Sudan University of Science and Technology

College of Postgraduate Studies

GC-MS Analysis And Antimicrobial Potency of Five Sudanese Medicinal Plants

تحليل الكروموتوغرافيا الغازية- طيف الكتلة وفعالية مضاد الميكروبات لخمسة نباتات طبية سودانية

A Thesis Submitted in Fulfillment for the Requirements of the Ph.D. Degree in Chemistry

By

Abdel Rahman Ibrahim Hassan Adam

(M.Sc.Chemistry)

Supervisor

Prof: Mohamed Abdel Karim Mohamed

August,2022

الاستهلال

بسم الله الرحمن الرحيم

وَقُلِ ٱعۡمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمۡ وَرَسُولُهُۥ وَٱلۡمُؤۡمِنُونَۖ وَسَتُرَدُّونَ إِلَىٰ عَٰلِمِ ٱلۡغَيۡبِ وَٱلشَّهَٰدَةِ فَيُنَبِّئُكُم بِمَا كُنتُمۡ تَعۡمَلُونَ ١٠٥

(التوبة- 105)

صدق الله العظيم

Dedication

To

my parents

wife

brothers and sisters

\

Acknowledgment

First of all, I would like to thank Almighty Allah for giving me the ability and strength to accomplish this work.

Iwould like to express my gratitude and respect to my supervisor Prof. Mohamed Abdel Karim for his interest, close supervision and continuous advice.

Thanks for the staff, Dept of chemistry, Sudan University of Science and Technology for all facilities.

Also thanks are extended to the technical staff of the Dept of Taxanomy, Medicinal and Aromatic plants Research Institute, Khartoum, Sudan for their kind help.

Deep thanks to my family for their infinite support.

Iwould spcially like to thank all the staff of Elsalam university for their infinite assistant.

Abstract

In this study, the oil from five potential medidinal plants have been extracted by maceration with n-hexane. The constituents of the oil have been characterized by the hyphenated technique: GC-MS.Furthermore, the antimicrobial activity of the oils has been evaluated using the agar diffusion bioassay. GC-MS analysis of *Cassia sieberiana, Kigelia africana* seed oil both of them dominated by 9,12-octadecadienoic acid(Z,Z)-, methyl ester and 9-octadcenoic acid (Z)-, methyl ester respectively, but 9,12,15-octadecatrienoic acid methyl ester only dominated in *kigelia Africana*. Also 9,12-octadecadienoic acid(Z,Z)-, methyl ester and 9-octadcenoic acid (Z)-, methyl ester appeared as a major component in *Acacia seyal, Ziziphus spina Christi, and Vitex doniana* in different percentage.The target oils were evaluated for their antimicrobial potential using the agar diffusion bioassay. The oils gave different antimicrobial responses.

المستخلص

في هذا البحث تمت دراسة خمسة نباتات طبية تنمو في السودان. تم استخلاص الزيوت من هذه النباتات عن طريق النقع باستخدام مذيب الهكسان العادي. تم تحديد مكونات هذه الزيوت بتقنية الكروموتوغرافيا الغازية- طيف الكتلة.ايضا اخضعت هذه الزيوت لاختبار مضاد الميكروبات. اوضح تحليل زيت نبات ام كشو ونبات الدمبلويا وجود احماض 9-اوكتاديكنويك و9,12 اوكتاديكنويك كمكونات اساسية اما حمض 9,12,15-اوكتاديكاترايونويك فقد ظهر فقط كمكون اساسي في زيت نبات الدمبلويا. كما ظهرت احماض 9- اوكتاديكنويك و9,12 اوكتاديكنويك كمكونات اساسية في زيت نباتات الطلح والسدر وام دقلقل بنسب متفاوتة. اجري اختبار مضاد الميكروبات للزيوت قيد الدراسة فاعطت اقطار منع متفاوتة.

Table of Contents

List of Tables

List of Figures

CHAPTER ONE
INTRODUCTION

Introduction

1.1 Essential oils

The use of plants as natural products for remedial, religious and esthetic purposes has a history refer to the appearance of humanity. Natural products researches became a modern idea to produce new chemical bioactive compounds which play main factor in preventing diseases[1]. It is known that plants produce these phytochemicals to protect themselves from pathogen and damages and contribute to the plants color, aroma and flavor[2]. Essential oils can be seen an important group of plants secondary metabolites which are utilized as food flavoring, cosmetics, perfumes and cure of illness[3,4]. Amore specific definition is proposed by international organization for standardization (ISO) which states " extract procured from raw material of natural origin i.e. by steam distillation, by processes that involve mechanical extraction from the epicarp of citrus, fruits or by physical extraction such as dry distillation following elution of the aqueous phase post extraction physical analysis provided no changes in its composition takes place[5,6].The term essential oils is also defined as a concentrated hydrophobic oily volatile liquids characterized by strong odor and produced by different plants parts such as flowers, peels, rhizomes, buds, seeds, leaves, twigs, barks, woods, fruits, roots and throughout the body of the plants[7,8].The essential oils are named due to mother plants from where they are isolated and the odor also resembles the organ from they are extracted[5].The name essential oils refer to their contents of the essence of the plants material, some of them obtained by animals and micro organisms[9]. During the ripening of plants the composition of essential oils alters, in young plants they contain mainly terpenic hydrocarbons and simpler molecules, while the reproductive organ contain etheric oils richer in oxygenated compounds. Although there are a large amounts of essential oils that physically and chemically characterized, about 150 of which on industrial scale[10,11] .

Essential oils are rarely colored and soluble in non polar or weakly polar organic solvants and of lower of density than water, with very few exception[12]. They may be oxidizable by light, heat, or air which change to the dark color, they need to be stored in a cool dry place in amber glass container[13,14].

The quality and quantity of essential oils relies on the climate, the soil tybe, the age and vegetable cycle stage, the biosynthesis method, chemotybes, as well as the plant organ[15]. An estimated 3000 essential oil, from about 2000 plants, are of great value and are utilized in a very large diversity of fields[15,16] .All plants possess principally the ability to produce volatile compounds as secondary metabolites, they are collected in non distinct cells or on the other hand in secretary organs for example glandular hairs, secretary ducts or in cavities. In minor cases, essential oils are not produced in the plants itself; however it is created through hydrolysis of a few compounds produced in the plant similar to the case in garlic or valeriana[17,18,19] .The biological function of essential oils create intensive scientific research and also lead to diverse industries due to their importance as active pharmaceutical compounds or natural preservatives[20]. Though the value of essential oils utilization is recurs to the source, quality, extraction technique. Essential oils show a modern application in the preparation of fragrance, beautifying agents, soaps,shampoos or cleaning gels. Another benefits of these oils is their usage as medicines or as carriers for drug delivery.The major utilization of essential oils is in the agro food business, both for refreshments and properties of food items. The usage of essential oils in the beauty care products, detergents, soaps and scent industry is of great concern from a financial point of view. The application of essential oils as perfumes and scents has extended on international level, because of the sufficient determination of the plants material and a suitable scientific methods employed for the extraction, these are the main reason for enhancing the nature of the volatile oils. In the case of

pharmaceutic and therapeutics, essential oils are used as pharmaceutics for their ability as therapeutic agents[21,22] .The bioactive properties demonstrates generally as antibacterial agents and antimicrobial activities against gram positive microbes[23].Essential oils are also utilized to improve sensory properties of drugs where the prime applying in pharmaceutics is aromatherapy. Moreover they can be used as balms copresses and creams, although oral utilization of essential oils of customized technique has been shown as a successful strategic for getting the assistive effects of these essence [24]. Essential oils are used as food products for pattern, confectionary sodas, and alcoholic drinks and also a part of agriculture and food industry for their antimicrobial, antiviral, antifungal, insecticidal, nematocidal and anticancer attributes[25,26] . Thereby they used as preservatives in food and as well as antibacterial and antioxidant activities[27,28,29] . Essential oils application as additives in food demand a detailed information of their properties including the inhibition of the microorganisms on target, due the particular antibacterial impact with food components[30].Food additives conserve the food storage life, while guarantee its quality and safety, in this way the definition of preservative can be stated by as a compounds that maintain or elongate the storage life of food products .as mention above the property of oils as food preservatives due to their versatile biological activities[31,32]. Plants mainly produce volatile oils and fixed oils, and there are some major difference between the two kinds of oils[33,34]….

.

Table (1.1) a comparison between volatile oil and fixed oil

Volatil oil	Fixed oil
Also called an essential oil	Also called as natural non volatile oil
Volatile oil can evaporate when placed under room temperature	Fixed oil do not evaporate at room temperature
They can be extracted by the distillation process	They require some specific techniques for extraction
There is no spot(no permanent stain) after evaporation	Some type of spot (permanent stain) left after evaporation
They are unable to undergo saponification	Fixed oil can be easily saponified
Mixtures of cleoptenes and stearoptenes are termed as volatile oils	Esters of higher fatty acids and glycerin are called as fixed oils
Posses high refractive index	Posses low refractive index
These are optically active	These are optically inactive
Their primary source is leaves, roots, in petals and bark	Their major source is seeds of the plant

1.2-Sources of natural essential oils

Essential oils are generally derived from one or more plant parts, such as flowers (e.g. rose, jasmine, carnation, clove, mimosa, rosemary, la vander), leaves (e.g. mint, ocimum spp., lemongrass, jamrosa), leaves and stems (e.g. geranium, patchouli, petitgrain, verbena, cinnamon), b ark (e.g. cinnamon, cassia, canella), wood (e.g. cedar, sandal, pine), r oots (e.g. angelica, sassafras, vetiver, saussurea, valerian), seeds (e.g f ennel, coriander, caraway, dill, nutmeg), fruits (bergamot, orange, lem on, juniper), rhizomes (e.g. ginger, calamus, curcuma, orris) and gum s or oleoresin exudations(e.g.balsam of peru, balsam of tolu, storax, m yrrh, benzoin).[35]

1.3-Chemical composition of essential oils

Essential oils are a mixture of volatile constitueunts produced by the s econdary metabolism of aromatic and other variety of plants.Compon ents found in essential oils generally contain volatile terpenes and hyd rocarbons[36,37].Every oil normally has a number of components depend ing on the oil under investigation. However, the most important active compounds are included in two chemical groups: terpenoids (monot erpenoids, and sesquiterpenoids and phenylpropanoids. These two gro ups originate from different precursors of the primary metabolism and are synthesized through separate metabolic pathways. Like all organi c compounds, essential oils are made up of hydrocarbon molecules an d can further be classified as terpenes, monoterpene, sesquiterpene an d diterpene[38,39].Other components of essential oils which include oxy genated compounds, phenols, alcohols, aldehyde, ketones, esters, la ctone, cumarines lactones, ethers and oxides[40].

1.3.1-Terpenoids

Terpene and terpenoids are the primary constituents of essential oils o f many species of plants and flowers[41].Within terpenoids the most im portant components of essential oils of the majortyof plants are prese nted in the monoterpenoid and sesquiterpenoids family[42].

Terpenes are important class of natural product secondary metabolites , comprise of five carbon isoprene units linked together in a head to ta il configuration, but can be constructed in other different types of stru cture with a degree of unsaturation, oxidation, reduction functional groups And ring closure, these hydrocarbons are termed as terpenoids

, which are occure biosynthetically in higher plants.They can also be found in insects and marine organisms. The name terpene refer to the word *turpentine*, a product of coniferous oleoresins.

The terpenes or terpenoids are classified or grouped according to the numb-er of isoprene units found in parent nucleus. Chemically isopre ne named as *2- methylbuta- 1,3-diene* and in industry used for the ma nufacture of Rubber.Thereby the simple class of terpenoids is hemiter penes consisting of single isoprene unit, their occurance is rare and is not biologically significant. Monoterpenes consist of two isoprene un its that can be built in a cyclic, monocyclic, and bicyclic forms and in different oxidation case. Sesquiterpenes are a class formed of thre e isoprene units, occurring in simple a cyclic to macro monocyclic rings as well as simple and complex bicyclic and tricyclic configurati on, though the structural variety of these class is due to the number of carbon skeleton. Diterpenes contain four isop-rene units. Their structu ral diversity ranges from simple a cyclic to compl-ix polycyclic rings. Triterpenes are consis of six isoprene units, and they numbering mo re than 4000 distributed in more than 40 different carbon sk-eletons, arising from the cyclization of an oxidized form of squalene the linea r parent triterpene and carotene.

The C_5 isoprene unit which can be linked " head to tail" to form linear chai-n or cyclized to form rings is regarded the building blocks of ter penes. Chemical and biological studies have revealed that the terpenoi ds posses a variety chemical, physical and biological activities. Biolo gically, the terpe-noids are most useful as anticancer, antimicrobial, c

ytotoxic and anti-inflammatory and analgesic activities[43].Terpenes are synthesized in the cytopl-asm of plants cells through the mevalonic acid pathway. Terpenoid are then oxidized derivatives of hydrocarbon terpenes such as aldehydes, ketones, alcohols, acids, ethers, and esters[44].

Terpenoids can be classified to four groups of compounds that include tr-ue terpenes, steroids, saponins, and cardiac glycosides. These types of natural products can be present in every class of living things, mainly in plants as components of essential oils[45]. Generally, only hemiterpenoids, the monoterpenoids, and sesquiterpenoids are volatile to be components of essential oils. The composition of oils is mainly represented by mono-, se-squi-, and even diterpene hydrocarbons and their respective oxygenated derivatives[46,47,48].

i) -Monoterpenes

Monoterpenes are compounds consist of the combination of two isoprene units linked by the head to tail binding .They are the main molecules that present in about 90% of some essential oils, thereby, they represent the unique odor of plants[14,49]. Monoterpenes in nature are mostly involved in pla-nt- animal and plant- plant interaction such as pollination, seed and fruit spreading, and allelopathic agents,Monoterpenes found in more than 30 known skeletons and can be divided into 3 groups: acyclic, monocyclic, and bicyclic[50]. Some examples of these compounds include geraniol, terpineol (found in lilacs), limonene (present in citrus fruits), myrecene (found in hops), linalool (present in lavender) and pinene (present in pinetrees) [51].They react easily to air a

nd heat sources, thereby citrus oils don't last long, since they are high in monoterpene hydrocarbons and have a quick reaction to air and are readily oxidized[52].

ii)- Sesquiterpenes

Sesquiterpenes are a class of terpenes that consist of three isoprene u nits and have the molecular formula $C_{15}H_{24}$ .These compounds forme d as acyclic or rings with specific combination. Biochemical modifica tion such as oxidation or rearrangement produce the related sesquiter penoids which are naturally found in plants and insects, as defense ag ents or pheromones[52].

Sesquiterpenes lactones are secondary metabolites that belong to the groupof C_{15} terpenoids. They are consist of three isoprene units. One of their methanol group, a part of the isoprene group was oxidized to group, was oxidized to lactones[53]. It forms an important group secon dary metabolites which act as an active products in plants defense, as antimicrobial and insecticides. This group of secondary metabolites demonstrates allelopathic prospective.

Recently, there is an increasing demand of sesquiterpene lactone, bec ause of their high therapeutic potential as cytotoxic and anticancer ag ent. thus Lactones were isolated from the members of asteraceae fam ily, while members of magnoliaceae, lauraceae, and apiaceae family were the more primitive representatives of sesquiterpene lactones. Lip ophilic solvents or supercritical fluid technology are used for the extr action of sesquiterpene lactones from plants material. The purification and structure elucidation was detected using chromatographic techni

ques and (NMR) and mass spectroscopy[54]. Some sesquiterpene sh ows an allelopathic potential and also represents antibacterial, antimic robial, antiviral, antiprotozoal, cytotoxic, and anticancer activity.

Farnesyl pyrophosphate, is an intermediate factor in the biosynthesis of sesquiterpenes as farnesene. Oxidation process provide sesquiterpe noids as farnesol and juvenile hormone. The wide variety of cyclic se squiterpene for example, absicisic acid, fumagillin, germacrene, dendr olasin, and trans monocyclo farnesol[54]. In addition to six membered ri ng such as in zingiberene, a component of the oil from ginger, cycliza tion of the chain ends can lead to macrocyclic rings such as humulen e[54].

Other type of sesquiterpene is a nine membered ring and cyclo butane ring model a classic bicyclic sesquiterpene caryophyllene. Additional unsatu –ration provides aromatic bicyclic sesquiterpenoids such as ve tivazulene and guaiazulene. Examples are caryophyllene, muurolene , petasin, car- otol, a vocettin, alkaloids of nuphor, and mycophenolic acid[54].

Athird ring (tricyclic) sesquiterpene is also possible and varied – exam ples are longifolene, copaene, patchoulol, illudins, hirsutic acid, corlol ins and santonen. Moreover there are four membered ring (tetracyclic), example, is gossypol and marasmic acid and pentacyclic compound s for example siccanochromenes[53].

iii)- Diterpenes

Diterpenes are a complex compounds of plants resins but are someti mes encountered as by products in the isolation of essential oils. Thes

e types of essential oils components are volatile due to their high mol ecular weight and less numerous than the mono – and sesquiterpenes. Thereby, they are difficult to extract by steam distillation and then yi elds in low amounts in distilled oil. However traditional extraction us ed distillation allows separat-ion and identification of diterpenes prese nt in essential oils[50]. Generally, molecules with molecular mass highe r than (300.a.m.u)can be considered as sign of unsuitable extraction c ondition or adulteration. Example of diterpenes are camphorene, caf estol, kahweol, cambrene, and taxideme[55].

1.3.2-Phenylpropanoids

Phenylpropanoids are mainly produced by plants for protection again st infections, ultraviolet irradiation, wounding and herbivores. They are synthesized from the amino acid phenylalanine, that is converted to cinnamic acid. Reduction of the carboxylic acid group present in t he cinnamic acid yields an aldehyde (e.g.cinnamaldehyde) and furthe r reduction produces monolignols such as phenylpropenes (euginol an d safrole). Natural and synthetic phenylpropanoids are under current medicinal uses for their pharmacological properties[56,57] Many bioactiv ity of these compounds including, anticonvalsant, anti-inflammatory and analgesic effects[58,59] .

1.3.3-Hydrocarbons

Hydrocarbons are the main category of compounds and are compose d enti-rely of carbon and hydrogen atoms, which vary greatly in size a nd complexity. They are very soluble in lipids (lipophilic), but are po

orly soluble in water. Simple hydrocarbons, such as alkanes, alkenes, and benzenoids, are called nonterpenoid hydrocarbons due to the fact that their biosynthesis not related to mevalonate or nonmevalonate pat hways[60].Those with open chain and do not have a closed or aromatic ring are classified as aliphatic, and include alkanee, alkenes, and alky nes. Aliphatic molecules are often found only in trace amount in ess ential oils, but oxygenated compounds have a considerable odors [46, 61].

Another class of hydrocarbons is known as the aromatic class. These compounds usually contain a benzene ring (C_6H_6) and include phenyl, benzyl, phenylethyl- and phenylpropyl compounds, as well as polycy clic structures, such as naphthalene and benzo[α]pyrene. The name " aromatic" derives from the first benzene derivatives isolated from pla nts, which were found to be pleasant smelling. Subsequently, howeve r, less pleasant derivatives were discovered[60].

1.3.4- Alcohols

Alcohols are the varied group of terpene derivatives found in essential oils[60]. Monoterpene alcohols are not large in number, but occur in a l arge number of essential oils. They are many sesquiterpene alcohols, but most of them are found in few essential oils. Alcohols are relative ly non toxic, nonmutagenic, and possess low irritancy and allergenic ity. Monoterpenic alcohols (monoterpenols) are good antiseptics, with antifungal properties. Menthol one examples of an alcohol, and is on e of the monoterpenic alcohols[61].

1.3.5- Esters

Esters are produced from the reaction of an alcohol with an acid in a process called esterification. They are common components found i n many various essential oils and are calming and relaxing and tend t o be fruity with therapeutic effects, which include being sedative and anti-spasmodic. Moreover acetate, awell known ester which is found bergamot, clary sage, lavender as well as petit is one of the useful co mpounds in essential oils[62]. Some ester also have anti-fungal and anti microbial activity like the antifungal properties in geranium oil [28].

1.3.6- Aldehydes

These compounds contain the formyl functional group, with an oxyge n atom double bonded to a carbon atom at the end of a carbon chain. The fourth bond is usually a hydrogen atom[46]. Aldehydes, which may considered as partially oxidized primary alcohol, are widely distribut ed as natural essential oil constituents. Aldehydes have a slightly fruit y odor when smelled on their own. The name of aldehydes end in"al" or aldehyde". Geranial and cumin aldehyde are examples of important aldehydes[60].

1.3.7-Ketones

Ketones are structurally similar to aldehydes and also possess a carb onyl group. Ketones can be produced by the oxidation of secondary al cohols. They are relatively stable compounds and are not easily oxidi zed further. The names of ketones generally end in" one" with one exc eption: Camphor. Carvone is one of the most well known ketones[60].

Ketones are often mucolytic and neuro-toxic when isolated from other constituents. They catalyze cell renewing, enhance the formation of ti ssue, and liquefy mucous. They are helpful with conditions such as dr y asthma, colds, flu and dry cough and are largely found in oils used f or the upper respiratory system. Essential oils that contain ketones inc lude Clary, Sage Hyssop, Idaho, Tansy, Rosemary and western red ce dar[63].

1.4-Methods of extraction of essential oils

Essential oils are used in a wide variety of consumer goods such as de tergents, soaps, toilet products, cosmetics, pharmaceuticals, perfumes, confectionery food products, soft drinks, distilled alcoholic beverage s (hard drinks) and insecticides. The world production and consumpti on of essential oils and perfumes are increasing very fast. Production t echnology is an essential element to improve the overall yield and qua lity of essential oil. Essential oils are obtained from plant material by several extraction methods[64,65] .

There are many techniques applied for the extraction of essential oils i ncluding: Hydrodistillation, steam distillation, solvent extraction, enfl eurage, cohobation, and maceration[65].

1.4.1 Hydrodistillation

Atraditional method of essential oil extraction is hydrodistillation whi ch is widely used method for extraction of essential oil. Water or hydr odistillation is one of the oldest and easiest methods[66] being used for t he extraction of essential oils[65].

Hydrodistillation is usually used for the extraction of essential oils fro m the aromatic and medicinal plant. The conventional method for the extraction of essential oils is hydrodistillation (HD), in which the esse ntial oils are evaporated by heating a mixture of water or other solven t and plant materials follo-wed by the liquefaction of the vapors in a c ondenser.The setup comprises also a condenser and a decanter to coll ect the condensate and to separate essential oils from water, respectiv ely[65].The principle of extraction is based on the isotropic distillation. I n fact, at atmospheric pressure and during extraction process (heating) , water or other solvent and oils molecules. Hydrodistillation (HD) is a variant of steam distillation, which is be spoke by the French Pharm acopoeia for the extraction of essential oils from dried plant There are three types of hydrodistillation: with water immersion, with direct va por injection and with water immersion and vapor injection. It is a mu ltilateral process that can be utilized for large or small industries. The distillation time depends on the plant material being processed. Prolo nged distillation produces only a small amount of essential oil, but do es add unwanted high boiling point compounds and oxidation product s[65].

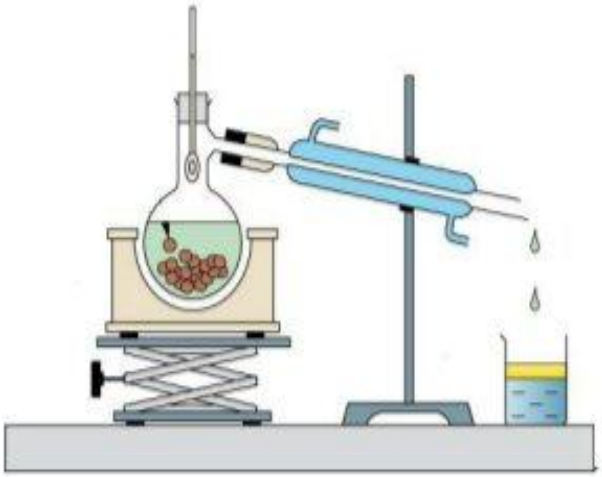

Fig.1.1: Schematic of apparatus for hydrodistillation

1.4.2-Steam distillation

Steam distillation is a separation or extraction process used mainly for a temperature-sensitive plant such as natural aromatic compounds. It once was a popular laboratory method for purification of organic com pounds but has become obsolete by vacuity distillation. Steam distilla tion is still important in certain industrial sectors,[66,67] . Steam distillati on is one of ancient and officially approved methods for isolation of e ssential oils from plant materials. The plant materials charged in the a lembic are subjected to the steam without maceration in water. The in jected steam passes through the plants from the base of the alembic to the top. Steam distillation is a method where steam flows through the material . This steam functions as agents that break up the pores of th e raw material and release the essential oil from it. The system yields a mixture of a vapor and desired essential oil. This vapor is then cond ensed further and the essential oil is collected[68] . The principle of this technique is that the combined vapor pressure equals th-e ambient pre ssure at about 100 °C so that the volatile components with the boiling

points ranging from 150 to 300 °C can be evaporated at a temp-eratur e close to that of water. Furthermore, this technique can be also carr-i ed out under pressure depending on the essential oils extraction diffic ulty[68].

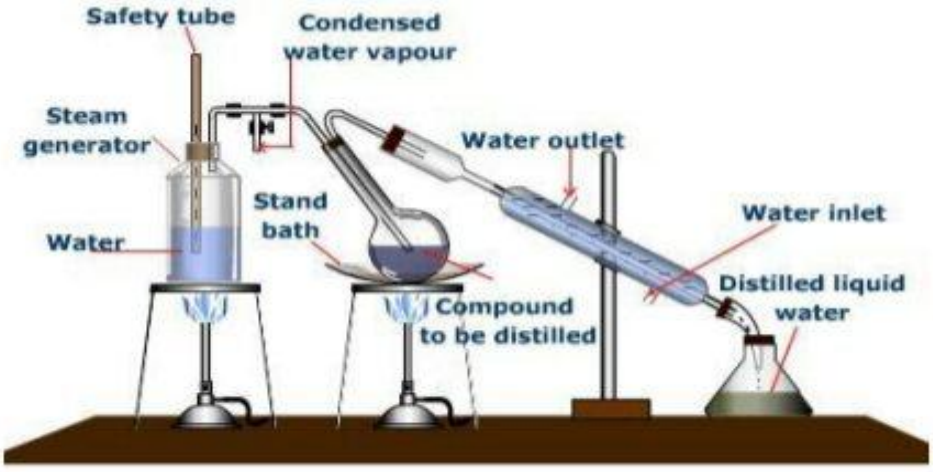

Fig.1.2 : Schematic of apparatus for Steam distillation

1.4.3-Solvent extraction

Solvent extraction, also known as liquid–liquid extraction or partitioni ng, is a method to separate a compound based on the solubility of its parts. This is done using two liquids that don't mix, for example, wate r and an organic solvent . In the solvent-extraction method of essentia l oils recovery, an extracting unit is loaded with perforated trays of es sential oil plant material and repeatedly washed with the solvent. Solv ent extraction is used in the processing of perfumes, vegetable oil, or biodiesel. Solvent extraction is used on delicate plants to produce hig her amounts of essential oils at a lower cost[69]. The most frequently ap plied sample preparation procedure in plant material analysis. The qu ality and quantity of extracted mixture are determined by the type of e xtra heat applied because of the method is limited by the compound

solubility in the specific solvent used. Although the method is relative ly simple and quite efficient, it suffers from such disadvantages as lon g extraction time, relatively high solvent consumption and often unsat isfactory reproducibility[70].

1.4.4 Soxhlet Extraction

A Soxhlet extractor is an apparatus intoduced by Franz von Soxhlet[71, 72] .It was originally designed for the extraction of a lipid from a solid material. Typically, a Soxhlet extraction is used when the desired com pound has a limited solubility in a solvent, and the impurity is insolub le in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a lar ger amount of material. Soxhlet extraction involves solid-liquid conta ct for the removal of one or several compounds from a solid by dissol ution into a refluxing liquid phase. In a conventional Soxhlet device, t he solid matrix is placed in a cavity that is gradually filled with the ex tracting liquid phase by condensation of vapors from a distillation flas k. When the liquid reaches a preset level, a siphon pulls the contents o f the cavity back into the distillation flask, thus carrying the extracted analytes into the bulk liquid[73]. This procedure is repeated until virtuall y complete extraction is achieved. There are several advantages of So xhlet extraction. The most important are that the sample is repeatedly brought into contact with fresh portions of the solvent. This procedure prevents the possibility of the solvent becoming saturated with extrac table material and enhances the removal of the analyte from the matri x. Moreover, the temperature of the system is close to the boiling poin

t of the solvent. This excess energy in the form of heat helps to increa se the extraction kinetics of the system. Soxhlet extraction has several disadvantages, including it requires several hours or days to perform; the sample is diluted in large volumes of solvent, and due to the heati ng of the distillation flask losses due to thermal degradation and volati lization have been observed[44].

1.4.5- Maceration

In this process, the whole powdered crude drug is placed in a stoppere d container with the solvent and allowed to stand at room temperartur e for a period of at least 3 days with frequent stirring until the soluble matter has dissolved. The mixture then is strained, the marc (the dam p solid material) is pressed, and the combined liquids are clarified by filteration or decantation after standing[35].

1.4.6-Cold Pressing method

The term cold pressed theoretically means that the oil is expeller-pres sed at low temperatures and pressure. Cold pressed method is one of the best methods to extract essential oils. This process is used for mos t carrier oils and many essential oils. This process ensures that the res ulting oil is 100%pure and retains all the properties of the plant. It is a method of mechanical extraction where heat is reduced and minimize d throughout the batching of the raw material. The cold pressed meth od is also known as scarification method. Cold pressed method is mai nly used for extracting essential oils from plants, flower, seeds, lemo n, tangerine oils. Essential oils are then separated from the material by centrifugation[74,75] .

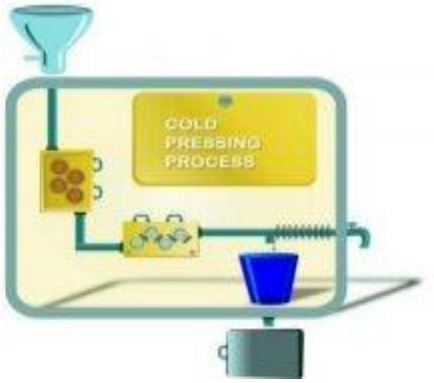

Fig. 1.3 : Cold pressing apparatus

Traditional methods of extraction are the most widelyutilized on co mmercial scale. However, new techniques have been developed which may not necessarily be widely used for commercial production of essential oils but are considered valuable in certain situations, such as alteration of their thermosensitive components or the extraction of essential oils for micro-analysis[35].

1.4.7 Supercritical fluid extraction (SFE).

Supercritical fluid extraction (SFE) is the process of separating one co mponent (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid ma trix, but can also be from liquids. Supercritical fluids have been used as solvents for a wide variety of applications such as essential oil extr action and metal cation extraction. In practice, more than 90% of all a nalytical supercritical fluid extraction (SFE) is performed with carbon dioxide (CO2) for several practice reasons. Apart from having relativ ely low critical pressure (74 bars) and temperature (32^{o}C), CO_2 is rela tively non-toxic, nonflammable, noncorrosive, safe, available in high

purity at relatively low cost and is easily removed from the extract[76]. The main drawback of CO_2 its lack polarity for the extraction of polar analytes[77]. extracts prepared by SFE yielded a higher antioxidant acti vity than extract prepared by other methods[78]. This extraction method produces higher yield, higher diffusion coefficient, and lower viscosit y. Many essential oils that cannot be extracted by steam distillation ca n be obtainable with carbon dioxide extraction. Nevertheless, this tech nique is very expensive because of the price of this equipment for this process is very expensive and it is not easily handled. Supercritical e xtracts proved to be of superior quality with better functional and biol ogical activities[79]. Furthermore, some studies showed better antibacter ial and antifungal properties for the supercritical product.

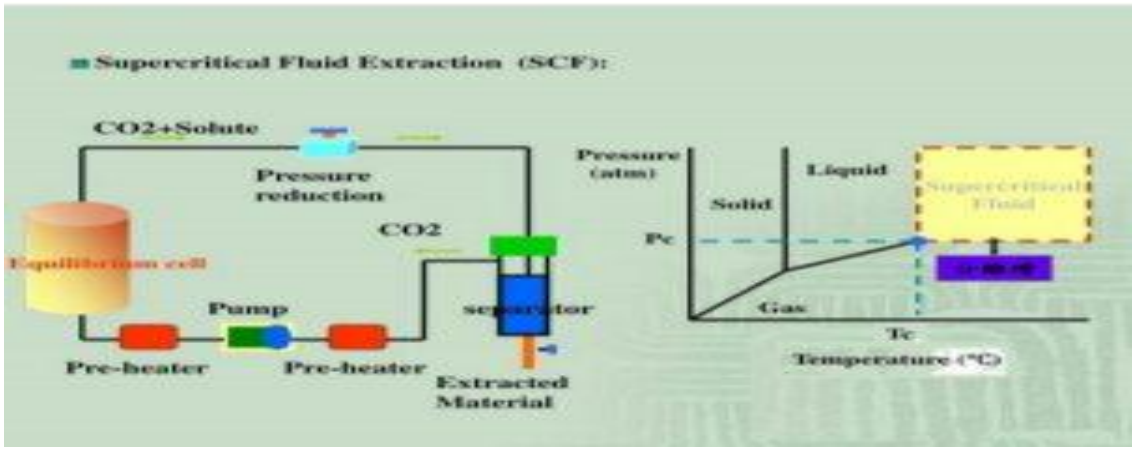

Fig. 1.4 : Scheme of supercritical fluid extraction (SFE)

1.4.8 Microwave-assisted hydrodistillation

Microwave-assisted hydrodistillation is regarded as an advanced hydr odistillation technique utilizing microwave oven in the extraction pro

cess[80]. Some researchers have successfully utilized a microwave ove n for the extraction of active components from plants[80]. The efficienc y of Microwave-assisted hydrodistillation is strongly dependent on th e dielectric constant of water and the sample[81]. Conventional techniqu es for the extraction of active constituents are time and solvent consu ming, thermally unsafe and the analysis of numerous constituents in p lant material is limited by the extraction step[82] .High and fast extracti on performance ability with less solvent consumption and protection offered to thermolabile constituents are some of the attractive features of this new promising microwave-assisted hydrodistillation technique . Application of microwave-assisted hydrodistillation in separation an d extraction processes has shown to reduce both extraction time and v olume of solvent required, minimizing environmental impact by emitt ing less CO_2 in atmosphere[83,84] and consuming only a fraction of the energy used in conventional extraction methods[85]. The use of microw ave-assisted hydrodistillation in industrial materials processing can pr ovide a versatile tool to process variety of materials under a wide rang e of conditions[84].

Microwave-assisted hydrodistillation is a current technology to extrac t biological materials and has been regarded as an important alternativ e in extraction techniques because of its advantages which mainly are a reduction of extraction time, solvents, selectivity, volumetric heatin g and controllable heating process[85].The principle of heating using mi crowave-assisted hydrodistillation is based upon its direct impact with polar materials/solvents and is governed by two phenomenon's: ionic

conduction and dipole rotation, which in most cases occurs simultaneously[86].

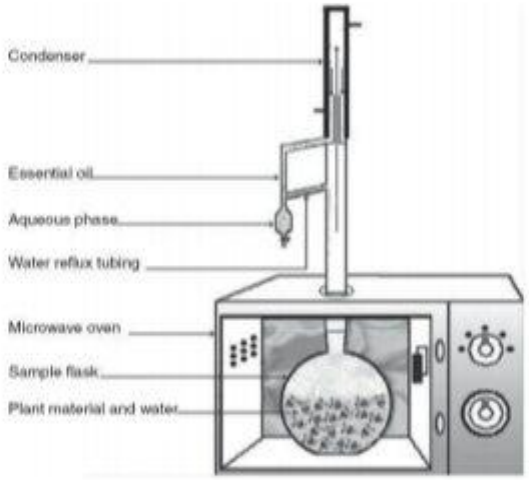

Fig.1.5: Apparatus of microwave-assisted hydrodistillation

1.4.9 Ultrasound-assisted extraction (UAE)

Ultrasound-assisted extraction (UAE) is a good process to achieve high valuable compounds and could involve the increase in the estimate of some food by-products when used as sources of natural compounds or plant material[87].The major importance will be a more effective extraction, so saving energy, and also the use of mean temperatures, which is beneficial for heat-sensitive components. This technique was developed in 1950 at laboratory apparatus[88]. Ultrasound allows selective and intensification of essential oils extraction by release from plant material when used in combination with other techniques for example solvent extraction and hydrodistillation[87].

Ultrasound technology has been featured as a valuable method in food engineering processes and plants[87]. In these applications the power ultrasound increases the surface wetness evaporation average and cau

ses oscillating velocities at the interfaces, which may affect the diffusi on boundary layer and generate rapid series of alternative expansions of the material affecting cluster transfer[89] The plants raw material is i mmersed in water or another solvent(methanol or ethanol or anyone o ther solvent) and at the same time, it is subjected to the work of ultras ound[90].This technique has been used for the extraction of many essent ial oils especially from the flower, leaves or seeds[91].

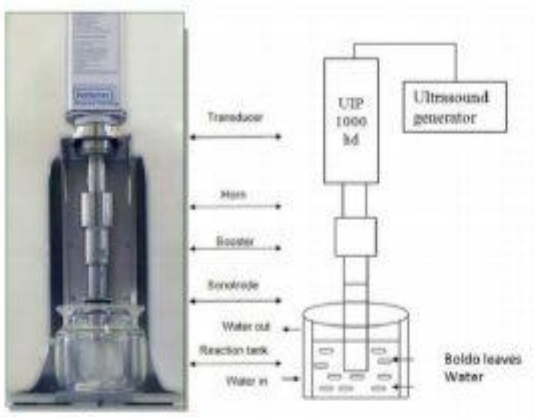

Fig.1.6 : Apparatus for ultrasound-assisted extraction

1.4.10 Solvent – free microwave extraction (SFME)

Solvent – free microwave extraction is suggested as a method for " green" extraction of edible essential oils from fresh plant material, at atmospheric pressure without addition of water or organic solvent[92] . This method is an original combination of microwave heating and hy drodistillation at atmospheric pressure. This techniques includes placi ng the plant material in a microwave reactor, without adding any solv ent or water.The internal heating of the water within the fresh plant m aterial inflates the plant cells and lead to break the glands and oleifero us receptacles. Acooling system outside the microwave oven condens

ed the distillate continually. The excess of water is refluxed to the extr action vessel that to retrieve *in situ* water to the plant material. At the end, essential oil is removed from the aqueous extract by decantation process[92].

The technique of (SFME) is neither a modified microwave- assisted e xtraction which uses organic solvent nor a modified hydroditillation p rocess with water coming from the fresh plant material[83,93]. The adva ntage, of (SFME) extraction include : increase the obtained essential o il, optimizing the essential oil composition, remove the waste of water treatment, and also contributes to limited time, and lower an energy c onsumption[83].

1.4.11 Microwave hydrodiffusion and gravity (MHG):

Is a new green technique for the extraction of essential oils. This gree n extraction technique is an original microwave blend microwave heat ing and earth attraction at atmospheric pressure. MHG was conceived for experimenter and processing scale applications for the extraction o f essential oils from different kind of material plants[94]. Microwave hy dro diffusion and gravity (MHG) become clear not only as economic and efficient but also as environment-friendly, not require solvent or water and as it does require less energy[95]. The performances and adva ntages of this technique are a reduction of extraction time (in the case of hydrodistillation it takes 90min or more but in this technique only 20min) and reducing environmental impact and power saving [94,95].

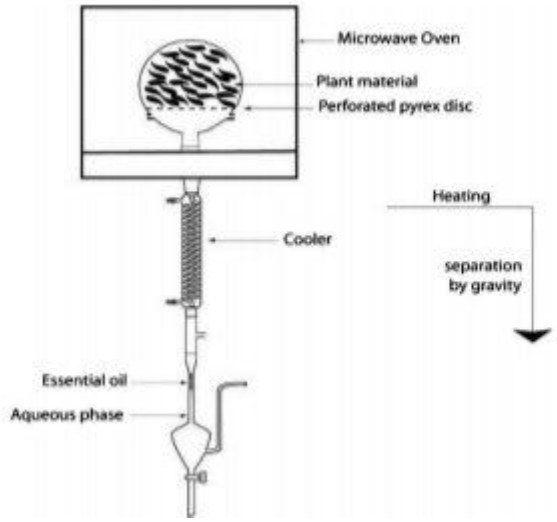

Fig.1.7: Apparatus for microwave hydro diffusion and gravity

1.5- Bioactivity of essential oils

previous studies on essential oils evaluate their pharmacological prop erties and toxicity in order to exploit possible alternative medicines[96].

1.5.1-Antioxidant activity

Essential oils are known to represent a large range of biological activi ty , one of the most important studied properties of essential oils is ant ioxidant activity. This could be explained by the damages of various free radicals which causes many metabolic diseases such as cancer, di abetes, arthritis, inflammation and Alzheimer[97,98]. Essential oils are ri ch natural sources of potential antioxidants that can be tested to preve nt damage of cells[99]. Antioxidants are substances that, in low concen tration inhibit the oxidation of the substrate[100]. Volatile compounds in essential oils, beside their protective antioxidant activity, can also act as prooxidant, affecting the cellular redox[14].

Although phenolic compounds are admitted as being responsible for t he antioxidant ability, recent studies demonstrated that volatile compo

nents could also individually or in mixture contribute to the whole ant ioxidant ability. Essential oils of lemon and balm(*Melissa officinalis L*) was found to offer the highest antioxidant activity[101].

1.5.2- Antimicrobial activity

Essential oils are recognized as antimicrobial agents, and are well repr esented in recent researches. Their antimicrobial activity depends on t he presence of active compounds and the interaction between various components which can have synergistic or antagonistic action. It also depends on the content, concentration, and susceptibility of micro or ganism[36,102]. The inactive components impact resorption, the rate of t he reaction, as well as biological activities of active compounds. The combination of both major and minor components can thus modify th e activity to exert significant synergisticor antagonistic effect[103,104].

The effect of essential oils against pathogens is as a result of the abilit y of the hydrophobic compounds that disrupt the microorganism cell membrane, which modify the cell morphology, alteration of membran e permeabilityand leakage of electrolytes[105]. The antimicrobial standa rd of various essential oils tested against food borne pathogens as well as spoilage microorganisms indicates a broad potential of their use in the food industry under strict evaluations to enhance their efficacies[106,107]. Essential oils show strong antimicr-obial activity towards food b orne pathogens, which can lead to use them as preservative or to incor porate in the food packaging as natural antimicrobial agents[108,109].

Essential oils have been subject to pharmacologic studies as well as v ario-us tests of their antimicrobial activities. The most common meth

ods are agar diffusion tests, serial broth or agar dilution tests, and vap or tests . These oils are thought to play arole in plant defence mechani sm acting[37]. Phytopathogenic microorganism[110,111]. The bacteria that c ause the most major clinical problems are *klebsiella and Enterobacter* species, *Staphylococcus aureus, Enterococcus faecium, Clostridium difficile, Acinetobacter baumannii, Pseudomonas aeruginosa and Esc herichia Coli* [112].

Generally essential oils are more active on gram- positive bacteria d ue to the presence of peptidoglycan layer which lies outside the outer membrane. In gram- negative bacteria, the outer membrane is compos ed of a double layer of phospholipids which is linked to the inner me mbrane by lipopolysacchride[113]. Several studies on the bioactivity of essential oils have revealed their antibacterial and antifungal potentia l on different pathogenic microorganism[114,115].

1.5.3 Antiviral activity

The effective agents that play a role against common pathogens are ne eded particulary for those resistant to traditional antiviral agents.The a bility of viruses to persist in fresh products, could lead to serious food borne problems[116]. Plants and plant-derived natural products provide a chance for new antiviral drugs. Many essential oils have been teste d for their antiviral activity. As conclusion in recent years, free viruse s are very sensitive to essential oils[117].

1.6 The target plants species

1.6.1 *Cassia sieberiana*.

Cassia sieberiana (Fabaceae) is a tropical deciduous small tree. It is characterized by bright yellow flowers that form into groups. It grows best in well drained, humid soils. *Cassia sieberiana* grows in groups of other plants, thus, they usually never grow alone[118].

This shrub native to Africa. Its distribution spans across Africa includ ing the southern part of the Sahel[119]. It also grows in wooded grasslan d and savannah, secondary bush, on lateritic soils, road sides, gravel a nd thickets, secondary forest, coastal scrub and sandstone plateau[120]. bark color is grey- black. The leaves are arranged in leaflets that conta in 7-10 pair of opposite leaves. The upper side of the leaf is moderatel y shiny while the bottom has very fine nerves with stipules that are de ciduous. *Cassia sieberiana* has both flowers and fruit. The flowers are very bright yellow during the dry season, The flowers are also arrang ed either uprightly or in pendulous racemes[119].

The leaves, roots and pods are widely used in traditional system of m edicine. Twigs are used against sleeping sickness. root is used for ti redness and also for body massage, A decoction of the bark, leaves or root is used for the treatment of dysentery, diarrhoea and vomiting the twigs are also used for the treatment of trypanosomiasis. Root barkis a natural remedy for the treatment of dysmenorrheal and gastric ulc er[120].

Seeds have been found to contain : protein (23.72%), crude fibre (10 .75%), potassium (252.33 mg/L) and magnesium (52.68 mg/L). Seed also containTannin, alkaloids, phenolic, oxalates, cardiac glycosides a nd flavonoids[121]. pulp (fruit) contain saponins, tannins, alkaloids, ster

oids, flavonoids, phlobatannins, cardiac glycosides, cyanogenic glyco sides and reducing sugars[122]. anthraquinones, sterols, steroidal glycosi des, tannins, triterpenes have also been reported in the root [123]. Root b ark and leaves contain : tannins saponosides, anthocyanosides, reduci ng compound, carbohydrates, flavonoids, and triterpenic steroids[125]. R oot bark extract showed the presence of saponins, flavnoids, anthraqui nones and tannins[126].

Fig. 1.8 *: Cassia sieberiana*

1.6.2 *Mimosa épineux*

Mimosa épineux is a thorny tree 6 to 17 m high in the family Mimosa ceae[127].Twigs are greenish and the leaves are alternating and bipinnat e, from 3 to 10 cm long with 3-7 pairs of pinnules. *Mimosa épineux* is a species that is Sahelo-Saharan and sudanoSahelian[127]. Fruits are re presented by narrow pods and cntain 6 to 10 seedsthat are brown whe n they are ripened. Flowers and fruiting usually take place in the seco nd half of the dry season, befor foliage. *Mimosa épineux* is found in lo w slopes and low ground and generally near rivers. This species has s

pread from Senegal to Cameroon and from Egypt to sudan and Somal ia[128].

Ethanolic extracts of leaves, root bark and trunk showed activity agai nst Klebsiella pneumoniae[129,134]. Methanolic extract of *Mimosa épine ux* leaves reduced the incidence of green mold (*Penicillium digitatum*) by 56.1% on fruits inoculated per injury. The methanolic extract co ntained gallic acid, salicylic acid, p- coumaric acid, caffeic acid, 3,4 d ihydroxy benzoic acid, ferulic acid[135]. Ethanolic extracts (leaves, bar k) and dichloromethane extract from the bark of *Mimosa épineux* sho wed an activity higher than 85% with respect to the enzyme acetylcho linesterase. Alkaloids are known to have many pharmacological prope rties, including inhibition of acetylcholinesterase enzyme activity and the author associate the activity with alkaloids[129]. A recent study sho wed that methanolic extract from the bark of *Mimosa épineux* showe d 100% mortality against *Biomphalaria Pfeifferi* at different doses use d[136]. Root extract has demonstrated antimicrobial activity against fungal and bacterial p athogens [137]. The cytotoxic study of the hydroethanolic extract of the stem bark of *Mimosa épineux* reduced the protein content of Bcl-xL a nd Bcl-2 which in turn promotes the intrinsic induction of apoptosis. I n addition, the phytochemical analysis of this extract shows that it is r ich in pro-apoptotic components such as flavonoids [138]. The structure of the gum of *Mimosa épineux* has recently been revised by methylati on analysis and nuclear magnetic resonance (NMR) spectroscopy. It h

as been found that this gum is more strongly branched than *A. senega l* and is composed of galactopyranosyl bound to 1,3. Galacturonic[139].

Fig. 1.9 :*Acacia seyal Del*

1.6.3 *Vitex doniana*

Vitex doniana (Verbenaceae) is one of the most important and widespread tree in savannh region[140]. Species of vitex are tropic and subtropic but other native in temperate Eurasia [141] .The name vitex refer to the latin word 'vieo' which mean to weave or to tie up[142]. Its also commonly known as black plum or african olive [143]. The genus *vitex* comprises around 150 species with sevral health benefits which have been well documented [144,145].

vitex species are deciduous and its leaves are opposite and renewed annually at the dry season, harvesting must be done after bush burning [145].The fruits contain vitamin A and B and can applied as ajam. The blackish pulp is rich in carbohydrate and minerals. The plant is treated for food, medicinal uses and a source of wood, fruits is sweet and the leaves are used as vegetable.

The fruits contain an essential oil, saponin, cineol and α. Pinen.The essential oil has an antibacterial activity [146], fruits also contain

flavonoid and iridoid glycoside[147]. Many researches are now available on the the benefits of *vitex doniana* in africa traditional system of medicine [148,149],

Different Phytochemicals detected in *vitex doniana* leaves such as alkaloids, flavonoids, terpenoids, saponin and tannins are bioactive compounds, they work with nutrients and dietary fibre to prevent occurrence of such deseases infection[150,151].

Several parts of *vitex doniana* are utlised by traditional medicine as aremedy of disorders such as rheumatism, hypertension, cancer and inflamatory conditions[152]. The leaf, the bark, dried and fresh fruit are used traditionally against conjunctivitis, headche, stiffness, measles, rash, fever, checkenpox, hemiplegia, respiratory diseases, rachitis, gasto-intestinal disorders, jaundice, kidney troubles, leprosy, liver disorders, bleeding after child birth and diarrhea [143,153].

Fig. 1.10 *:Vitex doniana*

1.6.4 *Ziziphus spina- christi*

Ziziphus spina-christi **(L.) Desf**. (Rhamnaceae) is a tropical evergreen tree of Sudanese origin with edible fresh or dried fruits[154,155].This plant grows in east Africa and West Asia including Egypt, Saudi Arabia, and south Iran[156]. It is a spiny tree that tolerates extreme heat and drought. It develops a very deep taproot system and has an amazing regenerative power. *Z. spina-christi* is covered in whitish-brown or pale grey bark which is deeply fissured and cracked, with a twisted trunk which branches widely, drooping at the ends to form a rounded, usually umbrella-shaped gown. The simple, alternate leaves are oval, becoming more pointed at the tips with three conspicuous veins running along the length. The leaves are hairless on the upper surface, with a fine downy covering of small hairs on the underside. Christ's thorn produces small, greenish-yellow flowers, which cluster tightly in the axils of the leaves and red-brown coloured small fleshy fruits that enclose a hard stone in the center.

The seeds are protected by hard woody coats called endocarp, which delay germination. To overcome this problem the seed coat has to be scarified before planting [155,156]. *Z. spina-christi* can be propagate by seeds, so it exhibits a broad genetic heterogeneity[157,158].

Z. spina-christi has many beneficial uses. The leaves are used as fodder for animals and the branches are used for fencing. The wood is used for construction and furniture. All parts of the plant (fruits, leaves, roots, bark) are used in traditional medicine[159,160]. Sinai and Negev's Bedouins have used the tea of fruits to increase milk production for nur

sing women and to treat liver problems[161]. In Sudan the twigs are use d externally to treat rheumatism and scorpion stings [162]. Moreover, in the United Arab Emirates, the boiled leaves are used to treat hair fall[15 5]. The methanolic extract of the stem bark reduces diarrhea in rats[161] whereas the methanolic extract of leaves protects against hepatic carci nogenicity in rats[162]. The butanolic extract of the leaves control the gl ucose level in rats safely[163]. The aqueous extract of the root bark has a n antinociceptive activity, and a central depressant effect in mice. The powder of the seeds showed high activity against *Escherichia coli an d Bacillus subtilis*[164]. Furthermore,it has been found that the hydro alc oholic extract of *Z. spina christi* fruit decreases the blood glucose leve l in dogs[165]. Also the aqueous extract of *Z.spina-christi* fruit decreases the neurotransmitter content in the brain of male albino rats[166]. The et hanolic extraction of fruits and the aqueous extract of the leaves show ed antiviral properties against *Herpes simplex* virus type1(HSV-1)[167]. Furthermore the hydro alcoholic extract of *Z. spina-christi* leaves ind uce contraction in the endothelium intact in the isolated rat aorta[156]. A ll parts of *Z. spina-christi* contain important nutrients and phytochemi cal compounds. The fruit is rich in carbohydrates[155]. The seeds contai n 28.5% lipid and 18.6% protein[164]. There are many studies that inves tigated the phytochemical constituents of *Z. spina christi.* which revea led beotulic and ceanothic acid[168], three cyclopeptide alkaloids: frana ganine, mauritine-C and sativanine-A[169], four saponin glycosides: Chr istinin A, B, C and D[170],beside several flavoniods from the leaves of *Z. spina-christi* [171]. Also dodeca acetyl prodelphinidin B3 has been is

olated from the dried leaves of *Z. spina-christi*[172]. Furthermore, twelv e flavoniods compounds were isolated from the methanolic extract of *Z. spina-christi* fruits[173] .In addition of a new peptide alkaloid spinan ine-A has been isolated from the stem bark of *Z. spina-christi*[174].

Fig. 1.11 :*Ziziphus spina Christi*

1.6.5 Kigelia Africana

Kigelia.Africana(**Lam)Benth**. Is a plant in the family Bignoniacea[175].

It is a tree growing up to 20 m tall or more. The bark is grey and smo oth at first, peeling on older trees. It can be as thick as 6 mm on a 15 c m branch. The wood is pale brown- yellowish, being not prone to crac king[175]. *K. Africana* is evergreen tree where rainfall occurs throughout the year, but deciduous where there is a long dry season. The leaves a re opposite or in whorls of three, 30-50 cm long, pinnate,with six to te n oval leaflets up to 20 cm long and 6 cm broad; the terminal leaflet c an be either present or absent. The flowers (and later the fruit) hang d own from branches on long flexible stems (2 - 6 m long). Flowers are produced in panicles; they are bell shaped (similar to those of the Afri can tulip tree but darker and more waxy), orange to reddish or purplis h green and about 10 cm wide. Individual flowers do not hang down b ut are oriented horizontally[176]. some birds are attracted to these flower

s and the strong stems of each flower make idea footholds. Their scen t is most notable at night indicating their reliance on pollination by ba ts, which visit them for pollen and nectar[177]. Flowers are bisexual, ver y large; pedicel up to 11-13.5 cm long up curved at tip; calyx shortly t ubular to campanulate, 2 - 4.5 cm long, suddenly widening and incurv ing upwards, limp 2-lipped, with the super or lip 2-lobed, the lower o ne 3-lobed and recurved. The fruit is indehiscent, with woody wall an d heavily marked with lenticels at the surface. It is grey- brown and m any seeded when matured. Seeds are obovoid, *ca*.10 mm x 7 mm with leathery testa, embedded in a fibrous pulp[178].The fruit is a woody ber ry from 30 - 100 cm long and up to 18 cm broad; weighs between 5 - 10 kg hangs down on a long rope-like peduncles[176].

The tree is found on riverbanks, along streams and on floodplains, als o in open woodland, from Kwazulu-Natal to Tanzania to sudan. The p lant is widely distributed in the south, central and West Africa *K. africana* grows along water courses, in riverine fringes, alluvial an d open woodland, high rainfall savanna, shrub land and in rain forest. It occurs on loamy red clay soils, sometimes rocky, damp or peaty, fr om sea level up to zoom altitude[178].

Various pharmacological examinations such as antibacterial, antiviral and antioxidant activities have been carried out[179].

The aqueous leaves extract of *K. africana* has been confirmed to poss ess antidiarrhoeal activity[180]. The traditional use as antileprotic has als o been reported[181].The plant has been reported for its antimalaria activ ities[182]. Wood extract possesses antimalarial activity against drug resi

stant strains of *Plasmodium falciparum* superior to chloroquine and q uinine[183].

The ethanolic extract of the stem bark was examined to show strong a nalgesic and anti-inflammatory activities. The extract components inh ibited the synthesis of prostagladins and other inflammatory mediator s which probably accounted for the analgesic and anti-inflammatory p roperties[184].The dried fruit and bark extract is established to be a stron g pain reliever when administered on painful joints, back and rheumat ism[185].

The extract of the plant has been shown to possess antioxidative prop erty which apparently makes it useful in the treatment of diseases esp ecially the liver-borne disease[186]. The ethnomedicinal plant bark is us ed for the treatment of rheumatism, dysentery and veneral diseases. It is also used as ringworm and tapeworm expellant, while other uses in clude treatment of haemorrhages, diabetes, pneumonia and toothache[1 87,188].Various chemical investigations have been carried out on **.***K. afri cana* and many chemical compounds mainly iridoids, naphthaquinon es, monoterpenoidnapht-haquinones, isocoumarins, lignans sterols an d flavonoids have been identified. An initial laboratory studies indicat ed the presence of two major naphthaquinones in stem bark aqueous e xtract showing activity against *B.subtilis, E. coli, P. aeruginosa, S. au reus* and yeast *C.albicans*[187,189]. Qualitative tests for the presence of pl ant seconddary metabolites such as carbohydrates, alkaloids, tannins, flavonoids, saponins and glycosides were carried out on the bark pow der[184]. Chemical analysis of the polar extract of fruit indicated the pre

sence of vermonosides[190]. Further investigation of the fruits yielded a new phenylpropanoid derivative identified as 6-p-coumaroyl-sucrose together with other known phenylpropanoid derivatives and flavonoid glycoside[191].

Fig. 1.12 *:kigelia Africana*

Aim of this study

This study was carried out to:

-Exrtact the oils from five plants of medicinal potential, namely: *Cass ia sieberiana, Acacia seyal, Vitex doniana, Kigelia Africana* and *Zizip hus spina Christi*.

- Characterize constituents of the oils by GC-MS technique.
- Evaluate the oils for antimicrobial activity.

2.1-Materials

2.1.1-Plant material

Seeds of *Cassia sieberiana, Acacia seyal, Vitex doniana, Kigelia Africana andZiziphus spina Christi* were collected from around damazin-Sudan. The plants were authenticated by the department of phytochemistry and taxonomy, Medicinal and Aromatic Plants

Research Institute ,Khartoum-Sudan.

2.1.2- Instruments

GC-MS analysis was conducted on a Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m length;0.25mm diameter;0.25 um thickness).

2.1.3-Test organisms

The studied oils were screened for antibacterial and antifungal activity using the standard microorganisms shown in table below

Table 2.1: test organism

Ser. No	Micro organism	Type
1	*Bacillus subtilis*	G+v
2	*Staphylococcus aureus*	G+v
3	*, Pseudomonas aeroginosa*	G-v
4	*Escherichia coli*	G-v
5	*Candida albicans*	fungi

CHAPTER TWO
MATERIAL and METHODS

2.2-Methods

2.2.1-Extraaction of oils

Powdered seeds of studied plant (500g) was exhaustively extracted with n- hexane by maceration. The solvent was removed under reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

The oil (2ml) was placed in a test tube and 7 ml of alcoholic sodium hydroxide were added followed by 7 ml of alcoholic sulphuric acid. The tube was stoppered and shaken vigorously for five minutes and then left overnight. (2ml) of supersaturated sodium chloride were added, then (2ml) of n-hexane and the tube was vigorously shaken for five minutes. Hexane layer was then separated.(5µl) of the hexane extract were mixed with 5ml diethyl ether. The solution was filtered and the filtrate (1µl) was injected in the GC-MS.

2.2.2 GC-MS analysis

The studied oils were analyzed by gas chromatography-mass spectroscopy. A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m, length; 0.25mm diameter; 0.25µm, thickness) was used. Helium (purity; 99.99%) was used as carrier gas. Oven temperature program is presented in table 2.2, and other chromatographic conditions are depicted in table 2.3:

Table 2.2: Oven temperature programe

Rate Time (min^{-1})	Temperature(^{o}C)	Hold time($min.^{-1}$)
- 4.00	150.0 300.0	1.00 0.00

Table 2.3 : Chromatographic conditions

Column oven temperature	150.0 ^{0}C
Injection temperature	300.0 ^{0}C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	139.3KPa
Total flow	50.0ml/min
Column flow	1.54ml/sec.
Linear flow	47.2cm/sec.
Purge flow	3.0ml/min
2.Spilt ratio	-1.0

2.2.3 Antimicrobial activity

i) Bacterial suspensions

One ml aliquots of 24 hours broth culture of the test organisms were aseptically distributed onto nutrient agar slopes and incubated at 37^{0}C for 24 hour.The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended is 100 ml of normal saline to product a suspension containing about 108 - 109 Colony forming units per ml. The suspension was stored in the refrigerator at 4^{0}C until used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique. Serial dilutions of the stock suspension were made in sterile normal saline in tubes and one drop volumes (0.02 ml) of the appropriate dilutions were transferred by adjustable volume micropipette onto the surface of dried nutrient agar plates. The plates were allowed to stand for two hours at room temperature for the drop to dry, and then incubated at 37^{0}C for 24 hours.

ii) Fungal suspensions

Fungal cultures were maintained on sabouraud dextrose agar incubated at 25^0C for four days. The fungal growth was harvested and washed with sterile normal saline, and the suspension was stored in the refrigerator until used.

iii) – Testing forAntimicrobial activity

The cup-plate agar diffusion method was adopted with some minor modification, to assess the antimicrobial activity of the oil. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45^0C in a water bath. 920 ml) Aliquots of the incubated nutrient agar were distributed into sterile Petri dishes, the agar was left to settle and in each of these plates which were divided into two halves, two cups in each half (10 mm in diameter) were cut using sterile cork borer (No 4), each one of the halves was designed for one of the compounds. Separate Petri dishes were designed for standard antibacterial chemotherapeutic, (ampicillin and gentamycin).The agar discs were removed, alternate cup were filled with o.1ml samples of each compound using adjustable volume micrometer pipette and allowed to diffuse at room temperature for two hours. The plate were then incubated in the upright position at 37^0C for 24 hours.The above procedure was repeated for different concentrations of the test compounds and the standard antibacterial chemotherapeutics. After incubation, the diameters of the resultant growth inhibition zones were measured in triplicates and averaged

CHAPTER THREE
RESULTS AND DISCUSSION

Results and Discussion

3.1 *Cassia sieberiana*

3.1.1 The GC-MS analysis of *Cassia sieberiana*

GC-MS analysis of *Cassia sieberiana* seeds oil showed 21 components being identified by retention time and mass fragmentation pattern table 3.1.

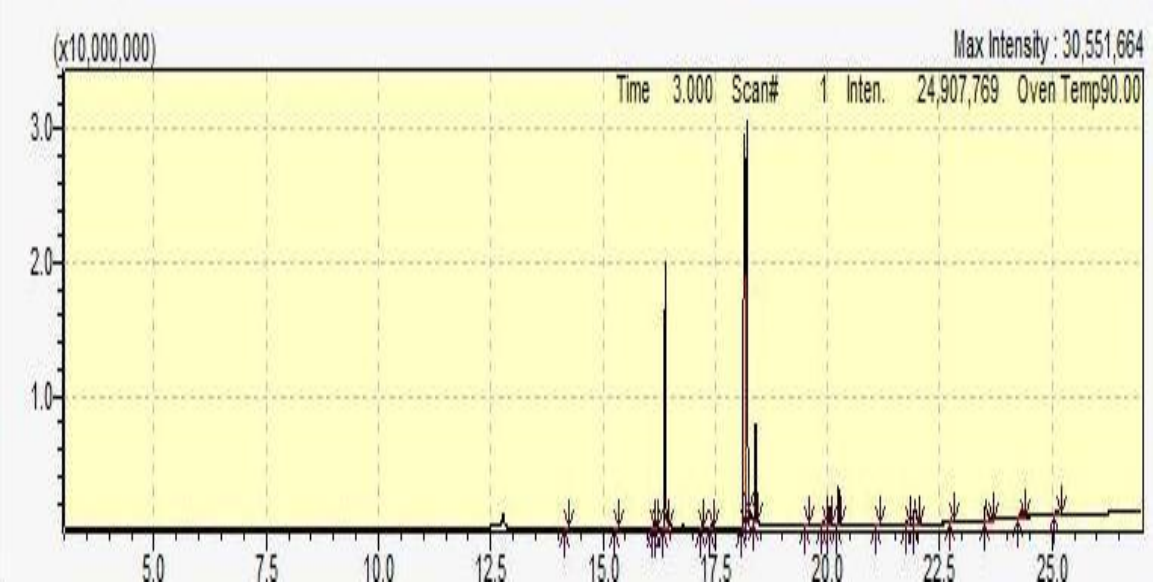

Fig.3.1: Total ions chromatograms

major constituents of the oil are:

i) 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester(37.12%)
ii) 9-octadecenoic acid (Z)-, methyl ester (29.07%)
iii) Hexadecanoic acid, methyl ester(16.54)%

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester is shown in Fig.3.2 The peak at m/z 294 (RT,18.154) is due to the molecular ion M^+ $[C_{19}\ H_{34}O_2]^+$, while the signal at m/z 263 is due to loss of a methoxyl group.

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig.3.3 The signal at m/z 296 (RT.18.202) accounts for the

molecular ion $M^+[C_{19}\ H_{36}O_2]^+$.the mass spectrum of hexadecanoic acid, methyl ester is shown in Fig 3.4. The peak at m/z 270 (RT.16.385) is due the molecular ion $M^+[C_{17}H_{34}O_2]^+$

Table 3.1: Constituents of the oil

No	Name	R.Time	Area%	Formula
1	Methyl tetradecanoate	14.171	0.11	$C_{15}H_{30}O_2$
2	Pentadecanoic acid, methyl ester	15.301	0.08	$C_{16}H_{32}O_2$
3	7-Hexadecenoic acid, methyl ester(Z)-	16.142	0.11	$C_{17}H_{32}O_2$
4	9-Hexadecenoic acid, methyl ester(Z)-	16.181	0.16	$C_{17}H_{32}O_2$
5	Hexadecanoic acid, methyl ester	16.385	16.54	$C_{17}H_{34}O_2$
6	cis-10-Heptadecenoic acid, methyl ester	17.195	0.10	$C_{17}H_{34}O_2$
7	Heptadecanoic acid, methyl ester	17.409	0.17	$C_{18}H_{36}O_2$
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.154	37.12	$C_{19}H_{34}O_2$
9	9-Octadecenoic acid (Z)-, methyl ester	18.202	29.07	$C_{19}H_{36}O_2$
10	Methyl stearate	18.398	5.73	$C_{19}H_{38}O_2$
11	Methyl 9.cis.,11.trans.t,13.trans.-octadecatrienoate	19.507	0.20	$C_{19}H_{32}O_2$
12	8,11,14-Eicosatrienoic acid, methyl ester	19.904	0.38	$C_{21}H_{36}O_2$
13	cis-11-Eicosenoic acid, methyl ester	20.043	1.40	$C_{21}H_{40}O_2$
14	Eicosanoic acid, methyl ester	20.244	2.52	$C_{21}H_{42}O_2$
15	Heneicosanoic acid, methyl ester	21.114	0.12	$C_{22}H_{44}O_2$
16	13-Docosenoic acid, methyl ester, (Z)-	21.772	0.22	$C_{23}H_{44}O_2$
17	Docosanoic acid, methyl ester	21.949	2.03	$C_{21}H_{42}O_2$
18	Tricosanoic acid, methyl ester	22.752	0.38	$C_{24}H_{48}O_2$
19	Tetracosanoic acid, methyl ester	23.525	2.28	$C_{25}H_{50}O_2$
20	Squalene	24.315	0.69	C30H50
21	Hexacosanoic acid, methyl ester	25.095	0.59	$C_{27}H_{54}O_2$

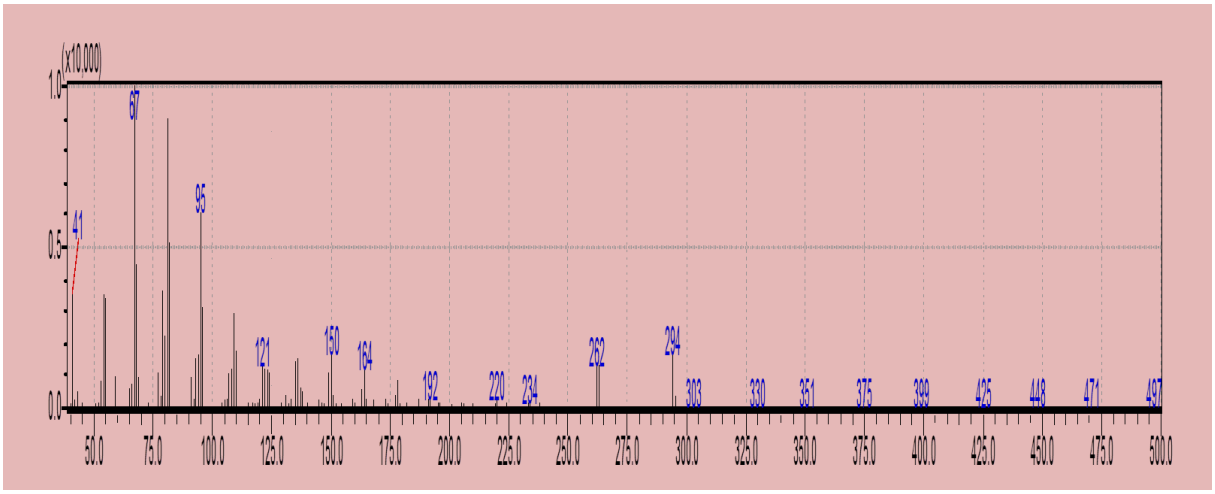

Fig.3.2: Mass spectrum of 9, 12-octadecadienoic acid methyl ester

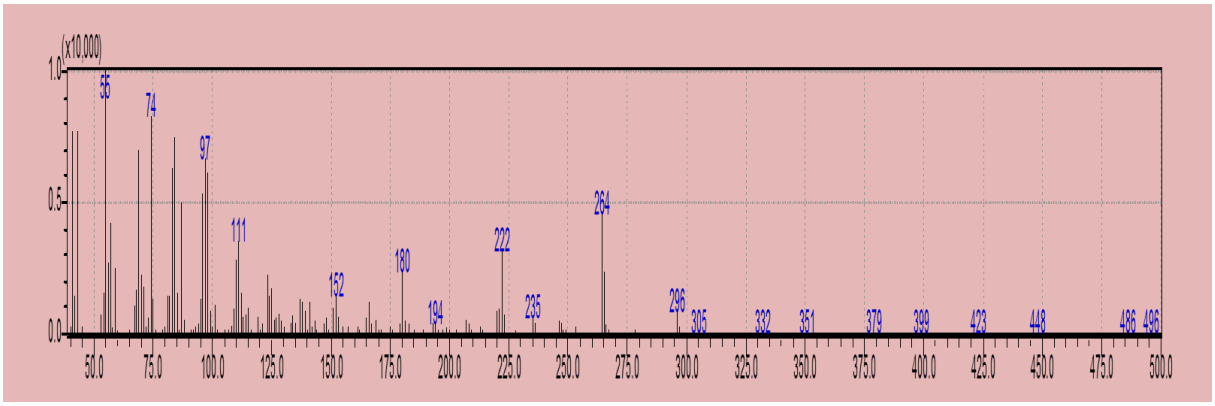

Fig.3.3.Mass spectrum of 9-octadecenoic acid methyl ester

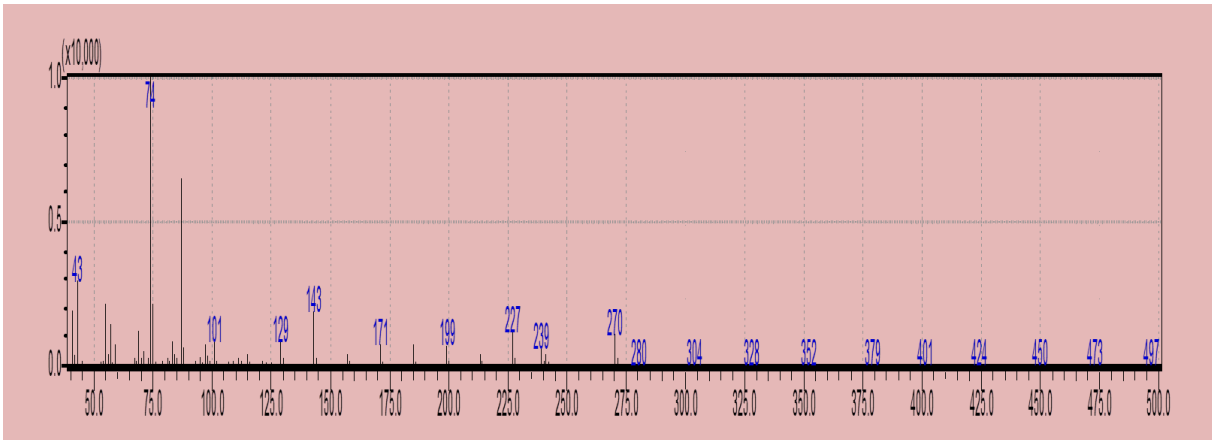

Fig. 3.4 :Mass spectrum of hexadecanoic acid, methyl ester

3.1.2 Antimicrobial activity

Cassia sieberiana seed oil was evaluated for antimicrobial activity against five standard human pathogens.The result s are presented in table 3.2 the oil showed weak activity against *Escherichia coli* and *Bacillus subtilis.* Ampicilin, gentamicin and clotrimazole were used as positive control, while DMSO was a negative control.

Table 3.2 Inhibition zones (mm/mg sample) of oil

Sample **Oil 100mg/ml**	**B.s**	**S.a**	**E.c**	**Ps.a**	**C.a**
Cassia *sieberiana*	10 10	- -	10 12	- -	- -

E.c*. Escherichia coli,* ***P.a****. Pseudomonas aerugenosa,* ***S.a****. Staphylococcus,*

B.s*. Basillus subtilis,* ***C.a****. Candida albicans.*

(inhibition zone-mm) > 18 mm : very active; 13-18 mm : moderate; 9-12mm : weak

Table 3.3 Inhibition zones of standard antibacterial agents

drug	Conc mg/ml	B.s	S.a	E.c	P.a
Ampicilin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamicin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table 3.4 Inhibition zones of standard antifungal agents

Drug	Conc mg/ml	A.n	C.a
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

3.2 Acacia seyal Del

3.2.1 The GC-MS analysis of *Acacia seyal*

Acacia seyal Del seeds oil was analyzed by GC.MS technique.The analysis showed 23 compounds (Table 3.5).

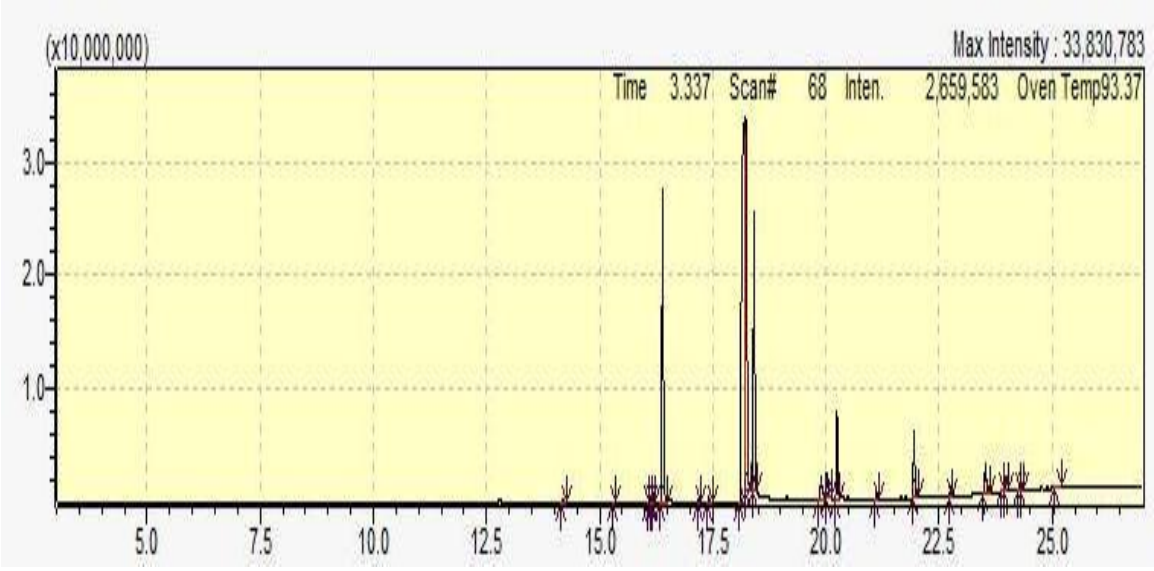

Fig. 3.5 : Total ions chromatograms

major constituents of the oil are:

i) 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester(38.25%)
ii) 9-octadecenoic acid (Z)-, methyl ester (20.59%)
iii) Hexadecanoic acid, methyl ester(15.19%)

iv) Methyl stearate (12.58%)

The mass spectrum of 9, 12-octadecadienoic acid, methyl ester is shown in Fig.3.6 The peak at m/z 294 (RT,18.200) is due to the molecular ion M^+ $[C_{19}\ H_{34}O_2]^+$, while the signal at m/z 263 is due to loss of a methoxyl group.

The mass spectrum of 9-octadecenoic acid methyl ester is presented in Fig.3.7 The signal at m/z 296 (RT.18.234) accounts for the molecular ion $M^+[C_{19}\ H_{36}O_2]^+$.the mass spectrum of hexadecanoic acid, methyl ester is shown in Fig 3.8. The peak at m/z 270 (RT.16.396) is due the molecular ion $M^+[C_{17}H_{34}O_2]^+$.

The mass spectrum of methyl stearate. The peak at m/z 298 (RT.18.416) account for $M^+[C_{19}H_{38}O_2]^+$.The peak at m/z 267 is due loss of methxyl.

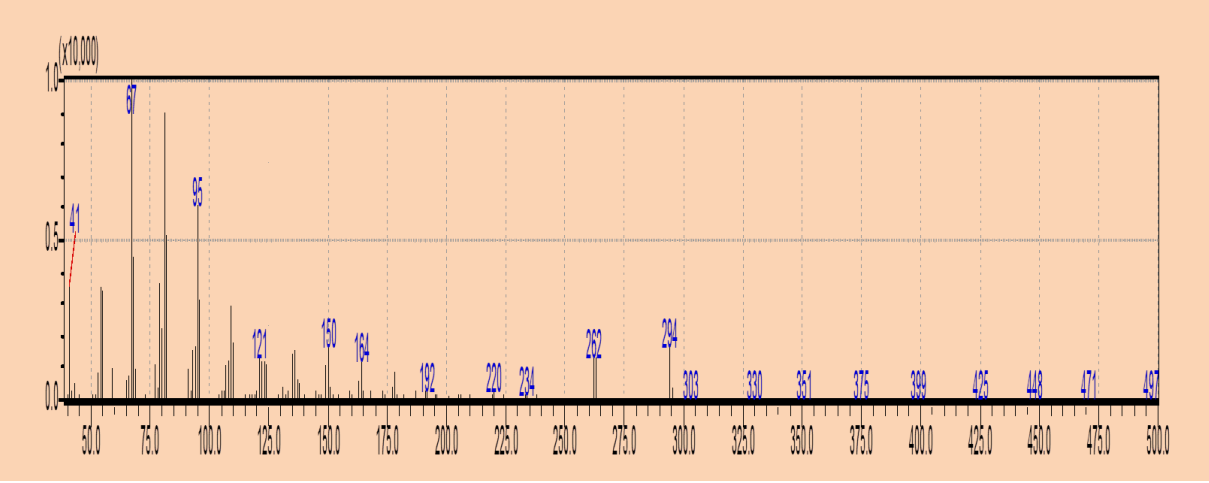

Fig.3.6: Mass spectrum of 9, 12-octadecadienoic acid methyl ester

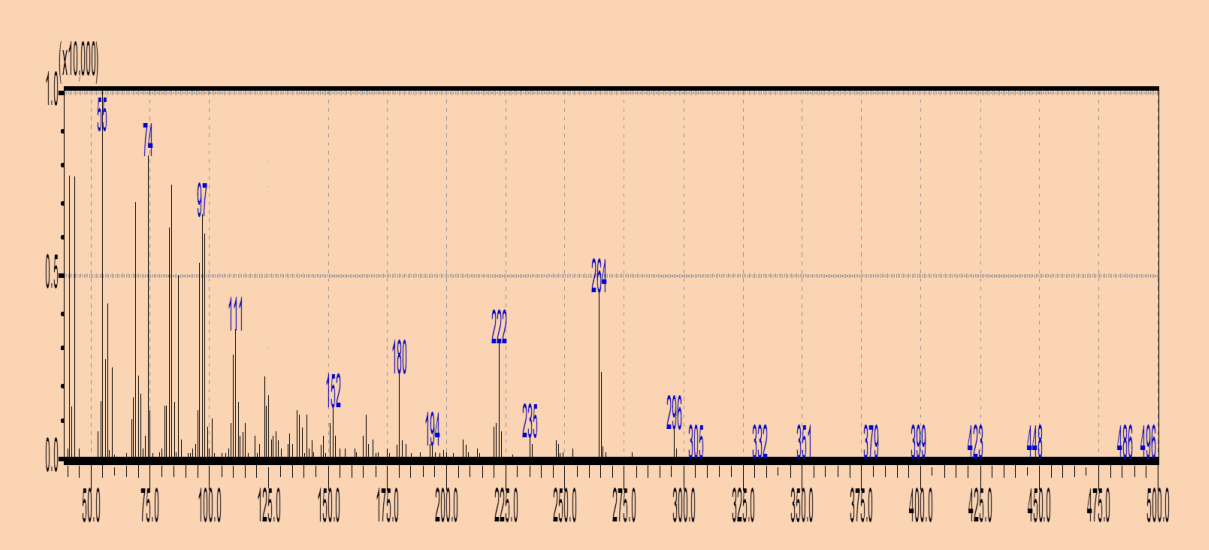

Fig.3.7. 9-octadecenoic acid methyl ester

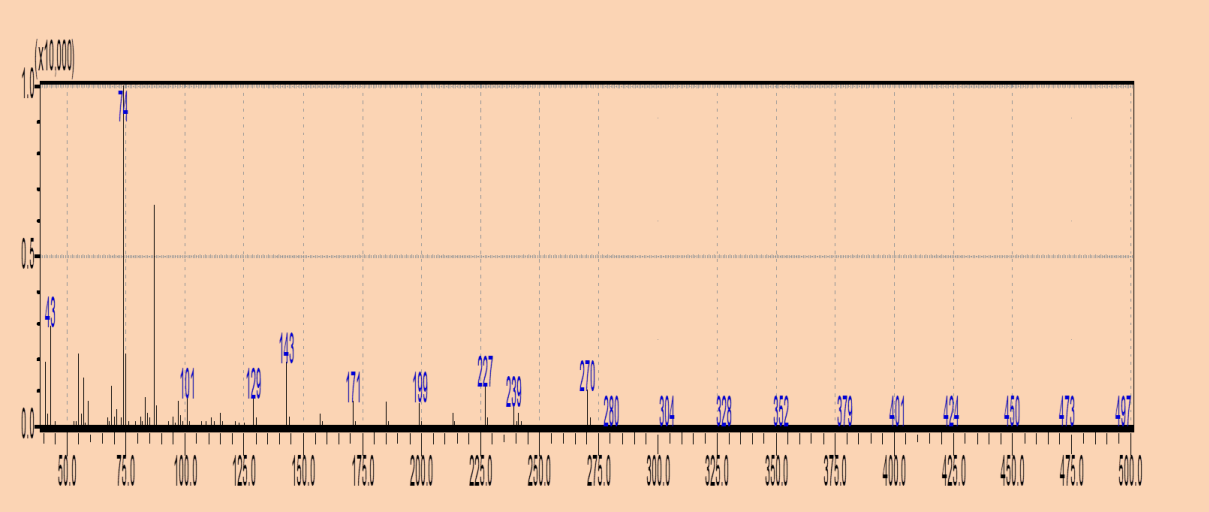

Fig. 3.8 :Mass spectrum of hexadecanoic acid, methyl ester

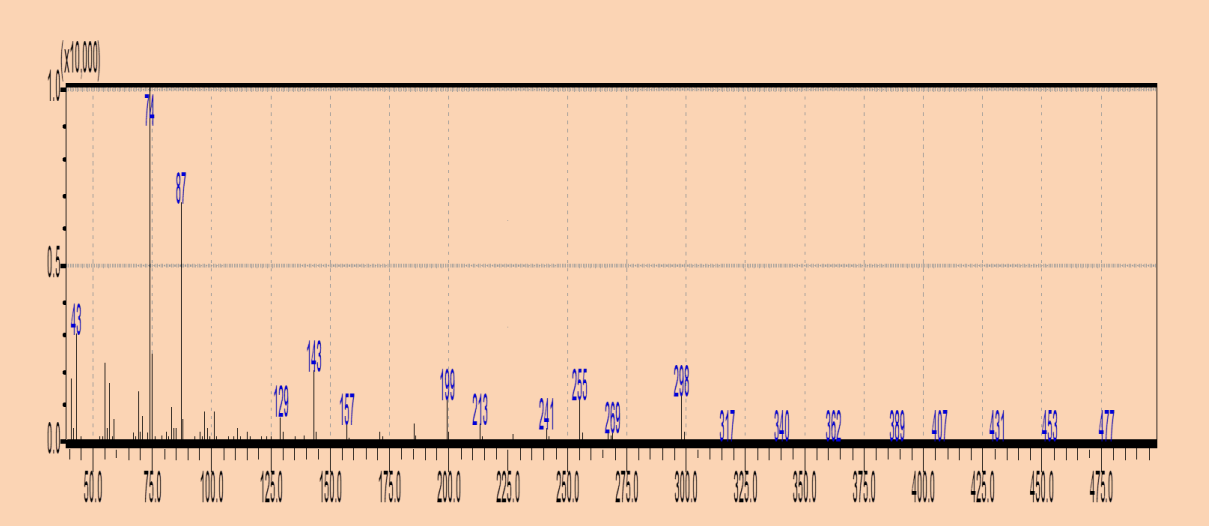

Fig 3.9: methyl stearate

Table 3.5: Constituents of the oil

No	Name	R.Time	Area%	Formula
1	Methyl tetradecanoate	14.180	0.20	$C_{15}H_{30}O_2$
2	Pentadecanoic acid, methyl ester	15.306	0.05	$C_{16}H_{32}O_2$
3	7,10-Hexadecadienoic acid, methyl ester	16.079	0.03	$C_{17}H_{30}O_2$
4	7-Hexadecenoic acid, methyl ester, (Z)-	16.139	0.11	$C_{17}H_{32}O_2$
5	9-Hexadecenoic acid, methyl ester, (Z)-	16.184	0.32	$C_{17}H_{32}O_2$
6	Hexadecanoic acid, methyl ester	16.396	15.19	$C_{17}H_{34}O_2$
7	cis-10-Heptadecenoic acid, methyl ester	17.199	0.16	$C_{18}H_{34}O_2$
8	Heptadecanoic acid, methyl ester	17.412	0.23	$C_{18}H_{36}O_2$
9	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.200	38.23	$C_{19}H_{34}O_2$
10	9-Octadecenoic acid (Z)-, methyl ester	18.234	20.59	$C_{34}H_{66}O_2$
11	Methyl stearate	18.416	12.58	$C_{19}H_{38}O_2$
12	Z,Z-3,13-Octadecadien-1-ol	19.892	1.31	$C_{18}H_{34}O$
13	cis-11-Eicosenoic acid, methyl ester	20.039	1.28	$C_{21}H_{40}O_2$
14	Eicosanoic acid, methyl ester	20.244	3.43	$C_{21}H_{42}O_2$
15	Heneicosanoic acid, methyl ester	21.112	0.21	$C_{22}H_{44}O_2$
16	Docosanoic acid, methyl ester	21.946	3.19	$C_{23}H_{46}O_2$
17	Tricosanoic acid, methyl ester	22.751	0.38	$C_{24}H_{48}O_2$
18	Tetracosanoic acid, methyl ester	23.525	1.79	$C_{25}H_{50}O_2$
19	.gamma.-Sitosterol	23.916	0.13	$C_{29}H_{50}O$
20	.beta.-Sitosterol	23.968	0.14	$C_{29}H_{50}O$
21	Pentacosanoic acid, methyl ester	24.279	0.08	$C_{26}H_{52}O_2$
22	Squalene	24.315	0.10	$C_{30}H_{50}$
23	Hexacosanoic acid, methyl ester	25.104	0.27	$C_{27}H_{54}O_2$

3.2.2 Antimicrobial activity

The studied oil was assessed for antimicrobial activity against five standard microbial strain. The inhibition zones are shown in table (3.6)Ampicilin, gentamicin and clotrimazle were used as positive control. The oil exhibited weak activity against *Bacillus subtilis*.

Table 3.6 Inhibition zones (mm/mg sample) of oil

Sample **Oil 100mg/ml**	**B.s**	**S.a**	**E.c**	**Ps.a**	**C.a**
	11	-	-	-	-
Acacia seyal Del	10	-	-	-	-

E.c*. Escherichia coli****,*** ***P.a****. Pseudomonas aerugenosa,* ***S.a****. Staphylococcus,* ***B.s****. Basillus subtilis,* ***C.a****. Candida albicans.*

(inhibition zone-mm) > 18 mm : very active; 13-18 mm : moderate; 9-12mm : weak

3.3 Vitex doniana

3.3.1 The GC-MS analysis of *Vitex doniana*

The qualitative and quantitative analysis of *Vitex doniana* oil was carried out by GC-MS.The GC-MS analysis revealed the presence of 13 components (3.7). Fatty acids constitute 99.72% of the bulk of the oil, while squalene (0.13%) and β-sitosterol (0.15%) appeared as minor constituents. The oil was dominated by9-octadecenoic acid (Z), methyl ester (35.51%) 9,12Octadecadienoic acid (Z, Z)-, methyl ester (24.17%) Hexadecanoic acid, methyl ester(17.92%) Methyl stearate (12.21%).

The mass spectra of the major constituents of the oil presented in figures (11-14). The molecular ion of 9-octadecenoic acid (Z), methyl ester $[C_{19}H_{36}O_2]^+$; 9,12Octadecadienoicacid (Z, Z)-, methyl ester $[C_{19}H_{34}O_2]^+$; Methyl stearate $[C_{19}H_{38}O_2]^+$; and Hexadecanoic acid, methyl ester $[C_{17}H_{34}O_2]^+$; appeared as expected at m/z 296; m/z 294; m/z 298 and m/z 270 respectively. These components hav retentions time : 16.839,16.769,17.014and 15.096 respectively.

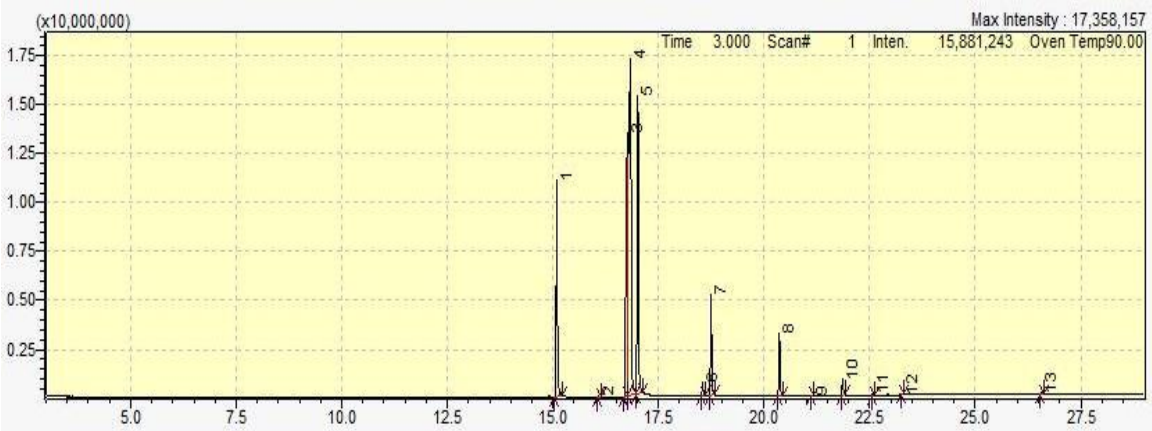

Fig. 3.10: Total ions chromatograms

Table 3.7: Constituents of the oil

No	Name	R. Time	Area%	Formula
1	Hexadecanoic acid, methyl ester	15.040	12.21	$C_{17}H_{34}O_2$
2	Heptadecanoic acid, methyl ester	16.045	0.07	$C_{18}H_{36}O_2$
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.685	24.17	$C_{19}H_{34}O_2$
4	9-Octadecenoic acid (Z)-, methyl ester	16.785	35.52	$C_{19}H_{36}O_2$
5	Methyl stearate	16.960	17.92	$C_{19}H_{38}O_2$
6	cis-11-Eicosenoic acid, methyl ester	18.505	0.69	$C_{21}H_{40}O_2$
7	Eicosanoic acid, methyl ester	18.700	5.04	$C_{21}H_{42}O_2$
8	Docosanoic acid, methyl ester	20.320	3.19	$C_{23}H_{46}O_2$
9	Tricosanoic acid, methyl ester	21.110	0.02	$C_{24}H_{48}O_2$
10	Tetracosanoic acid, methyl ester	21.820	0.85	$C_{25}H_{50}O_2$
11	Squalene	22.545	0.12	$C_{30}H_{50}$
12	Hexacosanoic acid, methyl ester	23.235	0.05	$C_{27}H_{54}O_2$
13	.beta.-Sitosterol	26.540	0.15	$C_{29}H_{50}O$

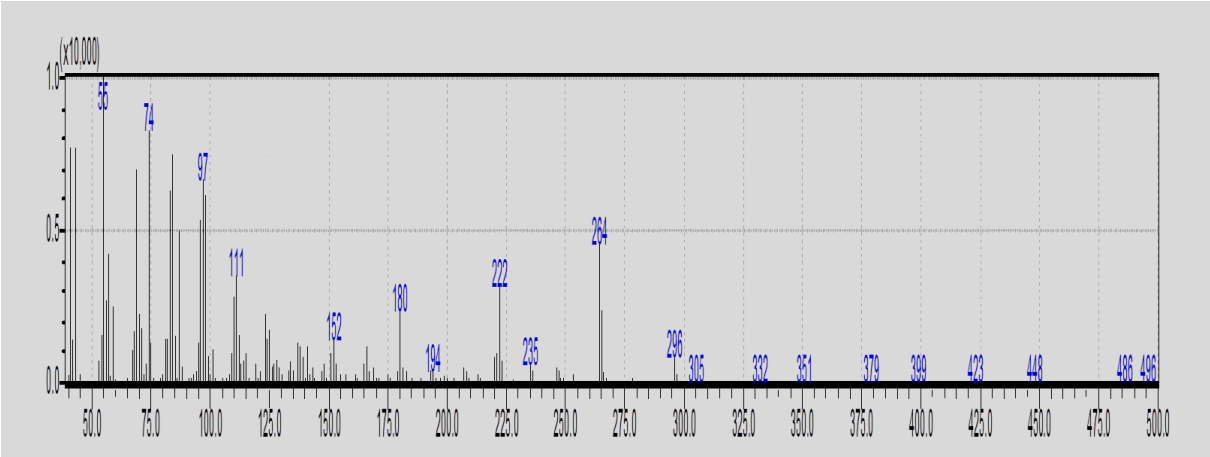

Fig.3.11: 9-octadecenoic acid (Z)-, methyl ester

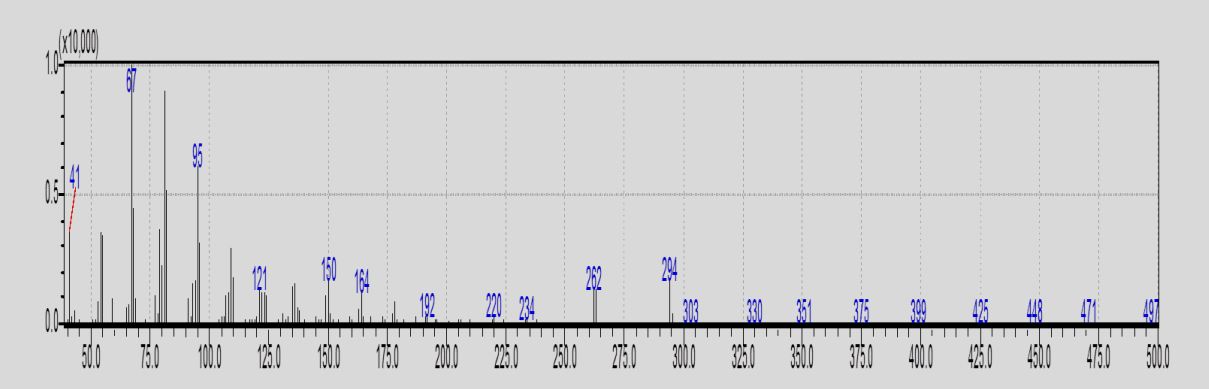

Fig.3.12 : Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

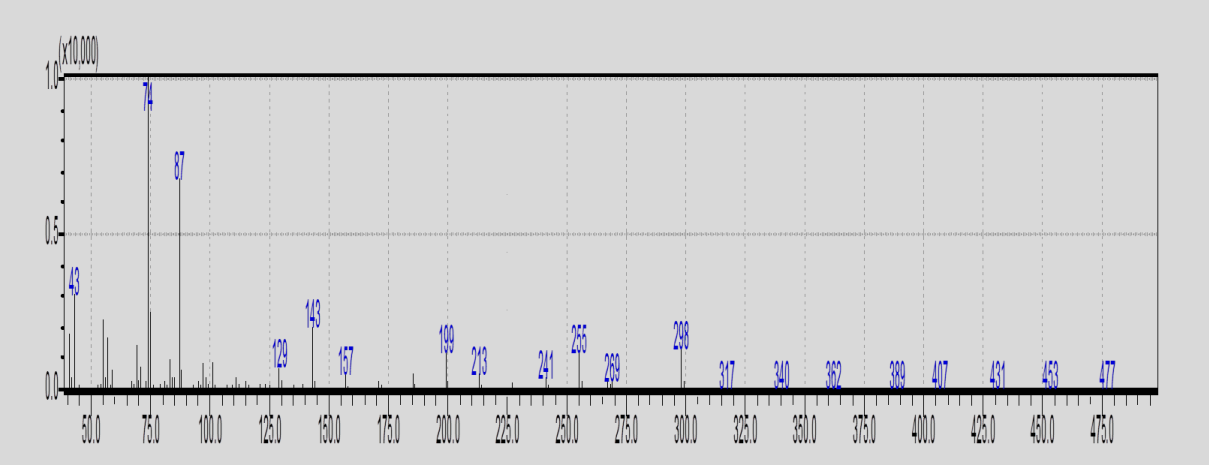

Fig.3.13: methyl stearate

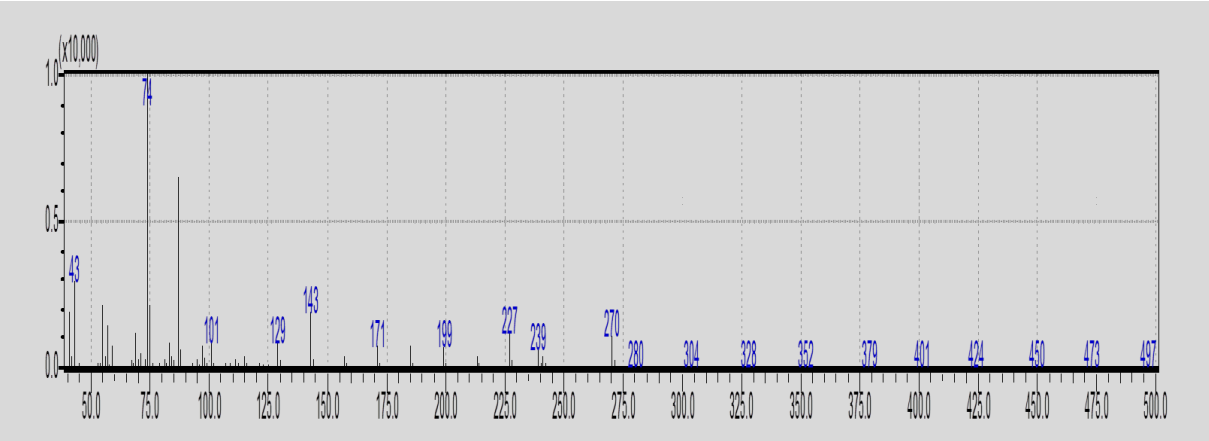

Fig. 3.14 :Mass spectrum of hexadecanoic acid, methyl ester

3.3.2 Antimicrobial activity

The antimicrobial activity of the oil was assyed and the average of the diameter of the inhibition zones were measured.Table (3.8). At a concentration of 100mg/ml, the oil was tested against. *Escherichia coli***,** *Pseudomonas aerugenosa, Staphylococcus, Basillus subtilis,* and *Candida albicans. Vitex doniana* oil showed significant activity against *Escherichia coli* . It also exhibited moderate activity against *Staphylococcus aureus. Basillus subtilis*, *Pseudomonas aerugenosa* and *candida albicans*. Ampicillin, gentamicin, and clotrimazole were used as positive control.

Table 3.8 Inhibition zones (mm/mg sample) of oil

Sample **Oil 100mg/ml**	**B.s**	**S.a**	**E.c**	**Ps.a**	**C.a**
Vitex doniana	13 15	12 16	15 17	12 14	12 13

E.c*. Escherichia coli****,*** ***P.a****. Pseudomonas aerugenosa,* ***S.a****. Staphylococcus,* ***B.s****. Basillus subtilis,* ***C.a****. Candida albicans.*

(inhibition zone-mm) > 18 mm : very active; 13-18 mm : moderate; 9-12mm : weak

3.4 *Ziziphus spina Christi*

3.4.1 GC-MS analysis of *Ziziphus spina Christi*

Eighteen constituents have been detected in *Ziziphus spina christi* by GC-MS analysis (Table 3.9).The total ion chromatograms is presented in Fig.3.15

Table 3.9: Constituents of the oil

No	Name	R. Time	Area%	Formula
1	Methyl tetradecanoate	14.186	0.16	$C_{15}H_{30}O_2$
2	7-Hexadecenoic acid, methyl ester, (Z)-	16.141	0.06	$C_{17}H_{32}O_2$
3	9-Hexadecenoic acid, methyl ester, (Z)-	16.186	0.11	$C_{17}H_{32}O_2$
4	Hexadecanoic acid, methyl ester	16.398	14.07	$C_{17}H_{34}O_2$
5	cis-10-Heptadecenoic acid, methyl ester	17.204	0.07	$C_{18}H_{34}O_2$
6	Heptadecanoic acid, methyl ester	17.416	0.16	$C_{18}H_{36}O_2$
7	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.190	22.60	$C_{19}H_{34}O_2$
8	9-Octadecenoic acid (Z)-, methyl ester	18.256	34.71	$C_{19}H_{36}O_2$
9	Methyl stearate	18.420	11.01	$C_{19}H_{38}O_2$
10	cis-11-Eicosenoic acid, methyl ester	20.047	5.43	$C_{21}H_{40}O_2$
11	Eicosanoic acid, methyl ester	20.249	5.16	$C_{21}H_{42}H_2$
12	Heneicosanoic acid, methyl ester	21.116	0.09	$C_{22}H_{44}O_2$
13	13-Docosenoic acid, methyl ester, (Z)-	21.775	0.12	$C_{23}H_{44}O_2$
14	Docosanoic acid, methyl ester	21.954	3.99	$C_{23}H_{46}O_2$
15	Tricosanoic acid, methyl ester	22.761	0.18	$C_{24}H_{48}O_2$
16	Tetracosanoic acid, methyl ester	23.539	1.38	$C_{25}H_{50}O_2$
17	Squalene	24.324	0.50	$C_{30}H_{50}$
18	Hexacosanoic acid, methyl ester	25.116	0.20	$C_{27}H_{54}O_2$

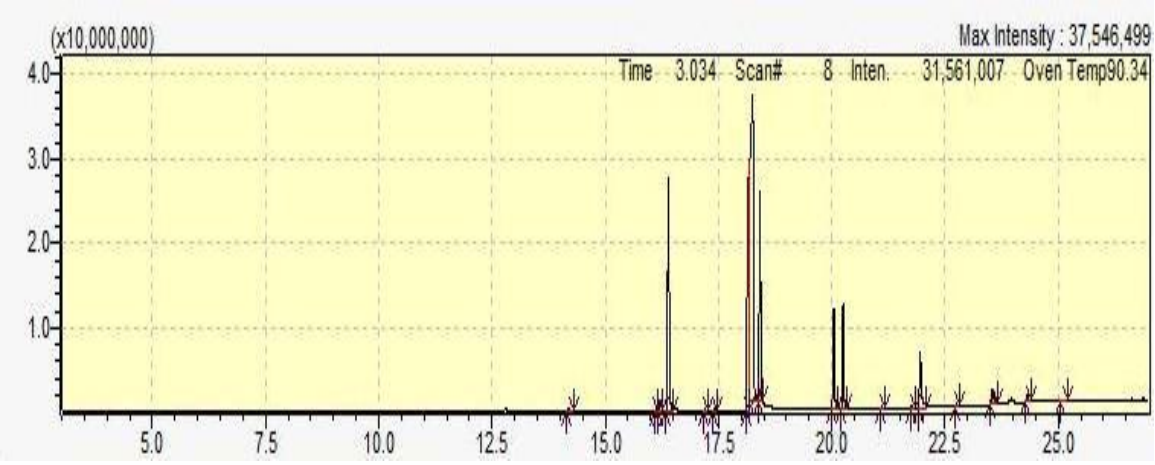

Fig 3.15: total ion chromatogram

The oil was dominated by:

i) 9-Octadecenoic acid (Z)-, methyl ester (34.71%)

ii) 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester(22.60%)
iii) Hexadecanoic acid, methyl ester(14.07%)

iv) Methyl stearate (11.01%)

In the mass spectra of 9-octadecenoic acid (Z)-, methyl ester (Fig 3.); 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (Fig.3.); Hexadecanoic acid, methyl ester (Fig.3.); Methyl stearate (Fig.3.3) the molecular ions appeared as expected at: m/z 296 (RT.18.256), m/z 294 (RT.18.190), m/z 270 (RT.16.398), m/z 298 (RT.18.420)respectively.

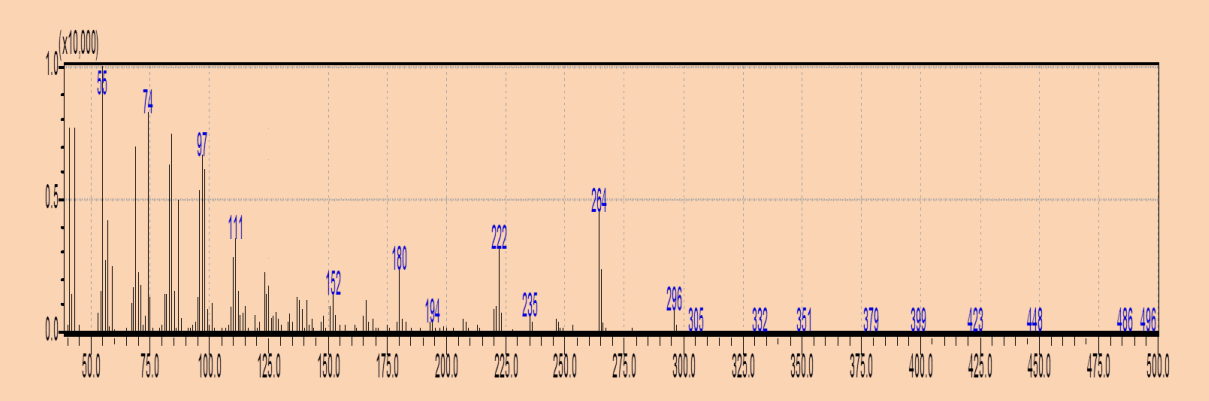

Fig.3.16. 9-Octadecenoic acid (Z)-, methyl ester

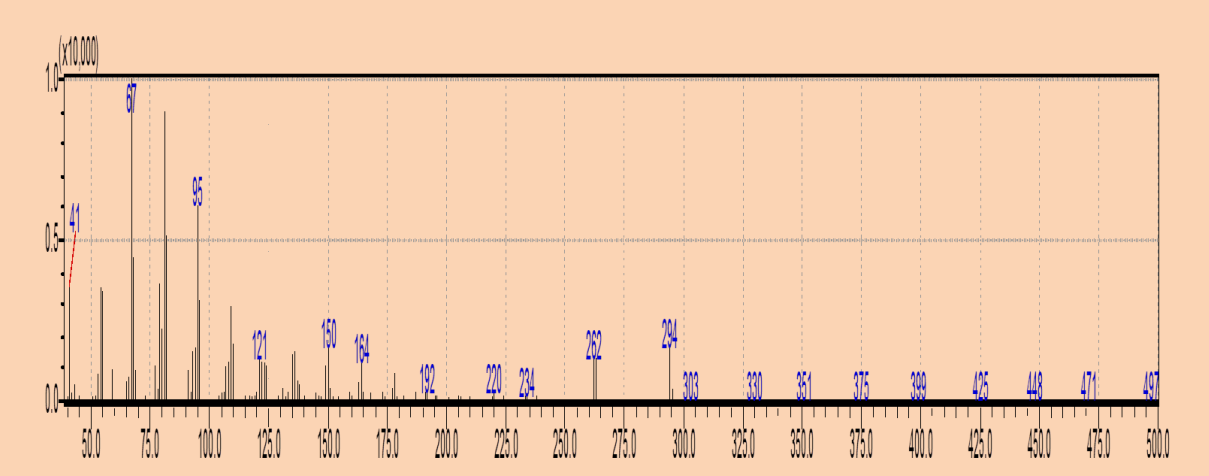

Fig.3.17: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

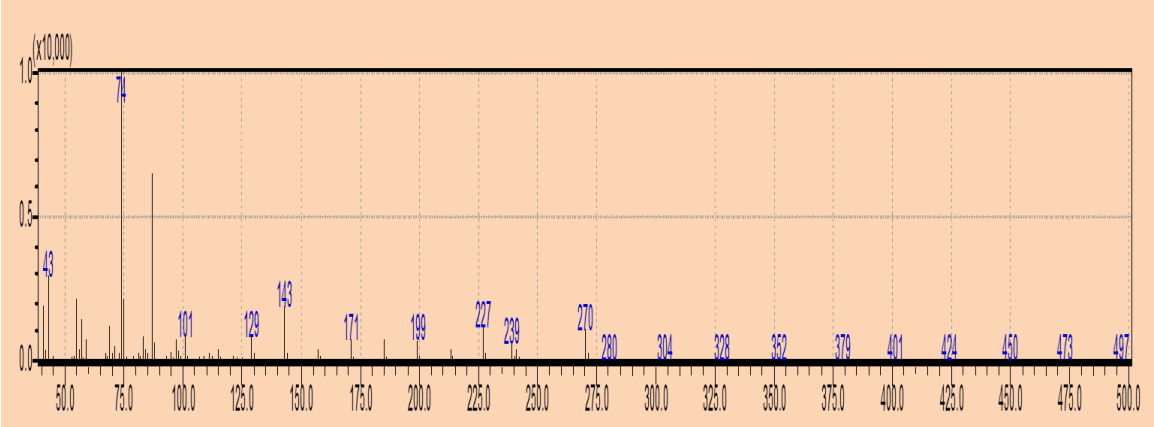

Fig. 3.18 :Mass spectrum of hexadecanoic acid, methyl ester

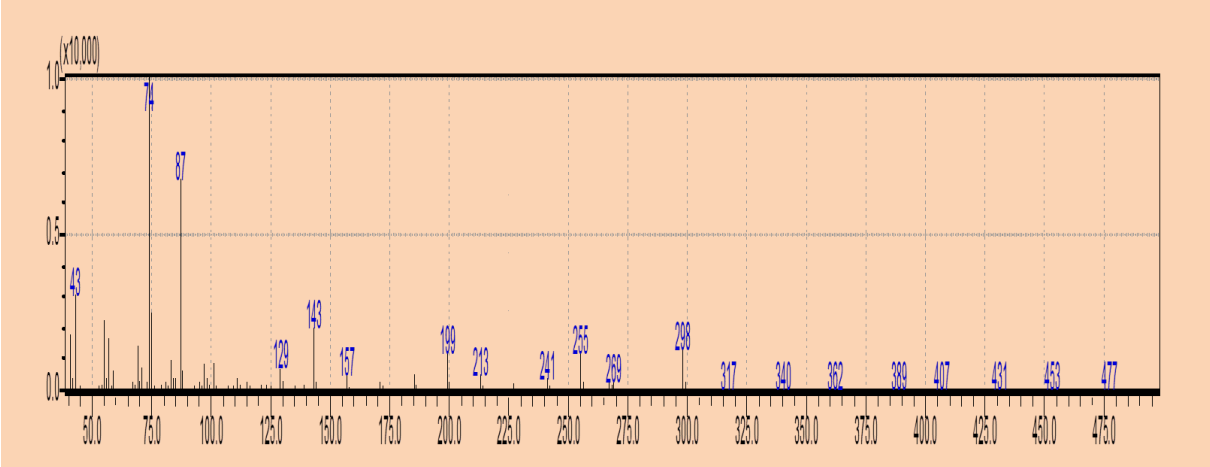

Fig 3.19: methyl stearate

3.4.2 Antimicrobial activity

Ziziphus spina christi seed oil was tested for antimicrobial activity against five standard microbial strain.The inhibition zones areshown in table 3.10. Ampicillin, gentamicin, and clotrimazole were used as positive control.The oil exhibited moderate activity against *Basillus subtilis*.

Table 3.10: Inhibition zones (mm/mg sample) of oil

Sample **Oil 100mg/ml**	**B.s**	**S.a**	**E.c**	**Ps.a**	**C.a**
Ziziphus spina	15	-	-	-	-
christi	15	-	-	-	-

E.c. *Escherichia coli***, *** ***P.a***. *Pseudomonas aerugenosa*, ***S.a***. *Staphylococcus*,

B.s. *Basillus subtilis*, ***C.a***. *Candida albicans.*

(inhibition zone-mm) > 18 mm: very active; 13-18 mm: moderate; 9-12mm: weak

3.5 Kigelia africana

3.5.1 The GC-MS analysis of *Kigelia africana*

Kigelia africana seeds oil was analyzed by GC.MS technique.The analysis revealed the presence of 16 components (Table 3.11)

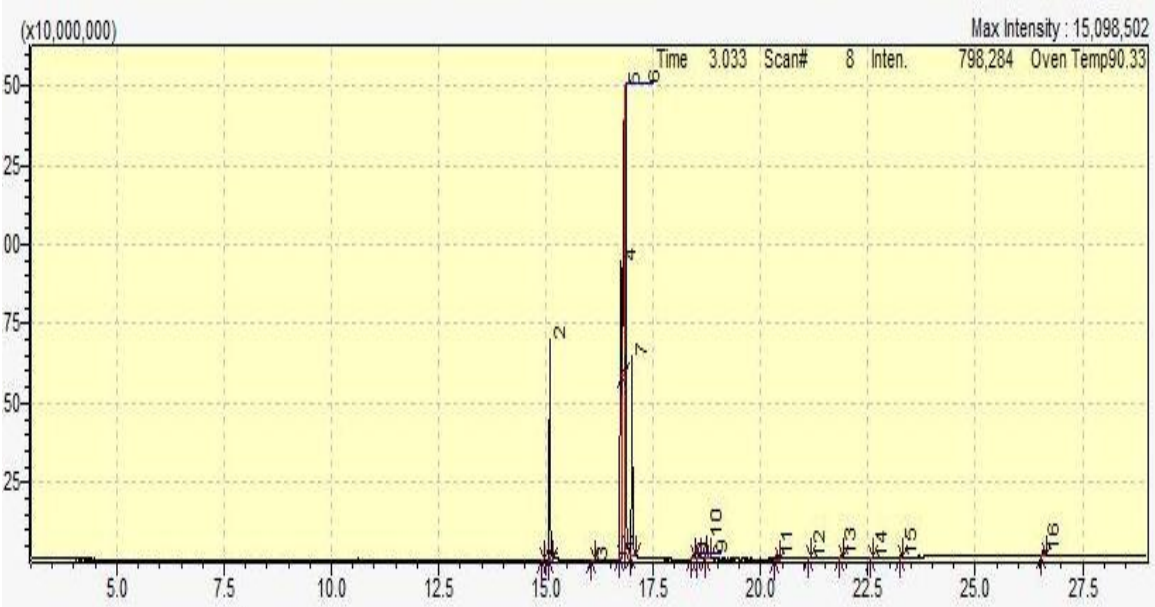

Fig:3.20: total ion chromatograms

Table 3.11: Constituents of the oil

No	Name	R. Time	Area%	Formula
1	9-Hexadecenoic acid, methyl ester, (Z)-	14.885	0.04	$C_{17}H_{32}O_2$
2	Hexadecanoic acid, methyl ester	15.045	12.94	$C_{17}H_{34}O_2$
3	Heptadecanoic acid, methyl ester	16.040	0.06	$C_{18}H_{36}O_2$
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	16.700	26.42	$C_{19}H_{34}O_2$
5	9-Octadecenoic acid (Z)-, methyl ester	16.775	22.93	$C_{19}H_{36}O_2$
6	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	16.840	22.74	$C_{19}H_{32}O_2$
7	Methyl stearate	16.965	11.51	$C_{19}H_{38}O_2$
8	11,14,17-Eicosatrienoic acid, methyl ester	18.385	0.42	$C_{21}H_{36}O_2$
9	cis-11-Eicosenoic acid, methyl ester	18.515	0.31	$C_{21}H_{40}O_2$
10	Eicosanoic acid, methyl ester	18.715	1.41	$C_{21}H_{42}O_2$
11	Docosanoic acid, methyl ester	20.320	0.35	$C_{23}H_{46}H_2$
12	Tricosanoic acid, methyl ester	21.105	0.03	$C_{24}H_{48}O_2$
13	Tetracosanoic acid, methyl ester	21.830	0.24	$C_{25}H_{50}O_2$
14	Hexacosanoic acid, methyl ester	22.555	0.04	$C_{27}H_{54}O_2$
15	Heptacosanoic acid, methyl ester	23.235	0.07	$C_{28}H_{56}O_2$
16	.beta.-Sitosterol	26.530	0.49	$C_{29}H_{50}O$

major constituents of the oil are

i)9, 12-Octadecadienoic acid (Z, Z)-, methyl ester(26.43%)

ii) 9-Octadecenoic acid (Z)-, methyl ester (22.93%)

iii) 9,12,15- Octadcatrienoic acid, methyl ester (22.74%)

iv) Hexadecanoic acid, methyl ester(12.94)%

v) Methyl stearate (11.51%)

The mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester is shown in Fig.3.21. The peak at m/z 294 with retention time 16.750 corresponds to the molecular ion $M^+[C_{19}\ H_{34}O_2]^+$ while the signal at m/z 263 is due to loss of a methoxyl group.The mass spectrum of 9-octadecenoic acid (Z)-, methyl ester is shown in Fig.3.22. The peak at m/z 296 with retention time 16.836 accounts for the molecular ion $M^+[C_{19}\ H_{36}O_2]^+$.The mass spectrum of

this compound (Fig:3.23) illustrated at the signal m/z 292 with retention time 16.853.this due to molecular weight 292.The peak at m/z 261 represent the loss of methoxyl. Fig. 3.24 presents the mass spectrum of hexadecanoic acid, methylester. The peak at m/z 2 70 with retention time 15.089 is due the molecular ion $M^{+}[C_{17}H_{34}O_{2}]^{+}$Fig.3.25 shows the mass spectrum of methyl stearate. The signalat m/z 298 (retention time:17.003) is due to the molecular ion $M^{+}[C_{19}H_{38}O_{2}]^{+}$.The peak at m/z 267 is due to loss of a methoxyl.

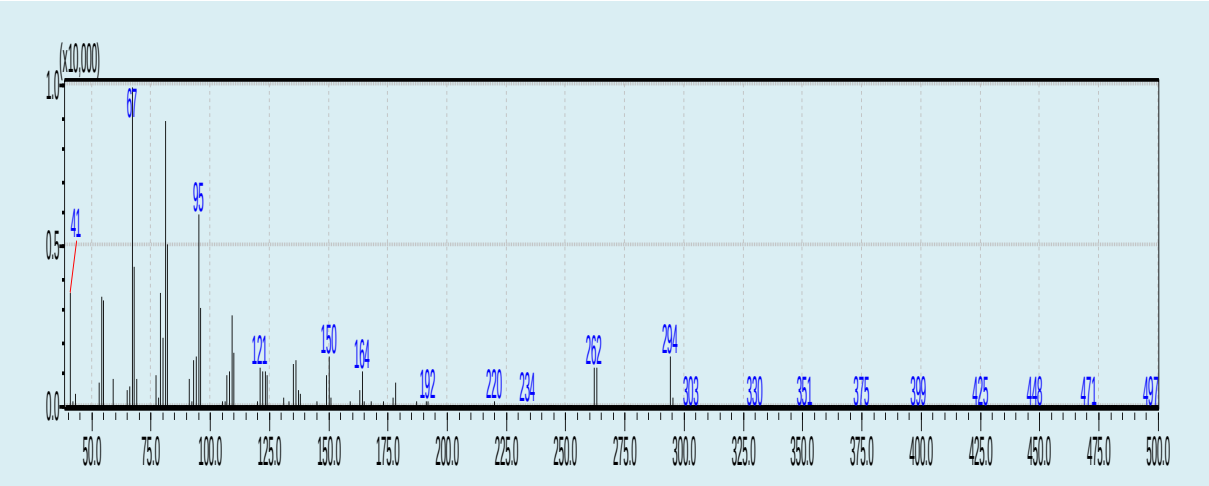

Fig.3.21: Mass spectrum of 9, 12-octadecadienoic acid (Z, Z)-, methyl ester

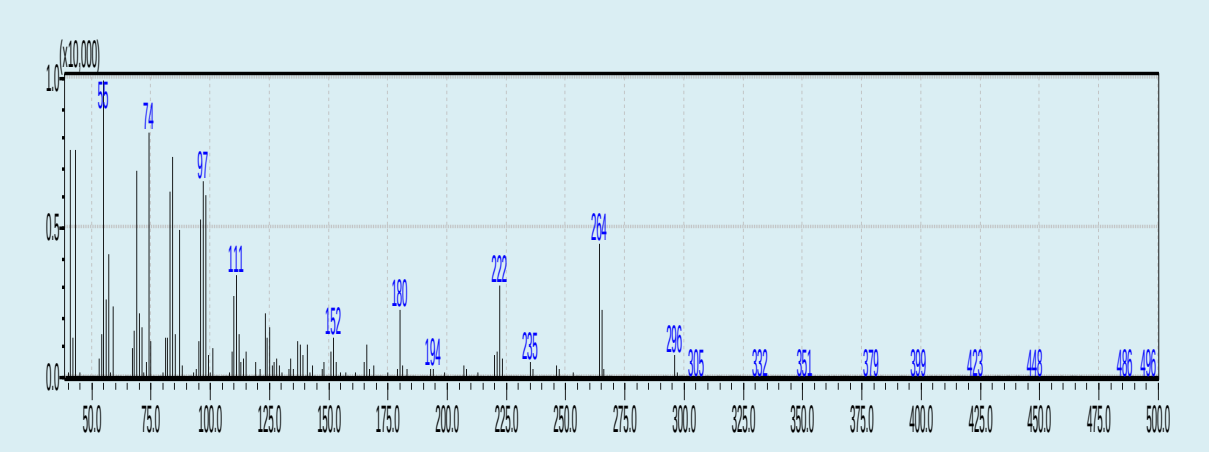

Fig.3.22: 9-octadecenoic acid (Z)-, methyl ester

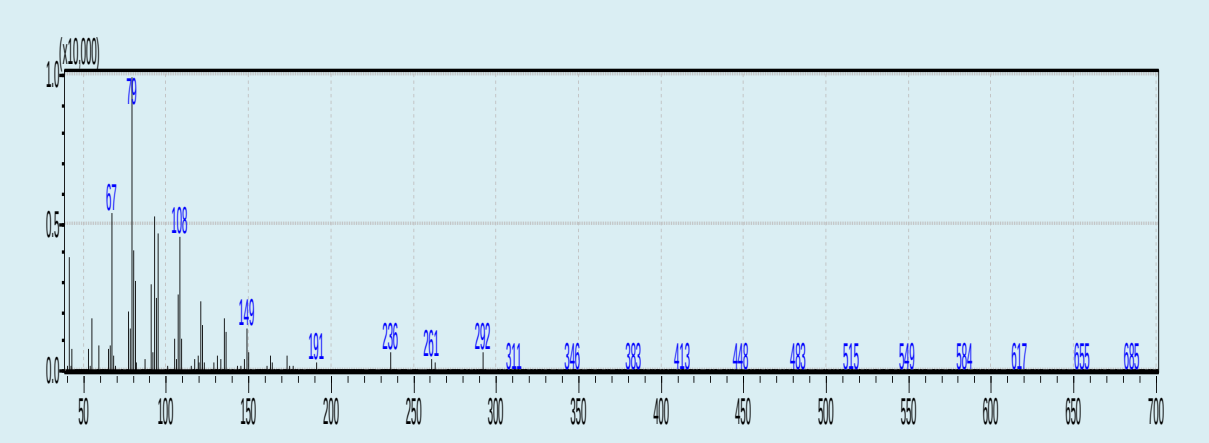

Fig:3.23: 9,12,15- Octadcatrienoic acid, methyl ester

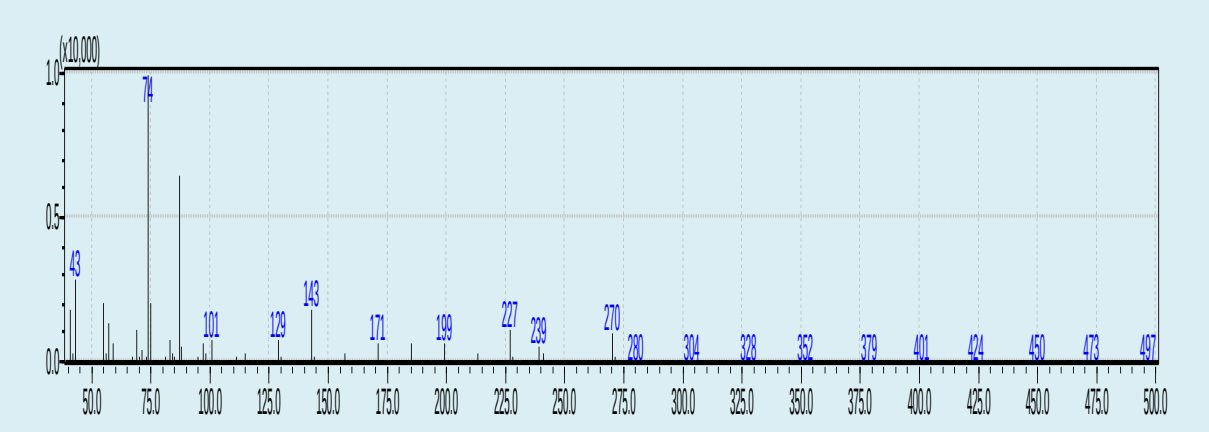

Fig.3.24: Mass spectrum of hexadecanoic acid, methyl ester

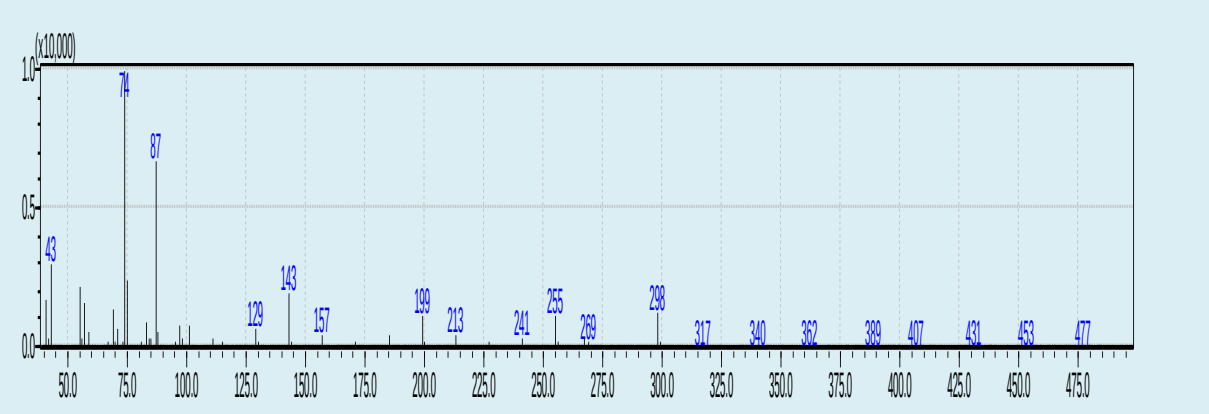

Fig:3.25. methyl stearate

3.5.2 Antimicrobial activity

Kigelia Africana seed oil was evaluated for antimicrobial activity against five standard human pathogens. The results are presented in table 3.12. The oil showed moderate activity against *Staphylococcus*

aureus and *Basillus subtilis*. Ampicilin, gentamicin and clotrimazole were used as positive control, while DMSO was a negative control.

Table 3.12: Inhibition zones (mm/mg sample) of oil

Sample **Oil 100mg/ml**	**B.s**	**S.a**	**E.c**	**Ps.a**	**C.a**
Kigelia africana	15 16	14 15	12 18	9 13	10 13

E.c*. Escherichia coli****, P.a****. Pseudomonas aerugenosa, S.a****. Staphylococcus, B.s****. Basillus subtilis, C.a****. Candida albicans.*

(inhibition zone-mm) > 18 mm : very active; 13-18 mm : moderate; 9-12mm : weak

Conclusion

This study was designed to contribute to knowledge in the field of evidence-based complementary medicine. The oils from the seeds of *Cassia sieberiana, Acacia seyal, Vitex doniana, Kigelia Africana* and *Ziziphus spina Christi* have been studied to establish evidence- based uses in folklore medicine.The oils were extracted by maceration with n-hexane and the constituents of the oils have been characterized by the hyphenated technique: GC-MS. Furthermore, the antimicrobial activity of the oils has been evaluated against five standard microbial strain: *Escherichia coli***,** . *Pseudomonas aerugenosa, Staphylococcus, Basillus subtilis,* and *Candida albicans*.The studied oils exhibited different antimicrobial responses.

Recommendations

The following future studies are recommended:

-The oils of the studied plants may be evaluated for some pharmacological effects including: antimlarial, antiviral, antidiabetic and antihypertensive activities.

-The bioactive molecules of target species, like steroids, alkaloids and terpenoids may be isolated and their structures elucidated by spectral tools.

REFERENCES

References

1-Saxena M, saxena J, Nema R, Singh D, Gupta A. Phytochemistry of medicinal plants. *Jornal of pharmacognosy and phytochemistry*. I(6): 168 - 182(2013).

2-Rao N: Phytochemicals in Indian foods and their potential in health promotion and diseases prevention- *Asia specific jornal of clinical nutrition* ; 12 (1) : 9 - 22(2003).

3- Caputi L, Aprea E. Use of terpenoids as natural flavouring compounds in food industry. Recent patents on food nutrition and agriculture . 24: 9-16(2011).

4-Djilani A, Dicko A. The therapeutic benefits essential oils In: Bouayed J, Bohn T, editors. Nutrition, Well - being and health – vol.7.Rijeka: Intech open; pp 155 – 179(2012.).

5- Mekm sonwa M -. Isolation and structure elucidation of essential oils constituents: comparative study of the oils of cyperus alopecuroides cyperus papyrus, and cyperus rotundus. (2000)

6- Turek C, Stintzing FC.. Stability of essential oils: A review comperhensive Revies in food Science and food safty; 12 (1): 40 - 53(2013).

7- Kumari S, Pundhir S, Priya P, jeena G, Punetha A, Chawlak, et al. .Essential oils reflecting terpene composition and variability in the plants kingdom.Database; 14: 1- 12.(2014)

8- Costa MA, Zia ZQ Davin LB, lewis NG. Chapter four toward engineeri-ng the metabolic pathways of cancer - preventing lignans in cereal grains and other crops. In: Romeo JT,editor. Recent

Advances in phytochemicals in human health protection nutrition, and plant defense vol- 33.New York:Springer Science - Business Media kluwer acadmic Publishers; pp. 67-87(1999).

9-Surburg H, Panten J.Common fragrance and flavor materials preparation, properties and uses- 6th ed. Germany: Wiley -VCH verlag Gmb H Co; pp. 84- 85(2016).

10- Nicolova I, Georgieva N.Effect of biological products on the population of a phids and chemical components in alfalfa, *Banat's Journal of Biotechnology* .9 (19), 38 – 46(2018).

11- Rahimian Y, Akbari SM, karami M, Fafghani M.. Effect of deffe-rent levels of fenugreek powder supplementation on performance, Influenza, sheep red blood cell, new castle diseases anti body titer and intestinal microbial flora on cobb 500 broiler chicks, Banat's Journal of Biotechnolo-gy 9 (19), 29 -37(2018).

12- Gupta V, Mittal P, Bansal P, khokra SL, Kaushik D.. Pharmacological potential of Matricaria reticula. International journal of pharmaceutical and Drug research.2: 67 – 71(2010).

13-Skold M, Karlberg At, Matura M, Borje A.The fragrance chemical β- caryophyllene- air oxidation and skin sensitization. Food and chemical toxicology; 44: 538-545(2006).

14- Bakkali F, Averbeck S, Averdeck D, Idaomar M. Microbial effects of essential oils, A review. Food and chemical toxicology.; 46: 446-475(2008).

15-Palazzolo E, Laudicina VA, Germana MA. Current and potential use of citrus essential oils. Current organic chemistry-;17: 3042-3049(2013).
16-Raut JS, Karuppayil SM. A status review on the medicinal properties of essential oils- Industerial crops; 62: 250- 264(2014).
17- Chlodwin F, Novak J.. Sources of essential oils.In: KHCB, Buch-bauer G, editors. Handbook of essential oils:Science technology and Applications London, UK: Taylor and francis Group(2010).
18- Evans WC.In: Trease and Evans, pharmacognosy E- book. Elsevier health Sciences(2009).
19- Franz. C, Novak J.. Sources of essential oils, hand book of essential oils Science, Technology, and Application,CRC Press, new York(2009).
20- Dreger M, wielgus K. Application of essential oils as natural cosmetic preservatives- Herba polonica; 59: 142- 156(2013).
21- Edris AE.. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents- a review- phytother. Res.;21(4): 308 – 23(2007).
22- Lawless J.The encyclopedia of essential oils: the complete guide to the use of aromatic oils in aromatherapy, Herbalism and well being: conari press, new York(2013).
23- Buchbauer G. Biological activities of essential oils- Handbook of essential oils: Science, technology, and application; 235-280(2009).

24- Boehm K, Bussing A, Ostermann T. A romatherapy as an adjuv-ant treatment in cancer- care - a descriptive systematic review. Afrj tradit complement altern Med, 9(4): 503 -18(2012).

25- Adorjan B, Buchbauer G. Biological properties of essential oils: an updated review- *flavours and fragrance journal*; 25(6): 407-26(2010).

26- Adlord ERK. Husnu can Baser Gerhard Buchbauer (EDs): Hand book of essential oils . Science, Technology and Applications. Chromatographia-;79(11-12): 791(2016).

27-Lopez- Reyes JG- Spadaro D, Prelle A, Garibaldi A, Gullino ML. Efficacy of plant essential oils on postharvest control of rots caused by fungi on different stone fruits in vivo- J Food prot; 7 (4):631-9(2013).

28- Lang G, Buchbaue G. A review on recent research results on essential oils as antimicrobials and Antifungal. A review. *Flavour and fragrance journal*; 27 (1): 13- 39(2010).

29- Dandlen SA, Lima AS, Mendes MD, Miguel MG, faleiro ML, Sousa MJ, . *Antioxidant activity of six Portuguese thyme species essential oils flavor and fragrance journal*; 25(3):150-5(2010).

30- Hyldgaard M, Mygind T, Meyer RL. Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Front Microbial- ;3 : 12(2012).

31-Burt S. Essential oils -their antimicrobial properties and potential applications in foods- a review-Int J Food Microbial;94(3):223-53(2004).

32- Jalali- Heravi M, Parastar H, Sereshti H. Towards obtaining more information for gas chromatography- mass spectrometric data of essential oils: an overview of mean field independent components analysis. J Chromatogr A.; 1217 (29): 4850-61(2010).
33- Remington JP. Rimington: The science and practice of pharmacy.Lippincott Williams and wilkins(2006).
34- BewleyJD, Black MJ, Halmer P. The encyclopedia of seeds technology and uses. CABi Puplishing(2006).
35-Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo, DevDutt Rakesh.. Extraction Technologies for Medicinal and Aromatic Plants. ICU- UNIDO, AREA Science park. Padriciano 99, 34012, Trieste, Italy(2008).
36- Bassole IHN and HR Juliani. Essential oils in combination and their antimicrobial properties. Molecules 17: 3989-4006(2012).
37- Xirley Pereira Nunes, Fabricio souza silva, Jackson Roberto Guedes da S Almeida, Julianeti Tolentino delima, luciano Aguusto de Araujo Ribeiro, Biological oxidations and antioxidant activity of natural products, in phytochemicals as nutraceuticals -Global Approches to their role in nutrition and health. Intechopen(2012.).
38- Baser KHC and F Demirci: Chemistry of essential oils flavours and fragrance:chemistry, Bioprocessing and sustainability, edited by Berge-r RG- Newyork- springer. 34-86(2007).
39- Tabanca N, Demirci B, Crockett SL, Baser KH, Wedge DE. Che-mical composition and antifungal activity of Arnica longifolia,

Aster hesperius, and chrysothamnus nauseosus essential oils- Journal of agricultural and food chemistry. 55: 8430-8435(2007.).
40- Husnuk, C Baser and F Demirci. Chemistry of essential oils, in flavours and fragrances, springer p 43-86(2007.).
41-Thimmappa R Geisler k, louveau T, O Maille P, osbourn A.Trite -rpene biosynthesis in plants ,Annual Review of plant Biology 65:22 5-257(2014)
42- Ludwiczuk A, K Skalicka and M Georgiev.Terpenoids In:Badal S, Delgoda R(eds) pharmacognosy: fundamentals, Application and strategied- Elsevier- London .P 233 -266(2017).
43- Silva J, Abebe W, Sousa SM, Duarte VG, Machado MIL, Matos FJA.. Analgesic and anti-inflammatory effects of essential oils of Eucalyp-tus. J Ethanopharmacol 89:277(2003).
44- Rassem HHA, Nour AH, yunus RM. Techniques for extraction of essential oils from plants: Areview -Australian Journal of Basic and Applied Sciences.; 10 (16):117-127(2016).
45- Harborne JB, Tomas – Bardenan FA. Ecological chemistry and Biochemistry of plant terpenoids - oxford: Clarendon(1991).
46- Bowles EJ.: The chemistry of Aromatherapeutic oils. 3rd ed. Crow's Nest and unwin; P. 236(2003).
47- Surburg H, Panten J. Common fragrance and flavor materials preparation, properties and uses- 5th ed. weiheim: Wiley VCH(2006).
48- Baharum SN, Bunawan H, GHani MA, Mustapha WA, Noor NM. Analysis of the chemical composition of the essential oils of

polygonum minus Huds.Using two dimentional gas chromatography - time- of flight mass spectrometry (GC-TOF MS). Molecules.; 15: 7006-7015(2010).

49- Loza- Ravera H.. Monoterpenes in essential oils: Biosynthesis and properties.Advance in experimental Medicine and Biology; 464:49-62(1999)

50- Mansoor K, lock wood GB. Chromatography, terpenoid. In: incyclopedia of separation Science.Academic press(2007).

51- Breitmaier E. Terpenes: flavors, fragrances, pharmaca, pheromones. John Wiley and sons(2006).

52- Swamy MK, Sudipta kumar Mohanty, uma Rani sinniah, Anuradha Maniyam. Evalution of patchouli (pogostemon cablin Benth) cultiva-rs for growth, yield and quality parameters. Journal of essential oils Beari-ng plants 18: 826- 832(2015).

53- Marin DP.. Pharmacological activity of sesquiterpene lactones. NNK. International Belgrade(2003).

54- Petrovic S, Maksimmovic Z, Kundakovic T. Prirucnik Za teorijskui parakticnu nastavu iz predmeta farmakognozija. Faculty of pharmacy, university of Belgrade(2009).

55- Isman MB, Wilson JA, Bradbury R. Insecticidal activities of commercial rosemary oils (Rosemarinus officinalis) against larvae of Pseudaletia unipuncta and trichoplusia ni. In relation to their chemical composition.pharmacutical Biology. ;46:82-87(2008).

56- Hahlbrock, k; S cheel, D. Physiology and molecular biology of phenylpropanoid metabolism. Annu.Rev. plant physiol. Plant Mol-Biol, 40, 347-369(1989).

57- Friedrich, H.. Phenylpropanoid constituents of essential oils. ,Lloydia, 39,1-7(1976).

58- Almeida RN, Agrade Fatima M, Maior FNS, souse DP.. Essential oils and their constituents: anticonvulsant activity – Molecules, 16, 2726 -2742(2011).

59- Da Silveira RDC, Andrade LN, De Oliveira RDRB, De Sousa DP. Areview on anti- inflammatory activity of phenylpropanoids found in essential oils- Molecules,19,1459- 1480(2014).

60- Tisserand, R.,Young, R., Essential Oil Safety: A Guide for Health Care Professionals. Elsevier Health. Sciences, United Kingdom(2013).

61- McGuinness, H., Aromatherapy Therapy Basics. Hodder and Stoughton, London(2003).

62- Arumugam G, M Swamy and U sinniah. Plectrathusamboinicus (Lour) Spreng:botanical phytochemical, pharmacological and nutriti -tionalsignifycance.Molecules21:369(2005).

63. Filomena Nazzaro, Florinda Fratianni, Laura De Martino,Raffaele Coppola and Vincenzo De Feo.Effect of essential oils on pathogenic bacteria. Pharmaceuticals 6: 1451-1474(2013).

64- Wang, L., C.L Weller,. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci. Technol., 17: 300-312(2006).

65- Dick, A.J., H.H.N. Starmans,. Extraction of secondary metabolites from plant material: a review. Trends Food Sci. Technol., pp: 191-197(1996).

66- Meyer-Warnod, B.,. Natural essential oils: extraction processes and application to some major oils. Perfume. Flavorist, 9: 93-10(1984)

67- Fahlbusch, Karl-Georg; Hammerschmidt, Franz-Josef; Panten, Johannes; Pickenhagen, Wilhelm Schatkowski, Dietmar; Bauer, Kurt; Garbe, Dorothea; Surburg, Horst,. "Flavors and Fragrances". Ullmann's Encyclopedia of Industrial Chemistry doi:10.1002/14356

007.a11-141(2003).

68- Rai,R. and B. Suresh,. *Indian Journal of Traditional Knowledge*, 3(2): 187-191(2004).

69- Chrissie, W.,"The Encyclopedia of Aromatherapy." Vermont: Healing Arts Press, pp: 16-21(1996).

70- Dawidowicz, A.L., E. Rado, D. Wianowska, M. Mardarowicz and J. Gawdzik,. Application of PLEfor the determination of essential oil components from Thymus Vulgaris L. Talanta, 76: 878-884(2008).

71-Harwood, Laurence, M., Moody, J. Christopher,. Experimental organic chemistry: Principles and Practice (Illustrated ed.).Wiley-Blackwell., pp: 122-125. ISBN 0-632-02017-2(1989).

72- Soxhlet, F., "Die gewichtsanalytische Bestimmung des Milchfett es". Dingler's Polytechnisches, Journal (in German), 232: 461-46(1879)

73- Michele Schantz, M., and S.B. Hawthorne,. Comparison of Supercritical Fluid Extraction and Soxhlet Extraction for the

determination of polychlorinated biphenyls in Environmental Matrix Standard Referenc Materials. *Journal of Chromatography* A, 816: 213-220(1998).

74- Arnould-Taylor, W.E., "Aromatherapy for the Whole Person." UK: Stanley Thornes, pp: 22-26(1981).

75- Geramitcioski T, Mitrevski V, Mijakovski V. Design of a small press for extracting essential oil according VDI 2221. In: IOP Conference Series: Material Science and Engineering, Novi Sad, Serbia. Vol, 393. Pp. 1-8(2018). DOI: 10.1088/1757899X/1/012131.

76- Rozzi, N.L., W. Phippen, J.E. Simon and R.K. Singh,. Supercritical fluid extraction of essential oil components from lemon-scented botanicals. Lebensm.- Wiss. U. Technol., 35: 319-324(2002).

77- Pourmortazavi, S.M. and S.S. Hajimirsadeghi,. Supercritical flui- d extraction in plant essential and volatile oil analysis. *Journal of Chromatography A*, 1163: 2-24(2007).

78- Fadel, H., F. Marx, A. El-Sawy and A. El-Gorab,. Effect of extraction techniques on the chemical compositon and antioxidant activity of Eucalyptus camaldulensis var. brevirostris leaf oils, 208: 212-216(1999).

79- Capuzzo, A., M.E. Maffei, A. Occhipinti,.S upercritical fluid extraction of plant flavors and fragrances. Molecules, 18: 7194-7238(2013).

80- Golmakani, M.T. and K. Rezaei,. Comparison of microwave-assisted hydrodistillation with the traditional hydrodistillation method in the extraction of essential oils from Thymus vulgaris L. Food Chemistry,109: 925-930(2008).

81- Brachet, A., P. Christen and J.L. Veuthey,. Focused microwave-assisted extraction of cocaine and benzoylecgonine from coca leaves. Phytochemical Analysis, 13: 162- 169(2002).

82- Mandal, V., Y. Mohan and S. Hemalatha, Microwave-assisted extraction-An innovative and promising extraction tool for medicinal plant research. Pharmacognosy Reviews, 1(1)(2007).

83- Lucchesi, M.E., F. Chemat and J. Smadja,. Original solvent free microwave extraction of essential oils from spices. *Flavor and Fragrance Journal.*, 19: 134-138(2004)

84- Ferhat, M., B. Meklati, J. Smadja and F. Chemat,."An improved microwave Clevenger apparatus for distillation of essential oils from orange peel", *Journal of Chromatography* A, 1112(1-2): 121-126(2006).

85- Farhat, A., Ginies, C. Romdhane,M and. Chemat,F."Eco-friendly and cleaner process for isolation of essential oil using microwave energy: Experimental and theoretical study", *Journal of Chromatography* A,1216(26): 5077-5085(2009).

86- Letellier, M., H. Budzinski, H. Charrier, L. Capes,S and A.M. Dorthe,. Optimization by factorial design of focused microwave-assisted extraction of polycyclic aromatic hydrocarbons from marine sediment. J. Anal. Chem., 364: 228-37(1999).

87- Bhaskaracharya, R.K., S. Kentish, M. Ashokkumar,. Selected applications of ultrasonics in food processing. Food Eng Rev., 1: 31-49(2009).

88- Vinatoru, M., An overview of the ultrasonically assisted extraction of bioactive principles from herbs. Ultrason. Sonochem, 8: 303-313(2001).

89- Garcı´a-Pe´rez, J.V., J.A. Ca´rcel, S. de la Fuente-Blanco, E. Riera-Francode Sarabia,. Ultrasonic drying of foodstuff in a fluidized bed: parametric study. Ultrasonics, 44: 539-543(2006).

90- Karim Assami, D.P.,Ultrasound-induced intensification and selective extraction of essential oil from Carum carvi L. seeds. Chem. Eng. Process. Process Intensif, 62: 99-105(2012).

91- Sereshti, H., Rohanifar, A.; Bakhtiari, S Samadi, S.Bifunctional ultrasound assisted extraction and determination of Elettaria cardamomum Maton essential oil. *Journal of Chromatography* A, 1238: 46-53. doi:http://dx.doi.org/10.1016/j. chroma.(2012).03.061.

92- Filly A, Fernandez X, Minuti M, Visioni F, Cravotto G, Chemat F.. Solvent- free microwave extraction of essential oil from aromatic herbs: From laboratory to pilot and idusterial scale. Food Chemistry. 150:193- 198(2014).

93- Kusama H, Mahfud M. Preliminary study: Kinetics of oil extraction from sandalwood by microwave-assisted hydrodistillation .*ASEAN Journal of Chemical Engineering*; 16: 62- 69(2015).

94- Abert Vian, M., X. Fernandez, F. Visinoni, F. Chemat, Microwave hydro diffusion and gravity, a new technique for extraction of essential oils. *Journal of Chromatography* A, 1190: 14-17(2008.).

95- Chemat, F., M.E. Lucchesi, Smadja,. J. Extraction sans solvant assistée par micro-ondes de produits naturels. EP Pat.,1 439 218 A1(2004).

96- Shabaan HAE, El-Ghorab AH,Shibamoto T. Bioactivity of essential oils and their volatile aroma components: Review. The *Journal of Essential Oil Research*.; 24(2):203-212(2012).

97- Moreira P, Smith MA, Zhu X, Honda K, Lee HG, Aliev G, et al. Since oxidative damage is a key phenomenon in Alzheimer's disease, treatment with antioxidants seems to be a promising approach for slowing disease profession. Oxidative damage and

Alzheimer's disease: Are antioxidant therapies useful? Drug News & Perspectives. ;18:13(2005).

98- Mimica-Dukic N, Bozin B, Sokovic M, Simin N. Antimicrobial and antioxidant activities of Melissa officinalis L. (Lamiaceae) essential oil. *Journal of Agriculture and Food Chemistry*. ;52:2485-2489(2004).

99-Yanishlieva-Maslarova N. Sources of natural antioxidants: Vegetables,fruits, herbs, spices and teas. In: Yanishlieva N, Pokorny J, Gordor M, editors. Antioxidant in food: practical application. Cambridge: Woodhead Publishing Ltd; .pp.201- 249(2001).

100- Halliwell B.The antioxidant paradox. Lancet. ;355(9210):1179-1180(2000).

101- Mimica-Dukic N, Orcic D, Lesjak M, Sibul F. Essential oils as powerful antioxidants: Misconception or scientific fact? In: Medicinal and Aromatic Crops: Production, phytochemistry, and Utilization.Washington, DC, USA: Ed ACS;.pp. 187- 208(2016).

102- Gallucci MN, Olivia M, Casero C, Dambolena J, Luna A, Zygadlo J, etal. Antimicrobial combined action of terpenes against the food-borne microorganisms Escherichia coli, Staphylococcus aureus and Bacillus cereus. *Flavour and Fragrance Journal*.;24(6):348-354(2009)

103- Pandey AK, Singh P, Tripathi NN..Chemistry and bioactivities of essential oils of some Ocimum species: An overview. *Asian Pacific Journal of Tropical Biomedicine*. ;4:682-694(2014).

104- Nascimiento MNG, Junqueira JGM, Terezan AP, Severino RP, Silva TS, Martins CHG, et al. Chemical composition and antimicrobial activity of essential oils from Xylopia aromatic (Annonaceae)
flowers and leaves. Revista Virtual de Quimica. 2018;10(5):1578-1590.

105- Zhang Y, Xiaoyu Liu,Yifei Wang, Pingping Jiang, SiewYoung Quek Antibacterial activity and mechanism of cinnamon essential oil against Escherichia coli and Staphylococcus aureus. Food Control 59: 282-289(2016).

106- Kavanaugh NL and K Ribbeck Selected antimicrobial essential oils eradicate Pseudomonas spp. and Staphylococcus aureus biofilms. Appl. Environ. Microbiol 78: 4057-4061(2012).

107- Prakash B, Singh P, Yadav S, Singh SC, Dubey NK Safety profile assessment and efficacy of chemically characterized Cinnamomum glaucescens essential oil against storage fungi, insect, aflatoxin secretion and as antioxidant. Food and chemical toxicology 53: 160-167(2013).

108- Simionato I, Domingues FC, Nerín C, Silva F Encapsulation of cinnamon oil in cyclodextrin nanosponges and their potential use for antimicrobial food packaging. Food and Chemical Toxicology 132:110647(2019)

109- Maryam Nazari, Babak Ghanbarzadeh, Hossein Samadi Kafil, Mahdi Zeinali, Hamed Hamishehkar Garlic essential oil nanophytosomes as a natural food preservative: Its application in yogurt as food model. Colloid and Interface Science Communications 30: 100176(2019).

110-Angnes SIA Isolation, chemical characterization and evaluat-ion of insecticide property of essential oil Piper amplumKunt, in Masters IDissertação (chemistry) R.U.o. Blumenau, Editor. Blumenau-SC p. 88(2005).

111- de Almeida Cde F, Ramos MA, de Amorim EL, de Albuquerque
UP A comparison of knowledge about medicinal plants for three rural communities in the semi-arid region of northeast of Brazil. Journal of ethnopharmacology 127: 674-684(2010).

112- Espinoza J, Urzua A,Sanhueza WM, Fincheira P, Muñoz MLWilkens M.. Essential oil, extracts, and sesquiterpenes obtained from the heartwood of Pilgerodendron uviferum act as potential inhibitors of the Staphylococcus aureus NorA multidrug efflux pump. Frontiers in Microbiology. ;40:337-351(2019).

113- Bhavaniramya S, Vanajothi R, Vishnupriya S, Al-Aboody MS, Vijayakumar R, Baskaran D. Computational characterization of deleterious SNPs in Toll-like receptor gene that potentially cause mastitis in dairy cattle. Biocatalysis and Agricultural Biotechnology.;19: 101151(2019).

114- Hammer KA, Carson CF, Dunstan JA, Hale J, Lehmann H, Robinson CJ, et al. Antimicrobial an anti-inflammatory activity of five Taxandria fragrans oils in vitro.Microbiology and Immunology. ; 52:522- 530(2008).

115- Zore GB, Thakre AD, Jadhav S, Karuppayil SM. Terpenoids inhibit Candida albicans growth by affecting membrane integrity and arrest of cell cycle. Phytomedicine.;18:1181-1190(2011).

116- Birmpa A, Constantinou P, Dedes C, Bellou M, Sazakli E,Leotsinidis M, et al. Antibacterial and antiviral effect of essential oils combined with nonthermal disinfection technologies for ready-to-eat romaine lettuce. Journal of Nutrition, Food Research and Technology. ;1(1):24-32(2018).

117- Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial ,antifungal, antiviral, and cytotoxic properties-Anoverview.Forschende.Komplementärmedizin;16:79-90(2009).

118-Maydell,H.J.von,.Trees and shrubs of the Sahel, their characteristics and uses., no. 196(1986).

119- Hans,J.P. “Trees and Shrubs of the Sahel: Their characteristic and uses Print by Typo-druck,” HansJurgen Von Maydell, Ger., 1990. 2012

120-T. K. Lim, “Garcinia macrophylla,” in Edible Medicinal And Non-Medicinal Plants, Springer Science and Business Media, (2012):ISBN,9400717636,9789400717633 .

121- A. A. Olapade, O. A. Ajayi, and I. A. Ajayi, “Physical and chemical properties of Cassia sieberiana seeds.,” Int. Food Res. J., vol.21,no.2,(2014).

122- I. Toma, Y. Karumi, and M. A. Geidam,.“Phytochemical screening and toxicity studies of the aqueous extract of the pods pulp of Cassia sieberiana DC.(Cassia Kotchiyana Oliv.),” *African J. PureAppl.*Chem.,vol.3,no.2,pp.26-30,(2009).

123-. Sam G. H,. Mensah M. K, and Nyakoa-Ofori,N, ,. Pharmacognostic studies and standardization of Cassia sieberiana roots,” Pharmacogn. J., vol. 3, no. 21, pp. 12-17(2011).

124- Donkor,K, Okine,L.N. Abotsi, W. K. and Woode,E ,. “Antiinflammatory and anti-nociceptive effects of ethyl acetate fraction of root bark of cassia sieberiana dc in murine models,” Pharmacologia, vol. 4, no. 4, pp. 301–310(2013).

125- A. Traore, S. Ouedraogo, M. B. Belemlilga, A. Kabore, and I. P. Guissou,. “Phytochemical analysis and ovicidal activity of Cassia sieberiana, Guiera senegalensis and Excoecaria grahamii

extracts,”African J. Pharm. Pharmacol., vol. 11, no. 44, pp. 554-560, (2017).

126-. Nartey, E.T. Ofosuhen,M Kudzi,W.Agbale,CM.Antioxidant and gastric cytoprotective prostaglandins properties of Cassia sieberiana roots bark extract as an anti-ulcerogenic agent,” BMC Complement. Altern. Med., vol. 12, no. 1, p. 65(2012).

127-Thiombiano A, Schmidt M, Dressler S, et al. Catalogue des plantes vasculaires du Burkina Faso. Boissiera: mémoires des

Conservatoire et Jardin botaniques de la Ville de Genève. 65: 1-391(2012). Ref.:https://bit.ly/2IseAwI

128-Arbonnier M. Arbres, arbustes et lianes des zones sèches d'Afrique de l'Ouest. Versailles Ed Quae, MHNH. 574(2009).

129- Eldeen I, Van Staden J. Cyclooxygenase inhibition and antimycobacterial effects of extracts from Sudanese medicinal plants. S Afr J Bot. 74: 225-229(2008). Ref.:https://bit.ly/2vH3aCA

130-Abd el Nabi OM, Reisinger E, Reinthaler FF, Antimicrobial activity of Acacia nilotica (L.) Willd. ex Del. var. nilotica (Mimosaceae).JEthnopharmacol.37:77(1992).Ref.:https://bit.ly/2Q7nzYP

131- Neuwinger HD. African ethnobotany: poisons and drugs: chemistry, pharmacology, toxicology. CRC Press. 941(1996). Ref.: https://bit.ly/2IoD5er

132- Zingue S, Njuh AN, Tueche AB, In vitro cytotoxicity and in vivo antimammary tumor effects of the hydroethanolic extract of Acacia seyal (Mimosaceae) stem bark. BioMed Res Int. 13(2018).

133- Ododo MM, Choudhury MK, Dekebo AH. Structure elucidation of β-sitosterol with antibacterial activity from the root bark of Malva parviflora. SpringerPlus. 5: 1210(2012). Ref.: https://bit.ly/32VVCs7

134- Sen A, Dhavan P, Shukla KK,. Analysis of IR, NMR and antimicrobial activity of β-sitosterol isolated from Momordica charantia. Sci Secure J .Biotechnol.1:9-13(2012). Ref.: https://bit.ly/2vH3WiY

135-Mekbib SB, Regnier TJ, Sivakumar D. Evaluation of Ethiopian plant extracts, Acacia seyal and Withania somnifera, to control green mould and ensure quality maintenance of citrus (Citrus sinensis L.) Fruits. 64: 285-294(2009). Ref.: https://bit.ly/3aABvlU

136- Ismail MA, Koko WS, Osman EE. Molluscicidal Activity of Acacia seyal (Dell) Bark Methanolic Extract Against Biomphalaria

pfeifferi Snails. Int Biol Biomed J. 2: 73-79(2016). Ref.: https://bit.ly/2vLQv1c

137- Mekbib SB. In vitro anti microbial assay of selected medicinal plants against medically important plant and food born pathogens. J Medicinal Plants Studies. 4: 163-169(2016).

Ref.:https://bit.ly/3cCyXW8

138-. Zingue S, Michel T, Cisilotto J. The hydro-ethanolic extract of Acacia seyal (Mimosaceae) stem barks induced death in an ER-negative breast cancer cell line by the intrinsic pathway of apoptosis and inhibited cell migration. J Ethnopharmacol. 15 : 41-50(2018). Ref.https://www.ncbi.nlm.nih.gov/pubmed/29783017

139- Nie S-P, Wang C, Cui SW. A further amendment to the classical core structure of gum arabic (Acacia senegal). Food Hydrocolloids. 31: 42-48(2013). Ref.:https://bit.ly/2uYtQ1v.

140- Laakay. R.R.B.Wwinlanduse strategies for africa,building and experience with agroforests in Asia and latin america. Int. for Rav. 3, 1-10(2001)

141- David j. Mabberley. Mabberley's plants book, third edition, cambridge university press. UK(2008).

142- Umbreta quattrocchhi. C.R.C.World dictionary of plant name , volume 1, page 91, CRC press, Boca Raton, New York Washington, DCUS ,London, UK2000.

143- Olusola L, Zebulon SC, okoye Fu. affect of vitex doniana stem bark on blood pressure. NJ Nat prod Med(1997).

144-Burkill HM.Vitex. In:editor: The useful plants of west tropical Africa families, S-Z, Addenda, vol -5-2nd ed. Kew, Richmond, united kingdom, Royal Botanic gardins; p-272-5(2002).

145-Ky k. vitex doniana sweet. Record from database in loupe D, oteng - Amooko AA, Brink M, editors 7 (1). Wageningen, Netherland prota, plants resources of tropical Africa(2008).

146- Kastra KD, Kuffinec J, Blazevic,.The composition of essential oil of vitex agnus – castus planta medica, 52, 681(1992).

147- Kuruuzum -UZA, strock k, Demirezer lo, zeeck A, , Glcosidees from vitex aguns - castus -phytochemistry, 34,50 - 51(2003).

148- Bolza, E and W.G.Keating, Africa timber, the properties, uses and characteristic of 700 species Melbourn common in scientific and industrial research organization(1972).

149-Abdulrahman, F.I.and P.A onyeyili. phytochemical screening and pharmacological activities of stem bark of terminal avicennoids. Bull. Anim, health prod. Afri - 49 = 236 - 242(2001).

150-T johns, ," phytochemicals as evolutionary mediators of human nutritional physiology" *international jornal of pharmacognosy*, vol 34,no. 5,pp 327 - 334(1996).

151-W. J .," Health promoting properties of common herbs" A merican jornal of clinical Nutrition vol, 70, no.3, pp, 491-499(1999).

152-.Sofowora, A.E., The state of medicinal plants in Nigeria, university of Ibadan, Ibadan, Nigeria(1993).

153-M aunda p., Achigan- Dako E.G., Morimoto Y.. Biodiversity of Africa vegetables, in Shackleton, C .M . ,pasquini M. W. Drescher A.W.(Eds), African indigenous vegetables urban Agriculture, Earth scan London, UK. 65- 104(2009).

154- Dafni, A. Levy, S. and Lev, E. The Ethnobotany of Christ's thorn Jujube (Ziziphus spina-christi) in Israel, *Journal of Ethnobiology and Ethnomedicine*, 1:8. (2005),.

155- Saied AS, Gebauer H, Hammer K, Buerkert A. Ziziphus spina-christi (L) Willd: a multipurpose fruit tree. Gene Resources & Crop Evol (2008), 55: 929- 937

156- Godini, A. Kazem, M. Naseri, G. and Badavi, M. The Effect of Zizyphus spina-christi Leaf Extract on the Isolated Rat Aorta. J Pak Med Assoc, Vol.59, No. 8((2009)).

157- Sudhersan, C. and Hussain, J., In Vitro Propagation of a Multipurpose Tree, Z.ziphus spina-christi(L.)Desf. Turk J. Bot, 27:167-171(2003).

158- Singh, A.K. Sharma R.K. Singh N.K. Bansal K.C. Koundal K.R. and Mohapatra, T. Genetic diversity in ber (Ziziphus spp.) revealed by AFLP markers. Hortic Sci Biotechnol 81:205-210(2006),.

159- Shtayeh, A. M. S. Yaghmour M.R. Faidi Y. R. Salem, K. and Al-Nuri, M.A. Antimicrobial Activity of 20 Plants Used in Folkloric Medicine in the Palestinian Area. J. Ethnopharmacol 60:265-271(1998),.

160- Palevitch, D. Yaniv, Z. Dafni, A. and Friedman, J. (1985), Ethnobotanical Survey of the Flora of Israel as a Source for Drugs. Jerusalem: Ministry of science(1998),.

161- Adzu, B. Amos, S. Dzarma, S. Amizan, M. B. and Gamaniel, K. Evaluation of the Antidiarrhoeal Effects ofZizyphus spina-christiStem bark in Rats. Acta Tropica, 87: 245-250(2003),.

162- Abdel-Wahhab, M.A. Omara, E.A. Abdel-Galil, M.M. Hassan, N.S. Somaia, Nada, A. Saeed, A. and Elsayed, M. Zizyphus spina-

christi extract protects against aflatoxin B1-initiated hepatic cacenogenicity Afr. J. Trad. CAM , 4 (3): 248-256(2007),.

163- Abdel-Zaher, A.O. Salim, S.Y. Assaf, M.H. and Abdel-Hady, R.H. Antidiabetic Activity and Toxicity of *Zizyphus spina-chriti* Leaves. *Journal of Ethnopharmacology*, 101:129-138(2005),.

164- Nazif, N. M. Phytoconstituents of *Ziziphus spina-chriti* L. Fruits and their Antimicrobial Activity. Food Chem, 76:77-81(2002),.

165- Avizeh, R. Najafzadeh, H. Pourmahdi, M. and Mirzaee, M. Effect of Glibenclamide and Fruit Extract of *Zizyphus spina-christi* on AlloxanInduced Diabetic Dogs. Intern J Appl Res Vet Med, Vol.8, No. 2(2010),.

166- Waggas, A.M. and Al-Hasani, R. Effect of Sidr (Zizyphus spinachristi) Fruit Extract on the Central Nervous System in Male Albino Rats. American-Eurasian Journal of Scientific Research, 4(4):263-267(2009),.

167- Shahat, A.A. Pieters, L. Apers, S., Nazeif, N.M. Abdel-Azim, N.S. Berghe, D.V. and Vlietinck, A.J. Chemical and biological investigation on Zizyphus spina-christiL. phytother. Res. 15, 593-597(2001).

168- Ikram, M. and Tomillnson, H. Chemical Constituents of Z. spinachristi. Planta Medica, 29, 289(1976).

169- Shah, A.H. Ageel, A.M. Tariq, M. Mossa, J.S. and Al-Yahya, M.A. Chemical constituents of the Stem Bark of *Z. spina-christi.* Fitoterapia, LVII, 452-454(1986),.

170- Mahran, G. H. Glombitza, K.W. Mirhom, Y. W. Hartmann, R. and Michel, C.G. Saponins of *Z. spina-christi* Growing in Egypt. Planta Medica, 62, 160-165(1994).

171- Nawar, M.A.M. Ishak, M. Michael, H.N. and Buddrust, J. Leaf Flavonoids of *Z. spina-christi*. Phytochemistry, 23(9), 2110-2111

(1984).

172- Weinges, K. and Schick, HDodecaacetylprodelphinidin B3 from the dried leaves of *Ziziphus spina-christi*. Phytochemistry, Vol. 28, No. 2, pp. 505-507.(1995).

173- Pawlowaska, A.M. Camangi, F. Bader, A. and Braca, A. Flavonoids of Zizyphus jujubeL. and *Zizyphus spina-christi(L.)* Willd (Rhamnaceae) Fruits. Food Chemistry, 112: 858-862(2009).

174- Abdel-Galil, F. and El-Jissry, M.A. Cyclopeptide alkaloids from Zizyphus spina-christi. Phytochemistry, Vol. 30, No. 4, pp. 1348-1349(1991).

175- Roodt V Kigelia Africana in the shell Field Guide to the common Trees of the Okarango Delta and Moremi Game reserve. Gaborone, Botswana; shell Oil Botswana.LCCN: 9398015, LC: QK402.B6 R66 1992, Dewels: 582.1609883: 20-110(1992).

176- Joffe P Kigelia Africana (Lam) Benth. Pretoria National Botanical Garden(2003). (www.plantzafrica.com).

177- Del Hoyo J, Elliot A, Sargatal J eds. Handbook of the birds of the world, 4-415. Lynx Editions(1997).

178- Grace OM, Davis SD Kigelia Africana (Lam.) Benth. Record from protabase. Oyen LPA, Lemmens RHMJ Wageningen, Netherlands. Inmagic DB/Text Webpublisher PRO: 1 records(2002). (http://database.prota.org/search.htm).

179- Khan MR, Kihara M, Omoloso A. Antimicrobial activity of the alkaloaidal constituents of the root bark of *Eupamatia Laurina*. Pharm. Biol. 41: 277-280(2003).

180- Akah PA. Antidiarrhoeal activity of the aqueous leaf extract of kigelia africana experimental animal. J. Herbs Spices Med. Plants 4(2): 31-38(1996).

181- Lal SD, Yadar BK: Folk Medicines of Kurukshetra district (Haryana), India Econ. Bot. 37: 299-305(1983).

182- Weenen H, Nkunya MHH, Bray DH, Mwasumbi LB, Kinabo LS, Kilimali VAEB Antimalaria activity of Tanzanian medicinal plants. Planta Medica 56: 368-370(1990).

183- Carvalho LH, Rocha EMM, Raslan DS, Oliveira AB, Krettli AU. *InVitro* activity of natural and synthetic naphthoquinones against erythrocytic stages of the *plasmodium falciparum*. Braz. J. Med. Biol. Res. 21: 485-487(1988).

184- Owolabi OJ, Omogbai EK. Analgesic and anti-inflammatory activities of ethanolic stembark extract of kigelia Africana (Bignoniacea). Afr. J. Biotechnol. 6(5): 582-585(2007).

185- Hutching A, Scott AH, Lewis G, Cunningham AB. Zulu medicinal plants. An inventory University of Natal press, pietermaritzburg pp.53-54(1996).

186- Olaive MT, Rocha JB. Commonly used tropical medicinal plants exibit distinct *in vitro* antioxidant activities against hepatotoxins in rat liver. Exp. Toxical. pathol. 58(6): 433-8(2007).

187- Akunyili D, Houghton P. Monoterpenoids and naphthaquinone from kigelia pinnata phytochemistry 32: 1015-1018(1993.).

188- Kolodziej H. Protective role of kigelia Africana fruits against benzo (a) pyrene induced fore-stomach tumorigenesis in mice and against albumen induced inflammation in rats. Pharmacol. Lett. 213:67-70(1997).

189-Akunyili DN, Houghton PJ, Roman A. Antimicrobial activities of the stem of kigelia pinnata, J. Ethnopharmacol. 35:173-177(1991).

190-Picerno P, Autore G, Marzocco S, Meloni M, Sanogo R, Aquino RP Anti-inflammatory activity of verminoside from kigelia Africana and evaluation of cutaneous irritation in cell cultures and reconstituted human epidermis. J. Nat. Prod. 68(11): 1610-4(2005).

191-Gouda YG, Abdel-Baky AM, Darwish FM, Mohamed KM, Kasai R, Yamasaky K. Phenylpropanoid and phenylethanoid derivatives from Kigelia pinnata D.C. fruits. Nat. Prod. Res. 20(10); 935-9(2006).