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Chemical Characterization of Marhabaib (*Cymbopgon*) Leaves, Spikes and their Essential Oil

توصيف كيميائي لأوراق وسنابل المرحبيب وزيتهما الأساسي

A thesis submitted in partial fulfillment of
The degree of Master in Chemistry

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إستهلال

قال تعالى:

﴿ وَهُوَ الَّذِي أَنْزَلَ مِنَ السَّمَاءِ مَاءً فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُخْرِجُ مِنْهُ حَبًّا مُتَرَاكِبًا وَمِنَ النَّخْلِ مِنْ طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انْظُرُوا إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكُمْ لَآيَاتٍ لِقَوْمٍ يُؤْمِنُونَ ﴾

صدق الله العظيم

سورة الأنعام – الآية 99.

Dedication

I dedicate this work to:

My lovely parents,

Soul of my uncle (Abd Alrhman),

My sister (Asmaa), my brothers (Monjed) and (Mojahed).

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First, I thank Allah Almighty who gave me health, patience and strength to accomplish this work.

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List of contents

List of tables

List of figures

List of Appendixes

Abstract

Marhabaib or Maharaib as normally known in "Sudan" is an annual plant which wildly grows in many parts of the country. It is traditionally consumed for medicinal purposes or as flavoring additive. The aim of this study was to investigate the chemical composition of Marhabaib leaves and spikes for determination of their minerals content and chemical structures of the volatile oil constituents. The leaves samples were collected from Al-gadreef state and the spikes samples were obtained from Northern Darfur state. Inductively couple plasma spectroscopy was used for determination for mineral content of the plant leaves and spikes. The volatile oil of the leaves samples and spikes samples was extracted by steam distillation, the two extracted oils samples were analyzed by GC\MS spectroscopy. The ICP analysis of Sudanese Marhabaib samples showed that the plant was rich with the macro-nutrients and the essential trace minerals. Phosphorus and Iron showed considerable availability. Leaves sample showed P content as (2.05mg\100g) and Fe content as (1.075mg\100), whereas the spikes sample showed P content as (10.75 mg\100g) and Fe content as (1.25 mg\ 100g). The analysis did not show any presence for the toxic minerals Lead and Tin in the two samples. Arsenic showed very low concentration in the spikes sample and not detected in the leaves sample. Cadmium, Strontium, Barium and Aluminum showed significantly low concentration in the two samples. The GC\MS analysis showed the presence of forty eight constituents in the spikes oil sample, dominated by Pipreitone (35.96%), Elemol (20.45%), (+) - 2- Carene (8.18%), Guaiol (6.04%), β-eudesmol(5.97%), γ-eudesmol (4.28 %), Bulnesol (3.14%), Limonine (1.44%) and α-Eudesmol (1.37%). The Leaves oil sample characterization by GC\MC showed a presence of sixteen constituents, dominated by Piperitone (84.16%), followed by (+)-2-carene (5.75%), d–Limonene (2.67%) and α–terpineol (2.21 %). The minerals content for the leaves and spikes of the plant, as well as, the chemical constituents of the oil may encourage traditional use of Marhabaib leaves, spikes and essential oil for medicinal and nutritional uses without any possible risks.

المستخلص

المرحبيب أو المحريب كما يُعرف في السودان هو نبات حولي ينمو بريا" في كثير من بقاع القطر. ويستهلك للأغراض الطبية أو كمادة منكهة.الغرض من هذه الدراسة هو إستقصاء التكوين الكيميائي لأوراق وسنابل المرحبيب لتحديد المحتوى المعدني والتركيب الكيميائي لمكونات الزيت الطيار. أخذت عينات لأوراق المرحبيب من ولاية القضارف كما جُمعت السنابل من ولاية شمال دارفور.أُستخدم جهازالحث البلازمي المزدوج لقياس المحتوى المعدني في كل من الأوراق والسنابل. تم إستخلاص الزيت الطيار من أوراق وسنابل المرحبيب بالتقطيربالبخار. تم تحليل عينتي الزيت المستخلص بجهاز كروموتغرافيا الغاز ومطيافية الكتلة**.** أظهر التحليل بجهاز بلازما الحث المزدوج (ICP) أن عينات المرحبيب السوداني غنية بالمغذيات الكبرى والعناصر الأساسية . وكان محتوى الفسفور والحديد معتبرا" حيث كان الفسفور (2.05mg/100g) والحديد (1.075mg/100g) في عينة الاوراق والفسفور (10.75mg/100g) والحديد (1.25mg/100g) في عينة السنابل. أظهر التحليل خلو العينتين من العناصر ذات الخواص السمية خاصة الرصاص و القصدير. أظهر الزرنيخ تركيزا"منخفضا"في عينة السنابل ولم يظهر في عينة الأوراق. أظهر كل من الكادميوم، الأسترانشيوم ، الباريوم والألمونيوم تراكيز منخفضة جدا" في العينتين. التحليل بجهاز كرومتوغرافيا الغاز ومطيافية الكتلة أظهر ثمانية وأربعين مكونا" في زيت السنابل يغلب عليها البايبريتون (35.96%) ، الأيليمول (20.45%) ، (+)-2-كارين (8.18%) ، جوايول (6.04%) ، بيتا-إيوديسمول (5.97%) ، جاما- إيوديسمول (4.28%) ،بلنيسول ,(3.14%) ، الليمونين (1.44%) وألفا إيوديسمول (1.37%) . أظهرالتحليل وجود ستة عشر مكونا" لزيت الأوراق يغلب عليها وجود البايبريتون (84.13%) ، (+)-2-كارين (5.75%) ، الليمونين(2.67%) و تيربينول (2.21%). المحتوى المعدني لأوراق وسنابل النبات والمركبات الكيميائية التي يحتوي عليها الزيت ربما تشجع على إستخدام أوراق وسنابل وزيت المرحبيب للأغراض الطبية والغذائية دون أي مخاط محتملة.

Chapter one

Introduction

Chapter one

1-Introduction

1.1*Cymbopogon*

1.1.1 General

The name *Cymbopogon* is derived from the Greek words “kymbe” (boat) and “pogon” (beard), which is referring to the flower spike arrangement. It means boat – beard (Watson and Dallwitz; 2015, Shah et al; 2011). *Cymbopogon* genus is a number of herbs of the family (gramineae\ poaceae), which are known worldwide for their high essential oils content. They are widely distributed in many regions of the world. They are used for various purposes.The commercial and medicinal uses of the various *Cymbopogon* species are well documented. The chemo-types from this genus have been used as biomarkers for their identification and classification (Avoseh et al; 2015). The genus of this family has about 144 species (Pavlovic et al; 2017, Khanuja et al; 2005). The most known, common and well-studied species are three Sudanese species which are *Cymbopogon schoenanthus*, *Cymbopogon citratus* and *Cymbopogon nervatus*. The plant is widly growing in many areas in eSudan. Other two species that are cultivated in west and east of India which, are *Cymbopogon citratus* and *Cymbopogon flexuosus* .There are several other species of this genus including *Cymbopogon bombycinus* , *Cymbopogon ambiguus* , *Cymbopogon obtectus* , *Cymbopogon refractus* and *Cymbopogon nardus* (Nambiar and Matela; 2012). The commercial value for most of the *Cymbopogon* species is enhanced by their ability to grow in moderate and extremely harsh climatic conditions (Paladia etal; 2011). *Cymbopogon citratus* and *Cymbopogon schoenanthus* have many different common names. The Arabic common names are Ethkher , Tibn Makkah , Hashishat El Gamal , Halfa Bar , Sinabel Alarab , Abo Rekba , Marhabaib and Maharaib .The English comm x on names are Camel grass , Geranium grass, Camel hay , Sweet cane ,Sweet ruch and Citronella grass.The most famous common name for all species of the genus *Cymbopogon* is Lemongrass. The name Lemongrass is derived from the typical lemony odour of the essential oil (Al-snafi ; 2016 , Deshmukh et al; 2010, Joy et al; 2003). The plants of the genus *Cymbopogon* (Lemongrass) contain various phyto-constituents such as, flavonoids, phenolic compounds, terpenoids and essential oils, which m ay be responsible for the several biological activities (Shah et al; 2011).The Ethnopharmacology evidences showed a wide array of properties that justified their use for, pest control, cosmetics and as anti-inflammation agents. The plant may hold promise as potent anti-tumor and chemo preventive drugs, in addition to the infusion of leaves which is used against stomach trouble and as anti – spasmodic .(Avoseh et al; 2015 , ElGahazali et al; 1997). In some parts of Sudan and South Africa, these plants have a good application as roof thatches and grass broom (Shackleton et al; 2007). In the Sudan the plant is widely growing in many parts of the country.

1.1.2 Taxanomical Classcification

Table 1.1 Taxanomical classification of *Cymbopogon* speacies

Cymbopogon citratus According to (Oladeji et al;2019)	*Cymbopogon schoenanthus* According to (Al-Snafi;2016)
Kingdom *: plantae*	Kingdom : *plantae*
Division : *Magnoliophyta*	Division : *Magnoliophyta*
Class : *Liliopsida*	Class : *Liliopsida*
Order : *poales*	Order : *Cyperales*
Family : *poaceae*	Family : *poaceae*
Genus : *Cymbopogon*	Genus : *Cymbopogon*

1.1.3 Plant description

Cymbopogon citratus is a herb with short roots and simple leaves which are characterize by alternate, linear, with length ranging (5. 0 – 7.0 cm), width ranging (0.5 – 1.5 cm), sheathed with apex acute and parallel venation. The height of this herb may reach about 80cm (Eltahir and Aboalereash; 2010).

Cymbopogon schoenanthus is a herb with erect stems in tufted shape with a height reach about 90 cm to one meter. The leaves of this herb are simple, erect, alternate, sheathed , with length of (5.0 – 7.0 cm) and width of about 1cm . (El Ghazali et al; 1997).

Figure 1.1 Lemongrass parts (www.theida.com/Lemongrass).

1.1.4 Geoghrapic distribuation

The plants of genus *Cymbopogon*, belonging to the grass family (*poaceae*) which is distributed throughout, the warm regions of the world and Oceania (Bertea and Maffei; 2010). The species of this genus are indigenous in tropical and semi tropical areas of Asia and cultivated in South , Central America and Africa (Kummar et al; 2000). Wild *Cymbopogon citratus* of Asia is native to India and the other tropical or subtropical countries (Oladeji et al; 2019, Figueirinha et al; 2008). According to Al- sanfi (2016), *Cymbopogon schoenanthus* is native in some tropical areas of Asia and Africa including Iraq, Oman, Saudi Arabia , Yemen Sudan, Senegal, Somalia, Morocco, Mali, Mauritania, Niger, Nigeria, Algeria, Benin, Burkina Faso, Kenya, Chad, Djibouti, Ethiopia, Egypt, Libya and Togo.

1.1.5 Lemongrass species

1.1.5.1 *Cymbopogon citratus* (Sudan)

This is an aromatic perennial herb with rhizomes and short tufted fibrous roots (Fig 1.2). The length of this herb reach to 60cm. It has simple, alternate, linear leaves and has ability to grow in West, East and Central of Sudan. In Sudan the herb is utilized in treatment of some diseases such as kidney inflammations. The vernacular name of this plant is Marhabaib or Maharaib . The common names are Lemongrass, Citronella grass and fever grass. The synonyms of this grass is *Andropogone citratus* , Lemongrass stalk and *Cymbopogon proximus* (Shah et al; 2016). This grass is rich with essential minerals and essential oil contents including a large number of chemicals compounds with multiple and different properties that make the oil useful for many purposes (El Ghazalie et al; 1997). It contains considerable amount of flavonoids, essential oils, phenolic compounds and other phytochemical constituents which possesses pharmacological activities such as anti-obesity, anti-bacterial, anti-fungal, anti-nociceptive, anti-oxidants, anti-diarrheal and anti-inflammatory properties which could enhance health (Oladeji et al; 2019).

Figure1.2 *Cymbopogon citratus* with narrow and long leaves (www.theida.com/Lemongrass *Cymbopogon citratu*s/Lemongrass-3).

1.1.5.2 *Cymbopogon schoenanthus*

This is perennial grass with erect and tall stems,aromatic herb which has a hight ranger from 60 cm to 1m. It has inflorescence spikeltes highly branched about (5cm long). The leaves of this herb are alternative and linear (Fig1.3). It is widely spread in Northern, Estern, and Central regions of Sudan. The vernacular name of this specie is Maharaib or Hamarieb .It's common name is Camel's Hay grass with synonym *Anderpogon schoenanthus* (El Ghazalie et al; 1997). The plant is used in Sudan in treatment of gout, prostate inflammation, kidney diseases due to it inhibits kidney shrinkage and for stomach pains. This herb also consumed in salads and in preparation of meat recipes (El tahir and Abu Elreish; 2010, Khadri et al; 2008).

Figure 1.3 *Cymbopogon schoenanthus* of Sudan (Northern Darfur state)

1.1.5.3 *Cymbopogon nervatus*

This is an erect and perennial herb with height up to 1m and the leaves are rounded at the base, which is up to 10 mm width and it has glabrous culms as well as sessil spikelete which are narrowly elliptic, 4–5 mm length, Pedicelled spikelet narrowly lanceolate, 4.5–5 mm length (Fig 1.4).The synonym of this specie is (*Andropogon nervatus*) and in the Sudan it distributes in Eastern, Central and Western of the country which has vernacular name (Nal) .This specie is utilizing as roof thatches and as grass broom (El Ghazalie et al; 1997).

Figure 1.4 *Cymbopogon nervatus*

(www.tuninst.net/MMPD/TIL/famP/pixpoaceae/opp-p149-300.jpg)

1.1.5.4 *Cymbopogon citratus* (Indian species)

This is known as West Indian or American Lemongrass (Fig1.5). It is a stemless perennial grass with short rhizomatous roots, making large tussocks which seldom flowers under cultivation. The leaf is narrow, linear, glaucous, inflorescence rarely produced, long and narrow, with sessile and awnless spikelets, linear, lanceolate. The essential oil contains is (74-76%) Citral and exhibits poor Solubility in alcohol. *Cymbopogon citratus* is more commonly grown on soils with higher acidity than *Cymbopogon flexuosus* (Gupata et al; 1969 , Chandra and Naranyanan 1971).

Figure 1.5 *Cymbopogon citratus* or Lemongrass, a native plant in South Asia (Image ID: S2NBAW)

1.1.5.5 *Cymbopogon flexuosus*

According to Gupata et al; (1969) this is known as East Indian grass, Cochin or Malabar grass. *Cymbopogon flexuosus* is a tufted perennial grass with height up to 2 m. The leaves are linear with very large and highly branched inflorescence (Fig 1.6), the terminal drooping is bearing paired spikes on tertiary branched,the spikes bear spikelets in pairs in which one is sessile and the other pedicellate,the sessile spikelet is an awned , bisexual and floret, whereas the pedicellate is an awn less , staminate and also floret. Under this specie two varieties are identified based on the color of the stem. *Cymbopogon flexuosus* with reddish or purple stem and leaf sheath; The essential oil of this type contains more than 75-80% citral ,and it exhibits good solubility in alcohol and hence it is superior in quality .The geranial rich variants of *Cymbopogon flexuosus* with high oil contents could be useful as additional source of geraniol .The variety of east Indian *Cymbopogon flexuosus*, is the white *Cymbopogon flexuosus* this is a white grass and characterized by the white color of the stem. The essential oil contains less than 65-70% citral and it exhibits poor alcohol solubility and there for it considered inferior in quality (Chandra and Narayanan; 1971).

Figure 1.6 *Cymbopogon flexuosus* of East India
(https://www.swappsta.com/cymbopogon-flexuosus-east-indian-lemongrass)

1.1.6 Climatic conditions and cultivation

Plants of the genus "*Cymbopogon*" are flourish in sunny, warm, humid conditions of the tropic regions. Lemongrass grows well between (900 and 1250 m) from the mean sea level. The species of this genus produce the highest oil yield per ton of herbage when the annual rainfall averages are (2500 – 3000mm). *Cymbopogon citratus* is more droughts tolerant. In areas where rainfall is poor, it can be growing with supplemental irrigation. Temperature of (25-30^0 C) is optimum for maximum oil production, with no extremely low night temperature. Short periods above 30^0 C have a little effect on plant, but severely reduce oil content (Weiss; 1997).

1.1.6.1 Soil characteristics and irrigation

Cymbpogon species grow in wide variety of soils, ranging from rich loam to poor laterite. In sandy loam and red soil; it requires good manuring. Lemongrass can grow well over medium fertile soils and moderate irrigation. Calcareous and water – logged soils are not suitable for its cultivation (Farooqi and Sreeramu, 2001, Nambiar and Matela, 2012). All species grow in sandy soils have higher leaf oil yield and citral content. Although *Cymbopogon* species can be growing in almost all types of land, from very light sandy soil to upland laterites soils with pH 5.5 to 7.5 are utilized, Lemongrass will grow and produce average herbage and oil yields on highly saline soils. In pot trials *Cymbopogon flexuosus* grown in soils with electrical conductivity of 11.5,10, and 5.5mmhos/cm showed no significant reduction in herb quantity, oil yield and citral content was not affected by increasing salinity levels up to 15 mmhos /cm (Weiss, 1997). In case of drought, the crop should be irrigated every alternate day, for about a month after planting. It is recommended that 4 to 6 irrigations are given during the period from February to June for optimum yield. When soil moisture maintained at optimum range, it lead to significantly increased crop growth, herbage and essential oil yield .The essential oil quantity is not affected by soil moisture (Singh *et al*, 1997). In the Sudan the plant is wildly grows within a period of June to November.

1.1.6.2 Propagation

Plantation of Lemongrass can be established by planting of slips. Plant of genus *Cymbopogon* is generally propagated through seeds. Seeds are mixed with dry river sand and sown in the field at the rate of 20 to 25 kg/ha. Alternatively, seedlings can be raised in a nursery of the main field and then transplanted after 45 days. In this method (3-4 kg seeds/ha) is considered ideal. *Cymbopogon flexuosus* is propagated through seeds while *Cymbopogon citratus* is propagated through division of clumps. Propagation through vegetative means from selected clones cause considerable genetic heterogeneity which played very important role in the propagation of Lemongrass (Hussain et al; 1988).

1.1.6.3 Nursery and transplanting

According to (Farooqi et al; 1999), Lemongrass seeds have a dormancy of few weeks and they lose viability in few months. The seeds that collected during January and February are usually sown in the nursery during April to May. Germination is very poor if it sown after October. For one hectare of land, 1000 m^2 nursery has to be raised. The seeds are uniformly broadcasted on the beds at 3-4 kg/ha and covered with a thin layer of soil these seeds bed should be irrigated frequently. Seeds that germinate within 5-7 days, a spacing of 30 cm x 30 cm with a plant density of 111000/ha is recommended, a wider spacing of 60 cm x 45 cm for seedlings and 90 cm x 60 cm for slips has been recommended for fertile, irrigated land.

1.1.6.4 Weed control

The first (25-30 days) after planting (or harvest) is cosidere crop-weed competition period. For a good establishment of the crop, the field should be kept weed free for the initial period of (3-4 months) after planting. Once the crop is well established, it can compete with weeds. Generally, 2-3 weedings are necessary in a year. There are many herbicides which have very effective role in weed control process such as, diuron at (1.5 kg /ha) and oxyfluorfen at (1.5 kg /ha) (Hussain *et al*; 1988 , Duhan , Gulati; 1973 and Khosla ; 1979).

1.1.7 Lemongrass uses

1.1.7.1 General uses

In the Sudan leaves of Marhabaib are usually added in small amounts to drinking water and sometimes it used as a hot drink. Leaves and roots in many areas are boiled with water and this solution is given to women during the first days of birthing to relieve the pains of birthing process and help in removal of the waste from woman's womb after birthing. Sudan like South Africa rarely use *Cymbopogon* species in cosmetic, drug and perfumery, in western and eastern Sudan Marhabaib is utilized as roof thatches in (manufacturing of huts roof) and it also used as grass broom in which *Cymbopogon nervatus* (Nal) is a common species utilizing in these purposes.

Lemongrass is used as food flavoring in which simple syrup made by steeping *herbs* with a hot water and sugar, this syrup can be used to enhance fruit salads or to make homemade soda by mixing it with seltzer. (Joy, 2003).

Economic uses which arising from their essential oils components such as citral, genariol and myrcene isolated from *Cymbopogon citratus* which are consider important raw materials in soap and detergent, food beverage, perfume confectionaries industries (Avoseh et al ; 2015 , Usha , 2012).

Cosmetically Lemongrass is used in improvement of the skin and keep it healthy due to it has ability to reducing acne and pimples and also can acts as a muscle and tissue toner (Joy, 2003).

1.1.7.2 Medicinal uses

The plants of genus "*Cymbopogon"* have been widely used in traditional medicine for several centuries to treat many health-related ailments. The majority of the world's population depends on traditional medicine for primary health care. There has been an increasing interest in medicinal plants and their active ingredients because of their potency and negligible adverse side effects (Mossa et al; 1987).

Tea of dried leaves of *Cymbopogon citratus* prepared either by decoction or infusion of the leaves in water is widely used as diuretic and it has no biochemical changes on the body in comparison with the ordinary tea (Wannmacher et al; 1990).

The genus "*Cymbopogon"* contain considerable amount of flavonoids, phenolic compounds and phytochemical constituents which possess many pharmacological activities like antispasmodic, hypotensive,anticonvulsant, analgesic , antiemetic , antitussive, antirheumatic, antiseptic , treatment of nervous and gastrointestinal disorders and fevers , anti-bacterial, anti-fungal, anti-oxidants anti-diarrheal, and anti-inflammatory properties which could enhance a health (Oladeji et al ; 2019).

Cymbopogon schoenanthu is used for treatments of gout, prostate inflammation, kidney diseases due to it inhibits kidney shrinkage and for stomach pains,fever , rheumatism , its used as digestive for treating intestinal spasm as well as for treating anorexia (El tahir and Abu Elreish , 2010, Khadri et al; 2008, Ben Othman et al,; 2013. Kpoviessi et al; 2014).

Agbafor and Akugwo - (2007) reported that *Cymbopogon citratus* helps in relieving stress and anxiety; high blood pressure is a common side effect of stress. Study carried out by previous scientists showed that aromatherapy eases relieves stress and anxiety. Combining aromatherapy with massage may bring greater benefits. Lemongrass and sweet almond massage oil are using during massage. Study participants who received a massage using the oil once a week for three weeks had lower diastolic blood pressure than those in the control group. Systolic blood pressure and pulse rate weren't affected.

Agbafor and Akugwo - (2007) reported that Lemongrass has ability to relieve pain caused by headaches and migraines. The researchers believe that a compound in Lemongrass called eugenol has similar abilities to aspirin**.**

In South Africa, *Cymbopogon* c*itratus* extract have been applied for treatment of oral thrush in patients who tested positive to (HIV/AIDS) and proved effective (Wright et al; 2009).

The institute of Science and technology in India showed that Lemongrass can be used as (anti-cancer) and as a good cleanser that helps to detoxify liver, pancreas, kidney, bladder and digestive tract (Joy, 2003).

1.1.8 Minerals content of Lemongrass

In a study carried out in West India by Fagbohun et al ; (2010) on *Cymbopogon citratus* reported that it contains some important minerals such as K, Na, Mg, Mn, Fe, Zn and P table (1.2).

According to Joy (2003) Lemongrass cultivated in different areas of India showed minerals contents as Na (0.74%), K (2.12%), Ca (0.36%), Mg (0.15%), P (0.07%), S (0.19%), Fe (126.73 ppm), Mn (155.82 ppm), Zn (35.51 ppm) and Cu (56.64 ppm).

Table 1.2 Mineral content of West Indian *Cymbopogon citratus* leaves

Mineral	Concentration(mg\100g)
Na	54.8
K	59.5
Ca	39.5
Mg	70
Fe	0.024
Mn	0.952
Zn	121
P	89.3

Source (Fagbohun et al, 2010).

According to Alsanfi ; (2016) *Cymbopogon schonenanthus* cultivated in Iraq at maturity satage reported that, minerals content on dry matter base were found to be Ca (0.49%), P (0.032%), K (0.48%), Mg (0.022%), Cu (23.0 ppm), Zn (3.5 ppm), Mn (2.7%) and Co (0.023ppm).

1.1.9 Essential oils

Essential oils are interesting natural products and among other qualities they possess various biological properties. The term “biological” comprises all activities occurring by mixtures of volatile compounds mainly (mono- and sesquiterpenoids, benzenoids, phenylpropanoids) exert on humans, animals and other plants. Natural essential oils are volatile, fragrant and have pleasant tasting, it is obtained from leaves, roots, flowers, seeds or fruits. Essential oils are low-volume high-value products used in perfumry, cosmetics, food beverages and pharmaceutical industries (Reische et al; 1998). They also have been used in both religion and medicine for thousands of years. The power of plant products (herbals and essential oils) lies in the combination of their elements, and the trace components. Many of these trace elements enable the herbals or oils to heal more efficiently and without the nasty side effects experienced when using the synthetic reconstructions (drugs or oils) that do not contain the trace minerals. The modern world is bringing aromatherapy into building designs and medical practices for this purpose(Ali et al; 2017). Essential oils of aromatic plants have been used by many cultures around the world centuries. Their uses vary between cultures, from religious to healing purposes. Nowadays essential oils have extensively applications in medicine, pharmacy, cosmetics and the food industry. Interest in essential oils has increased due to their high medicinal and economic value (Mathe, 2015).

1.1.10 Lemongrass essential oil

Essential volatile oil of both *Cymbopgon citratus* and *Cymbopogon schoenanthus* has yellow color. The color of Lemongrass oil is arranged from pale yellow to vivid yellow. The extraction process and the nature of the substances from which essential oils are extracted determine the color of the oil. Lemongrass oil has a thin consistency in which oil of *Cymbopogon citratus* extracted by steam distillation process was found to be has a thin consistency. The oil of two species oil have strong herbaceous earthy smell. The lemony flavor in the Lemongrass aroma makes it refreshing and lively (Flipoy et al, 1994). *Cymbopogon* oil plays an effective role against bacteria, flu and colds as well as it contains stimulating agents, tonics , diuretics, antispasmodisc, relaxing, soothing and balancing (Blanco et al ,2007. Cavalcanti et al, 2004).Previous studies were shown that the essential oil of plants of genus *Cymbopogon* can be effective against different agricultural insect pests (Lambrano et al; 2015 , Wang et al; 2016). Lemongrass oil has no adverse effects on the blood, liver function, kidney function, protein, carbohydrates and lipid metabolism on rats but studies have failed to detect mutagenic or toxicological reactions in humans (Leung and Foster, 1996).

Cymbbopogon essential volatile oils are characterize by the presence of many chemical constituents such as hydrocarbon terpenes, oxygenated terpenes, alcohols, ketones, esters, aldehydes as well as many flavonoids .The presence of these constituents in *C*ymbopogon oil give it a significant importance in the industrial , medicinal and pharmaceutical fields.

1.1.10.1 Antibacterial activity of Lemongrass oil

The chromatographic fraction of the essential oil in agar plate was active on *Bacillus subtilis, Escherichia coli, Staphylococus aureus* and *Salmonella paratyphi* and *Shigella flexneri*.These activities are shown in two of the three main components of the oil identified through chromatographic and mass spectrometric methods. The α-citral (geranial) and β-citral (neral) components in Lemongrass oil individually elicit an antibacterial action on gram-negative and gram-positive organisms, the third component, myrcene, did not show any observable antibacterial activity (Onawunmia et al, 1984).

1.1.10.2 Antifungal activity of Lemongrass oil

Lemongrass oil was reported to be active against dermatophytes such as *Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum and Microsporum gypseum*, and is among the most active agents against human dermatophytes. Other studies reported that Lemongrass oil is active against keratinophilic fungi, ringworm fungi and food storage fungi. Lemongrass oil is also effective as a herbicide and as an insecticide because of these naturally occurring antimicrobial effects (Wannissorn, 2005).

1.1.10.3 Chemical compositions of the essential oil of Lemongrass

The components of Lemongrass oil varies according to the geographical origin, but they agree in presence some compositions which are hydrocarbon terpenes, oxygenated terpenes, alcohols, ketones, esters aldehydes and flavinoids have been registered owing to most of these components are bioactive components and hence it's used as biomarkers as well as in industrial field.

Ali et al at; (2017) reported that the major components of the oil of *Cymbopogon citratus* leaves cultivated in in Khartoum are citral , neral, myrecene , greaniol , 1,3,4- tri methyl -3- cyclo hexene -1- carboxaldhyde , citranellol , geranyl acetate , bi cyclo [3.1.1]heptane- 2- caroxaldhyde – 6,6 di methyl and D- limonene and the predominant components were tabled in table (1.3).

Table 1.3 The major constituents of *Cymbopogon citratus* leaves oil (Sudan)

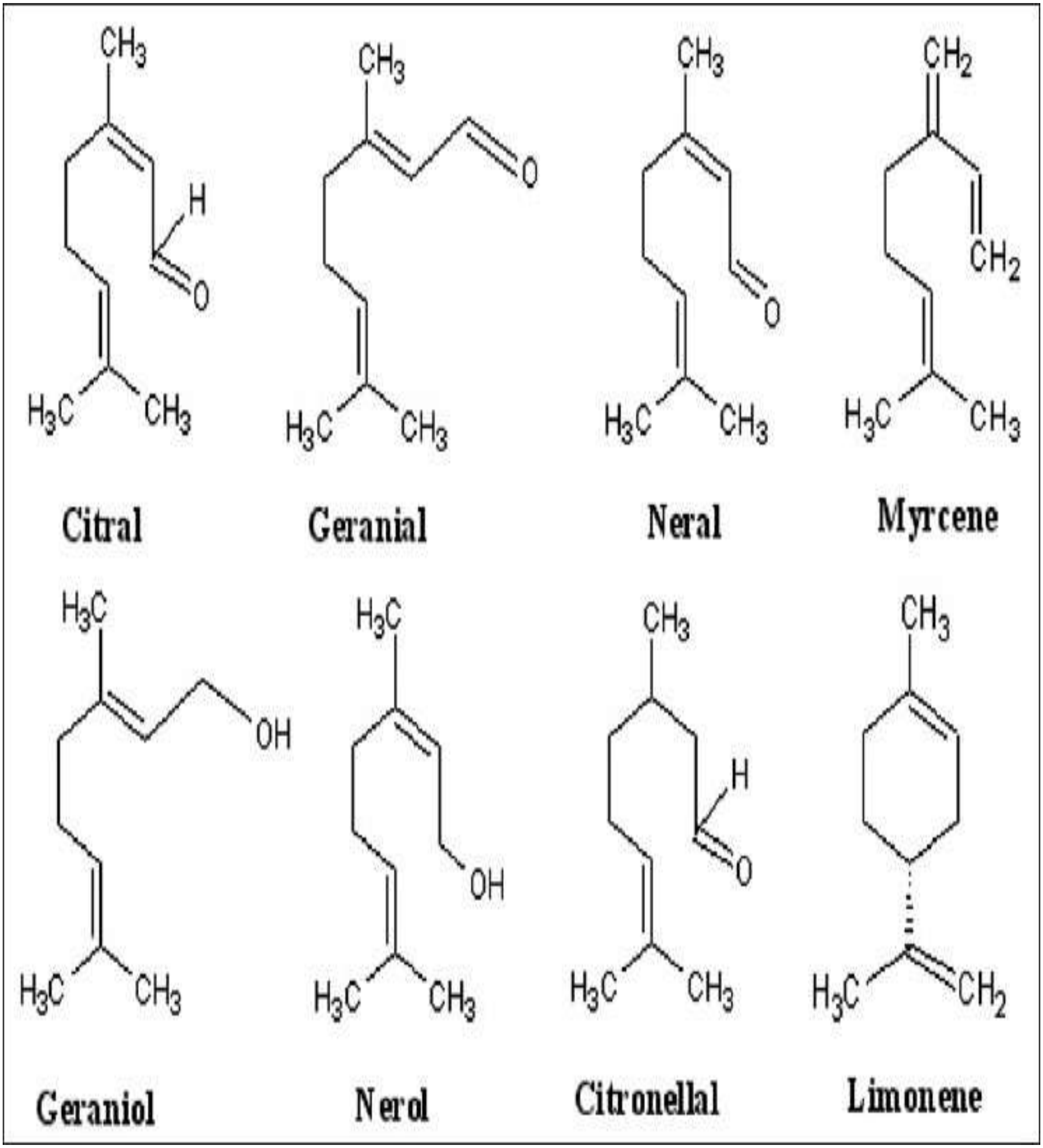
Components	Area (%)
β -Myrecene	11.28
Geraniol	5.54
D-limonene	0.03
Citral	34.80
Citranellol	1.34
Geranyl acetate	0.57
Neral	30.72
1,3,4 tri meth yl -3- cyclohexene -1- carboxaldhyde	2.20

Source (Ali et al at; 2017).

Shah et al ;(2011) reported that chemical constituents of (*Cymbopogon citratus)* essential oil from east Indian included terpenes, alcohols,ketones, aldehyde and esters a predominant constituents of these compounds are geranial (45.1–54.5%), neral (30.1–36.1%), geranyl acetate (0.1–4.0%), geraniol (0.2–3.8%), (d)-limonene (0.1–3.8%), caryophyllene oxide (0–1.6%), 6-methyl-5-hepten-2-one (0.3–1.4%) and linalool (0.4–1%).

Table 1.4 Chemical compositions of *Cymbopogon citratus* oil from East India

Constituent	Area (%)
Citral – α	40.8
Citral -β	32.0
Nerol	4.18
Geraniol	3.04
Citronellal	2.10
Terpinolene	1.23
Gerenyl acetate	0.83
Myrecene	0.72
Terpinol	0.45
Methylheptanone	0.2
Borneol	0.1- 0.4
Linalyl acetate	0.1
α - Pinene	0.07
β - Pinene	0.04
Limonene	Trace
Linalool	Trace
β- Cariophyllene	Trace



Source (Saleem et al; 2003).

Figure 1.7 Structures of some Indian *Cymbopogon citratus* oil constituents

Cymbopogon schoenanthus cultivated in Asfan area (Saudi Arabia) was subjected to GC\MS analysis the result showed different class of terpens in addition to phenolic compounds many of other components were shown included Pipretone , Cyclohexanemethanol , Elemene , alpha- Eudesmol , Beta – Eudesmol , gamma – Eudesmol , Elemol and Naphthalenemethanol as a major constituents of the essential oil (table 1.5).

Table 1. 5 Constituents of *Cymbopogon schoenanthus* oil (Suadi Arabia)

Constituent	Area (%)
Pipretone	14.6
Cyclohexanemethanol	11.6
Elemene	11.6
α_-Eudesmol	11.5
Elemol	10.8
β- Eudesmol	8.5
Naphthalenemethanol	7.1
♈- Eudesmol	4.2

Source (Hisham et al; 2016).

Study carried by Eihab et al; (2016) on oil of inflorescence of *Cymbopogon schoenanthus* cultivated in Sudan identified many compounds which represent 98.8% of the total oil. The oil investigated was characterized by a high content of oxygenated monoterpenes (50.8-75.5%). The dominant compound was piperitone (47.7-71.5%). The other abundant constituents were intermedeol (6.1-17.3%), δ-2-carene (4.5-10.0%) and elemol (5.2-9%) table (1.6).

Table 1.6 Compositions of *Cymbopogon schoenanthus* of essential oil (Sudan)

Constituent	Area (%)
Verbenene	0.2
ϒ- carene	10.0
α – pellanderene	Trace
α- terpinene	Trace
Limonene	0.1
Ocimene	1.5
Fenshone	Trace
Menth -2-en-ol	Trace
Thymol	0.3
α – selinene	0.6
Cuparene	0.5
Elemol	9.0
Caryophyllene oxide	0.3
ϒ -Eudesmol	1.8
β –Eudesmol	1.8
α – Edesmol	2.1
Intermedol	14.4
Pipretone	47.7

Source (Eihab et al; 2016)

1.1.10.4 Uses of Lemongrass oil

Lemongrass oil helps ease diarrhea; diarrhea is often just a bother, but it can also cause dehydration. Over-the-counter diarrhea remedies can come with unpleasant side effects — like constipation — leading some people to turn to natural remedies.And help slow diarrhea. The study showed that the oil reduced fjjecal output in mice with castor oil-induced diarrhea, possibly by slowing intestinal motility (Agbafor and Akugwo, 2007).

. A blend of Lemongrass, garlic, ginger and oil will be stable in the freezer during winter this paste can be fried until fragrant and then cooked down with a can of coconut milk strain to remove tough lemongrass fiber (for delicious sauce for noodle, vegetable or seafood (Joy, 2003).

Lemongrass oil has ability to reduce cholesterol; high cholesterol sometime increases the risk of heart attack and stroke. It's important to keep cholesterol levels stable Lemongrass oil serves to reduce high level of cholesterol in which it treats high cholesterol level and manage heart disease, Lemongrass oil was found significantly reduced cholesterol in rats which had been fed a high cholesterol diet for 14 days the positive reaction was dose-dependent, which means that its effects changed when the dose was changed (Agbafor and Akugwo ,2007).

According to Agbafor and Akugwo ;(2007) ,Lemongrass oil helps in regulate blood sugar and lipids;Lemongrass oil is used to reduce blood sugar in peoples who suffer from diabetes type - 2, according to study carried out by Agbafor and Akugwo ;(2007) which showed that a daily oral dose of 125 to 500 milligrams (mg) of Lemongrass oil for 42 days. Results showed that lemongrass oil lowered blood sugar levels. It also changed lipid parameters and that lead to monitoring good cholesterol levels.

Oil of plants of genus *Cymbopogon* capable to act as a pain reliever the chemo – types Lemongrass essential oil that is rich with citral is helps in ease relieves pain as it relieves inflammation and Lemongrass oil has ability to decrease arthritis pain of peoples who suffer from rheumatoid arthritis (Agbafor and Akugwo , 2007).

As a vaporizer, *Cymbopogon* oil works as an effective panacea against bacteria, flu and colds. It has stimulating agent, tonics, diuretic and antispasmodic. People suffering from urine problems can apply Lemongrass oils. In hot weather, this is the best oil to cool down the body temperature and to revive the mind and soul. In other health benefits it is used to improve digestion, nausea, menstruation problems as well as ailments like headaches, muscle cramps, spasms and rheumatisms (Blanco etal ; 2007).

Cymbopogon essential oils can be effective against different insect pests important in agriculture (Wang et al; 2016), but significant differences have also been revealed regarding the strength of effect, depending on plant species and applied concentration (Bossou et al; 2015).

Lemongrass oil is one of the most important essential oils being widely used for the isolation of Citral which is consider starting material for the preparation of ionones (isomeration compound) in which α-ionone is used in flavours, cosmetics and perfumes whereas β-ionone is used for the synthesis of vitamin A hence by indirect method it help in production of(A vitamin) (Joy , 2003).

1.1.11 Objectives of the study

- To investigate the availability of Macro, Micro and trace minerals in Marhabaib leaves and spikes using (ICP) instrumental analysis.
- To extract *Cymbopogon citratus* leaves and *Cymbopogon schoenanthus* spikes oil and determine the oil yield percentage.
- To identify the major compounds and constituents of Marhabaib leaves and spjkes oil.
- To show safe useing of Marhabaib in Sudanese houses, depending on the research findings.

Chapter two

Materials and Methods

Chapter two

Materials and Methods

2.1 Collection of samples

Fresh spikes sample of *Cymbopogon schenanthus* was harvested from the field, where the plant is wildly growing in Northern Darfour (Alkouma). Fig (1.3).

Cymbopogon citratus leaves were collected from (Algadareef state), where the plant is wildly growing .Fig (1.2).

2.2 Chemicals

- Nitric acid: Assay (69.72 %**),** Density (1.51g\cm^3), Acidity (pka, -1.37), Emsure Company – Germany.

- Anhydrous Sodium Sulphate: Assay (99%), (Alpha- Chem Company – India).

2.3 Instruments

- Gas choromtogphy- Mass spectrometer model (GC/MS-QP2010-Ultra) from japans (Shimadzu Company), serial number 020525101565SA and capillary column (Rtx-5ms-30m×0.25 mm×0.25µm).

- Inductively Coupled Plasma Optical Emission Spectrometer **(**ICP E - 9000, Shimadzu).

- Muffle furnace (4000, Shimadzu).

2.4. Methods of analysis

2.4.1 Extraction of volatile oils

200grams of dry *Cymbopogon schoenanthus* spikes and (300g) of *Cymbopogon citratus* leaves sample were accurately weighed respectively each sample was transferred to 500 ml round - bottomed flask. 250 ml of distilled water was added. The mixture was then boiled for four hours in distillation system. The volatile oil of each sample was then separated by separatry funnel. The extracted oils yields were calculated and each oil was kept for GC\MS analysis

2.4.2 ICP analysis of two *Cymbopogon* species

2gram of *Cymbopogon schoenanthus* spikes and 2 gram of *Cymbopogon citratus* leaves sample was ignited in a muffle furnace for four hours to white ash. 12 ml of concentrated nitric acid was added to each sample ash. The solution was then quantitatively transferred to into 25 ml volumetric flask and completed to the mark with distilled water. A sample from each solution was introduced through (ICP) sample introduction system. A full minerals content analysis was obtained. The obtained results were tabulated.

2.4.3 GC –MS Analysis of *Cymbopogon* oils

The qualitative and quantitative analysis of the oil samples was carried by GC\MS spectroscopy. The samples were prepared by diluting 1ml of the volatile oil by 1ml of ethyl alcohol and then the oil solution in ethyl alcohol was injected by using split mode, instrument operating in EI mode at 70eV. Helium as the carrier gas passed with flow rate 1.69 ml/min, the temperature program was started from 50C ^{0}with rate 7C ^{0}/min to 180C ^{0} then the rate was changed to 10C ^{0}/min until reaching 280C ^{0} as final temperature degree. The injection port temperature was 300C^{0}, .The ion source temperature was 200C^0 and the interface temperature was 250C^0.Eeach oil was analyzed by scan mode in the range of m/z 40-500 charges to ratio and the total run time was 28 minutes. Identification of components for the two samples was achieved by comparing their retention index and mass fragmentation patterns with those available in the library of the National Institute of Standards and Technology (NIST). The obtained results were recorded.

Chapter three

Results and Discussions

Chapter three

Results and discussion

3.1 The oil yield percentage

The yield percentages of oils of *Cymbopogon schoenanthus* spikes and *Cymbopogon citratus* leaves were found to be 1.5% v/w and 1.67 % v/wrespectively (table 3.1).

Table 3.1 Oil yield percentage compared with two previous studies

Oil source	Present study (2020)	Eihab Omer et al (2016) (Sudan)	Shah et al (2011) (India)
Spikes of *Cymbopogon schoenanthus*	1.5%	1.9%	_
Leaves of *Cynbopogon citratus*	1.67%	_	(0.2-0.5%)

The obtained yield percentage of spikes of *Cymbopogon schoenanthus* oil was found to be relatively low when it compared with that reported by Eihab Omer (Sudan, 2016). But the two yields may reflect the environmental similarities between the plant species of Sudan depending on climatic conditions and soil characteristics.

The obtained *Cymbopogon citratus* leaves yield percentage was significantly high when it was compared to the leaves oil yield reported by Shah (India, 2011) and that may due to variations in climatic conditions, topographical location and the method through it oils were extracted.

3.2 Inductively Coupled Plasma analysis of Marhabaib

The concentrations of minerals in Marhabiab sample of Iraq reported by Al-Sanfi (2016) were converted to the concentration (mg/100g) for a comparison purpose as in table (3.2)

Table 3.2 Minerals content of Iraq *Cymbopogon schoenanthus* spikes

Mineral	Concentration (mg/100g)
Ca	490
P	32
K	480
Mg	22
Cu	3.2
Mn	270
Zn	0.35
Co	0.0023

Source Alsanfi ; (2016)

3.2.1 Macro minerals in Marhabaib leaves

The inductively couple plasma analysis of Sudanese Marhabaib leaves showed that, the plant leaves were fairly rich with P (2.05mg/100g), Ca (0.425mg/100g), Mg (0.305 mg/100g), Na (0.085 mg/100g) and K (0.0625 mg/100g). The availability of these minerals may be described as significantly low, when compared to that reported by Fagbohun (2010), as P (54.8mg/100g), Ca (39.5mg/100g), Mg (70 mg/100g), Na (54.8 mg/100g) and K (59.5 mg/100g).this may reflect environmental varieties. Another variation was shown by the presence of Iodine (1.65mg/100g), Si (0.45mg\100g) and S (5.75mg/100g) Marhabaib spikes samples showed higher macro minerals content , when compared to that of the leaves. The spikes sample showed P (10.75mg/100g) , followed by S (5.0mg/ 100g), I (3.75 mg /100g) Si (0.85mg/100g), Mg (0.350mg/100g), Ca (0.325/100g), Na (0.190 mg / 100g) and K (0.155 mg /100g). Al- sanfi (2016), who studied *Cymbopogon* spikes in Iraq reported that high content of S (32mg/100g), Ca (490 mg/100g) and K (480 mg/ 100g) whereas the other minerals were not detected in this study. Both Marhabaib sample showed almost similar availability in (S), (Si) , (Ca) , (Na) , (K), (Mg) , (I) and relatively high availability of (P) exhibited in spikes sample comparing to leaves sample(table 3.3).

Table 3.3 Macro Minerals content of Marhabaib compared with some previous studies

Mineral	Concentration (mg/100)			
	Present study (Leaves)	(Fagbohun, 2010) (Leaves) India	Present study (Spikes)	ALSanfi(2016) (Spikes) Iraq
S	5.75	ND	5.00	32
p	2.050	54.80	10.750	ND
I	1.650	ND	3.750	ND
Si	0.450	ND	0.850	ND
Ca	0.425	39.5	0.325	490
Mg	0. 305	70	0.350	22
Na	0.085	54.8	0.190	ND
K	0.0625	59.5	0.155	480

3.2.2 Micro minerals in Marhabaib samples

The (ICP) analysis of Marhabaib leaves sample showed relatively high Fe content (1.075 mg/100g),followed by Zn (0.0975 mg/100g), Cr (0.075 mg/100g),Mn Cu (0.05 mg/100g), (0.0375 mg/100g) and These five minerals showed very low availability or even not detected in Fagbohun study (2010), which showed significant high occurrence of Zn only (121mg\100g), compaired with (0.0975 mg/100g) in the present study. Ni and Co as micro minerals were not detected in the two studies. Marhabaib spikes sample showed low concentrations of Fe (1.25 mg/100g), Zn (0.018mg/100g), Mn (0.0425 mg/100g), Cu (0.0675 mg/100g) and Cr (0.01 mg/100g). Most of these minerals were not detected in Al-Sanfi study (2016), in which Cu , Zn and Mn showed significant high occurrence of Cu (3.2 mg/100g) ,Zn (o.35mg/100g)and Mn(270 mg/100g) compaired with their availability in present study which are (0.018, 0.0675 and 0.0425 mg/100g) respectively.Two Marhabiab samples showed almost similar availability in S, Zn , Cu, Cr and Mn , in addition to that the two micro minerals Ni and Co were not detected in both samples table (3.4).

Table 3.4 Micro minerals content of Marhabaib samples compared with some previous studies

Mineral	Concentration (mg/100g)			
	Present study (Leaves)	Fagbohun(2010) (Leaves) India	Present study (Spikes)	Al-Sanfi (2016) (Spikes) Iraq
Fe	1.0750	0.024	1.25	ND
Zn	0.0975	121	0.018	0.35
Cu	0.0500	ND	0.0675	3.2
Cr	0.0750	ND	0.01	ND
Mn	0.0375	0.952	0.0425	270
Ni	ND	ND	ND	ND
Co	ND	ND	ND	ND

3.2.3 Toxic minerals in Marhabaib

The minerals which are normally classified to be of high toxicity Pb, As, Cd, Be, Ba, Sr, Sn and Al were either not detected or present in significantly low levels in the leaves and spikes samples. This finding was strongly agree with that reported by Fagbohun (2010) study on Indian and Al-Sanfi (2016) study table (3.5). Depending on the low levels of toxic content, Marhabaib can be considerable a fairly safe material for human consumption.

The variation which is representing in appearances or absence of some minerals with their different classifications in two samples can be attributed to difference in topographical location which involve many factors among them the type of soil .

The difference in amounts of some minerals or their absence may be due to difference in soil of specific regions in which the plants were cultivated.

Table 3.5 Toxic minerals content of Marhabiab compared with previous study

Mineral	Concentration (mg/100g)			
	Present study Leaves	Fagbohun(2010) (Leaves) India	Present study (Spikes)	Al-Sanfi (2016) (Spikes) Iraq
Pb	ND	ND	ND	ND
As	ND	ND	0.1625	ND
Cd	0.003	ND	0.003	ND
Be	ND	ND	0.003	ND
Ba	0.0035	ND	0.0035	ND
Sr	0.0275	ND	0.0275	ND
Sn	ND	ND	ND	ND
Al	0.0003	ND	0.0003	ND

3.3 GC-MS Analysis of *Cymbopogon schoenanthus* spikes oil

The GC-MS Analysis of *Cymbopogon schoenanthus* spikes oil showed presence of forty eight components (Fig 3.1, Table 3.6). The major constituents of this volatile oil were found to be representing above (83 %); which are [(oxygenated monoterpene Pipreitone (35.96%), (sesquiterpenes) Elemol(20.45%), monoterpene (+)-2–carene(8.18%), sesquitterpenes [guaiol (6.04 %) , β-eudesmol(5.97%) , γ-eudesmol (4.28 %) , α-Eudesmol (1.37%) and Bulnesol (3.14%)] and monoterpene Limonine (1.44%) , the remaining compounds representing less than (30 %) . (23) of these compounds were found to hydrocarbons compounds, they were found represent above (16 %) which are [(Tricyclene(0.02%) , α-Pinene (0.07%),Camphene (0.08%), 3,7,7,trimethyl-1,3,5-cyclohe pentatriene (0.06%).

p- mentha -2,8 diene (0.04%), α- mourronene (0.02%), (+)-2- carene (8.18%), (+)-4-carene(0.04%),β-cymene(0.135%),d-limonine(1.44%),α-bourbonene(0.11%), cyclohexane-1-ethenyl-1-methyl-2,4-bis(1-methylethyl(1.01%), santalene (0.12%), Iso-caryophyllene(0.74%), 1-H- cyclopropanapthalene (0.06%), Aromadendrene (0.19%), chamigren (0.2%), 2-isopropenyl-4,8-dimethyl-1,2,3,4,4a,5,6,7-octahydronaphthalene(1.47%),Eudesma4,11-diene(0.17),α–selinene(0.15%) , (+) –cuparene(0.97%), γ–muurolene(0.21%), delta -cadinene (0.63%)].And oxygenated compounds involve(+)-Limonine oxide(0.15%), L-fenchone (0.07%),(1,3,3 tri methyl bicyclo heptan-2-one)(0.35%), p -2- men then -1-ol(0.35%), 4-isopropyl -1- methyl -2-cyclohexene-1-ol (0.22%), isomenthone (0.05%),1-menthone(0,05%), cyclohexanone-5-methyl-2-(1-methyl ethyl)(0.18%) , α–phellandren-8-ol(0.18%), α–terpineol(0.94%), piperitone (p-menth-1-en-3-one)(35.96%),dihydroagarofuran(0.41%),Cedranone(0.07%),Epiglobulol(0.26%),elemol(20.45%),caryophylleneoxide(0.59%),2-napthalenemeth-an–ol-1,2,3,4,4a ,5,6,7,octahydroalpha,alpha,4a,8-tetramethyl(0.147%), gama eudesmol(4.28%), Bulnesol(5.97%), Eude-smol–4-(14)en-1-ol(betaeudesmol)(5.97%), guaiol(0.06%) , eudesm-7-(11)-en-ol(1.37%) ,4,6,6trimetylbicylo [3.1.1] hepta-3-en-2-yl) acetaldehyde(0.17%),α–terpineolacetate(0.03%), ethanol-2-(3,3-dimethylcyclo-hexylidene)(0.21%),2,6,10-dodecatrien-1-al, 12(acetoxy)-2-trimethyl (0.01%).

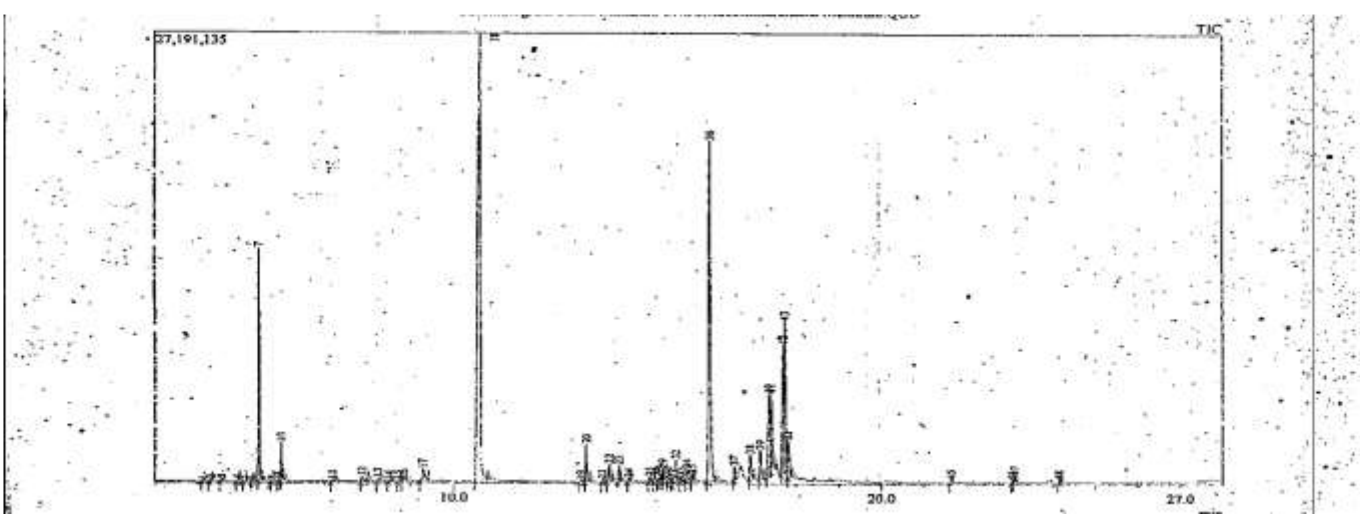


Fig (3.1) GC – MS Chromatogram of *Cymbopogon schoenanthus* spikes oil

Table 3.6 Chemical constituents of *Cymbopogon schoenanthus* spikes oil (GC-MS)

Peak No*	t_R	Area	Area%	Name
1	4.129	61860	0.02	Tricyclene
2	4.314	219727	0.07	α-Pinene
3	4.569	273604	0.08	Camphene
4	4.947	199848	0.06	3,7,7,trimethyl- 1,3,5- Cycloheptatriene,
5	5.109	121036	0.04	p- Mentha -2 ,8 diene
6	5.302	67344	0.02	α- mourronene
7	5.463	26821292	8.18	(+)-2-Carene
8	5.761	127974	0.04	(+)-4-Carene
9	5.922	413268	0.13	β– cymene(m-isoprobyltoluene)
10	5.988	4726381	1.44	D-Limonene
11	7.187	215387	0.07	L-Fenchone (1,3,3 tri methyl bicyclo heptan-2one)
12	7.873	1147529	0.35	P -2- menthen -1-ol
13	8.246	707959	0.22	4-isopropyl -1- methyl -2- cyclo hexene- 1-ol
14	8.500	149183	0.05	Isomenthone
15	8.716	179353	0.05	1- Menthone
16	8.838	579649	0.18	α– phellandren -8-ol
17	9.290	3078279	0.94	α–Terpineol

18	10.615	117935822	35.96	Piperitone (P-Menth-1-en-3-one)
19	12.972	371986	0.11	α –Bourbonene
20	13.087	4633996	1.41	2,4 diisopropenyl-1-methyl-1-vinyl cyclohexane
21	13.471	385721	0.12	Santalene
22	13.626	2427648	0.74	Isocaryophyllene
23	13.857	1971056	0.60	1H-Cyclopropa naphthalene
24	14.083	477527	0.15	(+) -Limonene oxide
25	14.573	618939	0.19	Dehydro Aromadendrene
26	14.693	671289	0.20	β– chamigrene
27	14.778	445479	0.14	2-Isopropenyl 4,8 dimethyl1,2,3,4,4a, 5,6,7, ,octahydro napthalene
28	14.840	549861	0.17	Eudesma-4,11-diene
29	14.886	1361017	0.41	Dihydro –β– agarofuran
30	14.992	501912	0.15	α– Selinene
31	15.094	233975	0.07	9-Cedranone
32	15.189	3190595	0.97	(+)- Cuparene
33	15.308	691270	0.21	γ-Muurolene
34	15.450	2055982	0.63	Delta- cadinene
35	15.572	842048	0.26	Epiglobulol
36	15.984	67084478	20.45	Elemol
37	16.556	1949644	0.59	Caryophyllene oxide
38	16.926	4502608	1.37	α-Eudesmol
39	17.166	4834961	0.47	2- Naphthalenemethanol,1,2,3,4,4a,5,6,7 octahydro- alpha ,alpha, 4a,8,tetramethyl

40	17.365	14047481	4.28	γ-eudesmol
41	17.429	10307114	3.14	Bulnesol
42	17.707	19570861	5.97	β- Eudesmol (Eudesmol -4-(14)-en- 11 -ol)
43	17.740	19812238	6.04	Guaiol
44	17.836	5869538	0.79	Eudesm -7 (11) -en -4-ol
45	21.631	409374	0.12	4,6,6 Trimetylbicylo[3.1.1]hepta-3-en-2- yl) acetaldehyde
46	23.059	239506	0.03	γ– terpineol acetate
47	23.113	684509	0.21	Ethanol, 2-(3,3-dimethylcyclohexylidene)
48	24.152	240144	0.01	2,6,10-Dodecatrien-1-al, 12-(acetoxy)-2,6,10-trimethyl

3.3.1Major Components of *Cymbopogon schoenanthus* spikes oil

The chemical compositions of *Cymbopogon schoenanthus* spikes oil of Sudan was almost similar to that of Borkinafaso Yantema et al; (2007). Hashim of Sudai Arabia (2016) reported relatively low Piperitone content (14.6%) compaired to this study (35.96%) , Eihab et al ; (2016) (47.7%) and Yantema et al; (2007)(42.0%) . This study showed highest content of Elemol (20.45%) compared with other previous studies (table 3.7).

2- Carene content of the spikes oil in this study (8.8%) was almost similar to that reported by Yantema et al; (2007) (8.2%) and Eihab as (10.0%) , whereas it is not detected in Saudi-Arabia study (2016) . β-Eudesmol and α-Eudesmol showed relatively high availability in *Cymbopogon schoenanthus* spikes oil of Saudi-Arabia as (8.5 and 11.5 %) respectively when compared with the other studies (table 3.7).

Guaiol characterized present study with percentage (6.04%) which is absent in other studies, Bulnesol has relatively high availability in present study compared with other studies. Limonine(1.44%) content was almost similar to that reported by Yantema et al; (2007) (1.5%) which are relatively high compared to other studies (table 3.7).

The variations in oil constituents percentages may attribute to some environmental and geographical factors such as climate, soil characteristics and cultivation procedures.

Table 3.7 Comparison between different *Cymbopogon schoenanthus* spikes oils

Component	Chemical formula	Present study (2020)	Eihab et al; (2016) Sudan	Hashim et al ;(2016) (KSA)	Yentema et al; (2007) Borkinafaso
Piperitone	$C_{10}H_{16}O$	35.96 %	47.7%	14.6%	42.0%
Elemol	$C_{15}H_{26}O$	20.45	9.0	10.8	6.2
2-Carene	$C_{10}H_{16}$	8.18	10.0	-	8.2
Guaiol	$C_{15}H_{26}O$	6.04	-	-	-
β-Eudesmol	$C_{15}H_{26}O$	5.97	1.8	8.5	1.8
γ-Eudesmol	$C_{15}H_{26}O$	4.28	1.8	4.2	-
Bulnesol	$C_{15}H_{26}O$	3.14	trace	-	0.2
Limonine	$C_{10}H_{16}$	1.44	0.1	-	1.5
α-Eudesmol	$C_{15}H_{26}O$	1.37	2.1	11.5	1.0

3.4 GC\MS Analysis of *Cymbopogon citratus* leaves oil

GC-MS analysis of *Cymbopogon citratus* leaves oil showed presence of sixteen (16) compounds . The dominant constituents were found to be Piperitone (84.13%), follwed by (+)-2-carene (5.75%) , d–Limonene and α– terpineol (2.21 %) (2.67%), these dominant compounds representing more than (94%). The oxygenated constituents oxygenated constituents are 2,5-Dimethylhex-5-en-3-yn-2-ol (0.03%), fenchone (0.1%), P- Menthen – 1 – ol (0.01%), 4- Isopropyl-1-methyl -2-cyclohexene –ol (0.7%), O-Menth-8-ene-4-methanol (0.65%) , Eudesm-7(11)-en -4- ol (0.68%), α– Eudesmol (0.76%), Globulol (0.64%), Caryophyllene oxide (0.12%) and Pipreitone (84.16%) which are represent more than (90%) of the total oil content. The hydrocarbons constituents which represent less more than (9%) which are (+)-2-Carene (5.75%), d-Limonine (2.67%), (+)–Cuparene (0.1%), Isocaryophyllene (0.12%) and γ-Muurolene (0.07%).

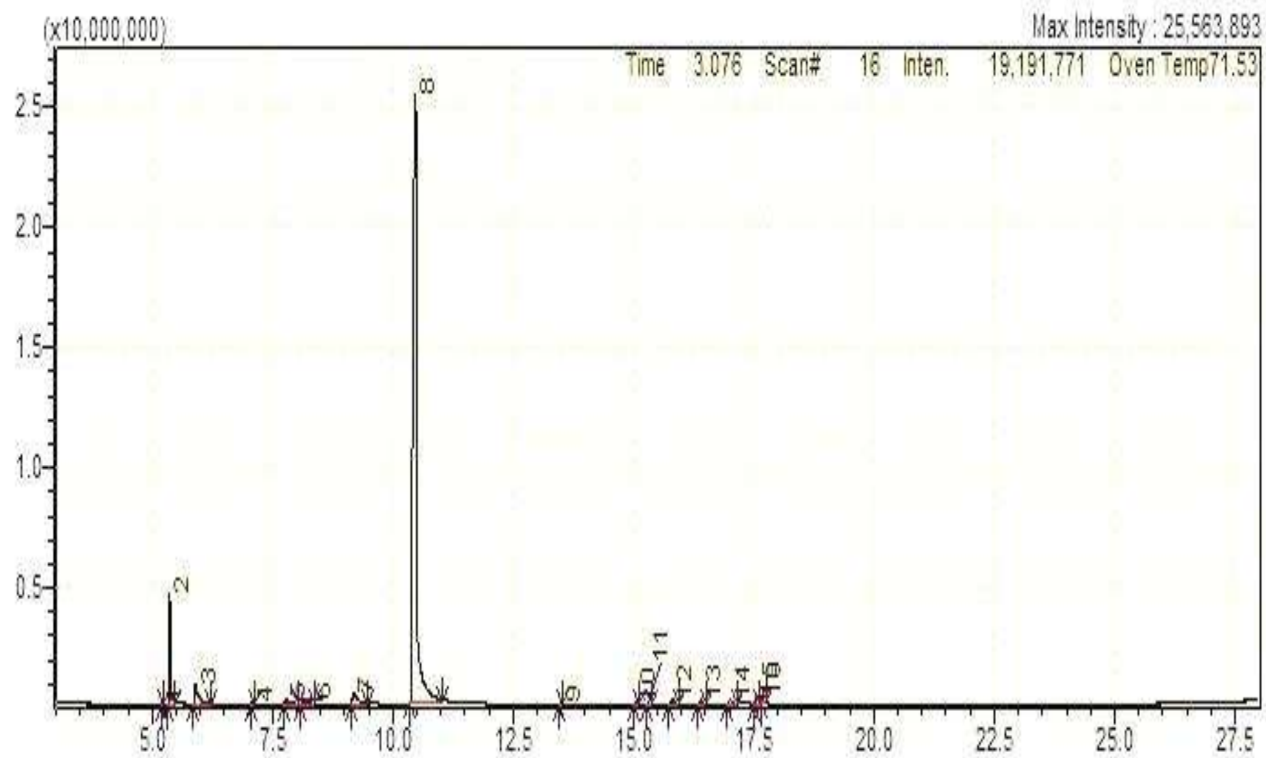


Figure 3.2 GC- MS Chromatogram of *Cymbopogon citratus* leaves oil

Table 3.8 Chemical constituents of *Cymbopogon citratus* leaves oil

PeakNo	(t_R)	Area	Area%	Name
1	5.206	55017	0.03	2,5-Dimethylhex-5-en-3-yn-2-ol
2	5.365	9602351	5.75	(+)-2-Carene
3	5.900	4462288	2.67	D-Limonene
4	7.079	174577	0.10	Fenchone
5	7.778	1685897	0.01	(E) – P- Menthen – 1 – ol
6	8.134	1164116	0.70	4- Isopropyl-1-methyl -2-cyclohexene –ol
7	9.183	3685783	2.21	α-Terpineol
8	10.474	14049612	84.16	Piperitone
9	13.488	204137	0.12	Isocaryophyllene
10	15.054	162680	0.10	(+) – Cuparene
11	15.317	124889	0.07	γ-Muurolene
12	15.808	1092391	0.65	O-Menth-8-ene-4-methanol
13	16.398	592775	0.35	Caryophyllene oxide
14	17.010	1140598	0.68	Eudesm-7(11)-en -4- ol
15	17.581	1275173	0.76	α– Eudesmol
16	17.670	1072640	0.64	Globulol

3.4.1 Major Components in *Cymbopogon citratus* leaves oil

On comparing the availability of the major constituents of *Cymbopogon citratus* leaves oil in this study with the previous studies carried in India (2011),Brazil

(2009) and Portogal (2020) , The major compounds in this study showed either very low percentages or absolutely not detected (Table 3.9).

The significant variations may reflect mainly the environmental differences between the four countries. Soil characteristics and cultivation procedures may also be additional factors that may lead to chemical composition variations of the different *Cymbopogon citratus* leaves oils.

The major constituent of Sudanese oil sample is Pipretone (84.16%) as predominant component where as its absent in *Cymbopogon citratus* leaves oil of India , Brazil, and with percentage (0.1%) in *Cymbopogon citratus* oil of Portugal, in which the predominant constituent in oils of pervious study were found to be either citral or its two isomer (geranial or neral). 2-Carene was detected in Sudanese sample only (5.75%) Limonine and Terpinol were detected with (2.67%, 2.21%) and showed low availability in other studies (table 3.9)

Table 3.9 Moajor components of *Cymbopogon citratus leaves oil*

Compoents	Chemica formula	Present study (2020)	Andrade et al (2009) (Brazil)	Shah et al (2011) (India)	Gago et al (2020) (Portugal)
Pipretone	$C_{10}H_{16}O$	84.16%	-	-	0.1%
2- Carene	$C_{10}H_{16}$	5.75%	-	-	-
Limonine	$C_{10}H_{16}$	2.67%	0.2%	trace	0.4%
α-Terpineol	$C_{10}H_{16}O$	2.21%	0.2%	0.45%	0.3%

pipretone is predominant component in both *Cymbopogon* species but in *Cymbopogon citratus Dc stapf* has percentsage (84 .16 %) which is greater than its percentage in other species (*Cymbopogon schoenanthus).* GC\MS analysis of oil of two *Cymbopogon* species showing that number of constituents in *Cymbopogon schoenanthus* oil which detected by this test greater than number of constituents detected in *Cymbopogon citratus Dc stapf* oil and that gives an indication about *Cymbopogon schoenanthus* oil is more rich by chemical composition than the last one species (*Cymbopogon citratus*), there are two constituents which were detected in both *Cymbopogon* species oils which are (+)_ carene (in case of oil of spikes of *Cymbopogon schoenanthus*) has high percentage (8.18 %) where as in *Cymbopogon citratus* oil is less than that (5.75 %) and in two oils its appears as major constituents, the compound D – limonene also appears in both species but it appears as major constituent in *Cymbopogon citratus* leaves oil with percentage (2.67%) where as in case of *Cymbopogon schoenanthus* spikes oil has percentage (1.44 %) . The *Cymbopogon schoenanthus* spikes oil is characterized by presence of elemol (20.45 %) guaiol (6.04 %) and gama – eudesmol (4.28 %) which appear as major constituents and they are absent in *Cymbopogon citratus* leaves oil . *Cymbopogon citratus* oil also has characteristic compound which appear as major conistiuents and it is absent in *Cymbopogon shcoenanthus* spikes oil which is α– terpineol (2.21 %) where as in *Cymbopogon schoenanthus* spikes oil does not appear as major constituents in addition to that it appear as α-Terpineol acetate (0.03%).

Table 3.10 Percentages of some compoenents in two of Marhabiab oils

Components	*Cymbopogon schoenathus*	*Cymbopogon citratus*
Pipretone	35.96%	84.16%
(+)-2-Carene	8.18 %	5.75 %
Limonine	1.44 %	2.67%
α– Eudesmol	1.37%	0.76%
Fnchone	0.07%	0.10%
(+)- Cuperene	0.97%	0.10%

3.5 Short summary about the major constituents of Marhabaib oil

3.5.1 Piperitone

Chemical formula: $C_{10}H_{16}O$ Molecular Weight: 152.23 g/ mol

Melting point: 232 to 233 °C Storage temperature: 2-8°C

Density: 0.9331 g/cm^3

IUPAC name : 6-Isopropyl-3-methyl-1-cyclohex-2-enone.

Pipretone is a monoterpene ketone which is a component of some essential oils. Both stereoisomers, the D-form and the L-form, are known. The D-form has a peppermint-like aroma and has been isolated from the oils of plants from the genera *Cymbopogon, Andropogon*, and Mentha.The L-form has been isolated from Sitka spruce. Piperitone is used as the principal raw material for the production of synthetic menthol and thymol. The primary source of D/L-piperitone is from Eucalyptus dives, produced mainly in South Africa.

3.5.2 (+)-2-Carene

Chemical formula: $C_{10} H_{16}$

Molecular Weight: 136.23 g/mol

Boiling Point: 167-168^{o}C

Optical activity: + 90.0^{o}C

Density: 0.862 g\ml at 25^{0}C

IUPAC name: Bicyclo[4.1.0] hept-2- ene ,3,7,7-trimethyl[4.1.0] hept-4-ene.

(+)-2- carene belongs to the class of organic compounds known as bicyclic mono-terpenoids.These are monoterpenoids containing exactly 2 rings, which are fused to each other. 2-Carene is considered to be a practically insoluble (in water) and relatively neutral molecule. Within the cell, 2-carene is primarily located in the membrane and cytoplasm.

3.5.3 β-Eudesmol

Chemical formula: $C_{15}H_{26}O$

Molecular Weight: 222.37g/mol

Melting Point: 72-74^{o}C

Boiling Point: 301-302^{o}C

Storage temperature: 2-8^{o}C

IUPAC name: 4*a*-methyl-8-methylidene-1, 2, 3, 4, 5, 6, 7, 8a octahydronaptha-len-2-yl] propan-2-ol.

Beta-eudesmol is a carbobicyclic compound that is substituted at positions 2, 4a, and 8 by 2-hydroxypropan-2-yl, methyl and methylidene group, respectively. It has a role as a volatile component. It is a carbobicyclic compound, a tertiary alcohol and a eudes-mane sesqit- terpenoid .It use as food additive (Includes spices, extracts, colorings, flavors, added to food for human consumption) and as food flavor (General flavoring agents used in foods, including condiments and Seasonings).

3.5.4 Guaiol

Chemical formula: $C_{15}H_{26}O$ Molecular Weight: 222.37 g/ mol

Melting point: 40-50^{o}C Density: 0.965-0.675 g/ cm^{3}

IUPAC name : 3, 8-Dimethyl-1,2,3,4,5,6,7,8-octahydro-5-azulenyl]-2-propanol

Guaiol or champacol is an organic compound, a sesquiterpenoid alcohol found in several plants, especially in the oil of guaiacum and cypress pine. It is a crystal-ine solid which is one of many terpenes found in Cannabis and it has been Associated with decreased anxiolytic activity.

3.5.5 γ-eudesmol

Chemical formula: $C_{15}H_{26}O$ Molecular Weight: 222.3663 g/mol

Boiling Point: 83-86^{o}C Density: 0.96 g/cm^{3}

IUPAC name: 4*a*,8-dimethyl-2,3,4,5,6,7-hexahydro-1*H*-naphthalenyl]prop2-ol

γ-eudesmol,is a member of the class of compounds known as eudesmane ,iso eudesmane or cycloeudesmane sesquiterpenoids. Eudesmane ,iso-eudesmane or cyclo-eudesmane sesquiterpenoids are sesquiterpenoids with a structure based on the eudesmane skeleton. It is practically insoluble (in water) and an extremely weak acidic compound (based on its pKa). Gamma-eudesmol is a sweet and waxy tasting compound and can be found in a number of food items such as rosemary, ginkgo nuts, mango, and common thyme, which makes gamma-eudesmol a potential biomarker for the consumption of these food products.

3.5.6 Limonene

Chemical formula: $C_{10}H_{16}$ Molecular weight: 136.238g/mol

Melting point: -74.35^{o}C boiling point: 176^{o}C

Density: 0.8411 g/cm^{3}

IUPAC name: 1- Methyl -4- (prop-1-en- 2-yl) cyclohex-1-ene.

Limonine is a colorless liquid aliphatic hydrocarbon classified as a cyclic monoterpene and it is the major component in the oil of citratus fruit peels. The D-somer occurring more commonly in nature as the fragrance of oranges, it used as flavouring agent in food manufacturing as well as in chemical synthesis as aprecursor to carvone and as a renewables –based solvent in cleaning products The less common L-isomer is found in mint oils and has a piny, turpentine-like odor. The compound is one of the main volatile mono terpenes found in the resin of conifers, particularly in the Pinaceae,and of orange oil .Limonene takes its name from French*limon* ("lemon").Limonene is a chiral molecule, and biological sources produce one enantiomer: the principal industrial source, citrus fruit, contains D-limonene (+) - limonine is obtained commercially from citratus fruit through two primary methods : centrifugal separation or steam distillation.

3.5.7 Elemol

Chemical formula: $C_{15}H_{26}O$ Molecular weight: 222.366g/ mol

Melting point: 51-52^{o} C Boiling point: 144-145^{o}C

IUPAC name: 4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexyl]propan-2-ol

Elemol is a sesquiterpenoid that is isopropanol which is substituted at position 2 by a 3-isopropenyl-4-methyl-4-vinylcyclohexyl group. It has a role as a fragrance and a plant metabolite. It is a sesquiterpenoid, a tertiary alcohol and an olefinic compound.it use as adsorbent (adhesion of molecules to a surface) and in air treatment (Air cleaners and anti-odor agents, air purifiers, air conditioners, air filters, general air care products).

3.5.8 α-Eudesmol

Chemicl formula: $C_{15}H_{26}O$ Molecular weight: 222.366

Melting point: 81-82^{o}C Boiling point: 299-302^{o}C

IUPAC name: 4*a*,8-dimethyl-2,3,4,5,6,8*a*-hexahydro--naphthalen-2-yl]propan-2-ol.

α-eudesmol is a sesquiterpenoid in which the eudesmane skeleton carries a hydroxy substituent at C-11 and has a double bond between C-3 and C-4. It has a role as a volatile oil component.It is eudesmane sesquiterpenoid,a member of octahydro naphthalenes and tertiary alcohol.

3.5.9 Bulnesol

Chemical formula: $C_{15}H_{26}O$ Molecular weight: 222.37g/mol

Boiling point: 314^{o}C Melting point: 69-70^{o}C

IUPAC name : 2-(3,8-dimethyl-1,2,3,3*a*,4,5,6,7-octahydroazulen-5-yl)propan-2-ol

It belongs to the class of organic compounds known as sesquiterpenoids. These are terpenes with three consecutive isoprene units. Bulnesol exists as a solid and is considered to be practically insoluble (in water) and relatively neutral. Within the cell, bulnesol is primarily located in the membrane and cytoplasm. Bulnesol is a spice tasting compound that can be found in herbs and spices. This makes bulnesol a potential biomarker for the consumption of this food product.

3.5.10 cuparene

Chemical formula: $C_{15}H_{22}$ Molecular weight: 202.335g/mol

Boiling point: 275^{o}C Density: 0.937g/cm^{3}

IUPAC name: 1-methyl-4-[(1R)-1, 2, 2-trimethylcyclopentyl]benzene

Organic compound lipid or lipid like molecule belongs to the class of organic compounds known as sesquiterpenoids. These are terpenes with three consecutive isoprene units.

3.5.11 Terpineol

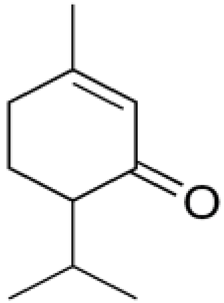
Chemical formula: $C_{10}H_{18}O$ Molecular weight: 154.253g/mol

Melting point: -35.9 to -28.2^{o}C Boiling point: 214 -217^{o}C

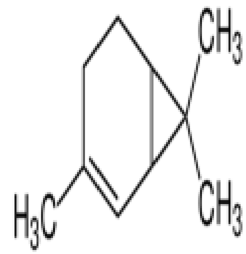
IUPAC names *p*-Menth-1-en-8-ol2-(4-Methylcyclohex-3-en-1-yl) propan-2-ol

Terpineol is a monoterpene alcohol that has been isolated from a variety of sources such as cajuput oil, pine oil, and petitgrain oil.There are four isomers: alpha-, beta-, gamma-terpineol, and terpinen-4-ol. Beta- and gamma-terpineol differ only by the location of the double bond. Terpineol is usually a mixture of these isomers with alpha-terpineol as the major constituent.

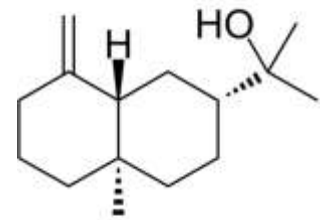
Terpineol has a pleasant odor similar to lilac and is a common ingredient in perfumes, cosmetics, and flavors. α-terpineol is one of the two most abundant aroma constituents of lapsang souchong tea; the α-terpineol originates in the pine smoke used to dry the tea.



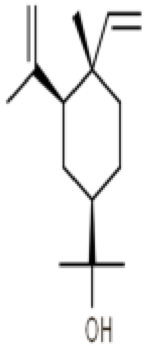
Pipretone



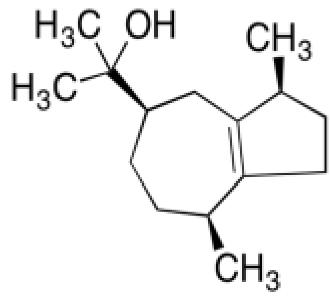
(+) – 2- Carene



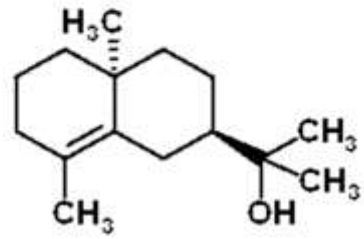
β-Eudesmol



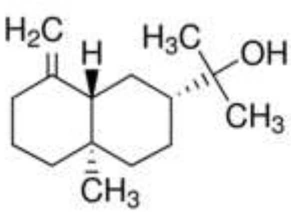
Elemol



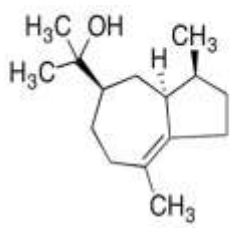
Guaiol



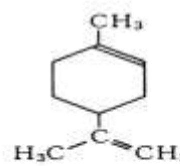
γ-Eudesmol



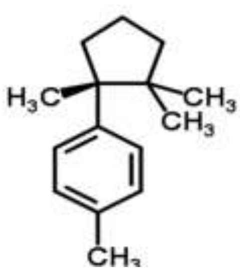
β-Eudesmol



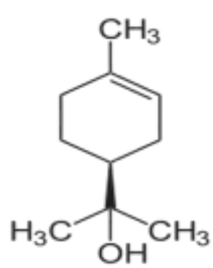
Bulnesol



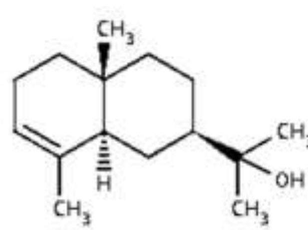
Limonine



Cuparene



Terpineol



α-Eudesmol

Figure 3.3 Structures of the major constituents of Marhabaib oil samples

Conclusion

*From human consumption sight of view, the *Cymbopogon* species of the Sudan may be described as highly safe as food additive, hot drink or medicinal herb.

*The plant should be given more interest as one of the aromatic medicinal plant of the Sudan.

*The Plant can be cultivated in a wide several parts of the country, for medicinal and economic pourpouses. The plant oils may need further characterization for possibility of its use for cosmetic purposes separately or as an additive.

*Marhabaib was found to be containing considerable amounts of macro nutrients, trace minerals and very low concentrations of toxic minerals this may encourage the traditional use of it without risks.

* The High essential oil content makes Sudanese Marhabaib a valuable source of the commercially important monoterpene, piperitone and many other classes of terpenes. Marhabaib samples were found to be containing considrablee amount of iodide there for study should be carried to show possibility of using this plant for thyroid disorder treatment.

Recommendations

*Some other properties such as moisture and ash contents of Marhabiab may need to be studied.

*The suitable environmental conditions for the plant growth may need to be studied for organized cultivation of this plant.

*Further researches are required to show the anti-oxidant and biological activities of the volatile oil of Sudanese Marhabaib.

* The analysis did not show measurable risks for Marhabaib consumption, but further research may be need to determine any possible side effects for it's use by women during pregnancy and lactation periods.

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Appendixes

Appendix (I): ICP Quantitive results of Marhabaib leaves sample

Comment						
1000mg/L <=						
1mg/L <=	S 2.3					
1ug/L <=	Al 120	B 4.2	Ba 1.4	Ca 170	Cd 1.2	Cr 2.7
	Cu 20	Er 3.1	Fe 430	Ho 1.1	I 660	K 25
	Mg 130	Mn 15	Na 34	P 820	Rb 690	Si 180
	Sr 1.1	Th 13	Ti 2.5	Tl 31	W 18	Zn 3.9
< 1ug/L	Li 0.23	Sc 0.39				
Not Detected ug/L	Ag < 1.8	As < 14 +	Au < 0.94	Be < 0.03	Bi < 5.2	Ce < 4.2
	Co < 1.9	Cs < 370	Dy < 1.1	Eu < 0.19	Ga < 2.5	Gd < 1.8
	Ge < 2.0	Hf < 4.6	Hg < 1.1	In < 19	Ir < 35	La < 0.79
	Lu < 0.22	Mo < 8.3	Nb < 2.5	Nd < 2.6	Ni < 4.6	Os < 13
	Pb < 6.2 +	Pd < 5.4	Pr < 3.2	Pt < 25	Re < 4.4	Rh < 6.6
	Ru < 4.5	Sb < 7.4	Se < 17	Sm < 3.7	Sn < 9.6	Ta < 5.1
	Tb < 2.9	Te < 14	Tm < 1.2	U < 20	V < 0.26	Y < 0.22
	Yb < 0.09	Zr < 0.69				

Instrument Name:Forensic

1

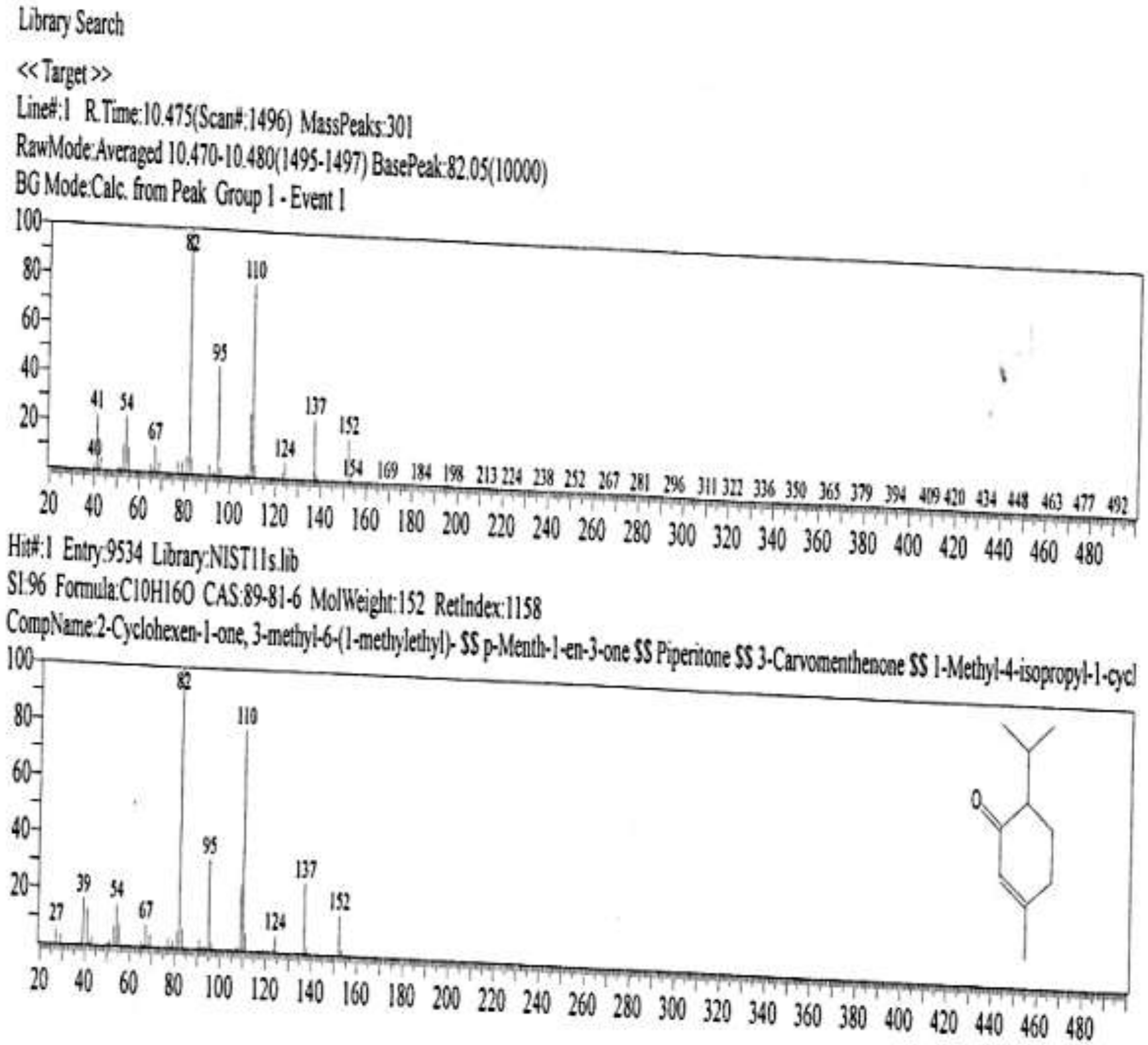
Appendix (II): ICP Quantitative results off Marhabaib spikes sample

Comment							
1000mg/L <=							
1mg/L <=	I 1.5	P 2.0	S 4.3				
1ug/L <=	Al 550	As 65	B 5.8	Ba 1.2	Ca 130	Cd 1.2	Cr 4.0
	Cu 27	Er 12	Fe 500	Ho 1.2	K 62	Mg 140	Mn 17
	Na 76	Pb 12 +	Rb 750	Si 340	Th 14	Ti 6.0	Tl 31
	Zn 7.2						
< 1ug/L	Li 0.94	Sc 0.18	Sr 0.45	V 0.37			
Not Detected ug/L	Ag < 1.8	Au < 0.90	Be < 0.03	Bi < 5.2	Ce < 3.9	Co < 1.9	Cs < 360
	Dy < 1.0	Eu < 0.18	Ga < 2.5	Gd < 1.5	Ge < 1.9	Hf < 4.6	Hg < 1.2
	In < 19	Ir < 35	La < 0.81	Lu < 0.23	Mo < 8.2	Nb < 2.6	Nd < 2.5
	Ni < 4.4	Os < 13	Pd < 5.5	Pr < 2.9	Pt < 26	Re < 4.2	Rh < 6.6
	Ru < 4.4	Sb < 7.3	Se < 17	Sm < 3.4	Sn < 9.7	Ta < 5.1	Tb < 2.7
	Te < 14	Tm < 1.2	U < 19	W < 17	Y < 0.19	Yb < 0.09	Zr < 0.68

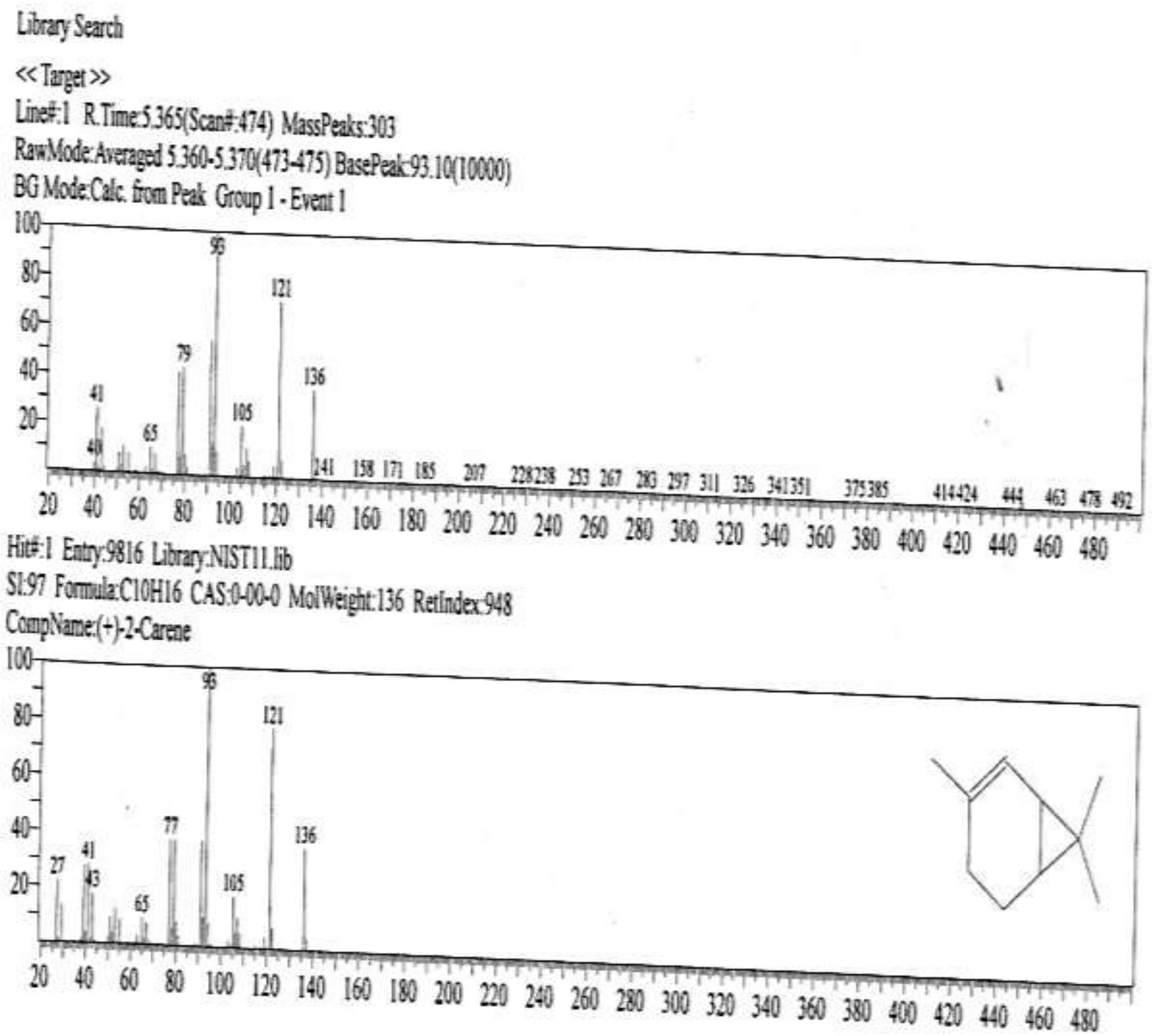
Instrument Name:Forensic

1

Appendix (III): Mass spectrum of Pipertone



Appendix (IV) : Mass spectrum of (+)-2-Carene



Appendix (V): Mass spectrum of D- Limonine

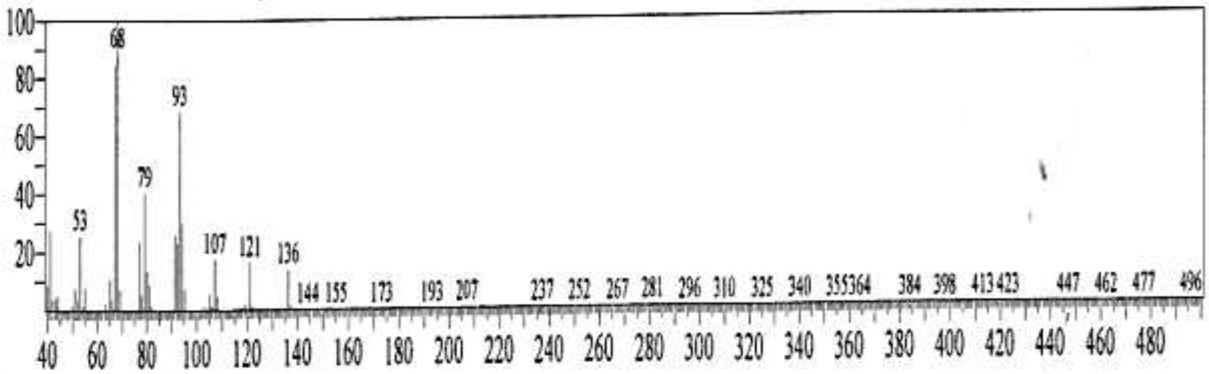
Library Search

<< Target >>

Line#:1 R.Time:5.900(Scan#:581) MassPeaks:279

RawMode:Averaged 5.895-5.905(580-582) BasePeak:68.05(10000)

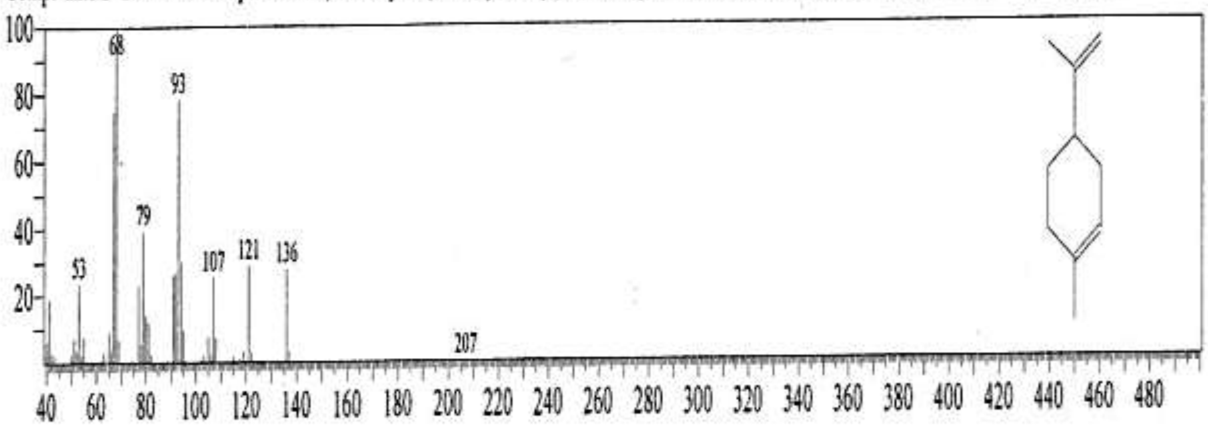
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:6621 Library:NIST11s.lib

SI:96 Formula:C10H16 CAS:5989-27-5 MolWeight:136 RetIndex:1018

CompName:D-Limonene $$ Cyclohexene, 1-methyl-4-(1-methylethenyl)-, (R)- $$ p-Mentha-1,8-diene, (R)-(+)- $$ (+)-(R)-Limonene $$ (+)-(4R)-Limonene



Appendix (VI): Mass spectrum of alpha- Eudesmol

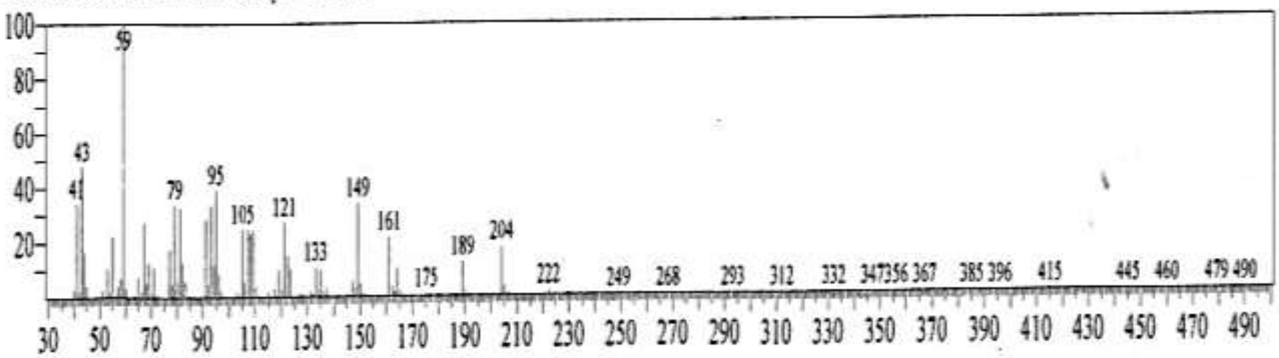
Library Search

<< Target >>

Line#:1 R.Time:17.580(Scan#:2917) MassPeaks:311

RawMode:Averaged 17.575-17.585(2916-2918) BasePeak:59.05(10000)

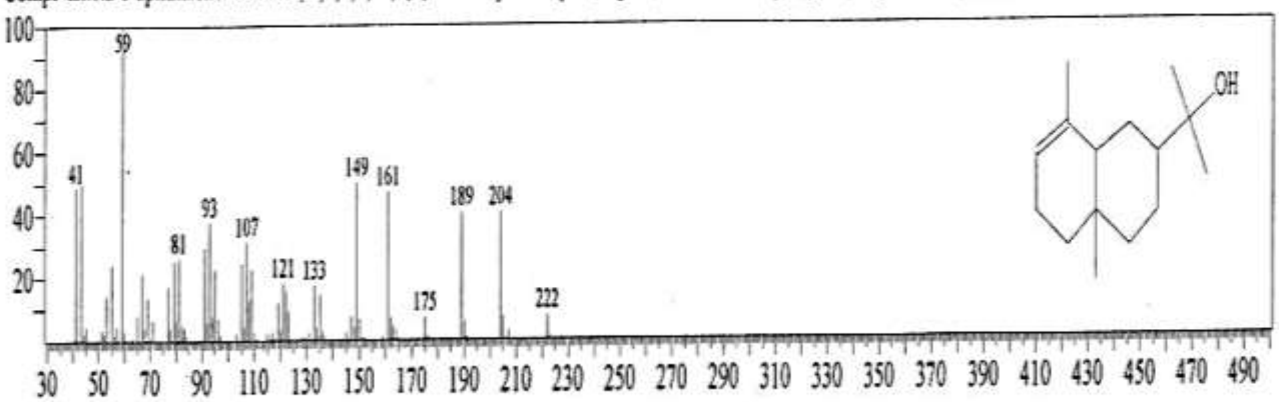
BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:2 Entry:20272 Library:NIST11s.lib

SI:90 Formula:C15H26O CAS:473-16-5 MolWeight:222 RetIndex:1598

CompName:2-Naphthalenemethanol, 1,2,3,4,4a,5,6,8a-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, [2R-(2.alpha.,4a.alpha.,8a.beta.)]- $$ .alpha.-Eudesmol $$



Appendix (VII): Mass spectrum of gamma- Eudesmol

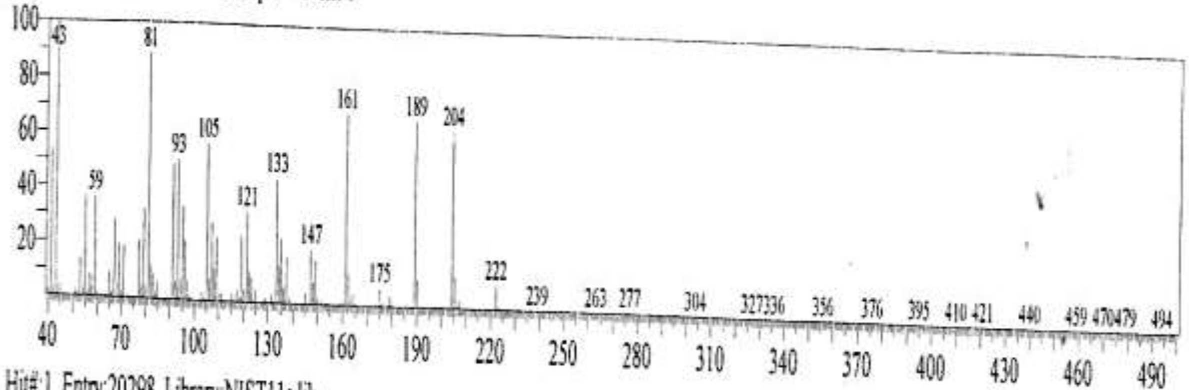
Library Search

<< Target >>

Line#:1 R.Time:17.165(Scan#:2834) MassPeaks:298

RawMode:Averaged 17.160-17.170(2833-2835) BasePeak:43.00(10000)

BG Mode:Calc. from Peak Group 1 - Event 1



Hit#:1 Entry:20298 Library:NIST11s.lib

SI:90 Formula:C15H26O CAS:1209-71-8 MolWeight:222 RetIndex:1626

CompName:2-Naphthalenemethanol, 1,2,3,4,4a,5,6,7-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-, (2R-cis)- $$ .gamma.-Eudesmol $$ .gamma.-Eudesmole $