Sudan University of Science and Technology

College of Postgraduate Studies

Chemical Constituents and Biological Activity of *Raphanus sativus* Oil

المكونات الكيميائية والفعالية البيولوجية لزيت الفجل

A Thesis Submitted in Partial Fulfillment of the Requirements of the M.Sc. Degree in Chemistry

By

Alyaa Abubaker Awad Osman

(B.Sc., Hons., Chemistry)

Supervisor

Prof. Mohamed Abdel Karim Mohamed

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Dedication

To

my father

To my mother

To my sisters and brothers

Acknowledgment

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

I would like to express my thanks , gratitude and respect to my supervisor Prof. Mohamed Abdel Karim for his interest ,close supervision and continuous advice. Thanks for the technical staff, Dept. of Chemistry, Sudan University of Science and Technology for their infinite support. Deep thanks to my family for their support.

Abstract

Raphanus sativus oil were extracted by hexan at room temperature.The oil was then analayzed by GC-MS.The analysis showed 15 components.The major constituent are: (z)13-Docosenoic acid,methyl ester(35.48%);9-octadecenoic acid(z)methyl ester(17.12%) ; Cis-11-Eicosenoic a cid,methyl ester(11.64%);9,12-octadecadienoic acid (z,z) methyl ester(10.77%); (Z,Z,Z) 9,12,15-oct adecatrienoic acid,methyl ester (7.14%); Hexadecanoic acid,methyl ester(5.48%).

Inc cup plate agar diffusion bioassay, the oil was assessed for antimicrobial activity against apanel of standard human pathogenic microbes.At a concentration of 100mg/ml,the oil showed partial activity against *Acinetobacter baumannii. Staphylococcus aureus ,Bacillus subtilis and Aspergillus flavus.*

مستخلص البحث

استخلصت بـذور نبات الفجل بالهكسان حيث تم استخلاص الزيت الثابت.
ثم حلل الزيت بتقنيه الكروماتوغرافيا الغازية - طيف الكتله والتي اوضحت وجود 15مكونا اهمها:

(z)13-Docosenoic acid,methyl ester(35.48%)

9-octadecenoic acid (z)methyl ester(17.12%)

Cis-11-Eicosenoic a cid,methyl ester(11.64%)

9,12-octadecadienoic acid (z,z) methyl ester(10.77%)

(Z,Z,Z) 9,12,15-oct adecatrienoic acid,methyl ester (7.14%)

Hexadecanoic acid,methyl ester(5.48%)

في اختبار مضاد الميكروبات وعند تركيز 100 mg/ml اعطى الزيت فعاليه جزئيه ضد:

Acinetobacter baumannii. Staphylococcus aureus ,Bacillus subtilis and Aspergillus flavus.

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Chapter One

Introduction

1-Introduction

1.1- **Gas chromatography (GC)**

GC (Gas chromatography) is a sensitive analytical tool used in various fields of science such as forensic, environmental, food, agriculture and petrochemical industries[5]. In gas chromatography, the separation is mainly achieved as a result of partitioning of analytes between the gaseous mobile phase and a static phase (stationary phase) while transporting the capillary column. A capillary GC column is coated with a thin film of liquid-like stationary phase which serves to retain the gaseous analytes transported by the mobile phase. The most commonly used mobile phases, referred to as carrier gases, include helium or hydrogen. Stationary phases are differentiated based on their polarity

In GC-MS differential partitioning of sample constituents occurs as a function of properties such as polarity and boiling points[6]. Those components which have greater affinity for the stationary phase spend more time in the column, whereas those with lower affinity spend less time in the stationary phase and thus elute earlier[3].

As far as instrumentation is concerned the GC equipment consists of a carrier gas supply, sample introduction unit (injector), capillary column, oven and a detector; the operation of the most important instrumental parts will be discussed briefly below.

GC instrument has an injection port that allows the volatiles to be introduced in vapour form via the carrier gas stream(like Helium) into the capillary column. The most common injector used in contemporary GC is the vaporising split/splitless injector. This injector was invented to prevent overloading of the capillary column due to its low volume and capacity, which may affect resolution. The sample is introduced into a heated chamber, where vaporisation occurs. Two modes of injection, split and splitless, can be used depending on the concentration of the target analyte. Split mode is mostly used when the analyte is present at high concentrations, while splitless is used when the concentration of the analyte is low[7]. Splitless injection requires effective utilisation of focusing mechanisms such as the solvent effect, cold trapping and stationary phase focusing to avoid injection band broadening.

Usually in GC machine , the capillary column is coated with a stationary phase that permits separation of different constituents of an analyte. Stationary phases in a capillary GC are differentiated according to their polarity. Non-polar stationary phases such as PDMS, sometimes with 5 to 50% phenyl PDMS groups added are commonly used for the separation of compounds ranging from non-polar to medium polar. In these phases, separation is governed primarily by differences in vapour pressure, since non-specific dispersion interactions occur between the analytes and the stationary phase. A polar phases have been used for the analysis of petrochemical samples and also for TDOs, since they

primarily contain hydrocarbons. On semi-polar (14% cyanopropylphenyl 86% PDMS) or polar phases, mostly PEG, selective interactions such as hydrogen bonding and dipole interactions occur, and compounds are separated according to their polarity. The column is housed in an oven for accurate temperature control. Since the separation of compounds in GC is primarily based on differences in the vapour pressures of compounds, temperature plays a crucial role.

In GC there must be a temperature programming, where an initial low oven temperature is increased as a function of time, is used to provide optimal resolution for a range of weakly and strongly retained analytes within an acceptable analysis time. Column dimensions such as length, internal diameter, film thickness and stationary phase are selected based on the analysis goals. Short (10-20 m) columns are used for fast separation of relatively simple mixtures. For complex samples, longer columns (50-60 m) provide improved separation efficiencies at the cost of longer analyses. Furthermore, reduction in the internal diameter (from e.g. from standard 0.25 mm i.d. to 0.1-0.18 mm) increases the efficiency per unit length and also provides higher optimal mobile phase flow rates, thereby allowing speeding up of the analysis. This is evident from the relationship between the column length, efficiency and optimal flow rate and the internal diameter: N = L H = L dc (1.1) $uopt = 2DM\ rc$ (1.2) Where N is the plate number, L is the length of the column, H is the height equivalent of a theoretical plate, uopt is the optimal mobile phase

linear velocity, DM is the diffusion coefficient of the analyte in the mobile phase and dc and rc are the column internal diameter and radius, respectively[6].

Another method to check the efficiency of separation in GC depends on the peak capacity. Peak capacity is defined as number of peaks that can theoretically be separated within the retention window[8]. The peak capacity of a chromatographic separation depends[9] on the plate number (N), the mobile phase linear velocity and the temperature.

Peak capacity can be calculated[8] using the following equation below:

$$nc = 1 + tg\ wav$$

Where:

- nc : is the peak capacity

-tg : denotes the gradient run time and

-wav : avarage peak width at baseline.

During a GC experiment, the oven ramping rate (˚C/min) affects the achievable peak capacity[10]. Slow ramping rates result in longer gradient times and generally higher peak capacities, although for very slow temperature programming rates peak widths increase and peak capacity decreases again. Evaluation of peak capacity in GC provides a measure

of the separation performance as well as the optimum conditions for better separation.

In chromatography detectors are extremely important and they should ideally obey certain characteristics such as adequate sensitivity, stability and reproducibility, linear response range to solute concentration over a wide dynamic range, as well as being reliable and easy to use.

However, a wide array of detectors are compatible with GC, such as the nitrogen phosphorus detector (NPD), atomic emission detector (AED), thermal conductivity detector (TCD), sulphur chemiluminescence detector (SCD), electron capture detector (ECD), FID and MS, amongst others. Detectors are normally selected depending on the analyte of interest and the analysis goals (i.e. selective detection of the target analytes or screening of unknowns, trace level analysis, etc). Some detectors are universal, meaning that they respond to any or most sample constituents, for example FID, MS and AED. In contrast, selective detectors respond to certain group of compounds, for example the NPD (for nitrogen and phosphorus containing compounds) and the ECD (for halogenated compounds). Among all these detectors, MS and FID are the most commonly used detectors for analysis of a wide range of samples containing organic compounds.

Such detectors are also most commonly used in petrochemical analysis. MS is the most powerful and universal detector that provides detailed

information about the identity of the chemical constituents, while FID only gives information about the quantitative chemical composition of the sample.

One of the most widely used detectors in gas chromatography is the so called flame ion detector(FID) is. Here, the effluent from the column is directed into a small air and hydrogen flame; ions formed in the combustion of organic compounds in the flame are detected. Detection involves monitoring the current produced by collection of these ions by the collector electrode. The response of the FID is related to the number of carbon atoms entering the detector, thus it is a mass sensitive detector. This detector is not sensitive towards non-combustible permanent gases such as CO2, SO2, NO2, etc.[1].

For qualitative and quantitative analyses[11-13], the FID is extensively used in a variety of fields. Being a mass sensitive universal detector for hydrocarbons, it can be used to estimate the mass % composition of hydrocarbon mixtures[14], and indeed has been used for this purpose in TDO analysis.

1.2-Mass spectrometry

Mass spectrometry measures the mass to charge ratio (m/z) of ions produced by the test sample. The technique of MS detection essentially involves:(i) ionization (ii) separation and (iii)detection.

During MS experiment, the test sample enters the mass spectrometer via the ionization source. Two types of ionization sources are used in GC ; (i) electron impact (EI) and (ii)chemical ionization (CI), with the former being more common.

In electron ionization, the molecules are bombarded with a high energy (70 eV) beam of electrons that ionise the molecules entering the ion source in the gas phase by removing an electron. Because the formed molecular ions are unstable under such low pressure conditions, they fragment easily, and may be identified according to the characteristic fragmentation patterns formed[3] .

During the second step, ions are separated according to their mass to charge ratio (m/z) in vacuum in the mass analyser. In this study, two of the most common mass analysers were used: quadrupole (q) and time-of-flight (TOF) systems. In quadrupole MS (qMS), separation according to mass to charge ratio is performed by changing the rf and dc voltages applied across the four rods comprising the quadrupole . This changes the field in the quadrupole and allows only ions of a particular m/z ratio through to the detector for a given rf/dc ratio. By varying this ratio, ions of different m/z ratios can be detected. The quadrupole mass analyser consists of four parallel rods around the flight path of the ions. On two opposite rods a radio frequency (rf) is applied, whilst on the remaining two a direct current (dc) voltage is applied. This results in a magnetic

field through which the ions travel which is changed continuously so that at any given setting of the rf and DC voltages only one ion will be resonant and arrive at the detector, while other ions are non-resonant and collide with the rods[3] . qMS instruments can be operated in one of two modes: full scan mode, which is used for identification of unknown compounds, and selected ion monitoring (SIM), which is used for analysis of target compounds and is more sensitive than scan mode. In time of-flight mass analysers , ions formed in the source are accelerated into a flight tube by application of an extraction field on a back-plate or repeller. Ions attain the same kinetic energy in this process, and are forced through the acceleration region into the field-free drift region[16]. Because all ions have the same kinetic energy, but different masses, the time taken by the ions to travel through the flight tube depends on their mass to charge ratios[17]. Lighter ions reach the detector earlier, while the heavier ones reach the detector last. TOFMS detectors are capable of high resolution acquisition and/or very fast acquisition speeds, which makes them the MS detector of choice for GC×GC. The final step of MS involves detection of ions. This is typically done in qMS detectors using an electron multiplier, whereas in TOFMS detectors multi-channel plates are more common.

1.3-Field portable GC-MS systems

GC-MS (Gas chromatography-mass spectrometry) is usually employed to identify unknown compounds based on their retention parameters, and interpretation of mass spectral fragmentation patterns[18].

A Combination of GC and MS is capable of detecting a wide range of constituents, providing high selectivity and sensitivity, as well as providing structural information about these compounds.[18,19]

Indeed a number of detectors have been used in confirming the existence of different constituents of samples but it is generally agreed that[18] confirmation of the presence of such constituents requires a further identification by MS[18,19].

Detection Portability Field portable GC-MS is desirable for organic compound detection and identification. Currently, such instruments have suffered from low sensitivity and low resolution compared to their traditional counterparts. Nevertheless a need exists for a portable GC-MS system. This is because of the need for immediate answers in certain

applications and also because in-laboratory confirmation runs the risk of losing volatile compounds during transportation. Field portable GC-MS systems have been successfully used in four major application categories:[20] environmental, diagnostics, forensics, and emergency response. When the technology was first used, field-portable referred to transportable, man-portable, and even better, hand-portable.

Field portable GC-MS systems have been defined as : *in-situ*, rather than on-site, analysis, (b) obtain information about transient events on a near real-time basis, (c) map out complex gradients and (d) accurately locate point sources.

In Field portable GC-MS systems there are several different types of detection methods that have been used as rapid screening techniques in the field. These include colorimetric, infrared, surface acoustic wave (SAW) sensors, and ion mobility spectrometry (IMS). However, these methods are effective only when used for analyzing specific classes of chemicals, particularly those having low background interferents.

Field portable GC-MS systems can provide detailed information about specific compounds with high sensitivity; these advantages make field-portable GC-MS more widely used than other methods[19].

When analyzing by GC-MS , a mixture usually is sampled, injected into the GC (sample introduction zone), separated into its components (separation zone), and detected (detection zone) using the MS. Finally the data are processed and analyzed (data analyzing zone). Each zone is separate, yet interrelated.

The miniaturization of GC-MS involves primarily the GC oven and MS analyzer. Researchers have used a variety of methods to design and construct field-portable GC-MS systems. The first portable GC-MS prototype was developed at Lawrence Livermore National Laboratory[21]. This system weighed 35 kg and was used to analyze hazardous substances. The laboratory is currently developing a newer version of this portable GC-MS system[22] based on a commercially available portable GC (Model 8610, SRI instruments, Torrance, CA, USA) and compact double-focusing MS[23,24], allowing separation of three samples

within 30 s using a 30 m long column, 2 s scan rate, resolving power of 125, and 10-150 Da mass range.

Back in 1964 , a GC-MS system has been designed[25] to be contained in a suitcase for field portable organic chemical analysis . This instrument weighed 27.3 kg, required a peak power consumption of less than 300 watts, had a mass range of 10-650 amu, a resolving power of no less than 1000, used hydrogen as carrier gas, and could be programmed to function up to 280 ˚C.

A Micro GC and a Paul quadrupole ITMS. has een integrated by Shortt et al.[26] .This equipment weighed only 5.4 kg (including turbomolecular and backing pumps), and required only 42 W of power. Although the mass range was narrow, all other aspects, such as mass resolution, sensitivity, etc., were all comparable to its commercial counterpart. Other systems include Hewlett Packard MSD based GC-MS systems[27,28] and a hand-portable prototype[29].

Several new approaches to portable GC-MS systems have been developed. Low thermal mass GC-MS is a novel approach that is well

suited for field GC-MS. This technique was first described by Hail et al.[30] Several configurations are currently available and their performances were evaluated by Sloan et al.[31]

One approach to field portable GC-MS is called transfer line GC (TLGC), in which a short, narrow-bore column is used as an interface between the MS and a sampling system, typically for a process that must be analyzed on line[32]. The column acts both as the flow restrictor for the MS pumping system and as the separation column for the sample.

The technique of GC-MS involves two steps for analyzing and identifying a sample. GC is a technique based on the partition (or adsorption) of analytes between a gaseous mobile phase and a liquid (or solid) phase immobilized on the surface of an inert solid in a small diameter tube (the column). A GC is an apparatus that is used to separate a mixture into its different components according to the interaction of the components with the liquid (or solid) stationary phase.

When separated, the components of the analyte arrive at the output of the GC where they are identified. A conventional GC requires a bulky

oven, which is power inefficient, heavy to carry, and has relatively slow temperature programming rates, usually allowing at most 75˚C/min. Miniaturizing GC usually involves miniaturizing the column oven, which has the largest thermal mass in a GC system. There are four possible ways of reducing the 5 mass of the traditional oven and thus increasing its maximum heating rate[33]: (a) build a smaller traditional oven or reduce the volume of a traditional oven by adding insulating material; (b) wrap a capillary column around a heated metal cylinder, thus allowing the components to take up a smaller volume; (c) micro-machine a capillary column into a small silicon wafer; and (d) sheath the capillary column with an electrically conducting material[34,35].

A compact GC probe for use in GC-MS has been designed by Yost et al.[30] . Aluminum-clad columns coiled around a 2.5 in. diameter Teflon spool were covered with Nextel glass braid insulation. Copper connector slides were used to electrically connect the ends of the columns, allowing them to be heated resistively. Because of the very low thermal mass of a column, it could be heated at a rate of 524 ˚C/min (with an

initial rate of 2400 ˚C/min) from 50 ˚C to 150 ˚C and cooled down at a rate of 165 ˚C/min (also with a initial rate of 2400˚C/min) from 150˚C to 50 ˚C. The maximum heating rate for a traditional GC oven from 50 ˚C to 150 ˚C is approximately 50 ˚C/min38, with an approximate cool-down time of 4 min for the same temperature range, or about 7 times longer. The total power required to resistively heat the 2.3 m column to 150 ˚C was approximately 35 W,23 whereas 2400 W is needed for a conventional oven under exactly the same conditions[36].

Resistively heated columns are useful for use in miniaturizing GC instrumentation. Another attractive method being used in the miniaturization of GC is microfabrication.

Investigators began working to produce a micro-GC on a silicon wafer. Sacks[37] produced a 3 m long microfabricated column that occupies 3.2 cm x 3.2 cm. Sixteen gaseous components were separated within 75 s using this column, and approximately 4900 theoretical plates were achieved.

However , Sacks[37] proposed that the theoretical plate value is below the actual plate number because the current theories to predict plate number

are difficult to use for channel geometries that are not round. Microfabrication typically produces channels that are not round, which are difficult to coat homogeneously. GC is used to resolve mixtures of compounds into less complex mixtures or into pure components, or it is used for sample preparation and/or clean up prior to final analysis. It can also provide retention data to be used as complementary information for positive identification of resolved components.

High correlation of retention data between an unknown and a selected reference compound on two columns of different polarity is sufficient for the positive identification of an unknown[38].

The Kováts retention indices have been effectively used in tabulating chromatographic retention data. The advantage of using Kováts indices instead of relative retention times, capacity ratios, or retention volumes is that Kováts indices are independent of the column dimensions, stationary phase, phase ratio, or coating film thickness.

Many techniques are employed to monitor analytes eluting from a GC. One method is the flame ionization detector (FID) which utilizes a hydrogen-oxygen flame that contains very few ions, and the ionization current increases when the particles of the eluting substances collide with high-energy particles. Thermal conductivity detection (TCD) is based on changes in the electrical conductivity of materials.

Other varieties of detectors including photoionization detector (PID), flame photometric detector (FPD), etc., are also widely used as detectors for GC.

Indeed very limited structural information was available from GC detectors until Gohlke[39] directed the GC effluent into an MS.

Mass spectrometry is simply the technique of "weighing" individual atoms and molecules to determine their masses or molecular weights[40]. Mass spectrometry could provide information about: (i) the elemental composition of samples of matter; (ii) the structures of inorganic, organic, and biological molecules; (iii) the qualitative and quantitative composition of complex mixtures; (iv) the structure and composition of solid surfaces; and (v) the isotopic ratios of atoms in samples[41].

During the GC-MS analysis, sample to be analyzed is typically ionized either by an ionizing agent in chemical ionization MS (CIMS), or a beam of high-energy electrons in electron ionization MS (EIMS); the ions undergo specific fragmentation, i.e., bonds break and new bonds form, from which the structure of the molecule can be interpreted.

The technique of gas chromatography-MS provides considerable information about many types of analytes, including volatile and semi-volatile organic compounds, and has resulted in a technique of considerable importance in the field of chemical analysis.

1.4-The target species- *Raphanus sativus* L.

The plant family of Cruciferae contains many important vegetables of economic importance. *Raphanus sativus* L. is originally from Europe and Asia. It grows in temperate climates at altitudes between 190 and 1240 m. It is 30–90 cm high and its roots are thick and of various sizes, forms, and colors . They are edible with a pungent taste. Salted radish roots (Takuan), which are consumed in the amount of about 500,000 tons/year in Japan, are essentially one of the traditional Japanese foods. The salted radish roots have a characteristic yellow color, which generates during storage.

This specie is used popularly to treat liver and respiratory illnesses[38]. The antibiotic activity of its extracts and its time persistence validates its effectiveness in microbial sickness as reported in traditional medicine. The root's juice showed antimicrobial activity against *Bacillus subtilis, Pseudomonas aeruginosa,* and *Salmonella thyphosa*. The ethanolic and aqueous extracts showed activity against *Streptococcus mutans* and *Candida albicans*. Aqueous extract of the whole plant presents activity against *Sarcinia lutea* and *Staphylococcus epidermidis*[39]. Aqueous extract of the leaves showed antiviral effect against influenza virus. Aqueous extract of the roots showed antimutagenic activity against *Salmonella typhimurium* .

Aim of this study

This study was undertaken to:

-Extract oil from the medicinally important species- *Raphanus sativus* L.

-Investigate the oil constituents by GC-MS.

-Screen the oil for antimicrobial activity.

Chapter Two

Materials and Method

2-Materials and Methods

2.1-Materials

2.1.1- Instruments

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

2.1.2-Test organisms

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

Table 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	*Candida albicans*	fungi

2.1.3-Plant material

Seeds of *Raphanus sativus* were purchased from the local Rhyad – Saudi Arabia and authenticated by direct comparison with a herbarium sample.

2.2- Methods

2.2.1-Extraction of oil

Powdered seeds of *Raphanus sativus* (500g) were exhaustively extracted with n-hexane at room temperature.The solvent was removed under reduced pressure and the oil was kept in the fridge at $4^{o}C$ for further manipulation.

The target oil was esterified as follows :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.

2.2.2- GC-MS analysis

The oil of *Raphanus sativus* was analyzed 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, while other chromatographic conditions are depicted in Table 3.

Table 2: Oven temperature program

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

Table 3: 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

3.2--Antimicrobial assay

3.2.1-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.

3.2.2-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.

3.2.3-Antimicrobial test

The paper disc diffusion method was used to screen the antimicrobial activity of plant extracts and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory Standards Guidelines (NCCLS, 1999). Bacterial suspension was diluted with sterile physiological solution to 10^8cfu/ ml (turbidity = McFarland standard 0.5). One hundred microliters of bacterial suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (Whatman No.1, 6 mm in diameter) were placed on the surface of the MHA and soaked with 20 µl of a solution of each plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the inverted position. The diameters (mm) of the inhibition zones were measured.

Chapter Three

Results and Discussion

3-Result and Discussion

The seeds of *Raphanus sativus* were extracted withn-hexane and the resulting fixed oil was analyzed by GC-MS.Constitents of the oil were identified by their retention time and mass fragmentation pattern where a mass spectrometer NST software was employed for identification purposes.

3.1-GC-MS analysis of *Raphanus sativus* oil

The GC-MS analysis of the target oil showed 15 components dominated by:

3.1.1-(z)13-Docosenoic acid,methyl ester(35.48%)

3.1.2-9-octadecenoic acid(z)methyl ester(17.12%)

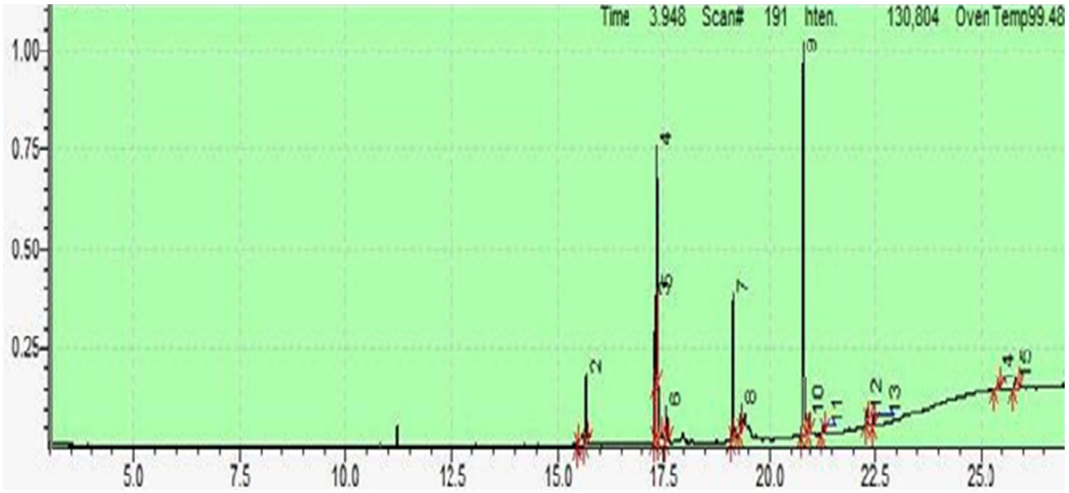
3.1.3-Cis-11-Eicosenoic a cid,methyl ester(11.64%)

3.1.4-9,12-octadecadienoic acid (z,z) methyl ester(10.77%)

3.1.5-(Z,Z,Z) 9,12,15-oct adecatrienoic acid,methyl ester (7.14%)

3.1.6-Hexadecanoic acid,methyl ester(5.48%)

The total ion chromatograms of *Raphanus sativus* oil is shown in Fig. 1, while the different constituents of the oil are displayed in Table 1.



Figure(1)Total ion chromatogram of Raphanus sativus oil

Table 1: Constituent of *Raphanus sativus* oil

Peak#	R.Time	Area	Area%	Name
1	15.472	61565	0.11	9-Hexadecenoic acid, methyl ester, (Z)-
2	15.664	3157853	5.48	Hexadecanoic acid, methyl ester
3	17.316	6204550	10.77	9,12-Octadecadienoic acid (Z,Z)-, methyl e
4	17.361	9856155	17.12	9-Octadecenoic acid (Z)-, methyl ester
5	17.385	4110173	7.14	9,12,15-Octadecatrienoic acid, methyl este
6	17.579	1415747	2.46	Methyl stearate
7	19.140	6705320	11.64	cis-11-Eicosenoic acid, methyl ester
8	19.338	1336160	2.32	Eicosanoic acid, methyl ester
9	20.786	20431171	35.48	13-Docosenoic acid, methyl ester, (Z)-
10	20.961	798998	1.39	Docosanoic acid, methyl ester
11	21.288	468825	0.81	Ethyl 9-hexadecenoate
12	22.312	695356	1.21	15-Tetracosenoic acid, methyl ester, (Z)-
13	22.468	544165	0.94	Tetracosanoic acid, methyl ester
14	25.432	767230	1.33	Olean-12-en-28-oic acid, 2.beta.,3.beta.,23-
15	25.844	1032770	1.79	Urs-12-en-28-al
		57586038	100.00	

The major components of *Raphanus sativus* oil are discussed below:

3.1.1-(z)13-Docosenoic acid,methyl ester(35.48%)

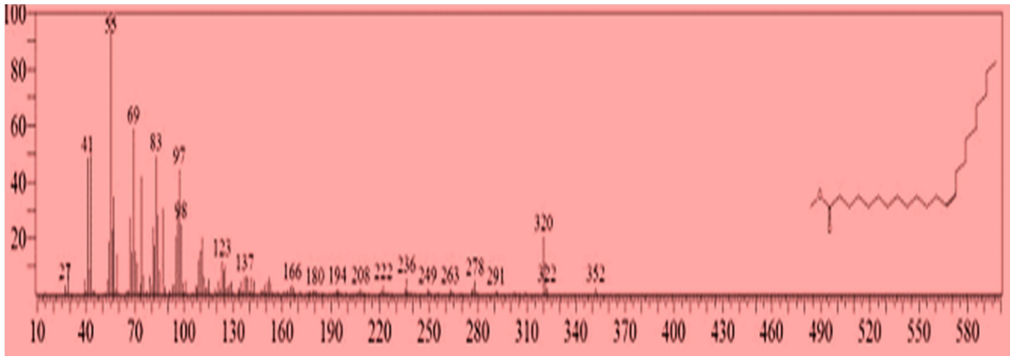


Fig. 2 :The mass spectrum of 13- docosenoic acid,methyl ester(z)

The peak at m/z 352, RT(20.786). corresponds to $M^{+[}C_{23}H_{44}O_2]^+$. The signal at m/z 321 is due to loss of a methoxyl.

3.1.2-9-octadecenoic acid(z)methyl ester(17.12%)

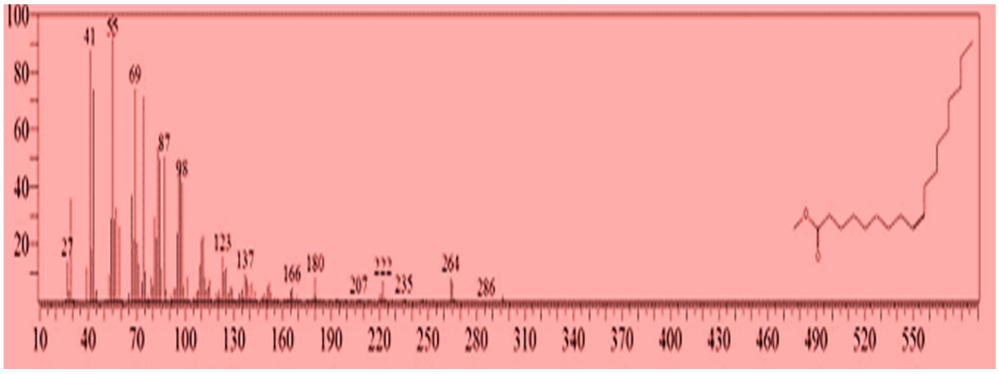


Fig. 3 : The mass spectrum of 9-octadecenoic acid(z)methyl ester

In the above figure, the peak at m/z 296,RT(17.316) corresponds to the molecular ion : $M^+[C_{19}H_{36}O_2]^+$, while the signal at m/z 265 accounts for loss of a methoxyl function.

3.1.3-Cis-11-Eicosenoic acid,methyl ester(11.64%)

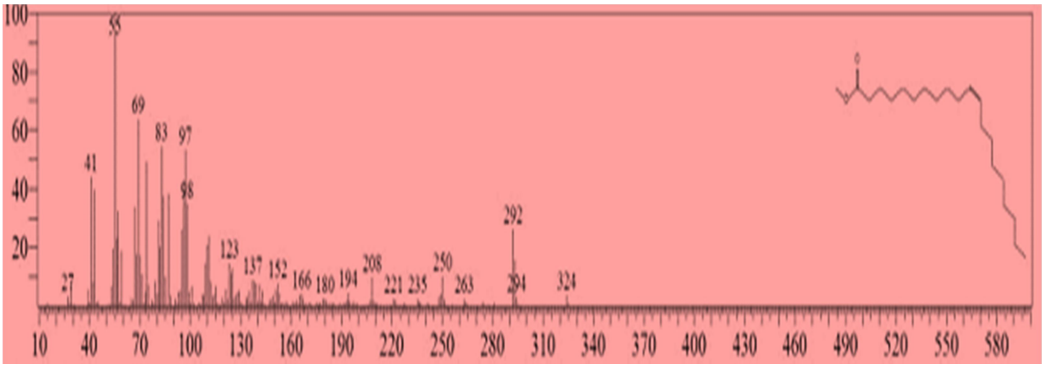


Fig. 4 : The mass spectrum of cis-11-eicosenoic acid,methyl ester

In Fig.4 ,the peak at m/z 324,RT(19.140) accounts for the molecular ion : $M^{+}[C_{21}H_{40}\ O_2]^{+}$.The signal at m/z 293 corresponds to loss of a methoxyl.

3.1.4-9,12-octadecadienoic acid (z,z) me thyl ester(10.77%)

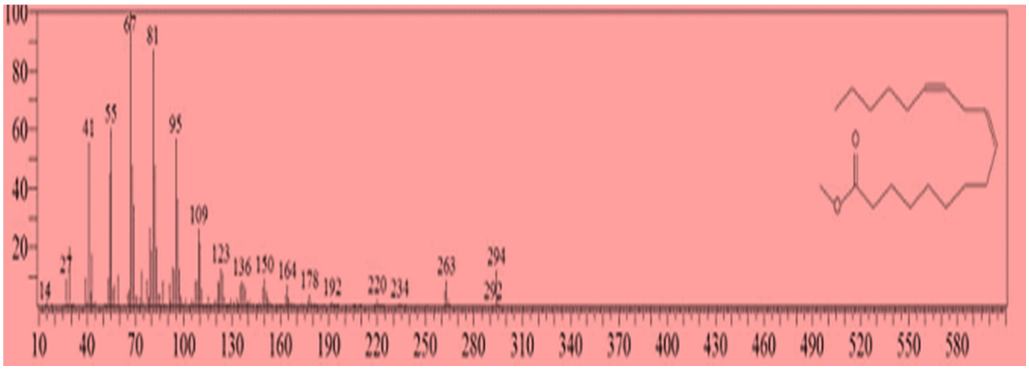
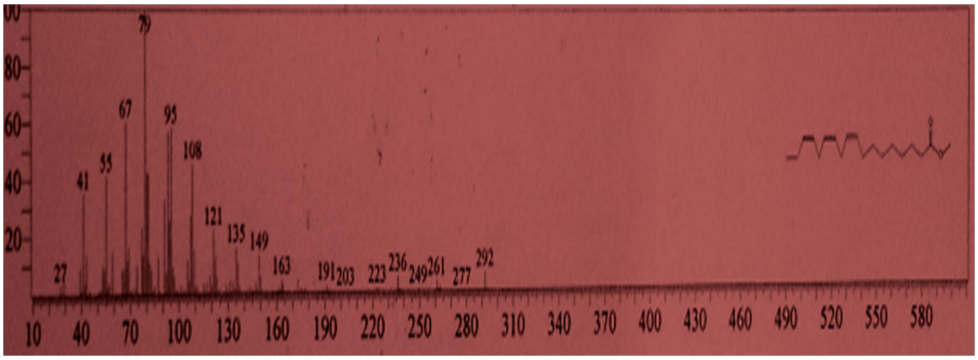


Fig. 5 : The mass spectrum of 9,12-octadecadienoic acid (z,z) methyl ester

The mass spectrum of 9,12-octadecadienoic acid (z,z) methyl ester is displayed in Fig.5. The peak at m/z 294,RT(17.316) corresponds to $M^{+}[C_{19}H_{34}\ O_2]^{+}$, while the signal at m/z 263 corresponds to loss of a methoxyl function.

3.1.5-(Z,Z,Z) 9,12,15-oct adecatrienoic acid,methyl ester (7.14%)



Figure(6)shows the mass spectrum of 9,12,15-octadecatrienoic acid,methyl ester

In Figure 6 ,the peak at m/z 292,RT(17.358) accounts for : $M^{+}[C_{19}H_{32}O_2]^{+}$.The signal at m/z 261 is due to loss of a methoxyl.

3.1.6-Hexadecanoic acid,methyl ester(5.48%)

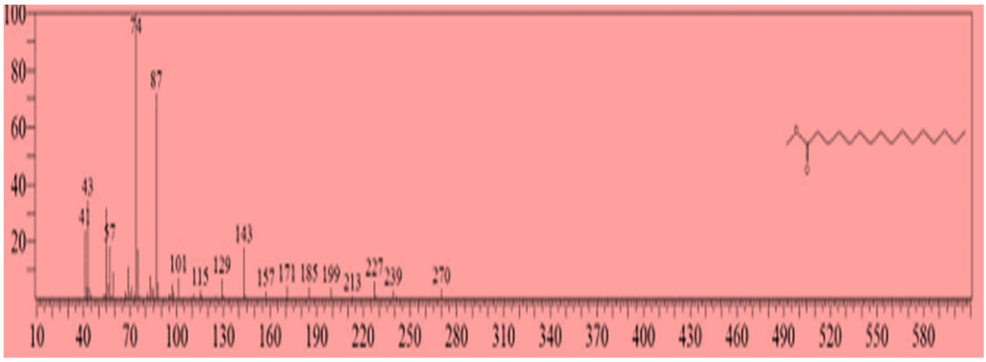


Fig. 7: The mass spectrum of hexadecanoic acid,methyl ester

The mass spectrum of hexadecanoic acid,methyl ester is displayed in figure 7. The peak at m/z 270,RT(15.664) is attributed to the molecular ion : $M^{+}[C_{17}H_{34}O_2]^{+}$.The signal at m/z 239 corresponds to loss of a methoxyl group.

3.2-Antimicrobia activity

Raphanus sativus oil was assessed for antimicrobial activity via the disc diffusion bioassay using five standard human pathogens.. The average of the diameters of the growth inhibition zones are shown in Table (2) .The results were interpreted in terms of the commonly used terms (<9mm: inative;9-12mm:partially active;13-18mm: active;>18mm:very active)**.**

At a concentration of 100mg/ml , the oil showed partial activity against *Acinetobacter baumannii, Staphylococcus aureus, Bacillus subtilis and Aspergillus flavus.*

Table 2 :Minimum inhibition zone (mm)

	Concn. mg/ml					
	oil			Amp	Kan	Nys
Strain	0%	50%	100%	10	10	10
Escherichia coli	-	$6_{\pm 0.1}$	$6_{\pm 0.7}$	$25_{\pm 0.4}$	$21_{\pm 0.3}$	N
Klebsiella pneumoniae	-	$6_{\pm 0.2}$	$7_{\pm 0.6}$	$22_{\pm 0.4}$	$24_{\pm 0.0}$	N
Acinetobacter baumannii	-	$7_{\pm 0.4}$	$10_{\pm 0.2}$	$22_{\pm 0.3}$	$16_{\pm 0.3}$	N
Pseudomonas aureginosa	-	$6_{\pm 0.1}$	$7_{\pm 0.6}$	$29_{\pm 0.3}$	$11_{\pm 0.3}$	N
Staphylococcus aureus	-	$6_{\pm 0.6}$	$9_{\pm 0.6}$	$32_{\pm 0.5}$	$19_{\pm 0.5}$	N
Bacillus subtilis	-	$6_{\pm 0.1}$	$8_{\pm 0.6}$	$19_{\pm 0.4}$	$21_{\pm 0.4}$	N
Candida albicans	-	$6_{\pm 0.1}$	$7_{\pm 0.6}$	N	N	$12_{\pm 0.5}$
Aspergillus flavus	-	$6_{\pm 0.7}$	$9_{\pm 0.6}$	N	N	$15_{\pm 0.5}$

-ve: gram negative, +ve: gram positive, C: colony forming, F: filameous, -: no activity, N: Not Valid

Conclusion

In this study, the fixed oil of *Raphanus sativus* was extracted from seeds. The oil was analyzed by GC-MS and 15 components were identified. Major constituent was:13-docosenoic acid methyl ester(35.48%). The oil was screened for antimicrobial activity and promising results were obtained.

Recommendations

The following recommendations may be taken in consideration:

- The extracted oil may be screened for other biological activities like antiinflammatory, antimalarial …etc.

-Other constituents of the target species may also be isolated and studied.

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