بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Study of Oil from Seeds of *(Adansoniadigitata) by* GC-M.S

دراسة زيتبذورنبات شجرة التبلدى بالكرومتوغرافيا الغازية ـ السائلة الملحقة بمطياف الكتلة

A Thesis Submitted in Partial Fulfillment of the Requirements of the Master Degree in Chemistry

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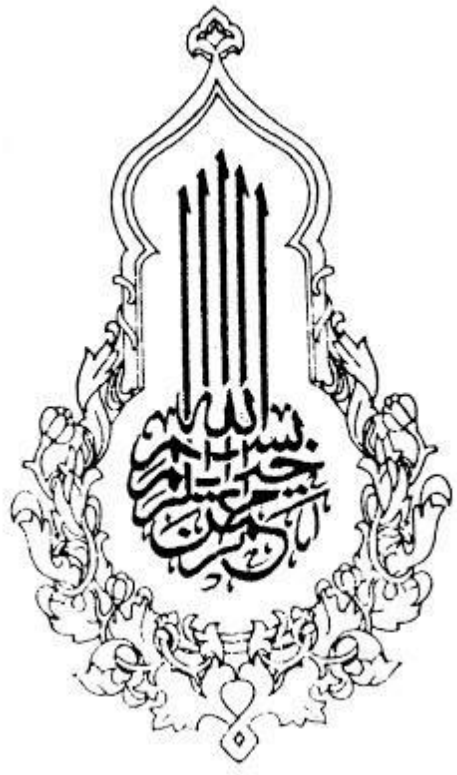

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿1﴾ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴿2﴾ اقْرَأْ
وَرَبُّكَ الْأَكْرَمُ ﴿3﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿4﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ) ﴿5﴾

[سورة العلق الآيات : 1-5]

Dedication

To

Mother and Father,

Brothers and Sisters

Acknowledgment

First of all my sincere thanks to Alla Almighty for helping me to complete this work. It is a pleasure to record my deep appreciation, and thanks to Prof. Mohammed abdelkarim for his wise guidance, which helped me to present this project in this shape. I am very grateful to the Staff of the Department of Chemistry–Sudan University of Science and Technology for all facilities.

Thanks to my family for their continual support.

Abstract

Phytochemical investigation of fruit pulp of *Adansonia digitata* gave positive reactions for : steroids,terpenes,flavonoids,tannins and glycosides.

GC-MS analysis of *Adansonia digitata*oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting of the observed fragmentation pattern.Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

The GC-MS analysis of the studied oil revealed the presence of 20 components. The oil contained the following components as major constituents:

-Oleic acid(Area:30.26%)

-9-Octadecenoic acid methyl ester(22.40%)

-9,12-Octadecadienoic acid methyl ester(18.73%)

الخلاصه

اوضحت نتائج المسح الفيتوكيميائى لثمار نبات التبلدى وجود : الاسترويدات, التربينات,الفلافونيدات, التنينات والجلايكوسيدات.

اخضع زيت بذور التبلدى للكروموتوغرافيا الغازية -السائلة الملحقة بمطياف الكتلة,وفسرت النتائج بالاستعانه ببيانات مكتبه (NIST) لاطياف الكتلة , كما وتم تاكيد التراكيب بدراسه نمط التفتت.وجد تطابق يصل الى 90-95% بين اطياف المكونات وبيانات المكتبة الملحقة بالجهاز.

اوضحت دراسات طيف الكتلة -الكروموتوغرافيا الغازية/السائلة وجود عشرون مكونا بالزيت اهمها:

-Oleic acid(Area:30.26%)

-9-Octadecenoic acid methyl ester(22.40%)

-9,12-Octadecadienoic acid methyl ester(18.73%)

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Chapter One

I-Introduction

1.1-*Adansonia digitata* L.

Adansonia digitata L.(Baobab*)* is widely distributed throughout the sub- Saharan Africa and western Madagascar areas and has many uses[1,2]. Most scientists believe that the name (Baobab) is derived from the Arabic name "buhibab" meaning fruit with many seeds[3]. The name *Adansonia digitata* was given by Linnaeus, the generic name honouring Michel Adanson who had been to Senegal in the eighteenth century and described Baobab[4]. A history of the African baobab is well documented in Baum[5]. Darwin documented baobab trees on the St Jago in the Cape Verde Islands in 1832 and he commented on their size and longevity[6]. *Adansonia digitata L.* is the most widely spread of the Adansonia species on the African continent which belongs to the family of Bombacaceae a sub family of the Malvaceae[7]. The African baobab is known by a very large number of local names: English (Baobab, Monkey bread tree, Ethiopian (Sour Gourd, Cream of Tartar Tree), Senegal (calabash fruit, Upside-down tree), French (Pain De Singe, Arbre Aux Calebasses), Portuguese (Cabaçevre), Arabic (Buhibab, Hamao-Hamaraya, Habhab, Hamar, Tebeldi,), Afrikans (Kremetart), Hausa (Kuka), Sotho (Seboi), Tswana (Mowana), Tsonga (Shimuwu), Venda (Muvhuyu)[8].Eight baobab species have been identified globally, and six species are endemic to Madagascar, the postulated centre of evolutionary origin of the genus Adansonia[9,10]. The African species, *Adansoniadigitata* is widely distributed

throughout the savanna woodlands of sub-Saharan Africa[10]. The only species not endemic to the African continent is *Adansonia gibbosa* (**A.Cunn**.) **Guymer exD.A.Baum** which is native to Australia[9,10]. In southern Africa, *Adansonia digitata* commonly occurs in Malawi, Zimbabwe, Mozambique, South Africa and Sudan, especially in the warm savanna areas [11-13]. In Sudan, the baobab is most frequently found on sandy soils and by seasonal streams 'Khors' in short grass savannas. It forms belts in central Sudan, in Kordofan, Darfur, Blue Nile, Upper Nile and Bahr El Ghazal[14]. It is often found associated with the tamarind(*Tamarindus indica* L.)[15].The different plant parts are widely used as foods, medicines and the bark fibres are also used[16]. The tree provides food, shelter, clothing and medicine as well as material for hunting and fishing. Every part of the baobab tree is reported to be useful[17].

The baobab tree (*Adansonia digitata* L.), is a fruit bearing tree species, characterized by a massive size of up to 25m height and 10 m trunk[18], and its bottle-shaped trunk which accumulates water[10,16]. It is regarded as the largest succulent[18]. During the leafless period, physiological processes such as photosynthesis take place in the green inner layer of the trunk and branches, utilizing water stored in the trunk[17].The tree produces an extensive lateral root system which can extend up to 5 m from the trunk and down to a depth of 10m[1].

The deciduous leaves which are 5–7 palmately compound can reach a diameter of 20 cm. The large and pure white bat-

pollinated bisexual flowers have five curled-black waxy petals and numerous stamens fused to form a central column[16]. The fruits, which are filled with reniform seeds embedded in the whitish acidic mealy pulp, are variable in size (up to 15 cm), and contains many seeds[10,16]. It is estimated that it takes between eight and twenty-three years before the baobab produces seeds, and the mature plant (over 60 years) can produce more than 160–250 fruits per year[12].

Work from carbon dating and examination of core samples from the stem have been used to estimate the age of baobabs. Although some baobab trees are reputed to be many thousands of years old, this is impossible to verify as the wood does not produce annual growth rings [10]. Few botanists believe these claims of extreme age. Research on radiocarbon dating of baobabs[19], as well as dating results presented by other researchers show that very large specimens are not necessarily among the oldest trees, and that medium sized individuals can also be very old[20].

A number of authors have pointed out that baobab recruitment is often underestimated because of the false predictions made about baobab growth rates.[21], as young baobabs grow faster relative to older trees. This was supported by Patrut et al.[22], who did radiocarbon dating of the "Grootboom" (meaning big tree) baobab in Namibia. Girth measurements have often been used as a rough guide to baobab population demography[23-25]The baobab tree shrinks in times of drought[26], and this affects age estimation by means of girth. Thus, caution should be taken when one uses girth

measurements in estimating the age of baobabs[27-29]. The baobab tree is a very long-lived species. It has been reported that it can survive for more than 400 years[30].

The different parts of *adansonia digitata* are widely used as foods, medicines and the bark fibres are also used[16]. Every part of the baobab tree is reported to be useful[17]

Leaves of *Adansonia digitata*

The leaves are staple food for many populations in Africa most especially the central region of the continent[31,2]. In Zimbabwe, they provide fresh vegetables that are substituted for the commercially grown leafy vegetables such as cabbages and lettuce[32]. In the northern part of Nigeria, the Hausas use the leaves for soup[31]. In Mali, the leaves are called "Lalo" and they are used in making sauce and they usually mix it with seeds of *Parkiabig lobosa*, onion, okra, pepper, ginger,sometimes meat, but more often fish.

The sauce is used with a thick porridge made from millet, sorghum or maize, but also for couscous and rice[33].

The leaves contain 13-15% protein, 60-70% carbohydrate, 4-10% fat and around 11% fibre and 16% ash[34]. Energy value varies from 1180-1900kJ/100g of which 80% is metabolized energy. The leaves are rich in pro-vitamins A and C. In terms of protein content and WHO standards, leaves of baobab can be rated 'good' in that they score well for 5 of the 8 essential amino acids. The highest level of pro-vitamin A was detected in young leaves especially when used as dried material[35], expressed in retinol equivalent it was between 9 and 27 mg/kg.

Baobab leaf is said to be an excellent source of calcium, iron, potassium, magnesium, manganese, molybdenum, phosphorus, and zinc[31].*Adansonia digitata* leaf could serve as a significant protein and mineral source in the staple food of the local population.

Powdered leaves are used as an anti-asthmatic and known to have antihistamineand anti-tension properties[16]. The leaves are also used to treat a wide variety of conditionsincluding fatigue, as a tonic and for insect bites, Guinea worm , internal pains, dysentery[16] and diseases of the urinary tract, opthalmia and otitis[16]. In Indian medicine, powdered leaves are similarlyused to check excessive perspiration[16]. Baobab leaves areused medicinally as a diaphoretic, an expectorant, and as a prophylactic against fever,to check excessive perspiration, and as an astringent. The leaves also havehyposensitive and antihistamine properties.

Fruits of *Adansonia digitata*

The fruit pulp is one of the most important parts of the tree thatis used as food. Ripped fruit pulps are removed from the fibers and seeds by kneading in cold water: the resulting emulsion is sieved. This is then added to thick grain preparations to make thinner gruels. The dry pulp is either eaten fresh or used to add to gruels on cooling after cooking and that is also a good way of preserving the vitamin content.[36,37]. It can also be frozen if ground to a powder[38].

The dry baobab fruit pulp has a slightly tart, refreshing taste and is very nutritious,with particularly high values for carbohydrates, energy, calcium, potassium (very high),thiamine, nicotinic acid and vitamin C (very high)[39]. The baobab fruitpulp is dry, acidulous and mealy, and rich in mucilage, pectins, tartarate and free tartaricacids.[16,10] Pulp sweetness is providedby fructose, saccharose and glucose contents. Fruit pulp is also acidic and this is due tothe presence of organic acids including citric, tartaric, malic, succinic as well as ascorbicacid[16]. When eaten raw, the pulpis a rich source

of calcium and vitamins B and C[40].It contains sugars but no starch, and is rich in pectins. The fruit pulp has a very high vitaminC content, almost ten times that of oranges. However, the vitamin C content of thebulk fruit pulp reportedly varies from 1623 mg/kg in one tree to 4991 mg/kg in another[35,17]..

A lot of studies have shown that *Adansonia digitata* L fruit pulp is rich in vitamins and minerals and contains a high amount of both the soluble and insoluble dietary fiber[17,34,41,42,39,43]. The vitamin C content present in the fruit is what contributes to its overall antioxidant capacity[44], and is a good source of polyphenols, including certain flavonoids[45], and tannins[46]. The fruit is of increasing nutritional interest because it may be a significant contributor to the daily intake of important nutrient[47,48].

The widest use in tradition medicine comes from the baobab bark as a substitutefor quinine in case of fever or as a prophylactic. A decoction of the bark deteriorates rapidlydue to the mucilaginous substances present. This process can be prevented by addingalcohol to the decoction[16]. Baobab bark is used in Europe as a febrifuge (antipyretic). Inthe Gold Coast (Ghana), the bark is used instead of quinine for curing fever [49]. In Indian medicine, baobab bark is used internally as a refrigerant, antipyretic andantiperiodic. It is used as a decoction, 30 g/l of water, boiled down to two thirds[16]. The activity of baobab bark as a febrifuge, however, has not beendetected in experimental malaria treatments, although it is both diaphoretic and antiperiodicThe bark, however, is certainly used for thetreatment of fever in Nigeria[48]. Moreover,

the barkcontains a white, semi-fluid gum that can be obtained from bark wounds andis used for cleansing sores [48]. the barkis used as an antidote to *Strophanthus* poisoning. In Congo Brazzaville, a bark decoctionis used to bathe rickety children and in Tanzania as a mouthwash for toothache. Furthermore, a new flavanonol glycoside was reported inthe root bark[48,50].

Seeds of *Adansonia digitata*

Seeds are used in cases of diarrhoea, and cough. Oil extracted from seeds is usedfor inflammed gums and to ease diseased teeth, Since seed oil isused to also treat skin complaints, it can be considered to have cosmetic applications aswell[16].

The Seeds are used as a thickening agent in soups, they are also fermented and used as a flavoring agent, or roasted and eaten as snacks [51,52]. When they are roasted, they are sometimes used as a substitute for coffee. In some cases, seeds are de-hulled by boiling, rubbing by hand, and then sun- drying the kernels before grinding. Fermentation of powdered de-hulled seeds is known to increase

protein digestibility. It also reduces the trypsin inhibition activity but increases tannin content[53]. The baobab seeds are ground with peanuts and water and sugar added to make a sauce used with porridge[54]. Seed pulp is sometimes known as monkey bread and is eaten and traded in different regions[32]. The seeds have an energy value of 1803 kJ/100g approximately 50% higher than leaves, moisture 8.1%, protein 33.7%, and fat 30.6%, carbohydrates 4.8%, fibre 16.9% and ash 5.9%[39]. The vitamin C content of the baobab seeds has not been researched extensively but they are known to contain high levels of lysine, thiamine, Ca and Fe[55].

Seed oil

Seeds are said to be a good source of oil.*Adansonia digitata* oil is golden yellow.The oil is extracted by pounding the seeds.[56]. The oils have been used for centuries by local communities for the purpose of food, medicine, cosmetic applications and production of lubricants, soaps and personal care products. The oils were used in topical treatment of various conditions such as hair dandruff,muscle spasms, varicose veins and wounds[57,58]. The baobab seeds oils contain high proportions of linoleic and oleic acid as well as palmitic and α-linolenic[57,59] acids. The baobab seed oil is an excellent source of

mono-and polyunsaturated fatty acids. The principal fatty acids are: linoleic and oleic acids, 39.42% and 26.07% respectively .Use of oil in cosmetics and biofuel has increased greatly as industry seeks natural alternatives[41],Seeds are used in cases of diarrhoea, and hiccough[16].

1.2- Essential oils

Essential oils also called volatile odoriferous oils, are aromatic oily liquids extracted from different parts of plants, for example: leaves, peels, barks, flowers, buds, seeds, and so on. They can be extracted from plant materials by several methods e.g. steam distillation. Among all methods, for example, steam distillation method has been widely used, especially for commercial scale production[60,61]. Essential oils have been widely used as food flavours[62]. Essential oils found in many different plants, especially the aromatic plants, vary in odor and flavour, which are governed by the type and amount of constituents present in oils. Additionally, the amount of essential oil from different plants is different and this determines the price of essential oil. Apart from aromatic compounds, indigenous pigments contribute to varying colours of essential oil. This can affect the applications as the ingredient in some particular foods. Essential oils have been known to possess antioxidant and antimicrobial activities, thereby serving as natural additives in foods and food products. It can be used as active compounds in packaging materials, in which the properties of those materials, particularly water vapour barrier property associated with hydrophobicity in nature of essential oils, can be improved. Almost any part of a plant may be the source of the oil, which could be

extracted and fully exploited for food applications or others. Modern technologies have been continuously developed to conquer the limitation of conventional methods, and to enhance the extraction efficacy. Due to the increasing attention in natural additives, essential oils from several plants have been used more widely, especially in conjunction with other preservations under concept of “hurdle technology.” Thus, essential oils can serve as the alternative additives or processing aid as green technology.

Several plants contain essential oils, however, parts of plants, which serve as the major source of essential oil can be different. Those include roots, peels, leaves, seeds, fruits, barks, and so on. Plant essential oils are usually the complex mixture of natural compounds, both polar and nonpolar compounds[63].In general, the constituents in essential oils are terpenes (monoterpenes and sesquerpenes), aromatic compounds (aldehyde, alcohol, phenol, methoxy derivative, and so on), and terpenoids (isoprenoids)[64,65]. Compounds and aroma of essential oils can be divided into 2 major groups: terpene hydrocarbons and oxygenated compounds.i)**Terpene hydrocarbons**

The hydrocarbons are the molecule, constituting of H and C atoms arranged in chains. These hydrocarbons may be acyclic, alicyclic (monocyclic, bicyclic, or tricyclic), or aromatic. Terpenes are the most common class of chemical compounds found in essential oils. Terpenes are made from isoprene units (several 5 carbon base units, C5), which are the combinations of 2 isoprene units, called a “terpene unit.” Essential oils consist of mainly monoterpenes (C_{10})

and sesquiterpenes (C_{15}), which are hydrocarbons with the general formula $(C_5H_8)_n$. The diterpenes (C_{20}), triterpenes (C_{30}), and tetraterpenes (C_{40}) exist in essential oils at low concentration[65]. Terpenoids (a terpene containing oxygen) is also found in essential oils[62]. Essential oils mostly contain monoterpenes and sesquiterpenes, which are $C_{10}H_{16}$(MW 136 amu) and $C_{15}H_{24}$ (MW 204 amu), respectively. Although sesquiterpenes are larger in molecules, structure and functional properties of sesquiterpenes are similar to the monoterpenes[66]. For diterpenes, triterpenes, and tetraterpenes, they have larger molecule than monoterpenes and sesquiterpenes, but they are present at very low concentration in essential oils[64].

ii)Oxygenated compounds

These compounds are the combination of C, H, and O, and there are a variety of compounds found in essential oils. Oxygenated compounds can be derived from the terpenes and termed "terpenoids." Some oxygenated compounds prevalent in plant essential oils are shown as follows:

- Phenols: thymol, eugenol, carvacrol, chavicol, thymol, and so on.
- Alcohols: Monoterpene alcohol: borneol, isopulegol, lavanduol, α-terpineol, and so on.
- Sesquiterpenes alcohol: elemol, nerolidol, santalol, α-santalol, andso on.
- Aldehydes: citral, myrtenal, cuminaldehyde, citronellal, cinnamaldehyde, benzaldehyde, and so on.

- Ketones: carvone, menthone, pulegone, fenchone, camphor, thujone,verbenone, and so on.
- Esters: bomyl acetate, linalyl acetate, citronellyl acetate, geranyl acetate, and so on.
- Oxides: 1,8-cineole, bisabolone oxide, linalool oxide, sclareol oxide, and so on.
- Lactones: bergaptene, nepetalactone, psoralen, aesculatine, citroptene,and so on.
- Ethers: 1,8-cineole, anethole, elemicin, myristicin, and so on.

Different constituents in essential oils exhibit varying smell or flavour[62]. Also, the perception of individual volatile compounds depends on their threshold.

Essential oils can be extracted from several plants with different parts by various extraction methods. The manufacturing of essential oils, and the method used for essential oil extraction are normally dependent on botanical material used. State and form of material is another factor used for consideration. Extraction method is one of prime factors that determine the quality of essential oil. Inappropriate extraction procedure can lead to the damage or alter action of chemical signature of essential oil. This results in the loss of bioactivity and natural characteristics. For severe cases: discoloration, off-odor/flavor as well as physical change such as the increased viscosity can occur. Those changes in extracted essential oil must be avoided. Extraction of essential oils can be carried out by various means, as follows:

i)Steam distillation

Steam distillation is the most widely used method for plant essential oil extraction[67]. The proportion of essential oils extracted by steam distillation is 93% and the remaining 7% can be further extracted by other methods[63]. Basically, the plant sample is placed in boiling water or heated by steam. The heat applied is the main cause of burst and break down of cell structure of plant material. As a consequence, the aromatic compounds or essential oils from plant material are released[68,69]. The temperature of heating must be enough to break down the plant material and release aromatic compound or essential oil. A new process design and operation for steam distillation of essential oils to increase oil yield and reduce the loss of polar compounds in wastewater was developed[63]. The system consists of a packed bed of the plant materials, which sits above the steam source. Only steam passes through it and the boiling water is not mixed with plant material. Thus, the process requires the minimum amount of steam in the process and the amount of water in the distillate is reduced. Also, water-soluble compounds are dissolved into the aqueous fraction of the condensate at a lower extent [63].

ii)Hydrodistillation (HD)

(HD)has become the standard method of essential oil extraction from plant material such as wood or flower, which is often used to isolate non-water soluble natural products with high boiling point. The process involves the complete immersion of plant materials in water, followed by boiling. This method protects the oils extracted

to a certain degree since the surrounding water acts as a barrier to prevent it from overheating. The steam and essential oil vapour are condensed to an aqueous fraction . The advantage of this technique is that the required material can be distilled at a temperature below 100 °C. [70]Ohmic-assisted HD (OAHD) is another advanced HD technique[71]. OAHD method had the extraction time of 24-75 min, while HD took 1 h for extraction of essential oil from thyme. No changes in the compounds of the essential oils obtained by OAHD were found in comparison with HD.

iii)Hydrodiffusion

Hydrodiffusion extraction is a type of steamdistillation, which is only different in the inlet way of steam intothe container of still. This method is used when the plant materialhas been dried and is not damaged at boiling temperature[72]. For hydrodiffusion, steam is applied from thetop of plant material, whereas steam is entered from the bottomfor steam distillation method. The process can also be operatedunder low pressure or vacuum and reduces the steam temperatureto below 100 °C. Hydrodiffusion method is superior to steamdistillation because of a shorter processing time and a higher oilyield with less steam is used[75].

iv)Solvent extraction

Conventional solvent extraction has been implementedfor fragile or delicate flower materials, which are nottolerant to the heat of steam distillation. Different solvents includingacetone, hexane, petroleum ether, methanol, or ethanolcan be used for extraction[74-76]. For general practice, thesolvent is mixed with the plant material

and then heated to extractthe essential oil, followed by filtration. Subsequently, thefiltrate is concentrated by solvent evaporation. The concentrate isresin (resinoid), or concrete (a combination of wax, fragrance, an essential oil). The concentrate, is then mixed with purealcohol to extract the oil and distilled at low temperatures. Thealcohol absorbs the fragrance and when the alcohol is evaporated,the aromatic absolute oil is remained. However, this method is arelatively time-consuming process, thus making the oils more expensivethan other methods[77].

v)Supercritical carbon dioxide

Conventional methods including solvent extraction and steam distillation have some shortcomings such as long preparation time and large amount of organic solvents[78]. Moreover, the losses of some volatile compounds, low extraction efficiency, degradation of unsaturated compounds, and toxic solvent residue in the extract may be encountered[79,80]. Therefore, supercritical fluids have been considered as an alternative medium for essential oil extraction. Carbon dioxide (CO_2) is the most commonly used supercritical fluid because of its modest critical conditions[81,82]. Under high-pressure condition, CO_2 turns into liquid, which can be used as a very inert and safe medium to extract the aromatic molecules from raw material. No solvent residue remains in the final finished product since the liquid CO_2 simply reverts to a gas and evaporates under normal atmospheric pressure and temperature[81].

vi)Subcritical water

The subcritical water or pressurized hot water has been introduced as an extractant under dynamic conditions (pressure high enough to maintain water under liquid state and temperature in the range of 100 to 374 °C). The efficiency (in terms of volume of essential oil/1 g of plant) of continuous subcritical water extraction was 5.1 times higher than HD method[83]. This method is quicker (15 min compared with 3 h), provides a more valuable essential oil (with higher amounts of oxygenated compounds and no significant presence of terpenes), and allows substantial savings of costs, in terms of both energy and plant material[84].

1.2.1- Role of essential oils as food additives

Essential oils from plants have been known to act as natural additives, for example, antimicrobial agents, antioxidant, and so on. Their activities vary with source of plants, chemical composition and extraction method. Due to the unique smell associated with the volatiles, this may limit the use of essential oil in some foods since it may alter the typical smell/flavour of foods.

1.2.2- Antimicrobial activity of essential oils

Plant essential oils has ability to protect foods againstpathogenic and spoilage microorganisms[85].Higherantimicrobial activity of essential oils is observed on Gram-positivebacteria than Gram-negative bacteria[70]. Lipophilic ends of hydrophopic acids incell membrane of Gram positive bacteria may facilitate the penetrationof hydrophobic compounds of essential oils[86]. On the other hand, the resistance of Gram-negative bacteria to essential oils is associated with the protecting role of extrinsicmembrane

proteins or cell wall lipopolysaccharides, whichlimits the diffusion rate of hydrophobic compounds through thelipopolysaccharide layer[62]. The activity of the essential oils is related to composition, functionalgroups, and synergistic interactions between components[87].

Plant essential oils have been known as antimicrobial agents. Thus, essential oils from some selected plants can beused as antimicrobial agents for food applications as well as otherpurposes; however, their activity depends on types of essential oilused[62].

1.2.3- Antioxidant activity

Several compounds in essential oils have the structure mimicking the well-known plant phenols with antioxidant activity. Among the major compounds available in the oil, thymol and carvacrol were reported to possess the highest antioxidant activity[88]. Essential oils have several modes of actions as antioxidant, such as prevention of chain initiation, free radical scavengers, reducing agents, termination of peroxides, prevention of continued hydrogen abstraction as well as quenchers of singlet oxygen formation and binding of transition metal ion catalysts[89]. With those functions, essential oils can serve as the potential natural antioxidants, which can be used to prevent lipid oxidation in food systems. Phenolics are organic compounds consisting of hydroxyl group (-OH) attached directly to a carbon atom that is a part of aromatic ring. The hydrogen atom of hydroxyl group can be donated to free radicals, thereby preventing other compounds to be oxidized. The antioxidant capability of phenolic compounds is mainly due to

their redox properties, which permit them to act as hydrogen donors, reducing agents, singlet oxygen quenchers as well as metal chelators[90].

The antioxidant activity is generally related with the major active compounds in essential oils such as eugenol in clove, m-thymol in thyme, and β-citronellol or β-citronellal in *citronella*[66]. However, the other antioxidant compounds in essential oils such as terpinene, (−)-camphor, (−)-bornylacetate, eucalyptol, and methylchavicol have been reported to exhibit antioxidant activity, but their amounts were probably too low to exhibit antioxidant activity[66]. Antioxidant activity varies with source of essential oils, The differences in antioxidative activity of different essential oils were mostly due to the differences in types and amounts of antioxidative components present in essential oils[62,91]. Antioxidative activity of essential oil is also affected by extraction method or solvents used. Nonpolarextracts showed less effective activities than polar extracts. Therefore,antioxidative activity of essential oil is strictly related withthe polarities of their phytochemicals[92]. Non-phenolicantioxidants of plant extracts might also contribute to theantioxidant activity[93].Additionally, the harvesting period of plant also determines theconcentration of the major oil components such as phenolic compounds,which are directly related with the antioxidant activity ofessentialoil[94].

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

Gas Chromatography-Mass Spectrometry (GC/MS) is the synergistic combination of two powerful analytic techniques. The gas

chromatography separates components of a mixture in time, and the mass spectrometer provides information that aid in the structural identification of each component[95].

Knowledge of the identity and relative amounts of the essential oils extracted from plants has great importance to several fields of basic and applied research in chemistry, biology and many other disciplines. Obtaining this knowledge requires overcoming many analytical challenges posed by these complex mixtures, because they normally present large variations in component amounts, chemical structures and functionalities. Gas chromatography (GC) is recognized as the most suitable technique to find out how many components and in what proportion there are in a complex matrix of volatile compounds. When it is coupled to mass spectrometry (GC-MS), additional information arises about each separated compound: molecular mass, elemental composition (when high resolution mass spectrometry is used), functional groups, and in certain cases, molecular geometry and spatial isomerism.

1.3.1- Gas chromatography

In a gas chromatographic system, the sample to be analyzed may be a liquid solution or a collection of molecules adsorbed on a surface, e.g., the solid-phase micro extraction (SPME) system. During the transfer into the GC, the sample is volatilized by rapid exposure to a zone kept at relatively high temperature (200-300°C) and mixed with a stream of carrier gas (Ar, He, N2, or H2). The resulting gaseous mixture enters the separation section, a chromatographic column, which in its current version is a fused-silica tubular capillary coated

internally with a thin polymer film. Upon their displacement through the column, analyte molecules are partitioned between the gas carrier stream (mobile phase) and the polymer coating (stationary phase), to an extent which depends mainly on their chemical structure. At the end of the separation section, the molecules reach a detection system in which a specific physical property (thermal conductivity) or a physico-chemical process (ionization in a flame, electron capture) gives rise to an electric signal which is proportional to the amount of molecules of the same identity. A data system permits to process these data to produce a graph of the variation of this detector signal with time (chromatogram). Thus, four principal sections are distinguishable in the chromatograph: introduction (injector), separation (chromatographic column), detection, and data handling units. Each section has its own function and its responsibility for the quality of the analysis and the results obtained. The injection system, for example, should ideally transfer the sample to the column quantitatively, without discrimination on molecular weights or volatility, and without chemical alteration (decomposition or isomerization). It is a critical step, especially for quantitative analysis. For correct GC operation, among other conditions, this gateway to the column should remain unpolluted, clean, inert, and leak-free. The main requirement for an analyte in GC is that it should be volatile enough to be present in detectable amounts in the mobile phase. Substances with low vapour pressure will not enter the chromatographic column, and will accumulate at the injection system, and may eventually clog its conduits. Very polar,

thermolabile, ionic and high-molecular weight compounds are notcompatible with regular GC analysis. Depending on the molecular structure of the analyte and the functional groups available, it is possible in some cases to obtain a chemical derivative which has a higher vapor pressure and is therefore more amenable to GC analysis. One of the most important characteristics of the chromatographic column is its resolution, or the ability to separate components with very similar distribution constants between the mobile and stationary phases (KD). Chromatographic resolution is a function of many operational parameters. Among them, the nature of the stationary phase, mobile phase, temperature, the size of the column, that is, its length (L), inner diameter (ID) and the thickness of the stationary phase (df). As the number of components in the matrix increases, and the structural similarity between its components grows (isomerism), longer columns are required for complete compound separation. Alternatively, for the same purpose, one can employ smaller internal diameter columns. Obviously, increasing the length of the column markedly increases the analysis time. So, as the analysis of polyaromatic hydrocarbons (PAHs) or controlled drugs is regularly accomplished using 30 m long columns, the separation of hydrocarbons in gasoline requires longer, 100 m columns[96].

1.3.2- . Mass selective detector

A mass spectrometer attached to the gas chromatograph is often referred to as "mass detector"or "mass selective detector" (MSD). The MSD consists of an ionization chamber which in thelarge majority of situations uses electron ionization or electron impact (El)

to provide theenergy to the analytes which could fragment and generate the ions to be detected. Chemicalionization (Cl) of positive ions or negative ions, is a complementary, mild ionization method.(CI) is used as an alternative when no molecular ions are registered in the mass spectra obtainedby El, since fragmentation is excessively intense, due to the high liability of molecular ions, M+• (extremely short lifetime, < 10-6 s)[36]. Ions formed in the ionization chamber are removedtherefrom by a series of electrodes that collimate (focus) and accelerate and direct them to amass analyzer. The potential energy created by the accelerating field (E) is converted intokinetic energy[97,98]. The analyzer separates ions according to their m/z ratio. Several types of massanalyzers may be used in the GC-MS equipment. Quadrupole (Q) and ion trap (lT) are themost common; in recent years, there has been an increase in the use of time-of-flight analyzers(TOF); lately, resonance ion-cyclotron analyzers with Fourier transform (FT-ICR) and orbitraphave become commercially available. The use of magnetic deflection mass analyzers is muchless frequent[96,99,100].The charm –from an analytical standpoint- of a mass selective detector, is its ability to operatein three modes of data acquisition, namely: universal, selective and specific. When a scan iscomplete (the MSD detector functions as a universal detector), the mass spectra obtained forall mixture components are the basis for recognizing or identifying them. The mass scanningrate depends on the type of analyzer used (quadrupole, ion trap, time-of-flight or magneticsector) and the mass range to cover. The mass range for the entire sweep is established

inaccordance with the nature of the sample, that is, the range of molecular weights of itscomponents. The low end of the scanned mass range for aliphatic compounds, alcohols,amines, etc., can be set to m/z 30-40 (lower masses are not recommended, as they correspondto background signals); the minimum mass for aromatics can be set to m/z 50. The upper endof the scan range should correspond to the molecular weight of the heaviest substance presentin the mixture, plus 40 - 50 units. If the mass range is chosen poorly, for example, if for the (GCMSD) analysis of low molecular weight, highly volatile compounds (molecular weight less than150 Da), a very wide mass range is set, e.g., m/z 30-550, the number of spectra obtained perunit of time will be small, which affects the quality, reproducibility and reliability of theanalytical data[34-36]. On the other hand, high scan speeds demand fast response from theaccompanying hardware, to avoid loss of information.Selected ion monitoring, SIM, is the selective mode of operation of the MSD. Instead ofscanning a mass range, only a discrete set of m/z values is monitored. This permits to obtain amuch higher signal accumulation per unit time, which means that the sensitivity is greater(30-100 times) than when full scan is used. GC-MS-SIM analysis of an extract or a complexmatrix permits the selective detection of homologous molecules, isomers, or structuralderivatives, when the selected ion is a common feature in their mass spectra. However, thedetection becomes specific, highly selective, when the monitored ions constitute a uniquecombination which is found only in the mass spectrum of the target analyte. Besides its use in performing

quantitative analysis with higher selectivity and sensitivity, theSIM mode helps to reduce the possibilities of false positives in substance identification.Presumptive identifications are based solely on matching retention times of target analytesand standard compounds, but this result should be confirmed by GC-MSD-SIM, whencompounds of interest are in the mixture at trace levels[101].To setup a GC-MSD analysis in SIM mode, generally three characteristic "diagnostic" ions arechosen (one for quantification and other ones as "qualifiers"). The ion selection criteria attendthe following recommendations: 1) the ion´s abundance should be higher than 30%; 2) the massof the selected ion should be high, preferably, because low-mass ions are common to manysubstances; 3) the selected ion should be structurally representative of the molecule. Forexample, it could be a molecular ion, or a genetically-related fragment; 4) the selected ionshould not coincide with those from background (m/z 17, 18, 28, 32, 40, 43, 44), stationary phasebleeding (e.g.,, m/z 73, 147, 207, 281, 355), thermal degradation of the septum, or plasticizers(m/z 149). The partial ion currents of the selected ions are measured in a retention time windowin which the standard substance elutes (e.g., tR ± 0.5 min). Then the sample is analyzed underthe same GC-MS-SIM operational conditions used with the standard substance. When thereare several substances of interest, the same procedure is applied to each one and the selectedions are scanned only within the retention time window of its corresponding standardsubstance. The identity of the target analyte in the sample can be confirmed only when thefollowing 3 criteria are

satisfied: 1) the retention times of the analyte and the standard substancematch; 2) the S/N ratio is higher than 1:3 – 1:10; 3) the intensity ratios of the selected ions arethe same in the spectra of the analyte and of the standard substance. If the retention times arethe same (a necessary, but not sufficient condition), but the intensity ratios of the selected ionsin the spectra of the target compound and of the standard do not match, that is, are not identical,differ by more than 15%, the situation of a " false positive" is observed, i.e., the alleged presenceof the target analyte in the sample is not confirmed[102].

1.3.3- Principle of GC-MS

The mass spectrometer is a universal detector for gas chromatographs since any compound that can pass through a gas chromatograph is converted into ions in mass spectrometer. At the same time, the highly specific nature of mass spectrum makes the mass spectrometer a very specific gas chromatographic detector. Gas chromatography is an ideal separator, whereas mass spectrometry is excellent for identification. The aim of an interfacing arrangement is to operate both a gas chromatograph and a mass spectrometer without degrading the performance of either instrument. The problem is compatibility. One incompatibility problem is the difference in pressure required for the operation of a gas chromatograph and the"Principle" at high pressures, the latter is designed to run under high vacuum[103]. An associated problem is the presence of much carrier gas and little sample in the effluent from the gas chromatograph[104]. If the gas chromatograph is using packed column the flow of carrier gas may be in excess of

30ml/min, which would collapse the vacuum of the mass spectrometer. Therefore carrier gas must be substantially removed and various designs have to be developed[105].

1.3.4- Data analysis and interpretation

There are two basic strategies in GC- MS, for the identification of compounds. The first is the use of standard substances (certified reference material). However, not always all standards are available, many of them are not easily accessible for a large number of analytes. The second strategy is the combination of several approaches, among which are the following: (a) retention indices (RI), in conjunction with (b) experimental mass spectra (EI, 70 eV) and (c) their comparison with those of databases of retention indices obtained in columns of orthogonal polarity (polar and nonpolar) and of standard mass spectra (EI, 70 eV). The combination of several experimental parameters and data, i.e., retention times measured in both columns and mass spectra is mandatory for the structural identification of components in a mixture. The identification can be tentative (preliminary, presumptive) or confirmatory. Confirmation (positive or unambiguous) requires, in many cases, the use of a certified standard compound. Multiple analytes from complex mixtures, however, can have similar retention times or their mass spectra seem alike or have only very small quantitative differences (ion intensities). Limonene epoxides, xylenes, and many structurally similar terpenes, are examples of this situation. However, the possibility of simultaneous coincidence of both the retention indices calculated in both columns (polar and nonpolar) and

of mass spectra for two different substances, in fact, is very remote, almost unlikely[106]. For some cases, such as those that may have legal implications, i.e., environmental, forensic cases or disputes, control of doping agents in sports competitions, it is absolutely mandatory to use certified standard substances for identification and confirmation. The analysis of an essential oil, perfume, aroma, and fragrance fractions (or any other complex mixture), in order to quantify and identify its components, done by one-dimensional chromatography, should comply with the following conditions[106]:

(1) using preferably long capillary columns (50, 60 m)

(2) performing the analysis in two capillary columns with orthogonal phase (e.g., DB-1 or DB-5 and DB-WAX)

(3) obtaining experimentalmass spectra EI (70 eV) and doing a comparative search, preferably, on various mass spectradatabases (e.g., NIST, Wiley, Adams)

(4) calculating linear retention indices in two columns,polar and nonpolar.

(5) using standard compounds for further structural confirmation.

The combination of all these parameters allows confirmatory identificationof the mixture components. In GC- MS analysis it is very important to ensure that the chromatographicpeaks are "homogeneous", as the co-elution of various substances can lead tostructural miss -assignments.

The large number of fragment ions (cations and cation-radicals) in the mass spectrum is due,firstly, to their formation from molecular ions which have very different excesses of internalenergy. All molecular

ions with internal energies lower than the potential energy of appearanceof an ion fragment with the lowest formation activation energy will be recorded in the massspectrum as not dissociated molecular ions. Their intensity in the spectrum depends on themolecular structure and, particularly, their ability to delocalize (stabilize) the positive charge,which allows the ion M+• to exist for longer time than is required for its detection (ca. > 10-5 s)in a mass spectrometer. One of the major limitations of the electron ionization technique liesin the fact that molecules that are ionized, should be in the vapor phase, i.e. being volatilizable without undergoing thermal decomposition, prior to their ionization. Even so, many of thevolatilizable and thermostable molecules do not exhibit molecular ions in their mass spectra,only fragment ions, something that limits obtaining information on molecularweight. In fact,less than 10 % of all existing molecules are suitable for analysis by mass spectrometry byelectron ionization. The excluded cases are highly polar species (e.g., salts, amino acids), thoseof high-molecular weight (e.g., proteins, nucleic acids, polymers), and thermolabile compounds(e.g., sugars). In some cases, chemical derivatization of the molecule permits toincrease its volatility and thermal stability and decrease itspolarity[106].

1.3.5- Applications of GC/MS

(GC-MS) is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample. GC is used to separate the volatile and thermally stable

constituents in a sample whereas GC-MS fragments the analyte to be identified on the basis of its mass[106,107].

Advantages of GC-MS requires the analyte to have significant vapor pressure between 30 and 300°C. GC presents an insufficient proof of the nature of the detected compounds. The main features of GC-MS include:enhanced molecular ion, improved confidence in sample identification, significant increase in range of thermally labile and low volatility samples , much faster analysis, improved sensitivity particularly for compounds that are hard to analyze and the many other features and options provide compelling reasons to use the GC-MS in broad range of areas [108,109]. It could use in:

i- Environmental monitoring

GC-MS has become a highly recommended tool for monitoring and tracking organic pollutants in the environment. The cost of GC/MS equipment has decreased whereas the reliability has markedly increased. Chloro-phenols in water and soil, polycyclic aromatic hydrocarbons (PAH), unleaded gasoline, dioxins, dibenzofurans, organo-chlorine pesticides, herbicides, phenols, halogenated pesticides are conveniently screened by this technique. It can be used to screen the degradation products of lignin in bio-mass research, pesticides in spinach. Analysis of decacyclene, ovalene and even C_{60} degradation analysis of carbamazepine and its metabolites in treated sewage water and steroid can be done without derivatization[110,111].

ii- Food, beverage, flavor and fragrance analysis

Foods and beverages have several aromatic compounds existing naturally in native state or formed while processing. GC-MS is

exclusively used for the analysis of esters, fatty acids, alcohols, aldehydes, terpenes etc. GC-MS is also used to detect and measure contaminants, spoilage and adulteration of food, oil, butter, ghee that could be harmful and should to be controlled and checked as regulated by governmental agencies. It is used in the analysis of piperine, spearmint oil, lavender oil, essential oil, fragrance standards, perfumes, menthol, allergens, olive oil, lemon oil, peppermint oil, strawberry syrup, butter triglycerides, residual pesticides in foods[112,113].

iii)Forensic and criminal cases

GC-MS can analyze the particles from suspect to correlate his involvement in case. The analysis of fire debris using GC-MS can be established. It is the key tool used in sports anti-doping laboratories to test athlete's urine samples for prohibited performance-enhancing drugs like anabolic steroids. It is also commonly used in forensic toxicology to find poisons, steroids in biological specimens of suspects and[victims113,114,115].

iv)Biological and pesticides detections

GC-MS is exclusively used in bio-analysis of blood, urine for the presence of barbiturates, narcotics, alcohols, residual solvents, drugs like: anesthetics, anticonvulsant, antihistamine, anti-epileptic drug, sedative hypnotics and narcotics. This technique could be used for detecting adulterations, fatty acid profiling in microbes, presence of free steroids, blood pollutants, metabolites in serum, organo-chlorinated pesticides in river water, drinking water, soft drinks, pesticides in sunflower oil etc.[116].

v)Security and chemical warfare agent detection

Explosive detection systems have become a part in almost all international airports.Here, GC-MS is an essential part of chemical analysis unit. For enhancing capability in homeland security and public health preparedness, traditional GC-MS units with the transmission quadrupole mass spectrometers, as well as those with cylindrical ion trap (CIT-MS) and toroidal ion trap (T-ITMS) mass spectrometers have been modified for field portability and near real-time detection of chemical warfare agents (CWA) such as sarin[117], soman, and VX[118].

vi)Astrochemistry and geochemical research

Several GC-MS have left earth for the astrochemical studies. Two were taken to Mars planet by the Viking program. Scientist analyzed the atmosphere of Venus with GC-MS. The Huygens probe of the Cassini-Huygens mission landed one GC-MS on Saturn's largest moon-Titan[119]. The material in the comet 67P/Churyumov-Gerasimenko was analyzed by the Rosetta mission with a chiral GC-MS in 2014. Significantly enhanced molecular ions, major isomer and structurally significant mass spectral peaks, extended range of low volatility hydrocarbons that are amenable for analysis and unique isotope ratio information make GC-MS valuable for organic geochemical applications[120].

vii)Medicine and pharmaceutical applications

Dozens of congenital metabolic diseases called as "inborn error of metabolism" are now detectable in newborn by screening tests using gas chromatography–mass spectrometry. GC-MS can determine

compounds in urine even in minor concentration. These compounds are normally not present but appear in individuals suffering from metabolic disorders. This is easy, effective and efficient way to diagnose the problem like in case of genetic metabolic disorders by a urine test at birth. In combination with isotopic labeling of metabolite, the GC-MS is used for determining metabolic activity. Most applications are based on the use of ^{13}C labeling and the measurement of ^{13}C-^{12}C ratios with an isotope ratio mass spectrometer (IRMS); an MS with a detector designed to measure a few selected ions and retention values as ratios. GC-MS is useful to detect oils in creams, ointments, lotion etc. It is widely used in pharmaceutical industries for analytical research and development, quality control, quality assurance, production, pilot plants departments for active pharmaceutical ingredients (API), bulk drugs and formulations[121]. It is used for process and method development, identification of impurities in API. It is an integral part of research associated with medicinal chemistry (synthesis and characterization of compounds), pharmaceutical analysis (stability testing, impurity profiling), pharmacognosy, pharmaceutical process control, pharmaceutical biotechnology etc.[122].

viii) Petrochemical and hydrocarbons analysis

Significantly enhanced molecular ions that are always observed, isomer and structurally significant mass spectral peaks and extended range of low- volatile hydrocarbons that are amenable for analysis including waxes up to $C_{74}H_{150}$ makes the GC-MS a most valuable technique. Broad range of petrochemicals, fuels and hydrocarbon

mixtures, including gasoline, kerosene, naphthenic acids, diesel fuel, various oil types, transformer oil, biodiesel, wax and broad range of geochemical samples can be analyzed by GC-MS[123].

ix)Clinical toxicology

Enhanced molecular ions, extended range of compounds amenable for analysis, superior sensitivity for compounds and faster analysis are the main attractive features of the clinical toxicology. The toxin and venoms are identified by GC-MS. It is extensively used in clinical toxicology[124].

x)Academic research As a unique and powerful technology the GC-MS provides a rare opportunity to perform the analysis of new compounds and for characterization and identification of synthesized or derivatized compound. It is widely used in pure and applied sciences like chemistry, polymers, nanotechnology and biotechnology etc. It yields useful information that can be used in global research publication [125,126].

xi-Industrial applications

GC-MS is used in industries for the analysis of aromatic solvents, inorganic gases, amino alcohol in water, impurities in styrene, glycol, diols, xylene, allergens in cosmetics etc. GC-MS is used for the characterization of formic acid in acetic acid for industrial use. In industries acetic acid is important intermediate in coal chemical synthesis. It is used in the production of polyethylene, cellulose acetate and polyvinyl as well as synthetic fiber and fabrics[127].

xii)Energy and fuel applications

GC-MS is used for the analysis of aromatic solvents, sulphur, impurities in polypropylene, sulphur in methane, natural gases, 1,3 butadiene, ethylene, gas oil, unleaded gasoline, polyethene, diesel.oil, , polyethylene, diesel, modified biomass, grafted polymers etc.[128]. GC-MS has triggered a new area of research and taken to new heights of impactful presentation and characterization of compounds by its wide range of applications[129,130].

Conclusions: GC-MS is an advanced technique that cannot be compared with other modern analytical equipments. It has broad range of applications that caters to academic research, quality control aswell as industrial applications. Its concise, efficient, automated system gives fast, reproducible and effective results that serve a key role in advancement of science and technology.

Aim of this study

This study was aimed to:

-Screening the seed of the medicinally important species Adansonia digitata for secondary metabolites.

-Extraction of oil from targeted species.

-Conducting a GC-MS analysis for extracted oil.

-Screening the oil for its antimicrobial activity.

Chapter Two

2-Materials and Methods

2.1-Materials

2.1.1-Plant material

Fruits of *Adansonia digitata* were purchased from the local market-Khartoum and kindly authenticated by the Department of Phytochemistry and Taxonomy, National Research Center, Khartoum-Sudan.

2.1.2- Instruments

A Shimadzo GC-MS-QP2010 Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 µm, thickness)was used**.**

2.1.3-Test organisms

Adansonia digitata oil was screened for antibacterial and antifungal activities using the standard microorganisms shown in table(2.1).

Table 2.1: Test organisms

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

2.2- Methods

2.2.1- Preperations of reagents for phytochemical screening.

2.2.1.1-Flavonoid test reagents

- Aluminium chloride solution

(1 g) of aluminum chloride was dissolved in 100 ml methanol

- Potassium hydroxide solution

(1 g) of potassium hydroxide was dissolved in 100 ml water.

-Ferric chloride solution

(1 g) of ferric chloride was dissolved in 100 ml methanol.

2.2.1.2- Alkaloid test reagents

Maeyer reagent

- **Mercuric chloride solution**: 1.36 g in 60 ml. water.

- **Potassium iodide solution** : 5 g in 10 ml. water

The two solutions were combined and then diluted with water up to 100 ml.

-Wagner reagent

(1.27 g) iodine and(2 g) of potassium iodide in (100 ml) water.

2.2.1.3- Preparation of plant extract for phytochemicalscreening

(100 g) Of powdered air- dried fruit pulp of *Adansonia digitata* were extracted with 95% ethanol (soxhlet) until exhaustion. This prepared extract(PE) was used for phytochemical screening.

2.2.2- Phytochemical screening

The prepared extract of the plant was screened for major secondary constituents.

2.2.2.1 Test for unsaturated sterols and for triterpenes

(10 ml)of the (PE) was evaporated to dryness on a water bath, and the cooled residue was stirred with petroleum ether to remove most of the coloring materials. The residue was then extracted with 10 ml chloroform. The chlorform solution was dehydrated over anhydrous sodium sulphite . (5 ml) portion of the solution was mixed with(0.5 ml) of acetic anhydride, followed by two drops of concentrated sulphuric acid.

2.2.2.2- Test for flavonoids

(20 ml) of the (PE) were evaporated to dryness on water bath. The cooled residue was defatted with petroleum ether and then dissolved in 30 ml of 30% aqueous methanol and filtered. The filtrate was used for the following tests:

- To 3 ml. of filtrate a fragment of magnesium ribbon was added, shaken and then few drops of concentrated hydrochloric acid were added.

- To 3 ml. of the filtrate few drops of aluminium chloride solution were added.
- To 3 ml. of the filtrate few drops of potassium hydroxide solution were added.

2.2.2.3- Test for alkaloids

(10 ml) of the (PE) were evaporated to dryness on a water bath and 5 ml of 0.2N hydrochloric acid were added and the solution was heated with stirring for 10 minutes, then cooled and filtrated.

Filtrate was divided into two portions:

To one portion a few drops of Maeyer reagent were added., to the other portion few drops of Wagner reagent were added.

2.2.2.4- Test for tannins

(10 ml) of (PE) were evaporated to dryness and the residue was extracted with n-hexane and then filtrated. The insoluble residue was stirred with n-hexane and (10 ml) of hot saline (0.9% w/v of sodium chloride and freshly prepared distilled water) were added. The mixture was cooled , filtrated and the volume adjusted to 10 ml. with more saline solution. (5 ml) of this solution were treated with few drops of ferric chloride solution.

2.2.2.5 -Test for saponins

(1g) of dried powdered plant material was placed in a clean test tube. (10 ml) of distilled water were added and the tube was stoppered and vigorously shaken for about 30 seconds, and allowed to stand.

2.2.3-Extraction of oil from seeds of *Adansonia digitata*

Powdered seeds of *Adansonia digitata* (500g) were exhaustively extracted with n-hexane (soxhlet).The solvent was removed under

reduced pressure and the oil was kept in the fridge at 4^{o}C for further manipulation.

2.2.4-Esterification of oil

A Methanolic solution of sodium hydroxide was prepared by dissolving (2g) of sodium hydroxide in 100ml methanol.A stock solution of methanolic sulphuric acid was prepared by mixing (1ml)of concentrated sulphuric acid with (99ml) methanol.

The oil(2ml) was placed in a test tube and 7ml of alcoholic sodium hydroxide were added followed by 7ml 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 normal hexane were added and the tube was vigorously shaken for five minutes.The

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 vial.

2.2.5- GC-MS analysis

The oil of the seeds of *Adansonia digitata*was analysed by gas chromatography – mass spectrometry.A Shimadzo GC-MS-QP2010

Ultra instrument with a RTX-5MS column (30m,length ; 0.25mm diameter ; 0.25 μm, thickness)was used.Helium (purity; 99.99 %) was used as carrier gas.Oven temperature program is given in Table 2.1, while other chromatographic conditions are depicted in Table 2.2.

Table 2.1: Oven temperature program

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

Table2.2: Chromatographic conditions

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

2.2.6-Antimicrobial assay

2.2.6.1-Preparation of 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°C for 24 hours.

The bacterial growth was harvested and washed off with sterile normal saline, and finally suspended in 100 ml of normal saline to produce a suspension containing about 10^8-10^9colony forming units per ml.The suspension was stored in the refrigerator at 4°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°C for 24 hours.

2.2.6.2-Preparation of fungal suspensions

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

2.2.6.3-Testing for antibacterial activity

The cup-plate agar diffusion method was adopted with some minor modifications, to assess the antibacterial activity of Schiff bases and their Mannich bases. (2ml) of the standardized bacterial stock suspension were mixed with 200 ml of sterile molten nutrient agar which was maintained at 45°C in a water bath. (20 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 0.1 ml samples of each compound using adjustable volume microtiter pipette and allowed to diffuse at room temperature for two hours. The plates were then incubated in the upright position at 37°C 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

3-Results and Discussion

3.1-Phytochemical screening

Phytochemical screeningof *Adansoniadigitata* fruit pulp gave positive reactions for: steroids,flavonoids,tannins,terpenes and glycosides.

Table 3.1:Phytochemical screening of *Adansoniadigitata* fruit pulp

Species	Flavonoids	Tannins	Steroids	Terpenes	Glycosides
Adansoniadigitata	+ve	+ve	+ve	+ve	+ve

3.2-The GC-MS analysis of *Adansoniadigitata* essential oil

GC-MS analysis of *Adansoniadigitata*oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern.Comparison of the mass spectra with the database on MS library revealed about 90-95% match.

3.2.1- Constituents of oil

The GC-MS spectrum of the studied oil revealed the presence of 20 components(Table:3.2).The typical total ion chromatograms(TIC) of hexane extract are shown in Fig.3.1.

Table:3.2: Constituents of *Adansoniadigitata* oil

Peak#	R.Time	Area	Area%	Name
1	8.425	676572	0.71	Butylated Hydroxytoluene
2	10.825	126889	0.13	Methyl tetradecanoate
3	12.054	27758	0.03	Pentadecanoic acid, methyl ester
4	13.066	95749	0.10	9-Hexadecenoic acid, methyl ester, (Z)-
5	13.308	14336990	14.95	Hexadecanoic acid, methyl ester
6	14.190	152276	0.16	7,10-Hexadecadienoic acid, methyl ester
7	14.259	150678	0.16	cis-10-Heptadecenoic acid, methyl ester
8	14.549	95651	0.10	Heptadecanoic acid, methyl ester
9	15.138	1996748	2.08	Methyl 2-octylcyclopropene-1-heptanoate
10	15.432	17963750	18.73	9,12-Octadecadienoic acid (Z,Z)-, methyl e
11	15.496	21479658	22.40	9-Octadecenoic acid, methyl ester, (E)-
12	15.783	3375120	3.52	Methyl stearate
13	16.006	29016065	30.26	Oleic Acid
14	16.821	1964348	2.05	cis-10-Nonadecenoic acid, methyl ester
15	17.843	536804	0.56	l-(+)-Ascorbic acid 2,6-dihexadecanoate
16	17.886	254628	0.27	cis-11-Eicosenoic acid, methyl ester
17	18.169	755491	0.79	Eicosanoic acid, methyl ester
18	19.373	886628	0.92	Phenol, 2,2'-methylenebis[6-(1,1-dimethyle
19	19.890	1736483	1.81	9-Octadecenoic acid, 1,2,3-propanetriyl est
20	20.063	276517	0.29	2-Ethylbutyric acid, eicosyl ester
		95904803	100.00	

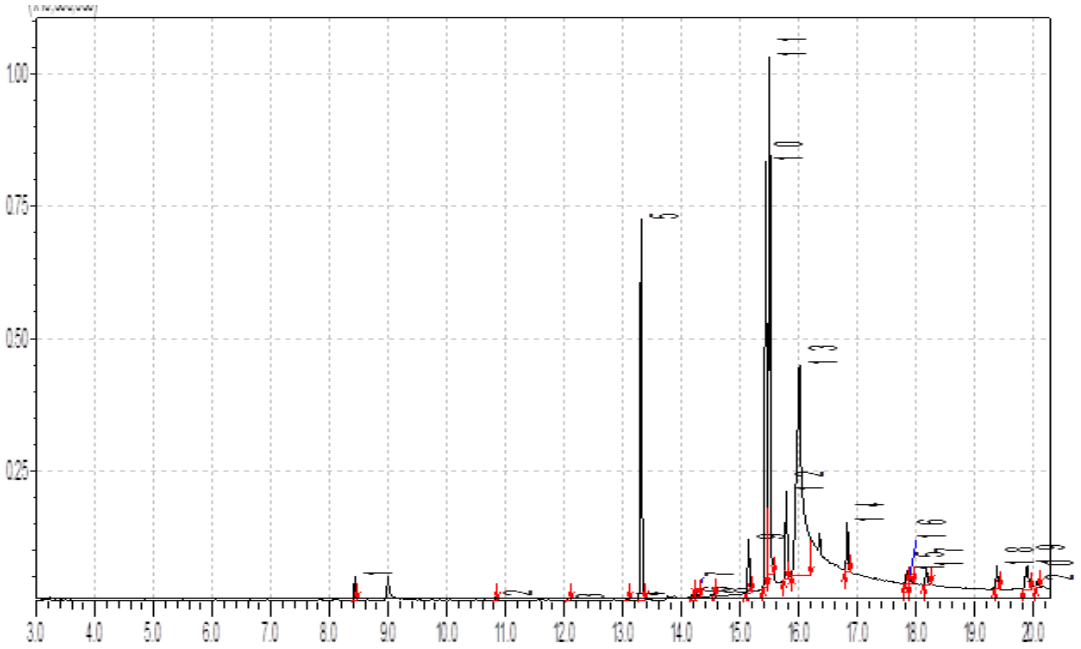

Fig.3.1:Cromatograms of *Adansoniadigitata* seed oil

The following acids and esters were detected in the chromatogram:

Methyl tetradecanoate(0.13%)

The EI mass spectrum of methyl tetradecanoateis shown in Fig. 3.2.The peak at m/z 242, which appeared at R.T. 10.825 in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{30}O_2]^{+}$ .The peak at m/z211 corresponds to loss of a methoxyl function.

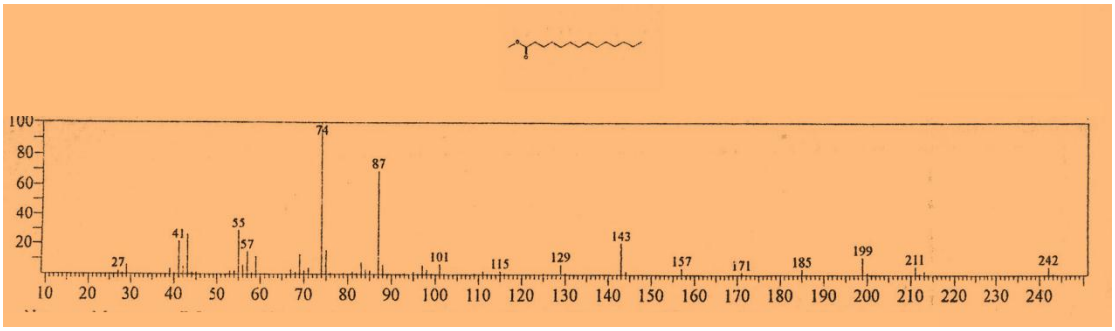

Fig. 3.2: Mass spectrum of methyl tetradecanoate

Pentadecanoic acid methyl ester

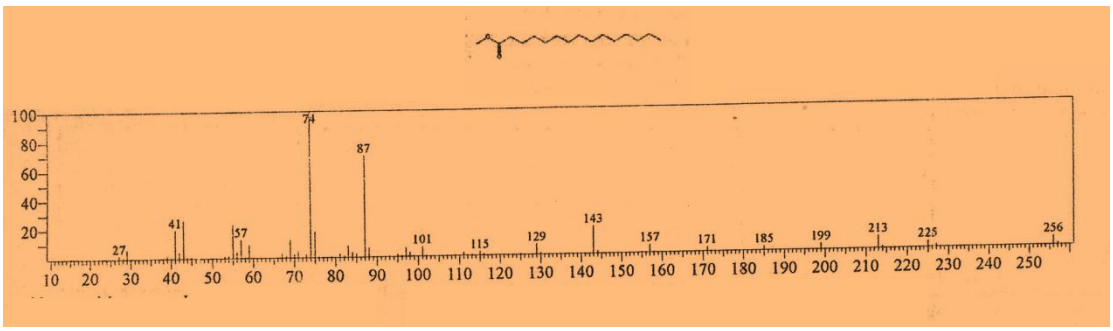

Fig. 3.3: Mass spectrum of pentadecanoic acid methyl ester

The EI mass spectrum of pentadecanoic acid methyl esteris shown in Fig. 3.3.The peak at m/z 256, which appeared at R.T. 12.054 in total ion chromatogram, corresponds to $M^{+}[C_{16}H_{32}O_2]^{+}$ .The peak at m/z225 corresponds to loss of a methoxyl function.

9-Hexadecanoicacid methyl ester

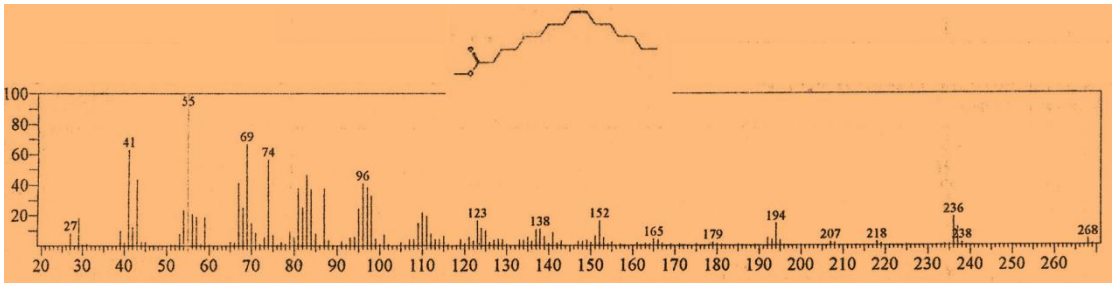

Fig. 3.4: Mass spectrum of 9-Hexadecanoic methyl ester

The EI mass spectrum of 9-hexadecanoic acid methyl ester is shown in Fig. 3.4.The peak at m/z 268, which appeared at R.T. 13.066 in total ion chromatogram, corresponds to $M^{+}[C_{17}H_{32}O_2]^{+}$ .The peak at m/z237 corresponds to loss of a methoxyl function.

Hexadecanoicacid methyl ester

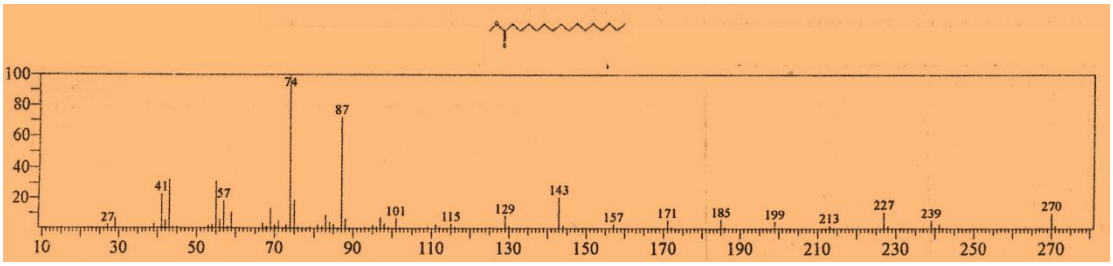

Fig. 3.5: Mass spectrum of hexadecanoic methyl ester

The EI mass spectrum of hexadecanoic acid methyl ester is shown in Fig. 3.5.The peak at m/z 270, which appeared at R.T. 13.308 in total ion chromatogram, corresponds to $M^+[C_{17}H_{34}O_2]^+$ .The peak at m/z239 corresponds to loss of a methoxyl function.

7,10-Hexadecanoic acid methyl ester

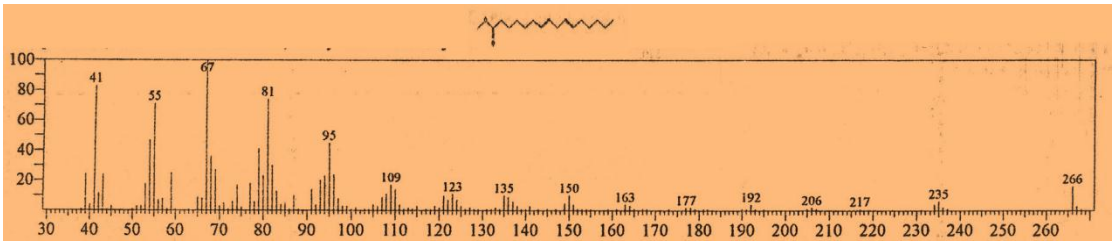

Fig. 3.6: Mass spectrum of 7,10-hexadecanoic methyl ester

The EI mass spectrum of 7,10-hexadecanoicacid methyl ester is shown in Fig. 3.6.The peak at m/z 266, which appeared at R.T. 14.190 in total ion chromatogram, corresponds to $M^+[C_{17}H_{30}O_2]^+$ .The peak at m/z235 corresponds to loss of a methoxyl function.

Cis—10-Heptadecanoic acid methyl ester

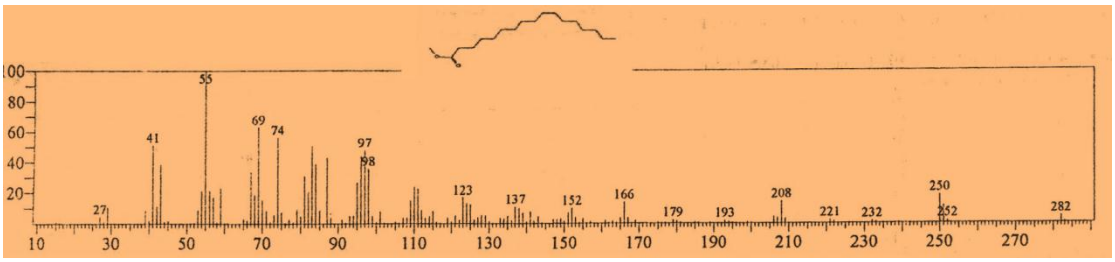

Fig. 3.7: Mass spectrum of cis-10-heptadecanoic methyl ester

The EI mass spectrum of Cis—10-heptadecanoic acid methyl ester is shown in Fig. 3.7.The peak at m/z 282, which appeared at R.T. 14.259 in total ion chromatogram, corresponds to $M^+[C_{18}H_{34}O_2]^+$ .The peak at m/z251 corresponds to loss of a methoxyl function.

Heptadecanoic acid methyl ester

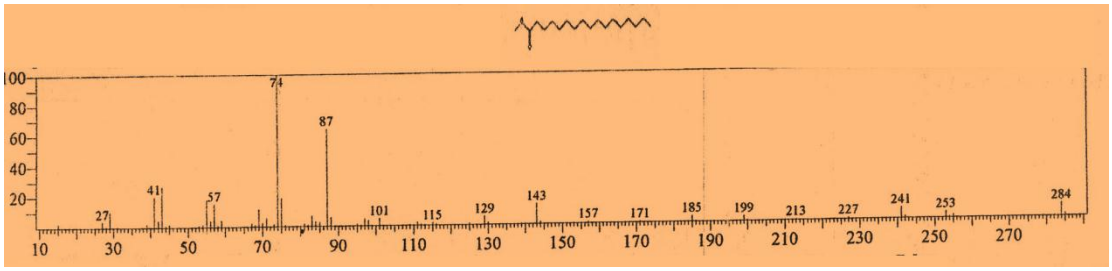

Fig. 3.8: Mass spectrum of heptadecanoic methyl ester

The EI mass spectrum of heptadecanoic acid methyl esteris shown in Fig. 3.8.The peak at m/z 284, which appeared at R.T. 14.549 in total ion chromatogram, corresponds to $M^+[C_{18}H_{36}O_2]^+$ .The peak at m/z253 corresponds to loss of a methoxyl function.

Methyl 2-octylcyclopropene-1-heptanoate

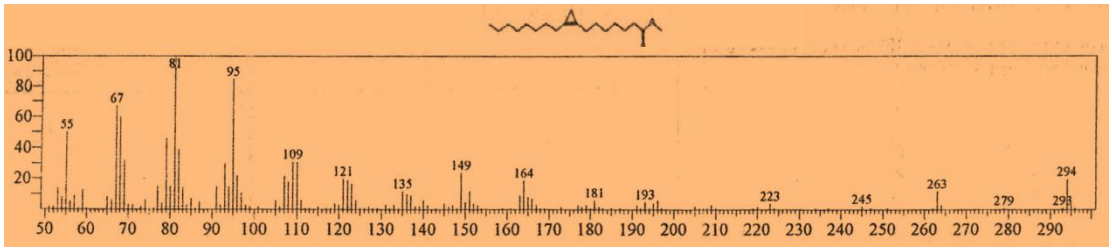

Fig. 3.9: Mass spectrum of methyl 2-octylcyclopropene-1-heptanoate

The EI mass spectrum of methyl 2-octylcyclopropene-1-heptanoate is shown in Fig. 3.9.The peak at m/z 294, which appeared at R.T. 15.138 in total ion chromatogram, corresponds to $M^+[C_{19}H_{34}O_2]^+$ .The peak at m/z263 corresponds to loss of a methoxyl function.

9,12-Octadecanoic acid methyl ester

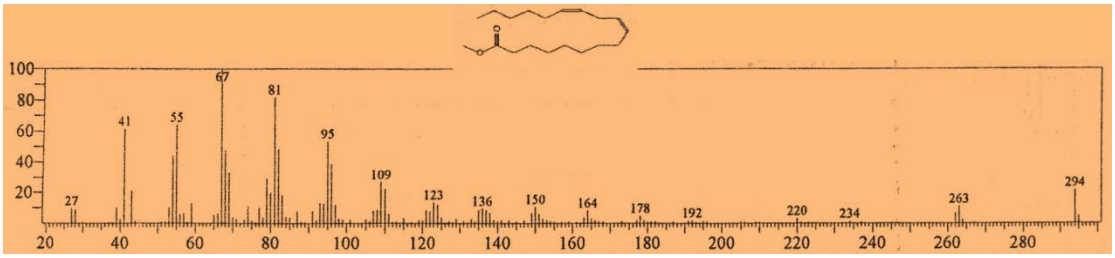

Fig. 3.10: Mass spectrum of9,12-octadecanoic acid methyl ester

The EI mass spectrum of 9,12-octadecanoic acid methyl ester is shown in Fig. 3.10.The peak at m/z 294, which appeared at R.T. 15.432 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{34}O_2]^{+}$ .The peak at m/z263 corresponds to loss of a methoxyl function.

9-Octadecanoic acid methyl ester

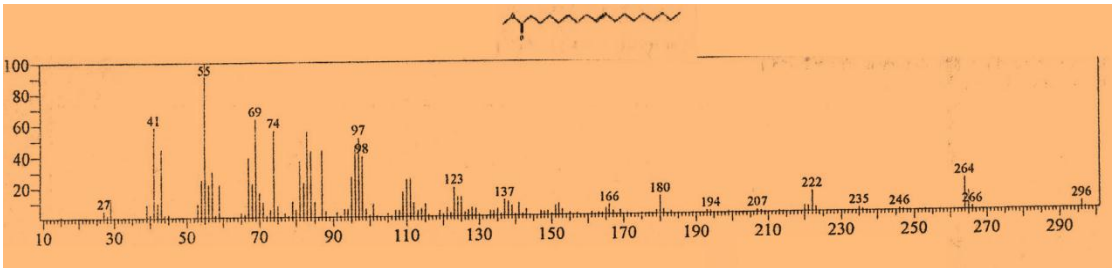

Fig. 3.11: Mass spectrum of9-octadecanoic acid methyl ester

The EI mass spectrum of 9-octadecanoic acid methyl esteris shown in Fig. 3.11.The peak at m/z 296, which appeared at R.T. 15.496 in total ion chromatogram, corresponds to $M^{+}[C_{19}H_{36}O_2]^{+}$ .The peak at m/z265 corresponds to loss of a methoxyl function.

Methyl stearate

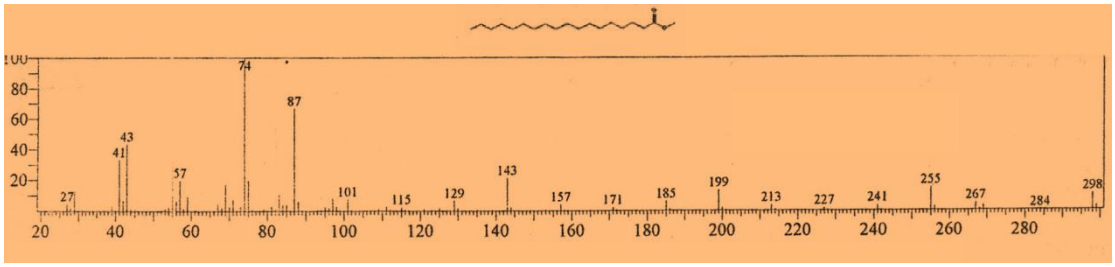

Fig. 3.12: Mass spectrum ofmethyl stearate

The EI mass spectrum of methyl stearateis shown in Fig. 3.12.The peak at m/z 298, which appeared at R.T. 15.783 in total ion chromatogram, corresponds to $M^+[C_{19}H_{38}O_2]^+$ .The peak at m/z267 corresponds to loss of a methoxyl function.

Oleic acid

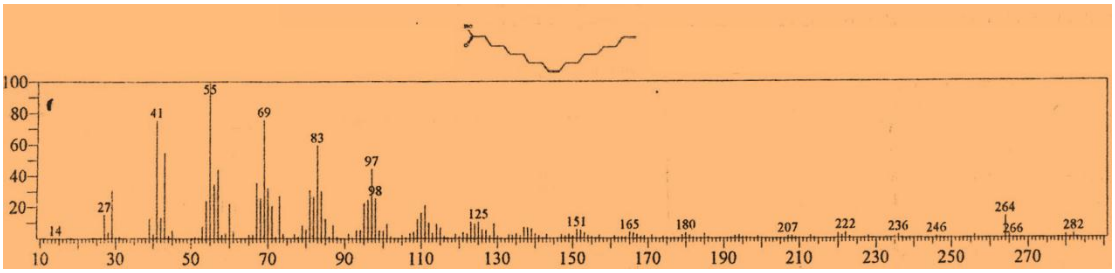

Fig. 3.13: Mass spectrumoleic acid

The EI mass spectrum of oleic acidis shown in Fig. 3.13.The peak at m/z 282, which appeared at R.T. 16.006 in total ion chromatogram, corresponds to $M^+[C_{18}H_{34}O_2]^+$ .The peak at m/z222 corresponds to loss of a methylenecarboxy function.

Cis-10-Nonadecanoic acid methyl ester

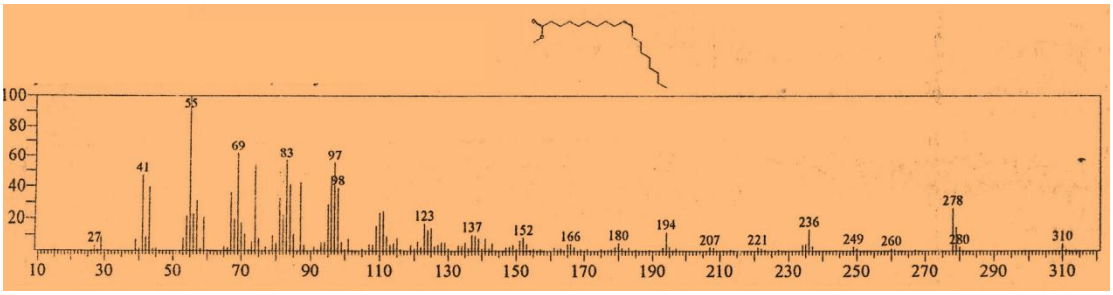

Fig. 3.14: Mass spectrum Cis-10-Nonadecanoic acid methyl ester

The EI mass spectrum of Cis-10-nonadecanoic acid methyl ester is shown in Fig. 3.14.The peak at m/z 310, which appeared at R.T. 16.821 in total ion chromatogram, corresponds to $M^+[C_{20}H_{38}O_2]^+$ .The peak at m/z180 corresponds to loss of a methoxyl function.

(+)-Ascorbic acid -2,6-dihexadecanoate

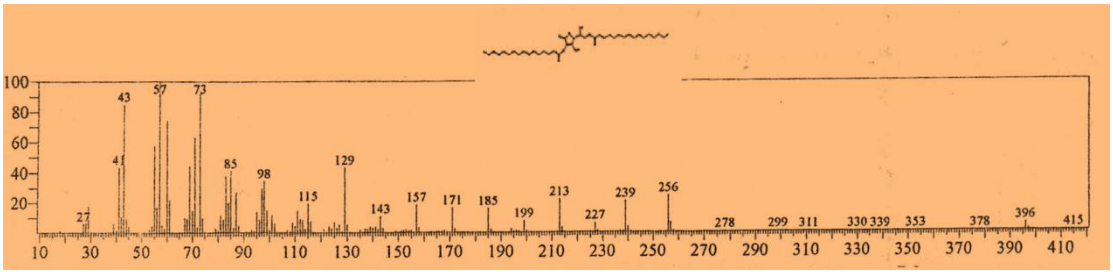

Fig. 3.15: Mass spectrum of (+)-Ascorbic acid -2,6-dihexadecanoate

The EI mass spectrum of (+)-ascorbic acid -2,6-dihexadecanoate is shown in Fig. 3.15.The peak at m/z 652, which appeared at R.T. 17.843 in total ion chromatogram, corresponds to $M^+[C_{38}H_{68}O_8]^+$ .The peak at m/z211 corresponds to loss of a methoxyl function.

Cis-11-Eicosenoic acid methyl ester

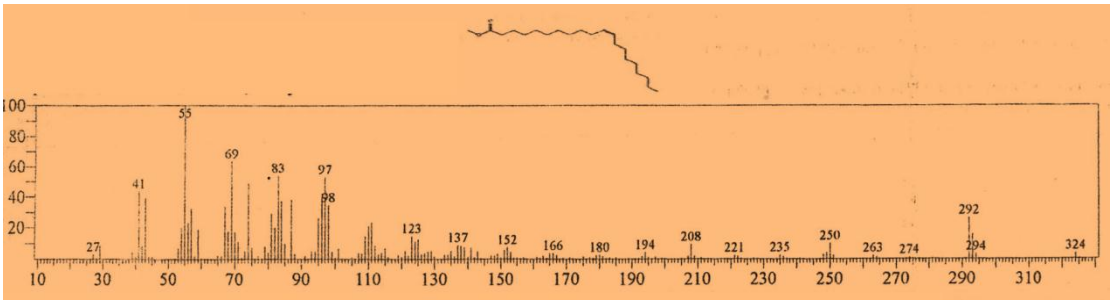

Fig. 3.16: Mass spectrum of Cis-11-Eicosenoic acid methyl ester

The EI mass spectrum of Cis-11-eicosenoic acid methyl esteris shown in Fig. 3.16.The peak at m/z324, which appeared at R.T. 17.886 in total ion chromatogram, corresponds to $M^+[C_{21}H_{40}O_2]^+$ .The peak at m/z293 corresponds to loss of a methoxyl function.

Eicosanoic acid methyl ester

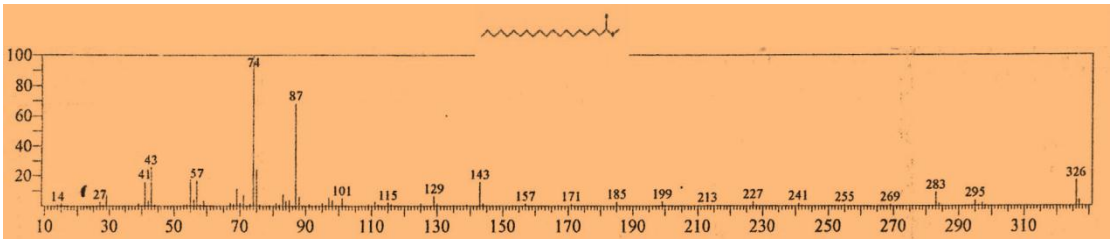

Fig. 3.17: Mass spectrum of eicosanoic acid methyl ester

The EI mass spectrum of eicosanoic acid methyl esteris shown in Fig. 3.17.The peak at m/z326, which appeared at R.T. 18.169 in total ion chromatogram, corresponds to $M^+[C_{21}H_{42}O_2]^+$ .The peak at m/z295 corresponds to loss of a methoxyl function.

1,2,3-Prpanetriyl-9-octadecanoic acid ester

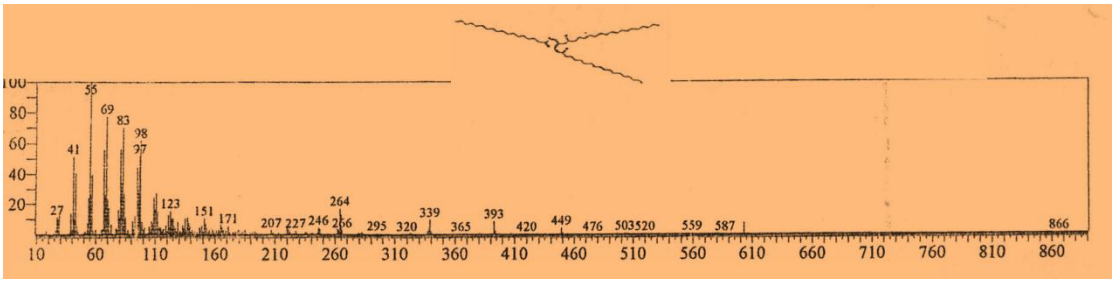

Fig. 3.18: Mass spectrum of 1,2,3-prpanetriyl-9-octadecanoic acid ester

The EI mass spectrum of 1,2,3-prpanetriyl-9-octadecanoic acid ester is shown in Fig. 3.18.The peak at m/z884, which appeared at R.T. 19.890 in total ion chromatogram, corresponds to $M^+[C_{57}H_{104}O_6]^+$

2-Ethylbutyric acid eicosyl ester

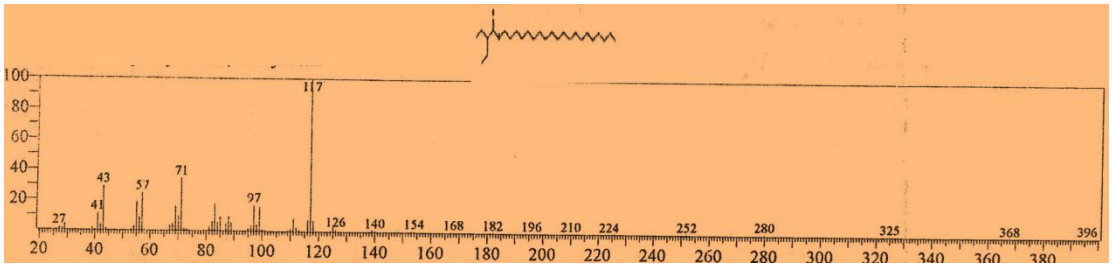

Fig. 3.19: Mass spectrum of 2-ethylbutyric acid eicosyl ester

The EI mass spectrum of 2-ethylbutyric acid eicosyl esteris shown in Fig. 3.19.The peak at m/z396, which appeared at R.T. 20.063 in total ion chromatogram, corresponds to $M^+[C_{26}H_{52}O_2]^+$.

Butylatedhydroxytoluene

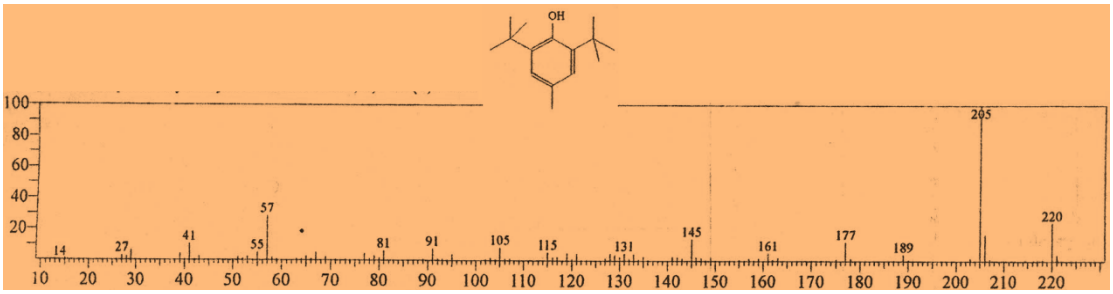

Fig. 3.20: Mass spectrum of butylatedhydroxytoluene

The EI mass spectrum of butylatedhydroxytolueneis shown in Fig. 3.20.The peak at m/z 220, which appeared at R.T. 8.425 in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{24}O]^{+}$ .The peak at m/z205 corresponds to loss of OH_2^{+}.

2,2`-Methylene-bis-[6-(1,1-dimethylethyl)-4-methyl]phenol

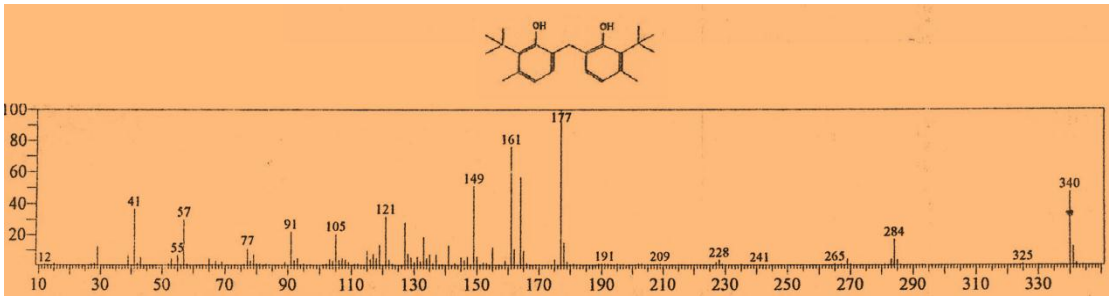

Fig. 3.21: Mass spectrum of 2,2`-Methylene-bis-[6-(1,1-dimethylethyl)-4-methyl]phenol

The EI mass spectrum of 2,2`-methylene-bis-[6-(1,1-dimethylethyl)-4-methyl]phenolis shown in Fig. 3.21.The peak at m/z 220, which appeared at R.T. 8.425 in total ion chromatogram, corresponds to $M^{+}[C_{15}H_{24}O]^{+}$ .The peak at m/z205 corresponds to loss of OH_2^{+}.

3.3-Antimicrobial activity

The oil was screened for antimicrobial activity against standard organisms. The average of the diameters of the growth inhibition zones are shown in Table (3.3) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially

active;13-18mm: active;>18mm:very active).Tables (3.4) and (3.5) represent the antimicrobial activity of standard antibacterial and antifungal chemotherapeutic agents against standard bacteria and fungi respectively.

Table (3.3) : Antibacterial activity of *A.digita*oil :M.D.I.Z (mm)

Drug	Conc.(mg/ml)	Ec	Ps	Sa	Bs	Ca	An
A.digitata oil	100	-	15	24	15	9	13

Table (3.4) : Antibacterial activity of standard chemotherapeutic agents :M.D.I.Z (mm)

Drug	Conc. mg/ml	Bs.	Sa.	Ec.	Ps.
Ampicillin	40	15	30	-	-
	20	14	25	-	-
	10	11	15	-	-
Gentamycin	40	25	19	22	21
	20	22	18	18	15
	10	17	14	15	12

Table (3.5) : Antifungal activity of standard chemotherapeutic agents against standard fungi

Drug	Conc. mg/ml	An.	Ca.
Clotrimazole	30	22	38
	15	17	31
	7.5	16	29

- Sa.*: Staphylococcus aureus*
- Ec.*: Escherichia coli*
- Pa.: *Pseudomonas aeruginosa*
- An.: *Aspergillusniger*
- Ca.: *Candida albicans*

- *Bs.: Bacillus subtilis*
- M.D.I.Z: Mean diameter or growth inhibition zone (mm). Average or two replicates, inhibition zone >=15: sensitive, <15: resistant.

The oil showed activity against all test organisms,but it was partially active with the fungus *Candida albicans*.Significant activity against *Staphylococcus aureus*was observed.

Conclusion

Phytochemical screeningof *Adansoniadigitata* fruit pulp gave positive reactions for: steroids,flavonoids,tannins,terpenes and glycosides.

GC-MS analysis of *Adansoniadigitata*oil was conducted and the identification of the constituents was initially accomplished by comparison with the MS library (NIST) and further confirmed by interpreting the observed fragmentation pattern.Comparison of the mass spectra with the database on MS library revealed about 90-95% match.The GC-MS spectrum of the studied oil revealed the presence of 20 components.

Recommendations

1-The extracted oil may be evaluated for anti-inflammatory,antimalarial and antispasmodic activities.

2-Other bioconstituents from the studied species may be isolated and evaluated for their biological potential and their structures may be elucidated via spectral tools.

References

REFERENCES

1. Diop, A.G., Sakho, M., Dornier, M., Cisse M, Reynes M.,*Fruits*, **61**, 55(2006).

2. Gebauer, J., El-Siddig, K., Ebert, G.,*Gartenbauwissenschaft*,**67**, 155(2002).

3.Diop, A.G., Sakho, M., Dornier, M., Cisse, M.,Reynes, M.,*Fruits*,**61**:55(2005)

4. Adanson, M.,*HistAcad Roy Sci* (Paris),**1771**, 218(1971).

5. Baum, D.A.,*Annals of the Missouri Botanical Garden*,**82**, 440(1995)

6. Armstrong, P.,*Trees in South Africa*,**28**: 92(1977).

7. Baum, D.A., Small, R.L., Wendel, J.F.,*SystBiol*, **47** 181(1998).

8. Chevalier, M.A.,*SociétéBotanique de France*,**53**, 480(1906).

9. Drake, E., *A Book of Baobabs*, Aardvark Press, Cape Town, South Africa(2006).

10. Wickens, G.E., Lowe, P.,*The Baobabs: Pachycauls of Africa, Madagascar and Australia*. *Springer*ISBN, **978**, 166358049London(2008).

11. Adesanya, S.A., Idowu, T.B.,Elujoba, A.A.,.PlantaMedic*a***54**: 374(1988).

12. UNCTAD (United Nations Conference on Trade and Development), Market brief in the European union for selected natural ingredients derived from native species(2005). *Adansonia digitata*.http://www.biotrade.org/ResourcesPublications/biotradebrief-baobab

13. Lamien-Meda, A., Lamien, C.E., Compaoré, M.M.Y., Meda, R.N.T., Kiendrebeogo, M., Zeba, B., Millogo, J.F.,Nacoulma, O.G., *Molecules* **13**:581(2008).

14. EL AMIN, H. M.,*Trees and Shrubs of the Sudan*, Ithaca Press, UK, ISBN,**0863721168**(1990).

15. PURSEGLOVE, J. W.,*Tropical Crops*:Dicotyledons,Longman Group Ltd, UK, ISBN **0582466660**,pbk(1982).

16. Sidibe, M., Williams, J.T.,*Southampton, UK*,**96p**(2002).

17. Gebauer, J., El-Siddig, K.,Ebert, G.,*Gartenbauwissenschaft***67** (**4**): 155(2002).

18. Coates-Pelgrave, K.,*Trees of Southern Africa*:Struik Publishers, Cape Town(1997).

19. Patrut, A., von Reden, K.F., Lowy, D.A.,Mayne, D.H., Elder, K.E., Roberts, M.L., McNichol, A.P.,*NuclInstr Methods*,**268**, 910 (2010)

20. Woodborne, S., Hall, G., Basson, S., Zambatis, G.,Zambatis, N., *Savanna Network Meeting*, (2010).

21. Dhillion, S.S., Gustad, G.,*Agricultural Ecosystem and Environment*, **101**: 85 (2004)

22.Patrut, A., Von Reden, K.F., Lowy, D.A., Alberts, A.H., Pohlman, J.W., Wittmann, R., Gerlach, D., Xu, L., Mitchell, C.S.,*Radiocarbon dating of a very large*, **27(11),** 1569(2007)

23. Wilson, R.T.,*African Journal of Ecology*,**26**, 197 (1988).

24. Swanepoel, C. M.,*African Journal of Ecology*,**31**, 220 (1993).

25. Barnes, R.F.W., Barnes, K.L.,Kapela, B.,*African Journal of Ecology*,**32**, 177(1994).

26. Wyk van, P.,*Struik,Capetown, South Africa*, **2**,272(1974).

27. Guy, G.L.,*Science Association*,**54**, 68 (1970).

28. Guy, P.R.,*African Journal Ecology*,**20**, 215 (1982).

29. Swart, E.R., *Nature*,**198**, 708 (1963).

30. Grubben, G.J.H., Denton, O.A.,*PROTA Foundation, Wageningen, **2***, 67(2004).

31. Yazzie, D., VanderJagt, D.J., Pastuszyn, A., Okolo, A., Glew, R.H.,*Journal of Food Composition and Analysis*,**7**, 189 (1994).

32. Dovie, D.B.K., Shackleton, C.M,,Witkowski, E.T.F.,*Joint FAO/ECE/ILO Committee on Forest Technology, Management and Training, Harvesting of Non-Wood Forest Products. Menemen-Izmir*, **2003**, 175 (2000).

33. Nordeide, M.B., Hatløy, A., Følling, M., Lied, E., Oshaug, A.,*Int J Food SciNutr*,**47**, 455 (1996).

34. De Caluwe, E., Halamova, K., Van Damme, P.,*Afrika focus*,**23**, 11 (2010).

35. Sidibe, M., Scheuring, J., Tembely, D., Sidibe, M.M., Hofman, P., Frigg, M.,*Agroforestry Today*,**8**, 13-15(1996).

36. Fleuret, A.,*Economic Botany*,**34**, 320 (1980).

37. Fleuret, P., Fleuret, A.,*Tanzania. Human Ecology*,**8**, 311 (1980).

38. Obizoba, I.C., Amaechi, N.,*Ecology of food and nutrition*,**29**, 199 (1993).

39. Arnold, T.H., Wells, M.J., Wehmeyer, A.S.,*Field, D.V. (eds.)*, Allen and Unwin,London, UK, 69-86 (1985).

40. Ajayi, I.A., Dawodi, F.A., Oderinde, R.A.,*La RivistaItalianadelleSostanze Grasse*, **80**, 41(2003).

41. Osman, M.A.,*Plant Foods Hum Nutr*,**59**, 29 (2004).

42. Oyeleke, G., Salam, M., Adetoro, R.,*J EnvSciTox Food Tech*,**1**, 32 (2012).

43. Magaia, T., Uamusse, A., Sjöholm, I., Skog, K.,*Springerplus*,**2**, 88(2013).

44. Lamien-Meda,A., Lamien, C.E., Compaoré, M.M., Meda, R.N., Kiendrebeogo, M., Zeba, B., *Molecules*, **13**, 581 (2008).

45. Gbago, O., Huaiyuan, Z.,Erasto, M., Mohamed D., Yuanda S.,*Journal of Food and Nutrition Research*, 2(6), 281 (2014)

46. Ghani, A., Agbejule, A.,*The state of medicinal plant research in Nigeria Nigerian Society of Pharmacognosy*, 181-185(1986)

47. Nour, A., Magboul, B., Kheiri, N.,*Trop. Sci*,**22**, 383 (1980).

48. Wickens, G.E., Chapter 15: *The uses of the baobab (Adansoniadigitata L.) in Africa. In*: Taxonomic aspects of Africaneconomic botany, editor, Kunkel, G., (1979).

49. Shukla, Y.N., Dubey, S., Jain, S.P., Kumar, S.,*Journal of Medicinal and Aromatic Plant Sciences*, **23**, 429 (2001).

50. Ramadan, A., Harraz, F.M., El-Mougy, S.A.,*Fitoterapia*, **65**, 418 (1993).

51. Addy, E,O., Eteshola, E.,*Journal of Science, Food and Agriculture*,**35**, 437 (1984).

52. Palmer, E., Pitman, N.,*Cape Town, AA Balkema***4**, 723 (1972).

53..Addy, EO., Salami, L.I., Igboeli, L.C., Remawa, H.S.,*Plant Foods Hum Nutr*,**48**, 113 (1995).

54. Pele, J., Le Berre, S., *Le Cameroun agricole, pastoral etforestier*. **49**, (1967)

55.Abbiw, D.K.,*London: Intermediate Technology Publishers*,**23**, 714 (1990).

56- Glew, R.H., VanderJagt, D.J., Lockett, C., Grivetti, L.E., Smith, G.C., Pastuszyn, A., *Journal of Food Composition and Analysi*,**10**, 205 (1997)

57. Zimba, N., Wren, S., Stucki, A.,*International Journal of Aromatherapy*,**15**, 177 (2005).

58. Chivandi, E., Davidson, B.C., Erlwanger, K.H.,*Industrial Crops and Products*,**27**, 29 (2008).

59. Ezeagu, I.E., Petzke, K.J., Lange, E., Metges, C.C.,*Journal of the American Oil Chemists Society*,**75**, 1031 (1998).

60. Cassel, E., Vargas, R.M.F.,*J Mexican ChemSoc*,**50**,126 (2006).

61- Di Leo Lira, P., Retta, D., Tkacik, E., Ringuelet, J., Coussio, J.D., van Baren, C., Bandoni, A.L.,*IndCrops Prod*,**30**, 259 (2009).

62. Burt, S.,*Intl J Food Microbiol*,**94**:223 (2004).

63. Masango, P.,*J Cleaner Prod* ,**13**, 833 (2005).

64. Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M.,*Food ChemToxicol*,**46**, 446 (2008).

65. Mohamed, A.A., El-Emary, G.A., Ali, H.F.,*JAmSci*,**6**, 820(2010).

66. Ruberto, G., Baratta, M.T.,*Food Chem*,**69**,167 (2000).

67. Reverchon, E., Senatore, F.,*Flavour Frag J*,**7**, 227 (1992).

68. Perineau, F., Ganou, L., Vilarem, G.,*J ChemTechnolBiotechnol*,**53**,165 (1992).

69. Babu, K.G.D., Kaul, V.K.,.*Flavour Frag J*,**20**, 222 (2005).

70. Okoh, O.O., Sadimenko, A.P., Afolayan, A.J., *Food Chem*, **120**, 308 (2010).

71. Gavahian, M., Farahnaky, A., Javidnia, K., Majzoobi, M., *Innov Food SciEmergTechnol*,**14**, 85 (2012).

72. Vian, M.A., Fernandez, X., Visinoni, F., Chemat, F., *J Chromatogr A*, **1190**,7(2008).

73. Bousbia, N., AbertVian, M., Ferhat, M.A., Petitcolas, E., Meklati, B.Y., Chemat, F., *Food Chem*,**114**, 62(2009).

74. Areias, F., Valentao, P., Andrade, P.B., Ferreres, F., Seabra, R.M., *J Agric Food Chem*,**48**, 4(2000).

75. Pizzale, L., Bortolomeazzi, R., Vichi, S., U¨ beregger, E., Conte, L.S.,.*J Sci Food Agric*,**82**, 51(2002).

76. Kosar, M., Dorman, H.J.D., Hiltunen, R.,.*Food Chem*,**91**, 525 (2005).

77. Li, X.M., Tian, S.L., Pang, Z.C., Shi, J.Y., Feng, Z.S., Zhang, Y.M., *Food Chem*,**115**, 1114 (2009).

78. Deng, C., Yao, N., Wang, A., Zhang, X., *AnalyticaChimicaActa*,**536**, 237 (2005).

79. Gliˇsi´ca, S.B., Miˇsi´c, D.R., Stameni´c, M.D., Zizovic, I.T., Aˇsanin, R.M., Skala, D.U., *Food Chem*,**105**, 346 (2007).

80. Gironi, F., Maschietti, M., *ChemEngSci*,**63**, 651 (2008).

81. Hawthorne, S.B., Rickkola, M.L., Screnius, K., Holm, Y., Hiltunen, R., Hartonen, K., *J Chromatogr A*,**634**, 297 (1993).

82. Senorans, F.J., Ibanez, E., Cavero, S., Tabera, J., Reglero, G.,*J Chromatogr A*,**870**,491 (2000).

83. Jimenez-Carmona, M.M., Ubera, J.L., Luque de Castro, M.D., *J Chromatogr A*, **855**, 625 (1999).

84. Kubatova, A., Miller, D.J., Hawthorne, S.B., *J Chromatogr A*, **923**, 94(2001).

85. Friedman, M., *Washingto DC: American Chemical Society*, **39(4)**, 83 (2006)

86. Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E.,Warmington, J.R., Wyllie, S.G.,*ApplMicrobiol*,**88**, 170 (2000).

87. Dorman, H.J.D., Deans, S.G.,*J ApplMicrobiol*,**88**, 308 (2000).

88. Dapkevicius, A., Venskutonis, R., van Beek, T.A., Linssen, J.P.H., *J SciFood Agric*,**77**, 140 (1998).

89. Mao, L.C., Pan, X., Que, F., Fang, X.H., *Eur Food Res Technol*,**222**, 236(2006).

90. Kumar, R.S., Sivakumar, T., Sunderam, R.S., Gupta, M., Mazumdar, U.K., Gomathi, P., Rajeshwar, Y., Saravanan, S., Kumar, M.S., Murugesh, K., Kumar, K.A., *Brazil J Med Biol Res*,**38**, 1015S (2005).

91. Kordali, S., Kotan, R., Mavi, A., Cakir, A., Ala, A., Yildirim, A.,*J Agric Food Chem*,**53**, 9452 (2005).

92. Sarikurkcu, C., SabihOzer, M., Eskici, M., Tepe, B., Can, S., Mete, E., *Food ChemToxicol*,**48**, 1801 (2010).

93. Newman, D.J., Gragg, G.M., Holbeck, S., Sausville, E.A., *Curr Cancer Drug Targets*,**2**, 279 (2002).

94. Wu, Z., Li, H., Yang, Y., Zhan, Y., Tu, D.,*Ind Crops Prod***46**, 311(2013).

95. Fulton, G.,Kitson, Barbara, S., Larsen, Charles, N.,McEwen.,*Academic Press Inc Berlin*, **8(3)**, 294 (1996),

96. Chaintreau, A.,*Flavour and Fragrance Journal*,**16(2),** 136, (2001).

97. .Hubschmann, H.J.,*Handbook of GC-MS: Fundamentals and Applications*(2d Edition),Wiley-VCH ISBN**978**-3-527-31427-0 - Weinheim (2009).

98- Ashcroft, A.,*Royal Societyof Chemistry*,London(United Kingdom) ISBN **10**, (1997)

99. Ahuja, S., Jespersen, N.D.,*Elsevier*,**47**, 15 (2006).

100. Kandiah, M., Urban, P.L.,*Chemical Society Reviews*,**42**, 5299 (2013).

101. Kataria, S., Prashant, B., Akanksha, M., Premjeet, S., Devashish, R.,*International Journal of Pharmaceutical & BiologicalArchives*,**2(6)** 1544 (2011).

102. McMaster, M.,*GC/MS: A Practical User´s Guide*(2nd Edition)John Wiley and Sons, ISBN **978**-0-471-73657-8, New York (2011).

103. Willard, H., Hobart, Meritt,L.Lyme.,*CBS Publishers & Distributors P.NO*, **2**, 569(2011).

104. Philp, R.P., http://www.myanmarelibrary.com/elibrary/document/...2825.../ 9000681.pdf (2011).

105. Sahil, K., Prashan,t B., Akanksha, M., Premjeet, S., Devashish, R.,*International Journal Pharma& Biological Archives*, **2**, 1544 (2011).

106. Rowley, A.G.,*Evaluating Uncertainty for Laboratorie*,.A Practical Hand book, version **1.1**, (2001).

107- International Organization for Standardization Quality management systems-Fundamentals and vocabulary. *ISO9000:2000(E)*(2002).

108. ISO/IEC 17025 *General Requirements for Competence of Testing and Calibration Laboratories. Paragraphs, 5.5-5.6*(2005).

109. Bliesner, D.M,.*Validating Chromatographic Methods: A Practical Guide.* John Wiley and Sons,ISBN: **978**-0-471-74147-3(2006).

110. Amirav, A., Gordin, A., Poliak, M., Fialkov, A.B., J Mass Spectrom**43**, 141(2008).

111. Alon, T., Amirav, A.,*Rapid Commun Mass Spectrom*,**20**, 2579(2006).

112. Robert,Pn, Dr. Adams.,*Identification of Essential Oil Components By Gas Chromatography/Mass spectrometry* (4th edition), Allured Pub Corp.ISBN-13,**978**-1932633214(2007).

113. Stein, S.E., Scott, D.R.,*J Am Soc Mass Spectrom*,**5**, 859 (1994).

114. Handley, A.J., Adlard, E.R.,*Gas chromatographic techniques and Applications*,Sheffield Academic, Press, ISBN **1**-84127-118-7, 260 UK London(2001).

115. Thermo., Fisher., Scientific Pesticides Method Reference. 2nd edition, Austin, TX, USA(2011).

116. Eiceman, G.A.,*Gas Chromatography. In Meyers RA (ed) Encyclopedia of Analytical Chemistry: Applications, Theory, and Instrumentation*, WileyISBN **0-471**-97670-9Chichester(2000)

117. Kitson FG, Larsen BS, McEwen CN (1996) Gas chromatography and mass spectrometry: a practical guide, San deiagoAcademic Press, ISBN: **0124833853** 9780124833852, Boston (1996)

118. Niessen, W.M.A.,*Current practice of gas chromatography–mass Spectrometry*, ISBN: **0824704738** 9780824704735 New York, (2001)

119. Maurer, H.H., Pfleger, K., Weber, A.A.,*Forensic SciInt*, **181**, 47(2007).

120. Layloff, T., Nasr. M., Baldwin, R., Chaphart, M., *Pharm Technol*, **24**, 30 (2000)

121. Hsu, H.,Chien, C. S. J., Rockville, M.D.,*Food Drug Anal*,**2(3)**, 161(1994).

122. Grob, R.L., Barry, E.F.,*Modern Practice of Gas Chromatography*. (4th edition) John Wiley & Sons,ISBN**:13-**9780471229834, New Jersey (2004).

123. Giannelli, P.C., Imwinkelried, E.J.,*Scientific Evidence*,**2**, 122 (1999).

124. Kalachova., Analysis of emerging persistent organicpollutants using GC-MS/MS. SETAC, Berlin. (2012)

125. Ziegenhals, K., Hübschmann, H.J., Speer, K., Jira, W., *J Sep Sci*,**31**, 1779 (2008).

126. Cole J Introducing Auto SRM: MRM Simplicity for High Performance Results. Application Brief No. AB52298; Thermo Fisher scientific: Austin, TX, USA.(2013).

127. Lakshmi,HimaBindu, M.R., Angala.,Parameswari, S., Gopinath, C., *International Journal of Pharmaceutical Quality Assurance*,**4**, 42(2013)

128. Priya, V., Jananie, R.K., Vijayalaxmi, K.,*Int Research Journal of Pharmacy*,**3**, 150(2012)

129. Choi, M.H., Chung, B.C., *Korea Institute of Science and Technology, Seoul*, 34(2) 136, Korea (2014).

130. Major Applications of Supersonic GC-MS Available from URL http:// www.actlabs.com/page.aspx?page=368&app=228... (Accessed on 21-July-2011).