any organic compound on the plate. If get a TLC plate and leave it laying on your workbench as you do the experiment, you might drop or splash an organic compound on the plate.
- **You see a blur of blue spots on the plate as it develops:** Perhaps you used an ink pen instead of a pencil to mark the origin?
- **No spots are seen on the plate:** You might not have spotted enough compound, perhaps because the solution of the compound is too dilute. Try concentrating the solution, or spot it several times in one place, allowing the solvent to dry between applications. Some compounds do not show up under UV light; try another method of visualizing the plate (such as staining or exposing to iodine vapor). Or, perhaps you do not have any compound because your experiment did not go as well as planned. If the solvent level in the developing jar is deeper than the origin (spotting line) of the TLC plate, the solvent will dissolve the compounds into the solvent reservoir instead of allowing them to move up the plate by capillary action. Thus, you will not see spots after the plate is developed. These photos show how the yellow compound is running into the solvent when lifted from the developing jar.



Medicinal plants **lab 9**

Chromatography Techniques (part 2)

3- Column Chromatography (CC):

This technique consists of a powdered adsorbent as the stationary phase, placed in a vertical glass column. The mixture to be analyzed is placed on top of this vertical glass column. The solvent forming the mobile phase is poured from the top of the column and flow down the column. In most cases, either silica (SiO_2) or alumina (Al_2O_3) is added to the solvent which act as the stationary phase. As the solvent flows, the solvent elutes the sample through the column and the components of the mixture begin to flow at their respective rates down the column. However, this technique need large volumes of solvent and any careless additions can cause disturbance to the stationary phase, leading to poor separations.

4- Gel-Filtration Chromatography

Gel filtration (GF) separates proteins solely on the basis of differences in molecular size.Separation is achieved using a porous matrix to which the molecules, for steric reasons, have different degrees of access (smaller molecules have greater access and larger molecules less).

The matrix is packed into a chromatographic column, the sample mixture applied, and the separation accomplished by passing an aqueous buffer (the mobile phase) through the column. Protein molecules that are confined in the volume outside the matrix

beads will be swept through the column by the mobile phase and the sample is thus eluted in decreasing order of size. The protein zones eluted are detected by an in-line UV monitor and fractions are collected for subsequent specific analysis or further preparation steps.

GF is often used to condition samples prior to further purification by adsorptive techniques. It is also frequently used in the final polishing stage to remove a few remaining contaminants with surface properties similar to the target protein (e.g., for polishing monomers from oligomers).Three basic protocols are provided in this unit, corresponding to the major applications of gel filtration.

- GF is used for desalting or group separation in which the target protein and contaminating solutes differ substantially in molecular size.
- It is also used for protein fractionation , where proteins with only a small size difference must be separated.
- A third application is in characterizing the molecular dimensions of proteins . A Support Protocol is alsoprovided for calibrating GF columns to be used in estimating molecular size.

5- Electrophoresis

is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field. This electrokinetic phenomenon was observed for the first time in 1807 by Reuss (Moscow State University), who noticed that the application of a constant electric field caused clay particles dispersed in water to migrate. It is ultimately caused by the presence of a charged interface between the

particle surface and the surrounding fluid. Electrophoresis of positively charged particles (cations) is called cataphoresis, while electrophoresis of negatively charged particles (anions) is called anaphoresis.

6- Affinity chromatography:

It is a method of separating biochemical mixtures and based on a highly specific interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand. Affinity chromatography can be used to:

- Purify and concentrate a substance from a mixture into a buffering solution
- Reduce the amount of a substance in a mixture
- Discern what biological compounds bind to a particular substance
- Purify and concentrate an enzyme solution.
- The immobile phase is typically a gel matrix, often of agarose; a linear sugar molecule derived from algae.

Usually the starting point is an undefined heterogeneous group of molecules in solution, such as a cell lysate, growth medium or blood serum. The molecule of interest will have a well known and defined property which can be exploited during the affinity purification process. The process itself can be thought of as an entrapment, with the target molecule becoming trapped on a solid or stationary phase or medium. The other molecules in solution will not become trapped as they do not possess this property. The solid medium can then be removed from the mixture, washed and the target molecule released from the entrapment in a process known as elution. Possibly the most common use of affinity chromotography is for the purification of recombinant proteins.

7- High-performance liquid chromatography (HPLC;

formerly referred to as high-pressure liquid chromatography), 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 the column.

8- Gas Chromatography : (GC):

In all chromatography, separation occurs when the sample mixture is introduced (injected) into a **mobile phase**. 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.

The mixture of compounds in the **mobile phase** interacts with the **stationary phase**. Each compound in the mixture interacts at a different rate. Those that interact the fastest will exit (elute from) the column first. Those that interact slowest will exit the column last. By changing characteristics of the mobile phase and the stationary phase, different mixtures of chemicals can be separated. Further refinements to this separation process can be made by changing the temperature of the stationary phase or the pressure of the mobile phase.

GC has a long, thin column containing a thin interior coating of a **solid stationary phase** (5% phenyl-, 95% dimethylsiloxane polymer). This 0.25 mm diameter column is referred to as a capillary column. This particular column is used for semivolatile, non-polar organic compounds such as the PAHs we will look at. The compounds must me in an organic solvent.

The capillary column is held in an oven that can be programmed to increase the temperature gradually (or in GC terms, ramped). this helps our separation. 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.

As the compounds are separated, they elute from the column and enter a detector. The detector is capable of creating an electronic signal whenever the presence of a compound is detected. The greater the concentration in the sample, the bigger the signal. The signal is then processed by a computer. The time from when the injection is made (time zero) to when elution occurs is referred to as the **retention time** (RT). **Retention time** is the amount of time a compound spends on the column after it has been injected. If a sample containing several compounds, each compound in the sample will spend a different amount of time on the column according to its chemical composition i.e. each will have a different retention time. Retention times are usually quoted in units of seconds or minutes.

A basic chromatography instrument consists of the following:

1. A sample port or **injector** for introduction and vaporization of the sample;
2. A separating **column**, consisting of metal tubing packed with a solid material coated with a stationary absorbing liquid;
3. A **carrier gas**, usually N_2 or He, to sweep the sample through the column;
4. Flow control equipment to maintain a constant flow of **carrier gas** through the column;
5. The **detector** for measuring the quantity of a separated component;
6. **Ovens** and heaters for temperature control of the column, detector and injector;
7. An **integrator** or integrator/strip chart recorder combination to provide permanent record of the analysis.

What is the difference between HPLC and GC?

The major difference between GC and HPLC lies in the phases used. While in GC, compounds of a mixture are separated using a liquid (stationary) phase and a gas (mobile) phase, in case of HPLC the stationary phase is a solid while liquids make up mobile phase. Another difference lies in temperature control during the process. In GC, there is an oven to contain the column consisting of gas phase and it can control the temperatures when the gases are passing through the column. On the other hand, there is no such provision of temperature control in HPLC. The last difference pertains to the concentration of the compounds. In GC, it is the vapor pressure of the gases that decide the concentration of compounds whereas it is possible to increase or decrease the concentration of the compounds in HPLC.

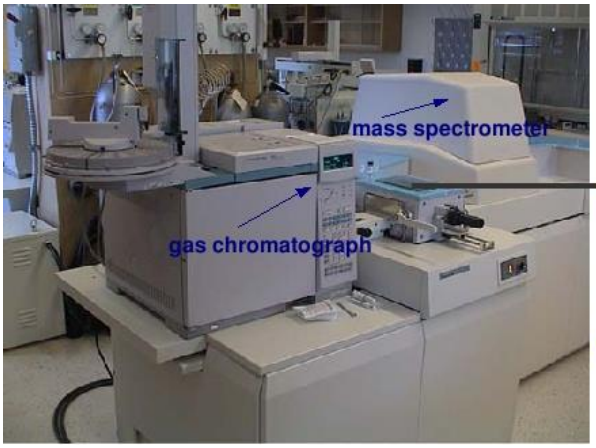
9- Gas Chromatography/Mass Spectrometry (GC/MS)

Introduction

combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. It is the principal method used to **detect** and **identify** compounds by exploiting the mass variations of different compounds.

A typical GCMS system performs six functions:

1. Compound separation by gas chromatography
2. Transfer of separated compounds to the ionizing chamber of the mass spectrometer
3. Ionization/ the molecules are ionized. Once the sample is fragmented it will then be detected, usually by an electron multiplier diode, which essentially turns the ionized mass fragment into an electrical signal that is then detected.
4. Mass analysis/ it is an analytical technique that ionizes chemical species and sorts the ions based on their mass-to-charge ratio
5. Detection of the ions by the electron multiplier and identification of components
6. Acquisition, processing and display of data by computer





MS Source

Methods

Gas Chromatography – Mass Spectrometry GC-MS analysis used a Shimadzu GC-2010Plus coupled with Shimadzu GCMS-Q2010 Ultra. Capillary column (InertCap 1MS, 0.25mm, 30m, 0.25µm, Gl Sciences, Japan), constant flow rate 1 ml/min;. Auto Injector: AOC-20i, Shimadzu. Injection volume: 5 µl. Column oven Temperature:100.°C. Oven temperature program:

Rate	Temperature (°C)	Hold Time (min)
	100.	3
15.00	240.	9
15.00	280	5
15.00	300	2

Identification of components

Identification of components of *C. officinalis* extract was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and computer matching with the (NIST mass spectral search program for the NIST/EPA/NIH mass spectral library version 2.0 f / 2008 and NIST08.LIB).

Results

The computer generates a graph from the signal , See the fallowing figure 1.

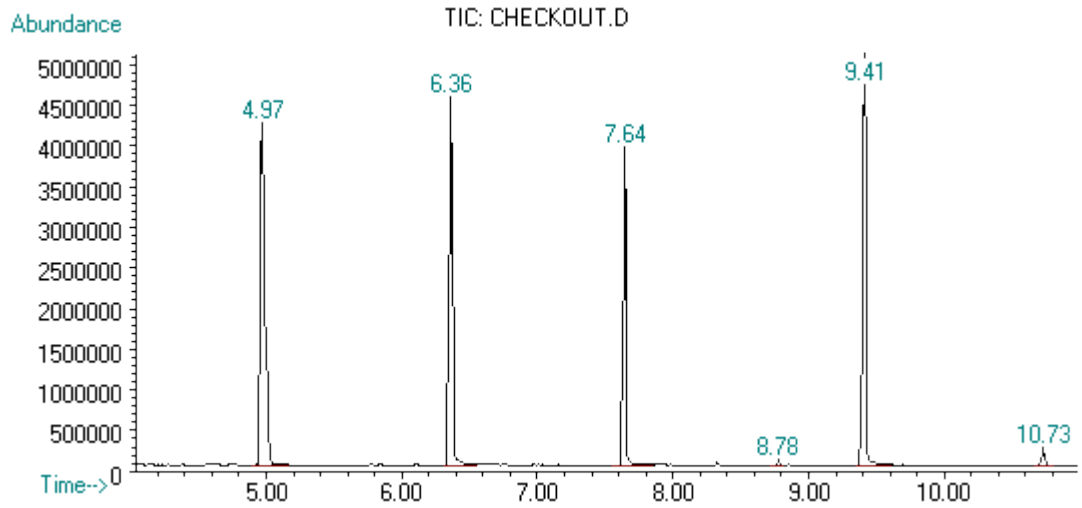


Figure 1: Chromatogram generated by a GC.

This graph is called a **chromatogram**. Each of the **peaks** in the chromatogram represents the signal created when a compound elutes from the GC column into the detector. The x-axis shows the RT, and the y-axis shows the intensity (abundence) of the signal. In Figure 1, there are several peaks labeled with their RTs. Each peak represents an individual compound that was separated from a sample mixture. The peak at 4.97 minutes is from dodecane, the peak at 6.36 minutes is from biphenyl, the peak at 7.64 minutes is from chlorobiphenyl, and the peak at 9.41 minutes is from hexadecanoic acid methyl ester.

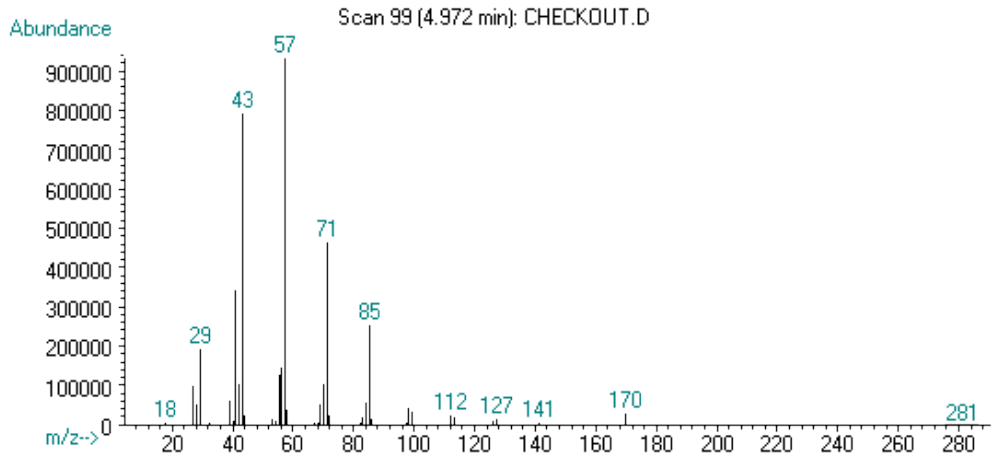


Figure 2: Mass-spectrum generated by an MS.

The computer records a graph for each scan. The x-axis represents the M/Z ratios. The y-axis represents the signal intensity (abundance) for each of the fragments detected during the scan. This graph is referred to as a **mass spectrum** (see Figure 2).

The mass spectrum produced by a given chemical compound is essentially the same every time. Therefore, the mass spectrum is essentially a fingerprint for the molecule. This fingerprint can be used to identify the compound. The mass spectrum in Figure 2 was produced by dodecane. The computer on our GC-MS has a library of spectra that can be used to identify an unknown chemical in the sample mixture. The library compares the mass spectrum from a sample component and compares it to mass spectra in the library. It reports a list of likely identifications along with the statistical probability of the match.

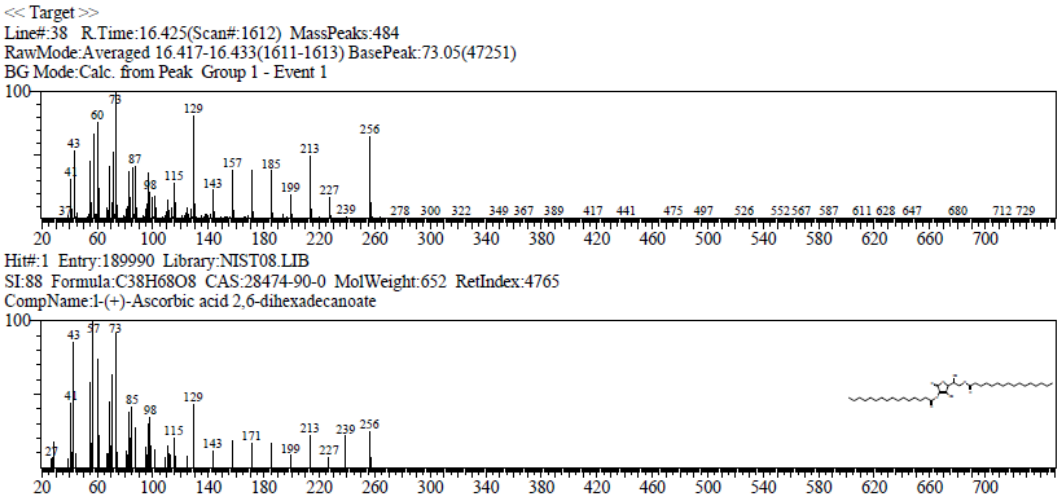


Figure 3: Mass-spectrum of standard ascorbic acid 2,d dihexanadecanoate in mass spectral library compared with expected compound that found in the oils of *C. colocynthis* by an MS.

We can arrange the data according the following tables which explain Chemical compositions of oil of *C. colocynthis* that analyzed by GCMS.

Table (1): Compositions of oil of *C. colocynthis*

P	**R.T.**	**Amount (%)**	**Name**	**Ch.F.**	**M.W.**
38	16.42	9.21	l-(+)-Ascorbic acid 2,6-dihexadecanoate	C38H68O8	652

RT: Retention time (min); CF: Chemical Formula; MW: Molecular weight.