CHAPTER ONE

INTRODUCTIN

I-Introduction

1.1- Gas Chromatography

A gas chromatography is a chemical analysis instrument used for separating and analyzing compounds of mixture that can be vaporized without decomposition into individual components. In some situations, GC may help in identifying a compound.[1, 2]

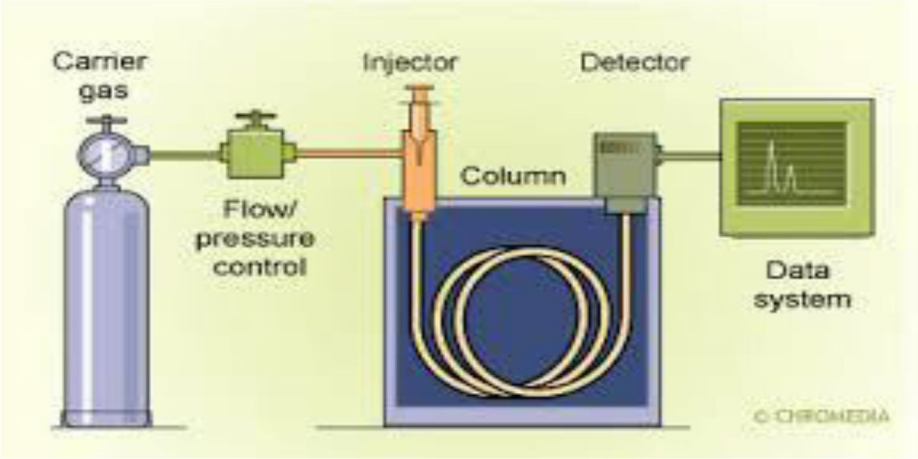
1.1.1- Principle of GC

In all chromatography ,separation occurs when the sample mixture is introduced (injected) into a mobile phase .In gas chromatography the mobile phase or "moving phase" is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen, The stationary phase is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column . The instrument used to perform gas chromatography is called a gas chromatograph (or "aerograph", "gas separator")[3].

When the sample mixture is injected the mixture of compounds in mobile phase interacts with the walls of the column, which is coated with a stationary phase. This causes each compound to elute at a different rate depending on their various chemical and physical properties. As the chemicals exit the end of the column,

the stationary phase in the column is to separate different components, causing each one to exit the column at a different time known as the retention time of the compound (RT). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, column length and the temperature.

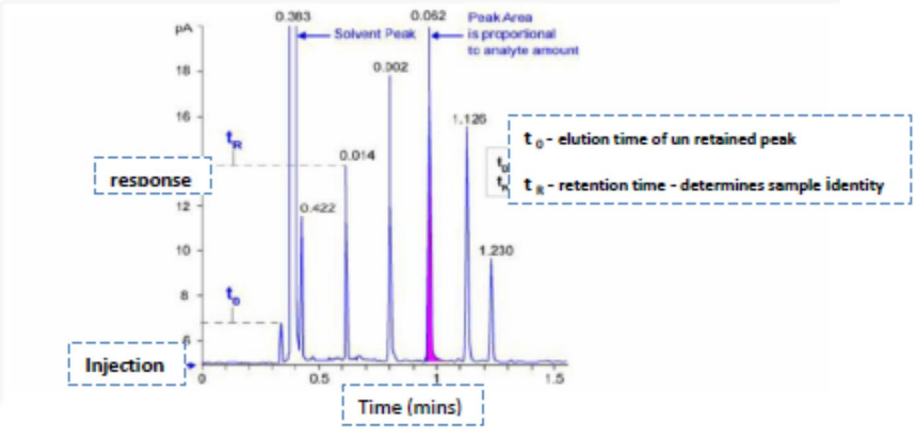
Generally, substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.[1]



A GC instrument

As the compounds are separated, they elute from the column and enter in detector. The detector is capable of creating an electronic signal whenever the presence of compound is detected .the greater is 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) during the operation, the computer generates a graph from the signals, this graph is called a chromatogram. Each peak in chromatogram represents an individual compound that was separated from a samplemixture.[4]



Chromatograms generated by GC

1.1.2-GC Advantages and disadvantages

Gas chromatography has several important advantages . GC techniques produce fast analyses because of the highly efficient nature of the separations achieved. It can be argued that modern GC produces the fastest separations of all chromatographic

techniques. A column has been produced to separate 970 components within a reasonable analysis time - proving that very complex separations may be carried out using GC.

By using a combination of oven temperature and stationary phase chemistry (polarity) very difficult separations may also be carried out – including separations of chiral and other positional isomers. GC is excellent for quantitative analysis with a range of sensitive and linear detectors to choose from[5].

GC is however limited to the analysis of volatile samples. Some highly polar analytes can be derivatized to impart a degree of volatility, but this process can be difficult and may incur quantitative errors.

A practical upper temperature limit for conventional GC columns is around 350-380 °C. Analyte boiling points rarely exceed 400 °C in GC analysis and the upper molecular weight is usually around 500 Da. Advantages of this technique include:

-Fast analysis.

-High efficiency – leading to high resolution.

-Sensitive detectors (ppb).

-Non-destructive – enabling coupling to mass spectrometers (MS) - an instrument that measures the masses of individual molecules that have been converted into ions, i.e. molecules that have been electrically charged .

-High quantitative accuracy (<1%RSD typical).

-Requires small samples (<1 mL).

-Rugged and reliable techniques.

-Well established with extensive literature and applications.

Disadvantages of **GC** include**:**

-Limited to volatile samples .

-Not suitable for samples that degrade at elevated temperatures (thermally labile).

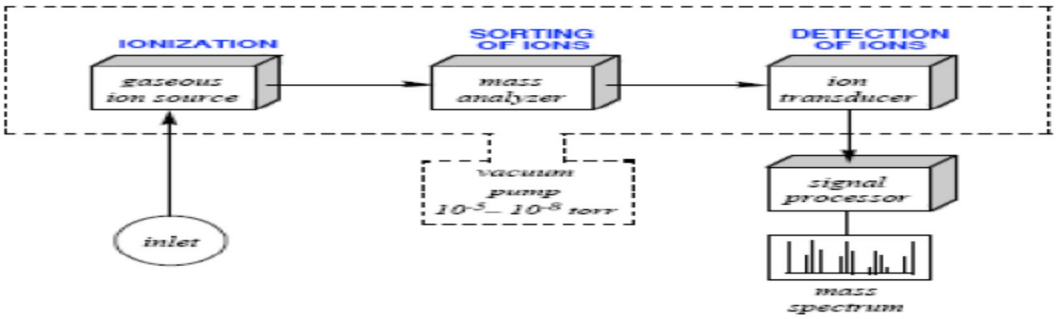
-Not suited to preparative chromatography.

-Requires MS detector for analyte structural elucidation (characterization).

-Most non-MS detectors are destructive[6].

1.2-Mass Spectrometry (MS)

Mass Spectrometry (MS) is an analytical technique that sorts ions based on their mass (or "weight"). A simple definition of a mass spectrometer is an analytical instrument that can separate charged molecules according to their mass–to–charge ratio.Mass spectrometry is primarily concerned with measuring the mass-to-charge ratio (m/z) and abundance of ions moving at high speed in a vacuum.



ASchematic of mass spectrometer

These ions are then separated according to their mass-to-charge ratio, typically by accelerating them and subjecting them to an electric or magnetic field: ions of the same mass-to-charge ratio will undergo the same amount of deflection. The ions are detected by a mechanism capable of detecting charged particles, such as an electron multiplier. Results are displayed as spectra of the relative abundance of detected ions as a function of the mass-to-charge ratio. The atoms or molecules in the sample can be identified by correlating known masses to the identified masses or through a characteristic fragmentation pattern[7].

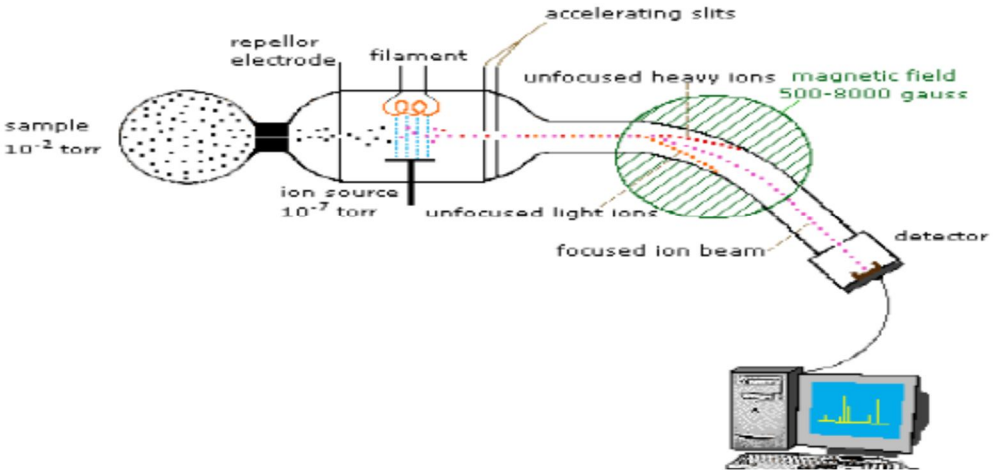
A mass spectrum (plural spectra) is a plot of the ion signal as a function of the mass-to-charge ratio. These spectra are used to

determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass-to-charge ratios[8].

1.2.1-Principle of mass spectrometry

The sample is vaporized, and then ionized by being bombarded by a beam of high energy electrons (usually at 70 eV). Electron beam knocks out an electron from the molecule of the injected sample, creating a molecular ion (which is also a radical cation because it has an unpaired electron and a positive charge).High energy electrons then give rise to more fragmentation where loss an electron weakens the bonds. while the collision gives it extra kinetic energy. The positively charged radical cations are then accelerated into an analyzer tube. The analyzer tube is surrounded by a curved magnetic field, which causes the path of the radical cations to be deflected in proportion to their mass-to-charge ratio (m/z). The flight path of the ions depend on their molecular masses, charges, and the strength of the magnetic field. Thus, at a given magnetic field strength, ions of only one specific mass collide with the detector and are recorded.

The strength of the magnetic field is varied in increments to produce a mass spectrum , which is a plot of m/z (on the x- axis) against relative abundance (on the y- axis). If we assume that all ions have a charge of +1, then the peaks give the mass ratios and their heights give the proportions of ions of different masses[9].



A Mass spectrometry instrument

1.2.2-Applications of mass spectrometry

Mass spectrometry has both qualitative and quantitative uses. These include identifying unknown compounds, determining the isotopic composition of elements in a molecule, and determining the structure of a compound by observing its fragmentation. Other uses include quantifying the amount of a compound in a sample or studying the fundamentals of gas phase ion chemistry (the chemistry of ions and neutrals in a vacuum). MS is now in very common use in analytical

laboratories that study physical, chemical, or biological properties of a great variety of compounds[10].

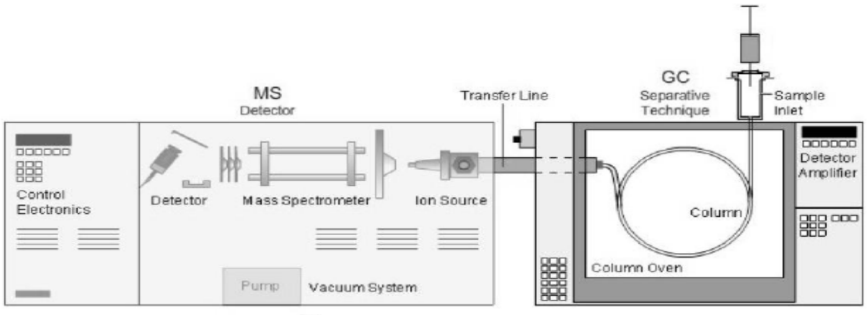
As an analytical technique it possesses distinct advantages such as: increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter, reduces background interference. The technique has excellent specificity where characteristic fragmentation patterns can identify unknowns or confirm the presence of suspected compounds. It provides information about molecular weight and isotopic abundance of elements[11].

A few of the disadvantages of the method is that often MS fails to distinguish between optical and geometrical isomers and the positions of substituent in o-, m- and p- positions in an aromatic ring. Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions[12].

1.3- Gas chromatography- Mass spectrometry (GC-MS)

(GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify

different substances within a test sample. GC can separate volatile and semi-volatile compounds with great resolution, but it cannot identify them. MS can provide detailed structural information on most compounds such that they can be exactly identified, but it cannot readily separate them. GC/MS- is a combination of two different analytical techniques, gas chromatography (GC) and mass spectrometry (MS). It is used to analyze complex organic and biochemical mixtures[13]. The GC-MS instrument consists of two main components. The gas chromatography portion separates different compounds in the sample into pulses of pure chemicals based on their volatility[13,14] by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column[13]. Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge ratio (m/z). These spectra can then be stored on the computer and analyzed [14].



ASchematic diagram of a GC- MS

Carrier gas is fed from the cylinders through the regulators and tubing to the instrument. It is usual to purify the gases to ensure high gas purity and gas supply pressure.

In the instrument ,the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column.Gas chromatography uses a gaseous mobile phase to transport sample components through columns either packed with coated silica particles or hollow capillary columns containing, the stationary phase coated onto the inner wall. Capillary GC columns are usually several meters long (10-120 m is typical) with an internal diameter of 0.10-0.50 mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal diameter[15].

Gas chromatography have ovens that are temperature programmable, the temperature of the gas chromatographic ovens typically range from 5°C to 400°C as low as -25°C with cryogenic cooling [16].

1.3.1-Advantages of GC-MS

GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. The cost of GC/MS equipment has decreased whereas the reliability has markedly increased. The determination of chloro-phenols in water and soil, polycyclic aromatic hydrocarbons (PAH), unleaded gasoline, dioxins, dibenzofurans, organo-chlorine

pesticides, herbicides, phenols, halogenated pesticides, sulphur in air is very convenient to be screened by this technique. It can be used to screen the degradation products of lignin in bio-mass research, pesticides in spinach[17,18]. Analysis of decacyclene, ovalene and even C60 degradation analysis of carbamazepine and its metabolites in treated sewage water and steroid can be done without derivatization [19, 20].

Foods and beverages have several aromatic compounds existing naturally in native state or formed while processing. GC-MS is exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. GC-MS is also used to detect and measure contaminants, spoilage and adulteration of food, oil, butter, ghee that could be harmful and should to be controlled and checked as regulated by governmental agencies. It is used in the analysis of piperine, spearmint oil, lavender oil, essential oil, fragrance reference standards, perfumes, chiral compounds in essential oils, fragrances, menthol, allergens, olive oil, lemon oil, peppermint oil, straw berry syrup, butter triglycerides, residual pesticides in food and wine [21,22].

In criminal cases GC-MS can analyze the particles from suspect to correlate his involvement in case. The analysis of fire debris using GC-MS can be established by American Society for Testing Materials (ASTM) standard for fire debris

analysis. It is the key tool used in sports anti-doping laboratories to test athlete's urine samples for prohibited performance enhancing drugs like anabolic steroids. It is also commonly used in forensic toxicology to find poisons, steroids in biological specimens of suspects or victims [23,24].

In biology , GC-MS is exclusively used in bio-analysis of blood, urine for the presence of barbiturates, narcotics, alcohols, residual solvents, drugs like anesthetics, anticonvulsant, antihistamine, anti-epileptic drug, sedative hypnotics, narcotics and food items. This technique could be used for detecting adulterations, fatty acid profiling in microbes, presence of free steroids, blood pollutants, metabolites in serum, organo-chlorinated pesticides in river water, drinking water, soft drinks by head space, and pesticides in sunflower oil etc[25].

GC-MS is also involved in security affairs . Explosive detection systems have become a part of all international airports. GC-MS is an essential part of chemical analysis unit. For enhancing capability in homeland security and public health preparedness, traditional GC-MS units with the transmission quadrupole mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field

portability and near real-time detection of chemical warfare agents (CWA) such as sarin, soman26,27.

Several GC-MS have left earth for the astro- chemical studies. Two GC-MS instruments were taken to Mars planet by the Viking program. Scientist analyzed the atmosphere of Venus wi GC-MS. The Huygens probe of the Cassini-Huygens mission la one GC-MS on Saturn's largest moon, Titan. The material in the comet 67P/Churyumov-Gerasimenko was analyzed by the Rose mission with a chiral GC-MS in 2014.

Significantly enhanced molecular ions, major isomer and struct significant mass spectral peaks, extended range of low vola hydrocarbons that are amenable for analysis and unique isotope information make GC-MS valuable for organic geoche applications [28,29].

1.3.2-Medical and Pharmaceutical Applications

GC-MS finds many applications in medicine and pharmaceuticals. Dozens of congenital metabolic diseases called as "inborn error of metabolism" are now detectable in newborn by screening tests using gas chromatography–mass spectrometry. GC-MS can determine compounds in urine even

in minor concentration. These compounds are normally not present but appear in individuals suffering from metabolic disorders. This is easy, effective and efficient way to diagnose the problem like in case of genetic metabolic disorders by a urine test at birth. In combination with isotopic labeling of metabolite, the GC-MS is used for determining metabolic activity. Most applications are based on the use of 13C labeling and the measurement of 13C-12C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few selected ions and retention values as ratios. It is useful to detect oils in creams, ointments, lotion etc30.

GC-MS is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs and formulations. It is used for process and method development, identification of impurities in API. It is an integral part of research associated with medicinal chemistry (synthesis and characterization of compounds), pharmaceutical analysis (stability testing, impurity profiling), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology etc 31.

In petrochemical industries , significantly enhanced molecular ions that are always observed, isomer and structurally

significant mass spectral peaks and extended range of low volatility hydrocarbons that are amenable for analysis including waxes up to C74H150 makes the GC-MS a most valuable technique . Broad range of petrochemicals, fuels and hydrocarbon mixtures, including gasoline, kerosene, naphthenic acids, diesel fuel, various oil types, transformer oil, biodiesel, wax and broad range of geochemical samples can be analyzed by GC-MS 32.

GC-MS is also employed in clinical toxicology , where enhanced molecular ions, extended range of compounds amenable for analysis, superior sensitivity for compounds and faster analysis are the main attractive features of the clinical toxicology. Toxins and venoms are identified by GC-MS which is extensively used in clinical toxicology 33.

As far as Academic research is concerned, GC-MS provides a rare opportunity to perform the analysis of new compounds for characterization and identification of synthesized or derivatized compound. It is widely used in pure and applied sciences like chemistry, polymers, nanotechnology and biotechnology etc. It yields useful information that can be used in research publication 34-36.

1.3.3-Energy and fuel applecations

In the field of industry ,GC-MS is used for the analysis of

aromatic solvents, sulphur, impurities in polypropylene, sulphur in menthane, natural gases, 1,3 butadiene, ethylene, gas oil, unleaded gasoline, polyethene, diesel oil, unleaded gasoline, polyethylene, diesel, modified biomass, grafted polymers etc [37].

GC-MS has triggered a new arena of research and taken to new heights of impactful presentation and characterization of compounds by its wide range of applications 38 – 40.

1.4- Essential Oils(EOs)

Essential oil is a highly concentrated hydrophobic liquid of complex mixtures containing volatile aroma compounds and can be extracted from several parts of plant , for example ,leaves, peels, barks, flowers , buds, seeds, and so on which serve as the major source of essential oil41.

1.4.1- History of essential oils

Throughout history, the essential oils of plants were used in many cultures for their medicinal and therapeutic benefits. The Egyptians were renowned for using essential oils extensively in medical practice, beauty treatment, food preparation, and in religious ceremony. Frankincense, sandalwood, myrrh and cinnamon were considered very valuable cargo along the ancient caravan trade routes and were some-times exchanged for gold.

Borrowing from the Egyptians, the Greeks used essential oils in their practices of therapeutic massage and aromatherapy. The Romans also used essential oils to promote health and personal hygiene. Influenced by the Greeks and Romans, as well as Chinese and Indian Ayurvedic use of aromatic herbs, the Persians began to refine distillation methods for extracting essential oils from aromatic plants42.

Essential oil extracts were used throughout the dark ages in Europe for their antibacterial and fragrant properties.

More recently, the powerful healing properties of essential oils were rediscovered in 1937 by a French chemist, Rene Maurice Gattefosse, who plunged his badly burnt hand into a vat of lavender oil (mistaking it for water) and was surprised to see no injury or scarring. A French contemporary, Dr. Jean Valnet, used therapeutic-grade essential oils to successfully treat injured soldiers during World War II. Dr. Valnet went on to become a world leader in the development of aromatherapy practices42.

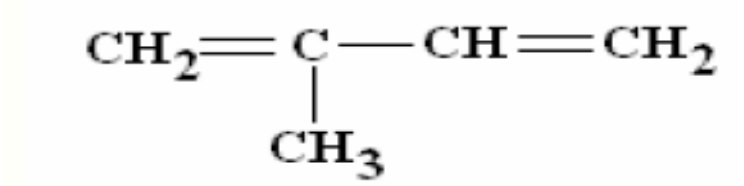
1.4.2-Chemical constituents of essential oils

In general, essential oils can be subdivided into two distinct groups of chemical constituents:

i)Volatile fraction

Hydrocarbons and oxygenated derivatives may constitute up to

90–95% of essential oil weight. The hydrocarbons are the molecules constituted of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bi cyclic, or tricyclic), or aromatic. Basic hydrocarbon found in plants are 90% terpenes made from isoprene units (several 5 carbon basic units, C5). A combinations of 2 isoprene units is called a “terpene unit.” Essential oils consist of mainly (mono-terpenes (have a structure of 10 carbon atoms and at least one double bond. The 10 carbon atoms are derived from two isoprene units.) and sesquiterpene (consisting of 15 carbon atoms) The di terpenes (C20), triterpenes (C30), and tetraterpenes (C40) exist in essential oils at low concentrations. Terpenoids (terpenes containing oxygen) is also found in essential oils43. 5% of oxygenated compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils. Oxygenated derivatives are: aliphatic aldehydes, alcohols, ketones, oxides, phenols and esters.isoprene



(Isoprene)

ii)Nonvolatile residue or fixed oils

Nonvolatiles comprises 1–10% of the oil, containing hydrocarbons, fatty acids, sterols, carotenoids, waxes, and flavonoids44.

1.4.3-Properties of essential oils

In plants the amount of essential oils is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods.

Essential oils are good source of several bioactive compounds which possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils can be improved41.

1.4.4- Pharmacological properties of essential oils

Essential oils have antiseptic properties and are active against a wide range of bacteria. Moreover, they are also known to be active against fungi and yeasts (Candida).The most common sources of essential oils used as antiseptics are: cinnamon, thymol; clover; eucalyptus; culin savory; lavender; citral,

geraniol; linalool. However, thymol and linalool are much more potent than phenol45.

When used externally, essential oils like (L'essence de terebenthine) increase microcirculation and provide a slight local anesthetic action. Till now, essential oils are used in a number of ointments, cream and gels, whereby they are known to be very effective in relieving sprains and other articular pains. Oral administration of essential oils like eucalyptus or pin oils, stimulate ciliated epithelial cells to secrete mucus. On the renal system, these are known to increase vasodilation and in consequence bring about a diuretic effect.

Essential oils from the Umbellifereae family and specially Mentha species and Verbena are reputed to decrease or eliminate gastrointestinal spasms. These essential oils increase secretion of gastric juices. In other cases, they are known to be effective against insomnia44.

1.4.5- Methods of Extracting Essential Oils

The way in which oils are extracted from plants is important because some processes use solvents that can destroy the therapeutic properties.

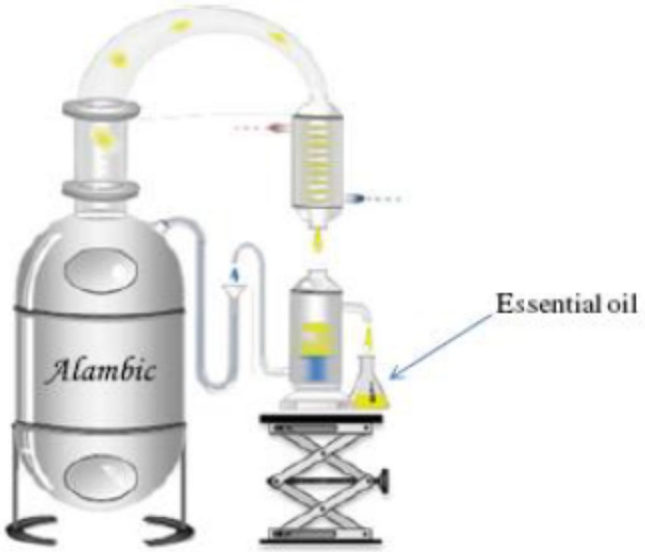
The value of the newer processing methods depends greatly on the experience of the distiller, as well as the intended application of the final product. Some of the extraction

methods are given below:

1.4.5.1-Steam Distillation

Steam distillation is one of ancient methods for isolation of EOs from plant materials. The plant materials are subjected to the steam without maceration in water. The injected steam passes through the plants from the base of the alembic to the top. The vapour laden with essential oils flows through a “swan-neck” column and is then condensed before decantation and collection in a Florentine flask . EOs that are lighter or heavier than water form two immiscible phases and can be easily separated. The principle of this technique is that the combined vapor pressure equals the ambient pressure at about100 °C so that the volatile components with the boiling points ranging from 150 to 300 °C can be evaporated at a temperature close to that of water.

Furthermore, this technique can be also carried out under pressure depending on the EOs’ extraction difficulty



A schematic representation of conventional recovery of essential oils

1.4.5.2- 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. The extracting solvent in flask is heated, and its vapors condense in condenser E. 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, 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 oil can be extracted with a

much smaller quantity of solvent. This affects 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 scale45.

1.4.5.3-Maceration

In this process, the whole or coarsely powdered plant material is placed in a stopper 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 standing45.

1.4.6 -Uses of essential oils

Essential oils have been used for thousands of years in various cultures for medicinal and health purposes. Essential oil uses range from aromatherapy, household cleaning products, personal beauty care and natural medicine treatments. Essential oil benefits come from their antioxidant, antimicrobial and anti-inflammatory properties.

The amount of essential oil from different plants is different and this determines the price of essential oil. Apart from

aromatic compounds, indigenous pigments contribute to varying colors of essential oil. This can affect the applications as the ingredient in some particular foods.

The Essential oils a good source of several bioactive compounds which possess antioxidant and antimicrobial activities, there by serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapor barrier property associated with hydrophobicity in nature of essential oils, can be improved.

Essential oils are used in perfumes, cosmetics, soaps and other products, for flavoring food and drink, and for adding scents to incense and household cleaning products and have been used medicinally in history. Medical applications proposed by those who sell medicinal oils range from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purposes. Claims for the efficacy of medical treatments, and treatment of cancers in particular, are now subject to regulation in most countries[46-48.]

1.5- *Pithecellobium dulce*

Pithecellobium dulce **(Roxb.) Benth**.(**Manila Tamarind**).Synonyms (*Acacia obliquifolia* **Mart. & Gall**.*Inga dulcis* **(Roxb.) Willd.;** *Inga leucantha* **K. Presl** *; Inga pungens*

Humb. & Bonpl. ex Willd. *; Mimosa dulcis* **Roxb**. *; Mimosa monilifera* **Bert.** *; Zygia dulcis* **(Roxb.) Lyons**. *;Albizia dulcis* **(Roxb.) F.Muell.** *;Feuilleea dulcis* **(Roxb.) Kuntz** *; Pithecellobium littorale* **Britton & Rose ex Record**) is a multipurpose tropical fruit tree used primarily for its fruits, which are eaten fresh or processed. and seeds are processed for non-food uses[49].

International Common Names:

- **English:** *blackbead; guayamochil; Madras thorn; poiss Sucre; sweet Inga*
- **Spanish:** *guamúchil; madre de fleche*
- **French:** *pois sucré, tamarin de Manille, tamarin d'Inde.*
- **Sudanese:** *tamarinde.*

Taxonomy

- **Domain:** *Eukaryota.*
- **Kingdom:** *Plantae.*
- **Phylum:** *Spermatophyta.*
- **Subphylum:** *Angiospermae.*
- **Class:** *Dicotyledonae.*

- **Order:** *Fabales.*
- **Family:** *Leguminasae* .
- **Subfamily:** *Mimosoideae.*
- **Genus:** *Pithecellobium.*
- **Species:** *Pithecellobium dulce***50**

Pithecellobium dulce is a small- to medium- sized semi-evergreen tree that grows up to 20m height. Crown is spreading but irregular. Trunk is short, about 1 m high, with crooked branches and somewhat shiny branch lets. Bark is grey and smooth in young trees, turning to slightly rough and furrowed in old trees. Bark exudes reddish-brown gum when injured. Leaves are bipinnately compound with a pair of pinnae, each with two leaflets that are kidney shaped and dark green in colour. Spines are present in pairs at the base of the leaf. New leaf growth and shedding of old leaves occur almost simultaneously, giving the tree an evergreen appearance. Inflorescence is about 10 cm long and 1 cm across, located at the end of the branches with 15 to 20 white flowers in round heads. Each flower is 0.3 to 0.5 cm long with hairy corolla and calyx. Fruit is a pod, 10 to 15 cm long, 1 to 1.5 cm wide, curled up tightly and reddish brown[50]

Pithecellobium dulce

Pithecellobium dulce

fruiting twige

Leaves Flower Seeds

Manila tamarind originated from a large central American area, stretching from southern California to Colombia and Venezuela. It was introduced to Indonesia and the Philippines

by the Portuguese and the Spanish. It was successfully planted in small areas in the south Sahelian and north Sudanese zones.

It is now widespread (planted and naturalized) in tropical regions where it can be found along rivers and roadsides, in dry thickets or forests, from sea level up to an altitude of 1800 m and in areas where annual rainfall ranges from 400 mm to 1500 mm. In Hawaii, it has been declared a weed50.

Manila tamarind grows in a wide range of soils and temperatures (it is nevertheless frost sensitive) and survives dry periods ranging from 3 up to 8 months. It prefers full sunlight but can withstand considerable shade [51].

Manila Tamarind contains: tannin, 25.36%; fixed oil, 18.22%,; olein. Seeds have been reported to contain steroids, saponins, lipids, phospholipids, glycosides, glycolipids and polysaccharides. Bark yields 37% tannins of the catechol type. Leaves yield quercitin, kaempferol, dulcitol and afezilin. Fatty acid analysis of seed extract yielded 9 saturated and 17 unsaturated fatty acids. Elemental composition yielded (mg/kg) arsenic 17.6µg/kg, copper 16.25, cadmium 3.48, iron 1.89, lead 0.19, magnesium 15.06, potassium 26.89, sodium 10.19, zinc 26.89. Total protein content was highest in the seeds (50.3-67.1%), followed by stems, roots, leaves, flowers, and fruits. **(53)** Ethanolic extract of fruits yielded ten compounds viz. (i)

2, 5, 6-trimethyl 1, 3-oxathiane, (ii) *trans*-3-methyl-2-N-propylthiophane, (iii) 2-furan carboxaldehyde-5-(hydroxymethyl), (iv) D-pinitol, (v) heptacosanoic acid, (vi) hexadecanoic acid, (vii) tetracosanol, (viii) 22-tricosenoic acid, (ix) methyl-2-hydroxy icosanoate and (x) stigmasterol. **(54)** Evaluation of seed protein flour showed a protein content of 39.22%, calcium 48 mg, and phosphorus 542 mg/100 g. Major amino acids were glutamic acid, arginine, aspartic acid, lysine,

valine, threonine and leucine. Ratio of essential to nonessential amino acid was 0.61. Total polyphenol content was 294 mg/100g 52-55.

Pithecellobium dulce is a most versatile medicinal plant,it has attracted a worldwide prominence in recent years, owing to its wide range of medicinal properties and diverse utility. All plant parts of the *P. dulce* elaborates a vast array of biologically active compounds and have been demonstrated to exhibit antidiabetic , locomotor, antivenom, free radical scavenging, protease inhibitor, antiinflammatory, antibacterial, antimycobacterial , abortifacient, spermicidal, anticonvulsant, antiulcer, antidiarrheal, antifungal**56.**

The plant is frequently used for bowel movements. The leaves, when applied as plasters is used for pain and venereal sores.

Salted decoction of leaves is a treatment for indigestion, it is also used as abortifacient. The bark is used in dysentery, dermatitis and eye inflammation. In Mexico, decoction of leaves are used for earaches, leprosy, toothaches and larvicide. In India, bark of the plant is used as astringent in dysentery and as febrifuge. Also it is used for dermatitis and eye inflammations [57].

Fruits and astringent and barks are claimed to treat ailments ranging from bronchitis, diarrhea, hemorrhages, sores, liver problems and spleen issues. Manila tamarinds are exceptionally high in vitamin C, which bolsters the immune system[58].

Aim of this study

This study was designed to:

-Extraction of fixed oil from seeds of the medicinally important *Pithecellobium dulce.*

- Analysis of extracted oil by GC-MS.

-Screening the oil for antimicrobial potency