1-Introduction

1.1- *Jateorhiza palmate*

Synonyms: :

Menispermum Palmatum (Lam);*Cocculus palmata* (Lam.) DC.

Menispermum Columba Roxb.; *Jateorhiza Columba* (Roxb.) Oliver;*Jateorhiza miersii* Oliver;*Chasmanthera Columba* (Roxb.) Bail.ex Diels [1]

Calumba root is a herbal medicine used for poor digestion, especially due to low stomach acid, diarrhea, gas, and loss of appetite. [2,3]

Taxanomy:

Kingdom : Plantae

(unranked):Angiosperms

(unranked): Eudicots

Order : Ranunculales

Family:Menispermaceae

Genus : Jateorhiza

Species : *J.palmata*

Colombo is a climbing plant, with a perennial root, formed of a number of fasciculated, fusiform, somewhat branched, fleshy, curved, descending tubers, of the thickness of an infant's arm, covered with a thin, brown epidermis, marked, especially toward the upper part, with transverse warts; internally they are deep yellow, inodorous, very bitter, filled with numerous, parallel, longitudinal fibers, or vessels. The stems, of which 1 or 2 proceed from the same root, are annual, herbaceous, about as thick as the little finger, simple in the male plant, twining, branched in the female, rounded and green; in the full-grown plant, below, they are thickly clothed with succulent, longitudinal hairs, which are tipped with a gland. The leaves are alternate and large; the younger ones thin, pellucid, bright-green, generally3-lobed, and upward gradually more numerous; the older ones remote, a span in breadth, nearly orbicular, deeply cordate, 5 to 7-lobed, the lobes entire, often deflexed, wavy on the surface and margin, dark-green above, paler beneath; hairy on both sides; the nerves, according to the number of lobes, are 3, 7, or 9, pale, connected by veins which, in themselves, are reticulated and are prominent beneath. The petioles are about as long as the leaves, rounded, glanduloso-pilose, and thickened below. The flowers are small, indistinct, arranged in the male plant in solitary, axillary, drooping, compound racemes, covered with glandular hairs, and with small, caducous bracts at the base; in the female they are also axillary, solitary, simple, spreading, but shorter than

those of the male. Sepals 6, glabrous; petals 6, in a single row; stamens 6; anthers terminal and 4-celled. The fruit is drupaceous, or berried, about the size of a hazel-nut, densely clothed with long, spreading hairs, and tipped with a black, oblong gland. The seeds are black, striated transversely, and subreniform . [5,6]

Calumba (also Columbo) root has long been in use under the name "kalumb”among the African tribes of Mozambique, who employed it as a remedy for dysentery and other diseases[7].Undoubtedly the drug was brought to them by the immediate knowledge of the Portuguese when they obtained possession of that country in 1508.[8]

Through the influence of their traders, knowledge of the drug was slowly diffused among the Europeans during the sixteenth and seventeenth centuries. The first definite information regarding Calumba root, however, dates from the year 1671, when Francisu Redi (1626-1697), physician to the Duke of Toscana, describing it under the name calumba made its medicinal virtues conspicuous[9] . In 1695 the celebrated Leeuwenhoek, in his work “Arcana Naturae,” recorded some chemical experiments that he had made with this root, which he calls radix indica, rays columba. He also introduced illustrations of crystals observed in the study of this drug. Contemporaneously with this physicist J.C. Semmendus (probably in 1689 or shortly before) mentioned calumba in his writings as occurring among drugs originating from India. This

author's work has become more prominent in a later edition (l722). [10] Valmont -Bomare in the 1764 edition of his dictionary describes

"calumbe" as the root of an unknown tree brought to us from India. He added that in Bengal this root is considered as specific in cases of colics, indigestion and against the effects of "mart-du-chien" which is the old French name for colchicum.

Not, however, until in close succession the treatises on calumba root by Gaubius (1771) , Cartheuser (1773) and Percival (1773).[11-13]

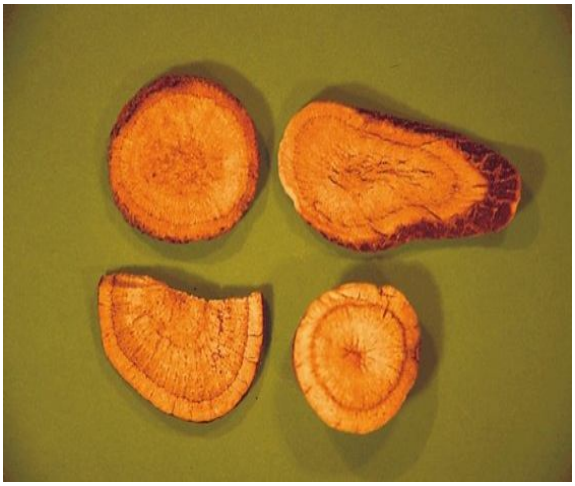
appeared was there much general distribution of knowledge concerning this drug. In this connection it is perhaps of interest to note that in a previous translation (dated 1755) of Cartheuser's Materia Medica

calumba root is not to be found. Through Percival's recommendation especially the drug rapidly gained entrance into European materia medica, and since about 1776 we find a record of it in many of the pharmacopeias of European countries. However, the geographical and botanical origin of calumba root as yet remained a mystery.

The Portuguese, as already stated, having had a monopoly of the trade in this article, seemed to have been careful not to disclose the origin of the drug and made it a custom to carry it to India and then to export it to Europe from Indian instead of African ports. Hence, for a long time the general impression prevailed

that the plant was a native of India and that the capital of Ceylon (Colombo) gave the drug its name. From about 1770, however, the suspicion that calumba root was of African origin had been gaining ground. [14]

The root is friable and readily reduced to a pale greenish-yellow powder, having a faintly aromatic odor, and an unpleasant, bitter taste, without the slightest acrimony or astringency. Alcohol, or boiling water, extracts its virtues. The bark has the strongest taste, which is readily taken up by water, alcohol, or ether. The central pith is almost mucilaginous. The powder soon spoils and becomes unfit for use, in consequence of absorbing moisture from a damp atmosphere. It is better to powder the root in limited portions, when required, keeping the powder in closely-stoppered bottles[14-16] .

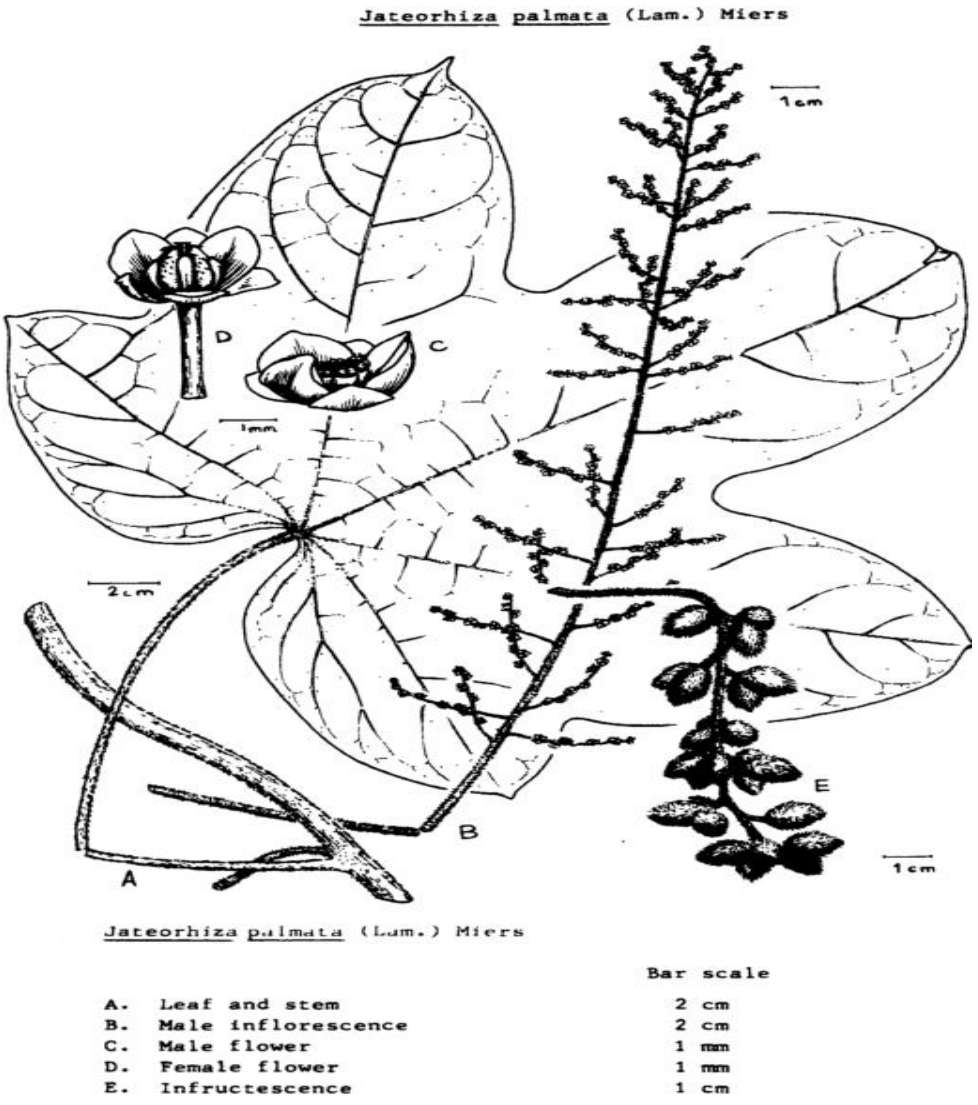


roots

leaf,flowers

Fruits

Rhizomes



male and female flowers

The name of this plant is with Latin and Greek base and implies[17] “root of medicinal virtues”. It has only two species : *Jateorhiza palmate* and *Jateorhiza calumba* all of them are

collectively known as Calumba root, Jateorhiza is native of tropical areas of Southern and Eastern Africa including Malawi. Jateorhiza palmata is named after the Latin “palmata” that refers to its palmately lobed leaves. Basal lobes mostly overlap and the male inflorence is smooth and Jateorhiza Calumba, The main difference from palmata is that the basal lobes of leaves are rounded but do not overlap and the male inflorescence is hispid.[18,19]

The American colombo (*Frasera Walteri*, Michaux., which is sometimes added to the genuine article, contains no starch, and is not, therefore, affected by iodine; but it contains tannic acid, and, therefore, becomes blackish-green when sulphate of iron is added to its decoction, and yields a precipitate with a solution of gelatin. root is said to have been an adulterant also. Both this and American columbo root can be detected by their widely divergent physical characteristics, so different from calumba. A wood, known as *false columbo,* or *columbo wood* (from *Cascinum fenestratum*., also of the Moonseed family, has been received into England from Ceylon.[19]

1.1.1Chemical composition

Information relevant to chemical characterisation of Jateorhiza was reviewed. The root as well as parts of the plant contain chemicals that have been extracted by world researchers, notably in the USA, Japan and Europe. The ARS (Agricultural Research

Services, Beltsville, Maryland, USA) alone have on record 119 distinct chemical activities for Jateorhiza chemical justification indicated by the works of global researchers[20] 2-3% .Alkaloids are stated to be present in calumba root but in more recent Studies on the quatemary alkaloids only 0.8-1.2% crude bases have been isolated . The alkaloids are proto berberines: of the total alkaloids, palmatine comprises between 50 and 96% and jatrorrhizine together with columbamine from 50 to 4%; berberine is absent . Nothing is known about the tertiary bases of the plant. More recently, the quaternary dimeric base-jatrorrhizine, formed from the monomer by ortho oxidative coupling, has been isolated ,it comprises a very small proportion of the alkaloid content of the root[21,22]. Non alkaloidal, diterpenoid bitter substances also occur in calumba root. The principal one is columbin (about 0.22%) and it is accompanied by the related substances chasmanthin and jateorhizin . The proto berberine alkaloids (including berberine) and their salts have antibacterial, as well as antifungal and antiprotozoal, properties; and they are effective in the treatment of cholera. The compounds also have anti-inflammatory and hypotensive activity . A partially purified bitter-substance fraction is reported to have synergistic effect on the antifungal activity of extracts and macerates of calumba root .Also reported to be present in the roots is 0.07-1.5% essential oil. Young roots contain more than old ones. On drying, most of the oil is lost .[23,24]

The root contains bitter matter (Furanoditerpenol, palmanin), yellow resinous extractive, volatile oil (up to 1%-mostly thymol), wax, gum, starch , woody fiber, and water. Wittstock, in 1830, discovered a bitter principle, which be named *colombin*, or *columbin*. If the genuine columbo be first moistened, it becomes black when in contact with tincture of iron; iodine added to a decoction of the root, forms the blue iodide of starch; a decoction of the root does not redden litmus paper, nor is there any precipitate (tannic and gallic acids) when gelatin, or sulphate or perchloride of iron are added to it; infusion of nut-galls, or tannic acid causes a precipitate. *Columbin* ($C_{42}H_{44}O_{14}$) may be obtained by treating columbo root twice or thrice successively with alcohol [18]

1.1.2-Tribal and herbal medicine uses

The root of this tropical plant is used in traditional medicine systems worldwide. It was first recorded in herbal medicine in 1671 when Portuguese traders took the plant from Africa back to Europe. Calumba root has long held a place in herbal medicine as a gentle but very effective digestive bitter. Bitters work on the principal that a bitter taste in the mouth signals the flow of digestive juices and bile to aid or speed up digestion processes. It is especially valuable in convalescence from acute fevers and other disorders in which there is lack of desire for food and poor digestion, with pain or without pain, immediately upon eating."

In Brazilian herbal medicine systems (where calumba is commonly cultivated as a medicinal plant) the root is used for poor digestion, low stomach acid, diarrhea, gas, and loss of appétit The bitter properties of the calumba are attributed to the herb's bitter principles and, to some smaller degree, to the isoquinoline alkaloids present in the herb. These isoquinoline alkaloids invigorate particular taste receptors present on the tongue, which, in turn, encourage the secretion of digestive juices. Calumba is considered to be among the bitterest plants existing and it has several things common with gentian. Nevertheless, calumba's bitterness is owing to a diverse assortment of elements. Dissimilar to several bitter herbs, which provide astringency , this herb has all the times been classified in the form of a ‘pure bitter'.Calumba is effective in preventing infections of the digestive system since it makes the stomach additionally acidic and, thereby, unreceptive to pathogens. This herb also augments the intensity of digestive secretions, in that way enhancing the breakdown as well as assimilation of ingested food by the body. In addition, calumba eases indigestion or dyspepsia, which especially occurs owing to shortage of digestive secretions and also due to diminished levels of stomach acids Calumba has an unadulterated bitter action which makes the herb a very valuable herbal medication for a feeble or under active digestive system as well as for poor appetite. This medication is particularly used to cure loss of appetite as well as

anorexia nervosa. Like in the instance of any other bitter herb, calumba is effective in treating several chronic ailments. Provided this medication is taken on a regular basis prior to meals, if possible in its tincture form, calumba strengthens the digestive system and, at the same time, augments the assimilation of nutrients by the body. Calumba is especially useful in treating chronic fatigue syndrome that is usually related to scarce production of stomach acids. Calumba is an effective remedy for treating dysentery and in East Africa this herb has been traditionally employed for this purpose as well as to force out worms from the body. While calumba ought to be usually avoided by pregnant women, often small doses of this medication have been prescribed to such women with a view to alleviate morning sickness However, this herb does not enclose tannins and, therefore, it may be used in iron preparation without any harm to treat anemia devoid of any apprehension of any precipitation caused by interaction in any artificial environment (in vitro).[25]

The root of calumba is the basis of ‘radix calumbae', a herbal remedy that was highly popular in earlier times and was imported by European countries from Tanzania and Mozambique. This medication was employed to treat diarrhea and dyspepsia, and is particularly appropriate for people having a weak stomach. People in Tanzania consumed the root of this herb to treat snakebites as well as in the form of a vermifuge (a

medication that expels worms). In fact, people belonging to the Zigua tribe in Tanzania continue to employ ‘radix calumbae' to cure ruptures and hernia. The scrapings of calumba roots are applied topically to scarifications or scratches made in abscesses with a view to mature them. The roots of this herb are believed to possess tonic properties by people all over south-eastern Africa and they take it to cure diarrhea and dysentery. People in India, take the roots of calumba in the form of a bitter tonic having anthelmintic and antipyretic attributes to cure gastric irritability as well as vomiting during pregnancy. Researchers in Japan conducted a study in 2002 with rats. They extracted a columbin chemical from calumba root They reported that this compound was able to prevent colon cancer stating: "These results indicate chemopreventive ability of dietary columbin against chemically induced colon tumorigenesis when fed during the initiation phase, providing a scientific basis for chemopreventive ability of columbin against human colon cancer.[26]

1.1.3-Products and usage of calumba root

Information from traditional healers and knowledgeable individuals indicted following products and uses for harvested Calumba root:

•Dried root pieces

•Powder

•Pills/tablets

•Fresh

Nutritional and pharmacological Attributes:

•Support for lost appetite

•Cure for many stomach ailme

•Treatment for snake bite

•Treatment against tumors

•Increasing blood level (an example of congruence with chemical

•Increasing permanent body immunity against diseases

•Mixture with many medicinal plants for cure of many different diseases and ailments.

•Remedy for headaches

•Treatment for colon cancer

•Remedy for chronic sores

•Remedy against nausea

•Increasing ability to bare children for barren couples

•Remedy against coughs

•Treatment for sexually transmitted diseases

•Remedy against cholera

•Remedy for malaria

•Treatment against tuberculosis. [27]

People using this herb or planning to use it ought to be aware of the side effects caused by it and take the necessary precautions to avoid them. Generally, there is no record of any side effect or perils caused by the therapeutic dosages of calumba. However, taking this herb in excessive doses may result in symptoms of

unconsciousness and paralysis. Breathing problems or tightness in your throat or chest ,chest pain ,Skin hives, rash, or itchy or swollen skin may be observed in case of allergy.[26]

1.1.4-Antibacterial activity of *Jateorhiza palmate*

The anti bacterial activity of *C.Fenestratum* is mainly due to the presence of the berberine pharmacological screening of an aqueose methanol(1:1) extract showed convulsant in clinical tests in Vietnam, the extract also showed distinct activity on staphylococcus aureus and streptococcus hemolyticus ,which may cause inflammation and infection especially in women after childbirth. The pharmacological effects of berberine have been fairly well investigated[27].

It has been found active against anumber of Gram-posative as well as Gram- negative bacteria and also against anumber of fungi. It was also effective against experimentally induced intestinal amoebiasis in rats and showed growth inhibition of Ehrlich and lymphoma ascites tumour cells. Beberine is also present in high concentration in other Menispermaceae species eiq ,in Arcangelisia flava which is used for similar complaints as C. Fenestratum.[28]

1.2-Gas chromatography

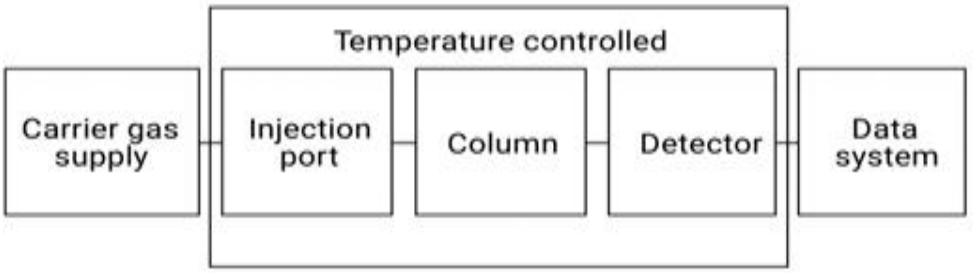
A gas chromatography is chemical analysis instrument used for separating and analyzing compounds of mixture that can be

vaporized without de composition into individual components. In some situations, GC may help in identifying compounds.

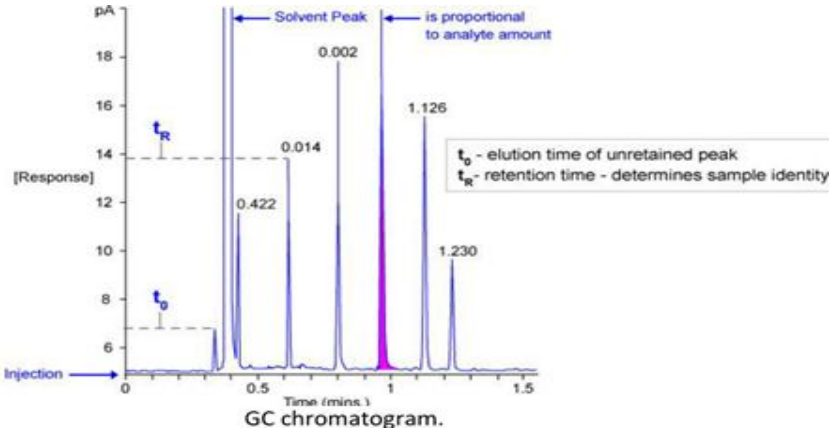
All forms of chromatography Involve the distribution, or partitioning, of a compound between a mobile phase and a stationary phase. In GC, the mobile phase is a gas and the stationary phase is an immobile, high molecular weight liquid which is deposited on or chemically bonded to the inner walls of a long capillary tubing .The term GLC(gas-liquid chromatography) is also used to refer to this separation technique. The capillary tubing through which the sample moves is called the chromatographic or GC column. Presently, most GC columns used for this work are manufactured from fused silica. They are generally 30-60m in length and have an internal diameter of about 0.2mm. by covering the outside surface of this capillary columns with a polymeric coating, these flexible fused silica GC columns are made more durable. The analysis of effluents for organic compounds requires extraction of the organics from the water matrix, concentration of the extract, separation of individual components of the organic extract by a GC column and detection of the separated components as they are eluted from the GC column. Complex mixtures of organic compounds effluents are extracted from effluents by using high-purity organic solvents. The low-volatility organic compounds extracted from an effluent sample can be concentrated to small volume (typically, 1.0ml or less) by removing the extraction

solvent through evaporation. This concentration step is necessary in order to obtain detection limits in the low part-per-billion(ppb:10-6g/l). Some compounds of concern may be more volatile than the extraction solvent and would be lost in this process. Such compounds are removed from the sample by directly purging the aqueous sample using an inert gas and collecting the purged volatile compounds on an adsorbent trap designed for this purpose .In either case, organic compounds from the sample are separated from the bulk aqueous matrix and concentrated for GC analysis. The organic compounds are introduced into the GC column by injecting a few micro liters (μ L) of the concentrated solvent extract into an injection port(non-volatile organics) or by heating the sorbent trap (volatile organics). An inert carrier gas(He,N_2,H_2), is used to sweep the extracted organic compounds, which are now in the vapor state, through the GC column. Compounds that have different solubilities in the liquid phase of the GC column will take different times to traverse the length of the column. For a specific set of experimental conditions, the time it takes a compound to travel through a GC column is a physical property of that compound-called its retention time. Generally higher molecular weight compounds will have greater retention times than lower molecular weight compounds. Also compounds that of the liquid phase will be more soluble in the phase and will have greater retention times than compounds less soluble in the

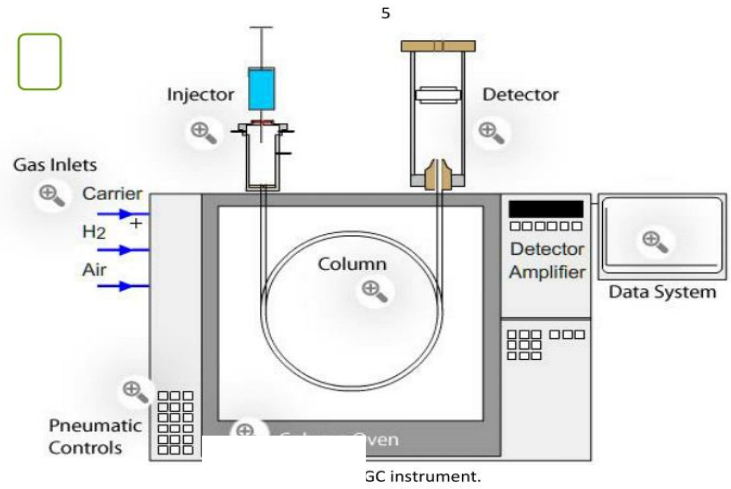
liquid phase. Therefore, organic compounds in a mixture can be separated from each other by using gas chromatography, and the retention times of these compounds can be used to assist in their identification. Some environmental samples are so complex that there are hundreds of compounds present in their concentrated organic extracts. There are currently no GC column available that can completely separate all components of such complex mixtures from each other. However, in most cases the principal sample components can be detected.[29-34]



Block diagram of gas chromatograph.



GC chromatogram.



GC instrument.

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[36].

By using a combination of oven temperature and stationary phase chemistry (polarity) very difficult separations may also be carried out _including separation of chiral and other positional isomers.GC is excellent for quantitative analysis with a range of sensitive and linear detectors to choose[35] .

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 column is around 350-380c. Analyte boiling points rarely exceed 400c in GC analysis and the upper molecular weight is usually around 500Da[37].

Advantages of this technique include:

-fast analysis.

-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.

-Sensitive detectors (ppb)

-High quantitative accuracy (less than 1%RSD typical).

-High efficiency-leading to high resolution.

-Requires small samples(less than 1ml).

-Reliable technique.

-Well established with extensive literature and applications.

Disadvantages of GC includes:

-Limited to volatile samples.

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

-Not suited to preparative chromatography.

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

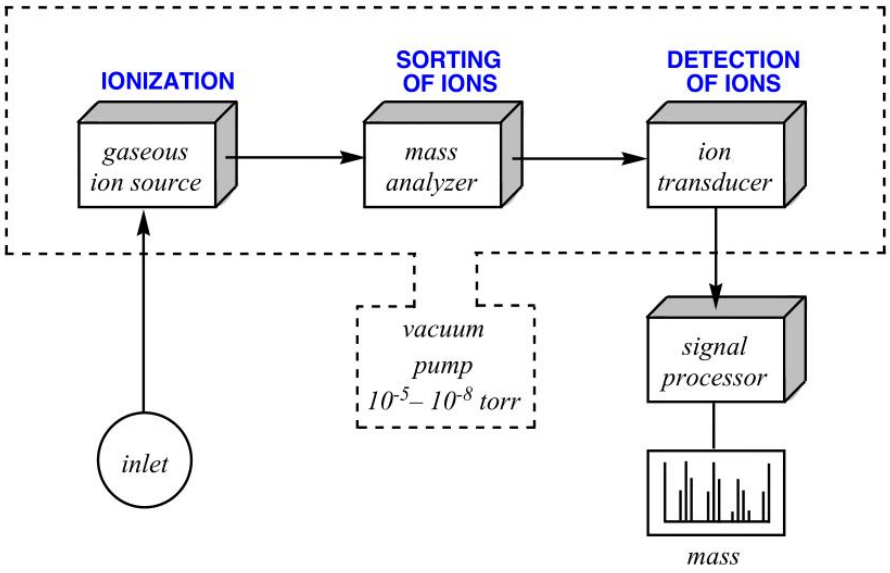
-Most non-MS detectors are destructive.[38]

1-3-Mass spectrometry (MS)

As the separated sample components elute from the GC column, they are monitored using any of a large number of detectors developed for this purpose. The most versatile of these detectors is the mass spectrometer (MS). When an MS detector is used to detect the compounds that elute from a GC column, molecules enter the source chamber of the mass spectrometer maintained under high vacuum, where they are bombarded by electrons. The energy transferred to molecules in this process causes them to ionize and dissociate into various fragment ions. Ions may be singly or multiply-charged. The positive ions formed are made to traverse an analyzer section, maintained at 10-5 to10-7 Torr. After the ions traverse the analyzer section where they are separated according to their mass-to-charge ratio (m/z), they are detected by an extremely sensitive device called an electron multiplier.

By plotting the abundance of ions detected versus their m/z, a mass spectrum is obtained. The mass spectrum of a compound is like a fingerprint that can be used to identify the original organic structure. It consists of a bar graph representation of the m/z of the ions and their abundances normalized to the most abundant ion (base peak).

Several different mass analyzers have been developed. One of the most common designs consists of a square array of four parallel metal rods. By controlling radio-frequency (RP and DC voltages to these rods) an oscillating electric field is generated and this allows ions to be filtered according to their m/z. At a specific setting of voltages, only ions of the desired m/z will have a stable trajectory and will be able to reach the electron multiplier. By changing the applied voltages in a specified manner, the mass spectrum of a compound can be generated as the ions of various m/z are scanned. The entire process is performed in about one second. This design is called a quadrupole mass analyzer.[36]



Schematic diagram of a mass spectrometer

1.3-principle of mass spectrometry

The core principle of MS is to determine the mass-to-charge ratio(m/z) of charged compounds. In principle, any charged (or can be charged) substance, which can be transferred into GC at the same time, can be detected by MS. Major development in recent decades is a great expansion of the molecular weight range of MS and the significant improvement of sensitivity. Meanwhile, the mass spectrometer becomes cheaper and easier to be operated[35].

The positively charged radical cations are then accelerated into an analyzer tube which 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[36].

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 ratio and their hights give the proportions of ions of different masses.[37]

1.3.1-Application of mass spectrometry

Mass spectrometry has both qualitative and quantitative use. 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 fundamental 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.

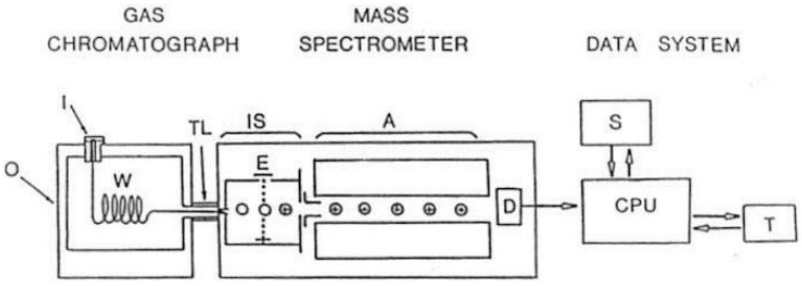
As an analytical techniques it possesses distinct advantage such as: increased sensitivity over most other analytical techniques because the analyzer, as a mass-charge filter, reduce background interference. The techniques has excellent specificity

where characteristic fragmentation patterns can identify unknowns or confirm the presence of suspected compounds. It provides information about molecular weight and isotopic abundance of element[37].

A few of the disadvantage of the method is that often MS fails to distinguish between optical and geometrical isomers and the position of the substitution in o-,m- and p-positions in an aromatic ring .Also, its scope is limited in identifying hydrocarbons that produce similar fragmented ions.[37]

1.4-Gas chromatography -mas spectroscopy

Gas chromatography-mass spectrometry (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.[37]

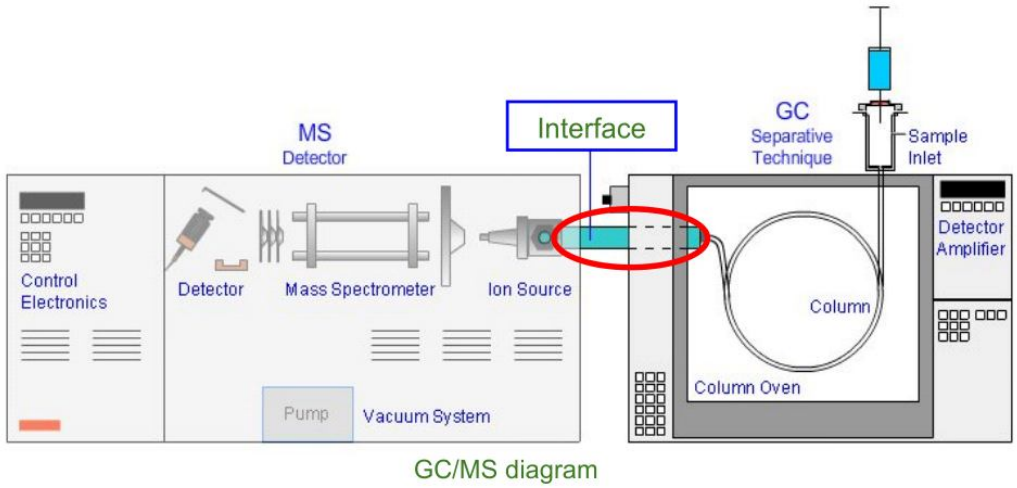


Schematic of a GC-MS System.

(O=Oven, I=Injector, W=WCOT Column, TL=Transfer Line, IS=Ion Source, E=Electron Beam, A=Analyzer, D=Detector, CPU=Central Processing Unit, T=Terminal, S=Data Storage Device)

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. 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 by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column .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[37] .

must be within the pumping capacity of the mass spectrometer



GC/MS diagram

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 injector , the sample is volatilized and the resulting gas is 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 0.10-0.50mm, whilst packed GC columns tend be 1-5 meters in length with either 2 or 4mm internal diameter . Gas chromatography has oven that is temperature programmable, the temperature of the gas chromatographic ovens typically range

from 5cto 400c but can go as low as -25c with cryogenic cooling[38].

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 bio-mass research, pesticides in spinach. analysis of decacyclene, ovalene and even C_{60} degradation analysis of carbamazepine and its metabolites in treated sewage water and steroid can be done without derivatization.

Food and beverage have several aromatic compounds existing naturally in native state or formed while processing. GC-MS is exclusively used for the analysis of esters, fatty acid, alcohols, aldehyde, terpenes, etc. GC-MS is also used to detect and measure contamination, 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 oil, fragrances, menthol, allergens, olive oil, lemon oil,

peppermint oil, straw berry syrup, butter triglycerides residual pesticides in food and wine.[38]

In criminal cases GC-MS can analyze the particles from suspect to correlate his involvement in case also GC-MS is the key tool used in sports anti-doping laboratories to test athletes urine sample 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.

In biological srudies GC-MS is exclusively used in bio- analysis of blood, urine for the presence barbiturates, narcotics, alcohol, residual solvent, drugs like anesthetics anticonvulsant, antihistamine anti epileptic druge, sedative hypnotics, narcotics and food items, fatty acid profiling in microbes, presence of free steroids, blood pollutants, metabolites in serum, organo-chlorinated pesticides in river water, drinking water, soft drinks, pesticides in sunflower oil etc[38].

GC-MS is also involved in security affairs .Explosive detection system 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. 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, soman. 1-4-3Medical and pharmaceutical Application[38].

GC-MS finds many applications in medicine and pharmaceuticals Relative to other metabolomics analysis techniques, gas chromatography mass spectrometry (GC/MS) is one of the earliest applied analysis techniques in metabolomics. The first paper on metabolomics (metabolic profiling) is derived from the application of GC/MS analysis in urine and tissue extracts. With the arrival of omics era and the proposing of metabolomics concept, people began to try using a variety of analytical techniques to obtain metabolomics data. These techniques include chromatography, capillary electrophoresis, mass spectrometry, nuclear magnetic resonance (NMR), infrared spectroscopy, and electro- chemical methods, etc. GC-MS is used in determining metabolic activity by using ^{13}C labeling and the measurement of ^{13}C- ^{12}C ratios with an isotopic ratio mass spectrometer(IRMS) . MS with a detector is designed to measure a few selected ions and retention values as ratios. It is useful to detect oils in cream, ointment, lotion etc. . GC/MS and NMR are the main technologies applied in the early development of metabolomics; in later stage, high-resolution liquid chromatography mass spectrometry (LC-MS) with fast scanning capability is widely used in metabolomics analysis; in recent years, people began trying to integrate a variety of analytical techniques in order to bring into play the advantage of a variety

of methods and to make up for the lack of a single analysis. However, there is no one technology that can perform quantitative and qualitative analysis for all the endogenous metabolites in the biological sample. GC/MS is the most mature chromatography mass spectrometry coupling technology, suitable for the analysis of metabolites with low polarity, low boiling point, or volatile after being derivatized. GC/MS has been one of the main analytical platforms in plant metabolomics due to the high resolution, high sensitivity, good reproducibility, a large number of standard metabolite spectra libraries, and the relative low cost[38].

GC-MS is widely used in pharmaceutical industries for analytical research to determine the active pharmaceutical ingredients(API). It is an integral part research associated with medicinal chemistry(synthesis and characterization of compounds) pharmaceutical analysis(stability testing, impurity, profiling), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology etc. GC-MS is also employed in clinical toxicology, where enhanced molecule ions, extended range of compounds amenable for analysis, superior sensitivity for compounds and faster analysis and the main attractive feature of the clinical toxicology. Toxins and venoms are identify by GC-MS which is extensively used in clinical toxicology[37].

1.3-Essential oils(Eos)

Essential oils are a mixture of volatile lipophilic (fat loving, i.e. ,soluble in fat) constituents, most commonly sourced from leaf, twig, wood pulp or bark tissue of higher plants, but also widely found in bryophytes, such as the liverworts . Although essential oils are only slightly soluble in water, the aqueous solubility of individual essential oil components varies with respect to polarity (magnetic activity). Generally,components with more polar functional groups are expected to be more soluble in water relative to other components.[38]

Aim of this study

This study was designed to :

- Extraction of fixed oil from seeds of the medicinally important *Jateorhiza palmate*

-Analysis of extracted oil by GC-MS.

-Screening the oil for antimicrobial potency.

1.5.1-History of essential oils

Essential oils are the oldest form of medicine known to man.

Essential oils are subtle, aromatic liquids extracted from the flowers, seeds, leaves, stems, bark and/or roots of trees, herbs, bushes & shrubbery through distillation. In the craft of alchemy, the soul of a plant is its oil, while its spirit is the plant's alcohol or tincture. According to ancient Egyptian hieroglyphics and Chinese manuscripts, priests and alchemists used essential oils to heal the sick. Essential oils were considered more valuable than gold in many ancient cultures. And, like gold, the desire for essential oils is strong when it comes to discerning individuals who want an organic, simple, strong, reliable alternative to synthetic medicines that often produce unhealthy side effects.

In the ancient egyptia, believed the sense of smell was the most important of all sensory abilities, more so than sight. They knew inhaling aromas amplified intrinsic "frequency" & transformation. The utchat eye pendant, shown here, found on the mummy of Tutankhamen, depicts the alchemy of spiritual nourishment both in the afterlife & in the created world. An ancient egyptian scroll, carbon dated 1500 B.C., reveals hundreds of Egyptian remedies using essential oils. When King Tut's tomb was opened in 1922, 50 alabaster mason jars for keeping oils were present. The invaluable oils were stolen long ago, by thieves who valued the oil more than the gold, which remained present in the tomb.

Also the Bible, refers to essential or anointing oils over 150 times. The word "anoint" comes from the Latin word inunctus and it means"to smear with oil", to make a person sacred, to set them apart & to dedicate them to serve a higher spiritual purpose. The Hebrew form of Messiah and the Greek form of Christ literally mean "anointed." The bible tells us a plague was stopped in part by preparing a sacred temple with aromatic oils. The New Testament shares gifts offered by the 3 wise men, and how baby Jesus received "gold", frankincense and myrrh. Some historians believe the "gold" refers to a valuable "liquid gold" known as balsam oil (Mt.2:1). The oil Mary used to anoint both feet of Jesus was equivalent in value to one year's wages (Jn.12:3).51

in Chinese, culture used essential oils for healing as far back as 3000 B.C., as proven by the discovery of Shen Nong Shi's herbal book, the oldest surviving medical book in China. Shen Nong Shi (above), is the father of agriculture who invented herbal medicine & he was the 1st Chinese herbal doctor. The ancient pages of his book contain over 350 herbs and their medicinal uses, including the application of essential oils. The Arabians, amassed a thriving frankincense trade in 500 B.C. where supply & demand was so high the trade route became famously known as "Frankincense Trail." In the marketplaces of modern Arabia, baskets still overflow with aromatic herbs, spices & oils. These items once came to Arabia atop camel caravans, requiring many months of transport under sheavy guard. The people of Arabia

utilize these fragrant treasures today, in much the same way as they did centuries ago. For generations, mothers and grandmothers have & continue to share & employ the rich heritage of natural healing remedies, known today as a unique form of Middle Eastern aromatherapy. 52

In the Greek & Roman, cultures adopted their healing methods from the Egyptians. One of the most well-known physicians of all time, Hippocrates, was a firm believer in treating his patients holistically. If you traveled back in time between 460 B.C. & 377 B.C. you would see him administering essential oils via aromatherapy & massage. Greece was known for its bath houses. Many used essential oils to create healing waters. The Ancient Romans

were great believers in hygiene to promote health & they always placed great stock in aromatherapy, the power of fragrances.

1-5-2Chemical 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%essential oil weight. The hydrocarbons are the molecule constitute of H and C atoms arranged in chains. These hydrocarbons may be cyclic, ali cyclic(monocyclic, bi cyclic, or tri cyclic), or aromatic Basic hydrocarbons found basic unit, C5). A combination of 2 isoprene units is called" terpene unit".

Essential oil 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 diterpenes(C20), tri terpenes(C30), and tetra terpenes(C40) exist in essential oils at low concentrations. 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, alcohol, ketones, oxides, phenols and esters.

(ii)Non volatile residue or fixed oils:

Non volatile comprises 1-10% of the oil, containing hydrocarbons, fatty acid, sterols, carotenoids, waxes, and flavonoids.53

1-5-3Properties of essential oils:

Until now, most studies indicated that anti-mutagenic properties may be due to inhibition of penetration of the mutagens into the cells inactivation of the mutagens by direct scavenging, antioxidant capture of radicals produced by a mutagen or activation of cell antioxidant enzymes inhibition of metabolic conversion by P450 of promutagens into mutagens or activation of enzymatic detoxification of mutagens for instance by plant extracts. Less known is a possible antimutagenic interference with DNA repair systems after induction of genotoxic lesions.

Some antimutagenic agents can either inhibit error-prone DNA repair or promote error-free DNA repair .

In plant 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, particular water vapor barrier property associated with hydrophobicity in nature of essential oils can be improved .52

1-5-3-1Pharmacological 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 antiseptic are: cinnamon, thymol, clover, eucalyptus, culin savory ,lavender ,citral, geraniol, linalool. However, thymol and linalool are much more potent than phenol.

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 there 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 Menth 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 insomnia .54,55

1-5-4Specificity of essential oils

The question of specificity of the different essential oils also arises. Very few studies have analyzed enough essential oils and biological endpoints to determine whether there is a specificity for different effects according to different oils or not. Clearly, it has been shown that the tested essential oils presented a specificity in the amplitude, but not in the mode of action, of the biological effects, i.e. cytotoxicity, cytoplasmic mutant induction, gene induction and antigen toxic effects. However, they did exhibit a specificity of the mode of action concerning production of ROS, probably due to differences in their actual composition corresponding to differences in compartmentation of the oxidative stress Concerning antigen toxicity, the tested essential oils showed the same protective activity. However, the

mode of protection differed, not according to the type of oil, but according to the mutagens, i.e. to the type of lesions induced and thus, to the type of their enzymatic recognition and processing leading to translational synthesis or late apoptosis/necrosis.56

1-5-5Method 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. In commercial practice volatile oils are removed from plant material by various methods, depending on the quantity of oil present, its commercial value, the stability of its aromatic constituents, and other factors. The tendency of some Constituents to undergo" changes when subjected to high temperatures calls for special methods of extraction whereby the final product is obtained without decomposition. Three methods are now used commercially:

(1)Extraction by expression;

(2)extraction by solution;

(3)extraction by steam distillation.

EXTRACTION BY EXPRESSION

A small group of volatile oils, of which orange, lemon, and bergamot oils are the most important, may be obtained by expression. The oils, present in the cells of the rind of the fruit,

are obtained by crushing or rupturing the cells and removing The oil by some suitable method. In southern Europe, where labor is plentiful, the oils were long obtained by absorption with small sponges. The fruit was cut in half, the peel was removed from the half section, turned inside out, thus rupturing

Most of the oil cells, and the oil was caught on and soaked up by a sponge held in The hand. Gradually machines that removed the oil from the peel with sponges Were designed, but it was still largely a hand process. The oil obtained by hand or Machine sponging is of high quality. Later, devices were introduced for handling The whole fruit mechanically for the production of the oil and the juice.

EXTRACTION BY SOLUTION

Extraction by solution involves the use of a substance That either dissolves or absorbs the aromatic constituents and the removal of

The constituents from it by further treatment. Three modifications of this method Are in use: (1) Extraction with cold solid fats;

(2) extraction with hot liquid fats;

(3) extraction with volatile solvents.

EXTRACTION WITH COLD SOLID FATS

The use of solid fats at ordinary temperatures for the extraction of perfume from flowers by absorption is know^ as the effleurage process. It has been replaced To some extent by the

more modern solvent-extraction method, but it is still used for the flowers of tuberose and jasmine. These flowers continue to produce valuable perfume for some time after they are picked and their oils are unfavorably affected by even moderate heat. The enfleurage process requires so Much hand labor that its use is necessarily limited to the production of flower oils of high market value. The equipment needed is very simple. It consists of many small wooden frames, each several inches high, about 16 inches wide, and slightly57

longer. A glass plate is fitted in the frame, and a layer of specially prepared fat is applied on both sides of the plate to absorb the odor from the flowers. This fat must be of the proper consistency, practically odorless, and of such composition that it will remain nonrancid for a long time under the conditions of use. A mixture of one part of purified tallow and two parts of lard has been widely used. When these frames are stacked one upon another, they provide many small, practically airtight compartments with a layer of fat on both the bottom and the top of the glass plate. The flowers, carefully cleaned and free from external moisture, are spread on the bottom layers of fat. The odorous constituents are absorbed by the fat on both top and bottom plate surfaces. After about 24 hours the flowers will have yielded most of their oil. They are carefully removed and a new charge of flowers is introduced. This procedure is continued throughout the harvesting period, after which the fat, saturated

with the flower oils, is removed. This product is known as pomade ; its value depends on the kind of flowers used and the degree of saturation. Although such pomades may be used directly in the preparation of perfumes, the general practice is to extract the oils from the fat with high-proof alcohol in special containers in a series of manipulations that assure complete extraction of the oils and subsequent removal of any extracted fat by refrigeration. The "pomade extracts," which represeut the true perfume of the flowers, may go into the trade as such or may be further processed by removal of the alcohol in a vacuum still at low temperature. The floral "absolutes" thus obtained may be purified by various means if the market value of the resulting product is high enough to justify the additional costs. The flowers removed from the fat in the enfleurage process retain some odorous constituents that are not sufficiently
volatile to be released from the flowers and absorbed by the fat. They can be dissolved with a suitable solvent to yield a useful product quite different from the pomade extracts and floral absolutes and of lower market value.

EXTRACTION WITH HOT LIQUID FATS

At one time widely used for extracting the oils from flowers other than tuberose And jasmine, extraction with hot liquid fats is now seldom employed commercially. It is cumbersome and the products obtained do not represent The true perfume of the

flowers. The flowers are immersed in a specially prepared fat. The mixture is heated To about 80° C. for about half an hour and then allowed to cool for an hour. It is finally reheated and then strained or filtered to remove the flowers. The

Proportion by weight of flowers to fat is about 1 to 4. New charges of flowers Are introduced until the total weight of flowers immersed and macerated is About twice the weight of the fat solvent used. The perfume-saturated fat Is sold as such, or an extract may be made from it with strong alcohol.

3.EXTRACTION WITH VOLATILE SOLVENTS

The volatile-solvent method is used to extract oil from flowers only. Other Parts of the plants would yield to the solvents large quantities of matter that Could not be removed from the oil and that would detract from the delicate Odor desired. This method involves the use of a process known as continuous extraction. After passing through the material, the solvent is volatilized, condensed, and again passed through the material. The process is repeated Until the entire charge is exhausted. At each passage of the solvent a portion Of the volatile oil is dissolved and passes off with the solvent. The advantage Of the method lies in the fact that only a relatively small quantity of he solvent

Has to be finally removed from the extracted oil. It requires special equipment, Well designed and constructed of good materials. Because of the delicate nature Of the floral products extracted and the hazards involved in the use of highly

flammable solvents, only skilled personnel can successfully operate such equipment. Equipment of one type in commercial use consists of a cylindrical, Upright extractor of copper or tinned sheet metal of about 300-gallon capacity, Usually arranged in batteries of three, with the concentrator, condenser, and

Solvent storage tank. Each extractor holds about 350 pounds of flowers; approximately 50 gallons of solvent is required for extracting 100 pounds Of the flowers. The extraction is carried out by subjecting each batch of flowers To at least three washings with the solvent. To begin the operation, the flowers In the first extractor are washed with a batch of solvent, which is then transferred Directly to the concentrator. The flowers are given a second washing with a new Batch of solvent, which is then pumped to the second extractor for the first wash And thence to the concentrator. A third batch of solvent used for the third washing of the flowers in the first extractor is used for the second washing in the second Extractor and then as the first washing of a third batch of flowers in the third extractor, from which it goes to the concentrator. Similarly, a new batch of solvent is first used for the third washing in the second extractor, then for a second Washing in the third extractor, on to a fourth batch of flowers as a first wash, and

Then to the concentrator. Thus the process continues with a minimum use of solvent. After the third washing has been

drained off the adhering solvent is removed by blowing a current of steam through the flowers and into the condenser For recovery of the solvent. The flowers are then discarded. The several washings Are usually concentrated in the still or evaporator by blowing steam into a jacket Beneath it until most of the Solvent is removed and recovered. The desired concentration is usually reached When the temperature in the evaporator is about 60° C. The extract is then transferred to a smaller vacuum still in which the rest of the solvent is completely Removed under greatly reduced pressure. This operation requires skill and care so That all traces of the solvent are removed without damage to the delicate floral product. The rotary extractor is also widely used. It consists of a heavily tinned Iron cylindrical drum, revolving on a horizontal axle. Inside are four perforated, cylindrical, horizontal compartments, which are charged with the flowers through openings at the ends. Enough solvent is placed in the drum to fill it about halfway up to the axle level. As the drum is slowly rotated the flowers are dipped into the solvent, which passes into the compartments through the perforations. As the compartments rise above the solvent level the solvent drains back. This continues with each revolution of the drum. When the extraction is complete the extract is draine off and steam is passed into the drum to distill off the solvent adhering to the flowers. The extract is then concentrated in the same way as that obtained with equipment of the other type. The rotary extractor

has several advantages. The solvent is more effective in extracting the odorous constituents than in the stationary extractors and the yield is therefore greater; much less solvent is required; the solvent loss is lower; the equipment requires less floor space and is less expensive The concentrated extracts as obtained by either method, when cooled, are usually solids, owing to the plant waxes and other constituents present. They are known as flower concretes. As they contain all the odoriferous principals, they represent the true fragrance of the flowers. The concretes may Be used as such in the manufacture of perfumes, or they may be further processed By dissolving the odoriferous constituents in 95 percent alcohol, thus removing

The insoluble waxes. The alcohol extract is concentrated in a vacuum still with great care to obtain the final product, known in the trade as flower-oil absolutes. The nature and quality of the solvent used are of the greatest importance. Most Satisfactory for the most expensive flowers is a high-grade petroleum ether, obtained by careful fractionation of gasoline. It volatilizes completely below 75° C, leaving no perceptible residue or odor. Benzene, highly purified so that It will

Completely volatilize at about 80°, may be used for extracting less valuable plant Materials if the presence of some coloring matter extracted by it is not objectionable. The foregoing discussion of solvent-extraction methods and equipment is intended only to provide a general understanding of the subject.

The detailed information necessary to make use of such methods even on a small Scale and to select the proper equipment must be obtained from experienced operators of the methods or from illustrated publications on the subject.

EXTRACTION BY STEAM DISTILLATION

The simplest and most economical method of removing volatile oils

From plant material is by distillation with a current of steam. This method Cannot be used for flowers having odors that are unfavorably affected By the action of steam. Most volatile oils, however, can be distilled by Steam without serious decomposition. The chief advantages of the method Are its simplicity, the comparatively brief time required for its operation, And the fact that large quantities of material can be handled at a small cost. It is the only method economically possible for the extraction of the great number Of volatile oils of only nominal value, for which the more tedious processes Would be impracticable. The steam-distillation method is based on the facts that Volatile oils are vaporized when the material containing them is subjected to a Current of steam and that, when the mixture of oil and water vapors is condensed, The oil separates as a liquid in a layer that may be readily removed from the water.

To accomplish this result it is necessary to supply a tub or retort in which the Plant material may be subjected to the action of

steam obtained from any convenient source, a suitable condenser for condensing the mixture of vapors, And a receiver in which the condensed water and oil may be collected.

the principle of this technique is that the combined vapor pressure equals the ambient pressure at about 100C so that the volatile components with the boiling points ranging from 150 to 300C can be evaporated at a temperature close to that of water.58

1-5-6uses of essential oils:

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

The amount of essential oils 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 bio active compounds which possess antioxidant and antimicrobial activities, there by severing 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, particular water vapor barrier

property associated with hydrophobicity in natural 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 rang from skin treatments to remedies for cancer and often are based solely on historical accounts of use of essential oils for these purpose. Claims for the efficacy of medical treatments, and treatment of cancers in particular, are now subject to regulation in most countries. 59

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