Napata College

Pharmacy program

Title "Investigation of Fixed and essential oils from Abelmoschus esculentus seeds extract and Assessment of their Antimicrobial and Antioxidant activities"

Research submitted in partial fulfilment for the requirements of B.Sc. degree in pharmacy

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بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ وَعَلَّمَ ءَادَمَ ٱلْأَسْمَآءَ كُلَّهَا ثُمَّ عَرَضَهُمْ عَلَى ٱلْمَلَٰٓئِكَةِ فَقَالَ أَنۢبِـُٔونِى بِأَسْمَآءِ هَٰٓؤُلَآءِ إِن كُنتُمْ صَٰدِقِينَ ﴿٣١﴾ قَالُوا۟ سُبْحَٰنَكَ لَا عِلْمَ لَنَآ إِلَّا مَا عَلَّمْتَنَآ ۖ إِنَّكَ أَنتَ ٱلْعَلِيمُ ٱلْحَكِيمُ ﴿٣٢﴾ ﴾

صدق الله العظيم

سورة البقرة ، الاية(31-32)

Dedication

This research is lovingly dedicated to our respective parents, sisters, and teachers who have been our constant source of inspiration.
They have given us the drive and discipline to tackle a task with enthusiast and determination. Without their love and support this project would not have been made possible.

Acknowledgement

we are very grateful to **DR. MUTAZ ELSHIEKH**, *a source of inspiration to us since we met him for first time. we would like to thank the staff of research lab Chemistry department for helping us to complete the research.*

We would like also to thank our college Napata college for her full support of research.

Abstract

Plants and plants derived materials represent the basis of many traditional medicine systems as Ayurvedic, Kampo, and many others that have been used successfully for healing ailments over millennia. Okra seed appears to be a good source of oil (20-40%) and rich (60-70%) in unsaturated fatty acid, especially oleic (20.3%) and linoleic acid (44.4%) as well as polyunsaturated fatty acids, which are essential for human nutrition. The results further showed that the seed oil has a good Antioxidant and anti-Microbial activity against some species of bacteria.

المستخلص :

تمثل المواد المشتقة من النباتات أساس العديد من أنظمة الطب التقليدي مثل الأيورفيدا والكامبو والعديد من الأنظمة الأخرى التي تم استخدامها بنجاح لعلاج الأمراض على مدى آلاف السنين. يبدو أن بذور البامية مصدر جيد للزيت (20–40٪) وغنية (60–70٪) بالأحماض الدهنية غير المشبعة وخاصة الأوليك (20.3٪) وحمض اللينوليك (44.4٪) وكذلك الأحماض الدهنية المتعددة غير المشبعة والتي ضرورية لتغذية الإنسان. وأظهرت النتائج كذلك أن زيت البذور له نشاط جيد كمضاد للأكسدة ومضاد للميكروبات ضد بعض أنواع البكتيريا.

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Chapter One

INTRODUCTION

1-1 Introduction:

Plants and plants derived materials represent the basis of many traditional medicine systems as Ayurvedic, Campo, and many others that have been used successfully for healing ailments over millennia. Nowadays, it is very interesting to emphasize that despite of the vast increase in manufacturing synthetic and/or semi-synthetic therapeutic agents to face the global demand for new drugs, approximately 80% of the world's inhabitants rely mainly on traditional herbal medicines for their health care .[21]The most important antimicrobial drugs in clinical usage are naturally derived -substances [22].

Plants have a wide range of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, battalions), vitamins (C and E), terpenoids (including carotenoids), and other endogenous metabolites (Cai et al, 2004). These natural antioxidants are spread in different plants parts (Al-Mustafa, 2008). Many studies indicate that phytosterols may have antiinflammatory activities and antioxidant properties (Onyango et al, 2009).[22]

An essential oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plant [5-8]. Essential oils are also known as volatile oils, ethereal oils , aethereal ,or simply as the oil of the plant from which they were extracted ,such as oil of clove [9-12].An oil is "essential " in the sense that it contain the "essence of " the plant's fragrance- the characteristic fragrance of the plant from which it is derived [13-17] . the term essential used here does not mean indispensable as with the terms essential amino acid or essential fatty acid which are so called since they are nutritionally required by given living organism [18.19] .

Okra (Abelmoschus esculentus) is the only vegetable crop of significance in the Malvaceae family and is very popular in the Indo-Pak subcontinent. In India, it ranks

number one in its consumption but its original home is Ethiopia and Sudan, the north-eastern African countries. It is one of the oldest cultivated crops and presently grown in many countries and is widely distributed from Africa to Asia, southern Europe and America. It is a tropical to subtropical crop and is sensitive to frost; low temperature, water logging and drought conditions and the cultivation from different countries have certain adapted distinguishing characteristics specific to the country to which they belong 1.

It is an oligo purpose crop, but it is usually consumed for its green tender fruits as a vegetable in a variety of ways. These fruits are rich in Vitamins, calcium, potassium and other mineral matters. The mature okra seed is a good source of oil and protein has been known to have superior nutritional quality. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid, which is essential for human nutrition. Its mature fruit and stems contain crude fiber, which is used in the paper industry.

1-2 Description:

Biological Name: Hibiscus esculentus, Abelmoschus esculentus.

Scientific Classification:

Kingdom : Plantae

Division : Magnoliophyte

Class : Magnoliopsida

(Unranked) : Rosins

Order : Malave's

Genus : Abelmoschus

Species: A. Esculentus

Binomial name : Abelmoschus esculentu **Other Names**:

Kacang Bendi, qiu kui, Okra, okura, Okro, Quiabos, Ochro, Quiabo, Gumbo, Quimgombo, Bamieh, Bamya, Quingumbo, Bamia, Ladies Fingers, Bendi, Bhindi, Kopi Arab

1-3 Chemical Composition:

Okra bast, a multicellular fiber was analyzed and the estimated average chemical compositions of OBF (Abelmoschus esculentus variety) are 67.5% acellulose, 15.4% hemicelluloses, 7.1% lignin, 3.4% pectic matter, 3.9% fatty and waxy matter and 2.7% aqueous extract. It is clear that the main constituents of OBF are a-cellulose, hemicelluloses and lignin and the rest are very minor in proportion, so render a little influence to the structure of OBF. Therefore, the structure of a-cellulose,

hemicelluloses and lignin and the mode of combinations that exist in between themselves are dominating the structure of OBF.

1-4 Ethno medicinal Uses:

Plants for a future cannot take any responsibility for any adverse effects from the use of plants. Always seek advice from a professional before using a plant medicinally. Antispasmodic; Demulcent; Diaphoretic; Diuretic; Emollient; Stimulant; Vulnerary Table 2. The roots are very rich in mucilage, having a strongly demulcent action. They are said by some to be better than marsh mallow (Althaea officinalis). This mucilage can be used as a plasma replacement.

An infusion of the roots is used in the treatment of syphilis. The juice of the roots is used externally in Nepal to treat cuts, wounds and boils. The leaves furnish an emollient poultice. A decoction of the immature capsules is demulcent, diuretic and emollient. It is used in the treatment of catarrhal infections, dysuria and gonorrhoea. The seeds are antispasmodic, cordial and stimulant. An infusion of the roasted seeds has sudorific properties 17, 189.

1-5 Other Uses:

Fibre; Paper; A fibre obtained from the stems is used as a substitute for jute. It is also used in making paper and textiles. The fibres are about 2.4 mm long.

When used for paper the stems are harvested in late summer or autumn after the edible seedpods have been harvested, the leaves are removed and the stems are steamed until the fibres can be stripped off. The fibres are cooked for 2 hours with lye and then put in a ball mill for 3 hours.

1-6 Litterateur review:

Functional and antioxidant properties of mucilage extracted from the pods of eight okra accessions grown in Benishangul-Gumuz region, Western Ethiopia, were evaluated. This study had shown that the mucilage contents of the pods of eight okra accessions ranged from 1.25 to 3.45 g/100 g.

The results also demonstrated that okra pod mucilage had potential sources of natural antioxidant.Fine structure, physicochemical and antioxidant properties of LM-pectins from okra pods dried under different techniquesinvestigates the effect of drying okra pods by different techniques [freeze-drying (FD), sundrying (SD), oven-drying (OD) and microwave-drying (MD)] on the molecular structure, physicochemical and antioxidant properties of the subsequently extracted OP. Remarkably, although the degree of methyl esterification ($\sim$41.1 %) remained similar among samples, the content of galacturonic acid (62.67– 68.77 %), average number molecular weight (MnI: 758.8–808.5 kDa, MnII: 20.9–24.2 kDa), and to a greater extent the apparent viscosity of an aqueous solution of pectin molecules, water holding capacity (0.21–10.71 g/g) and emulsifying activity (42.3–72.7 %) and stability (38.6–53.5 %), decreased with the drying temperature in the order of FD-OP > SD-OP > OD-OP > MD-OP. On the other hand, only FD-OP presented a higher proportion of galactan and/or arabinan side chains [(Ara + Gal) / Rha = 12.37%] compared to the rest of the samples, with values ranging from 7.79 to 9.17%. FD-OP and SD-OP resulted in lower DPPH and ABTS radical scavenging activities.

Abelmoschus esculentus (L.): Bioactive components' beneficial properties—Focused on antidiabetic role—For sustainable health applicationsThe main features of the okra, Abelmoschus esculentus (L.), are highlighted. The evaluation of interactions between biologically active compounds and other components of the food matrix can be considered as the first action in the investigation of potential

benefits of this annual herb. Moreover, updated examples of current and innovative directions in an integrated and multidisciplinary approach are discussed, with particular attention to chemometrics. Among the main effects attributed to okra, its antidiabetic property is the focus. Finally, the use of okra in different fields will be discussed.

Chapter Tow

METHODOLOGY

2-1 Methodology:

The essential oils of the target species may be obtained by hydro distillation. Once obtained, the oil will be analyzed by GC-MS to identify and quantify the phytoconstituents.

The antimicrobial activity is evaluated via cup plate agar diffusion bioassay. The antioxidant potential is estimated by measuring the capacity of test compound against stable DPPH radical.

2-1-1 Extraction method:

A- Maceration methods (for fixed oil)

The weighed plant material is macerated using (n-hexane) for three days (72 hours) and the extract is filtered, Then the extract is dried under air and kept at room temperature. The fixed oil is obtained by decantation [23.]

2-1-2 Gas Chromatography-MS

Gas chromatography (GC) is a widely applied technique in many branches of science and technology. For over half a century, GC has played a fundamental role in determining how many components and in what proportion they exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system. The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e, its mass spectrum. Mass spectra provide information on the molecular weight, elemental composition, if a high resolution mass spectrometer is used, functional groups present, and, in some cases, the geometry and spatial isomerism of the molecule.

GC–MS analysis is carried out using a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column HP-5MS (5% phenyl methyl siloxane, 30 m×0.25 mm, film thickness 0.25 μ, Agilent Technologies, USA) and coupled with a 5975B inert mass selective detector of the same company. The injector and interface are operated at 250 and 280 °C, respectively. The oven temperature is programmed as follows: from 70 to 225 °C at 5 °C min–1, then isothermally held for 10 min. Helium is the carrier gas at 1.0 cm3 min–1; the sample (10–3 cm3 of 1/100 diluted solution in diethyl ether) was injected in a pulsed split mode (split ratio 40:1). MS conditions were as follows: ionization voltage of 70 eV, acquisition mass range 35–500, scan time 0.32 s. Identification of components in the essential oil was based on retention indices relative to n-alkanes and computer matching with the Wiley 7NIST05 and EPA-NBS data library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature.[24]

2-1-2-1 Method of analysis:

GC/MS Conditions The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MSQP2010-Ultra) from japans 'Simadzu Company, with serial number 020525101565SA and capillary column (Rtx-5ms-30m 0.25 mm 0,25um).The sample was injected by using split mode, helium as the carrier gas passed with flow rate 1.61 ml/min, the temperature program was started from 60c with rate 10c/min to 300c as final temperature degree with 5 minutes hold time, the injection port temperature was 300c, the ion source temperature was 200e and the interface temperature was 250c. The sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 29 minutes. Identification of components for the sample was achieved by comparing their retention times and mass fragmentation patents with those available in the library ,the National Institute of Standards and Technology (NIST), results were recorded. **Sample preparation (Methylation):**

- Take 2ml from the sample in to test tube
- Add 7 ml from alcoholic NaOH that prepared by dissolve 2g sodium hydroxide in 100ml methanol
- Add 7 ml of alcoholic H_2SO4 1% prepared by mix 1ml Conc. H_2SO4+99 ml methanol
- Shake by vortex for 3 minutes Leave the contents to over night
- Add 2ml from supersaturated NaCl
- Add 2ml normal hexane and shake for three minutes and collect the hexane layer
- Take 5uL from hexane collected and dilute it with 5ml
- Add 1gram from sodium sulphate as drying agent syringe
- Filter through filter 0.45 um
- Transfer the filtrate directly to the GC-MS vial
- Inject Iul. directly to the GC-MS

2-1-3 Atomic absorption spectroscopy AAS

Atomic absorption spectroscopy, or AAS, is a technique for measuring the concentrations of metallic elements in different materials. As an analytical technique, it uses electromagnetic wavelengths, coming from a light source.

Distinct elements will absorb these wavelengths differently. It gives a picture of what concentrations of a specific element there is in whatever material, or liquid, is being tested.

Here we look at what AAS involves as an analytical technique, what it can measure, why it is useful, and the instruments involved in carrying it out

ASS has been an established method for analysis of materials for metallic elements for many years, it remains a benchmark technique. This is because it has greater sensitivity than other methods, with less limitations. For some liquid samples, it can provide direct analysis. It also works accurately with very small sample sizes, making it rapid, efficient and economical as a testing method.

2-1-3-1 Determination of element result by ASS

For the analysis of Na,Cr,Cd,Ca and Pb by ASS 500 ml of sample was immediatly filtred throug whatman filter paper and acidified with addition of concentrated nitric acid then the concentration of elements were measured directly using ASS.

2-1-4 Cub-plate agar diffusion assay

The cup-plate agar diffusion method is adopted with some minor modifications to assess the antimicrobial activity of the prepared extracts. Microorganism were thoroughly mixed with nutrient agar which was maintained at 45°C. (20 ml) aliquots of the inoculated agar are distributed into sterile Petri-dishes. The agar is left to settle and all in all plates 4 cups (10 mm in diameter) are cut using a sterile cork borer (No. 4) and agar discs are removed. The cups are filled with plant extracts, using automatic microliter pipette, and allowed to diffuse at room temperature for two hours. The plates are then incubated in the upright position at 37°C for 18 h. Two replicates were carried out for each extract against microorganisms. After incubation, the diameters of the resultant growth inhibition zones were measured, averaged and the mean values are tabulated[24].

2-1-4-1 Biological results for Anti-oxidant activity by DPPH:

The DPPH radical scavenging was determined according to the method of hamada et.al (1992). With some modification. In 96-well plate, the test samples were allowed to react with 2.2Di (4-tert-octylphenyl)-1-piery-hydrazyl staple free radical (DPPH)

for half an hour at 3*7C. The concentration of DPPH was kept as (300~M). the test sample were dissolved in DMSO while DPPH was prepared in ethanol. After incubation, decrease in absorbance was measured at 517nm using multiplane reader spectrophotometer. Percentage radical scavenging activity by sample was determined in caparison with a DMSO treated control group. All tests and analysis were run in triplicate.

2-1-5 DPPH Radical Scavenging Assay

The antioxidant activity is assessed on the basis of the radical scavenging effect of the stable 2, 2-Diphenyl-1-pircryl hydroxyl (DPPH) free radical activity by modified method (Yadav et al, 2012). This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen–donating antioxidant due to the formation of the non-radical form DPPH-H. The diluted working solutions of the test extracts are prepared in methanol. The stock solution of extracts (1000 µg/ml) are prepared by dissolving weighed amount of the crude extract in 99.9% methanol. Serial dilutions of the extracts are prepared from the stock solution using suitable diluted solutions (1, 5, 10, 20, 40, 50, 80, 100, 200, 300, 400, 500, 800 and 1000 µg/ml) and are applied according to the dilution calculation equation**.**

Ascorbic acid is used as a standard. DPPH (0.002%) is prepared in methanol. One ml of this solution is mixed with one ml of sample solution of each extract and the same procedure is done on standard solution (ascorbic acid). These solutions are kept in dark for thirty minutes at room temperature (25°C) and the optical density (absorbance) is measured at λ max 517 nm. The optical density (absorbance) is recorded and % inhibition is calculated using the formula:

Percent (%) inhibition of DPPH activity = [(Absorbance of blank – Absorbance of test sample) / Absorbance of blank] x 100.

Chapter Three

RESULT

3-1 GC-MS Result:

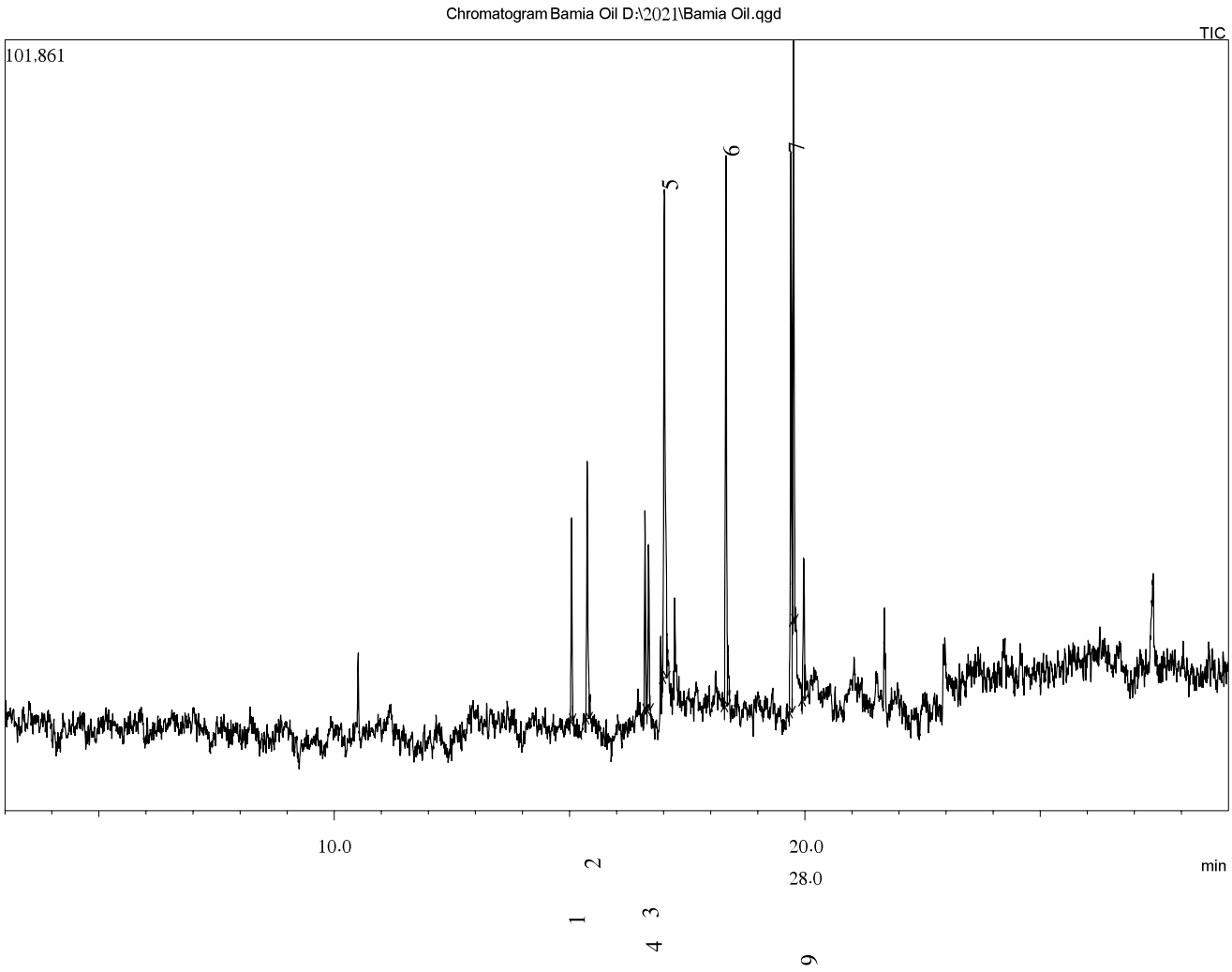

Table (1): Peak Report TIC

No	Name	R.Time	Area	Area%
1.	Hexadecanoic acid, methyl ester	15.045	39604	5.33
2.	Pentadecanoic acid	15.376	63935	8.61
3.	cis-11,14-Eicosadienoic acid, methyl ester	16.605	36827	4.96
4.	6-Octadecenoic acid, methyl ester, (Z)-	16.681	33700	4.54
5.	6-Octadecenoic acid, (Z)-	17.017	151644	20.43
6.	Hexadecanoic acid, 1- (hydroxymethyl)-1,2-e	18.323	126477	17.04
7.	(R)-(-)-14-Methyl-8hexadecyn-1-ol	19.703	132787	17.89
8.	9-Octadecenoic acid, 1,2,3- propanetriyl ester	19.761	122311	16.47
9.	Octadecanoic acid, 2,3- dihydroxypropyl este	19.975	35138	4.73
			742423	100.00

Library
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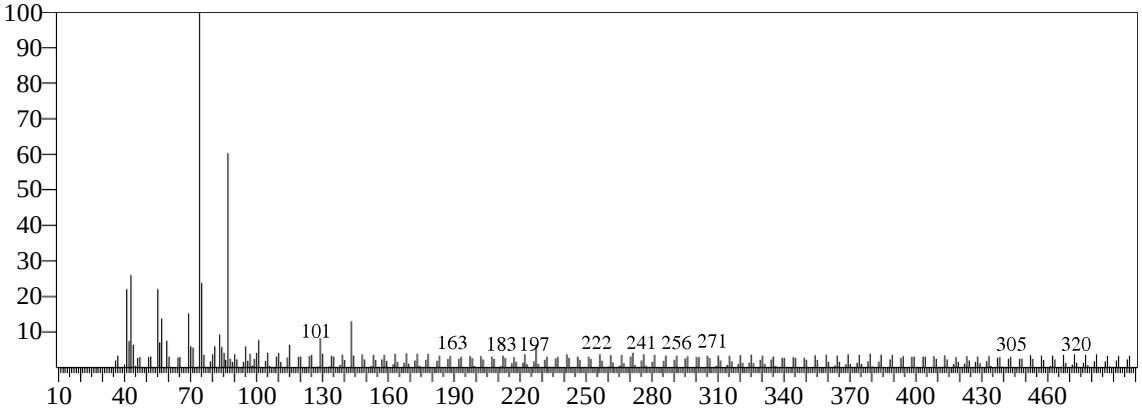

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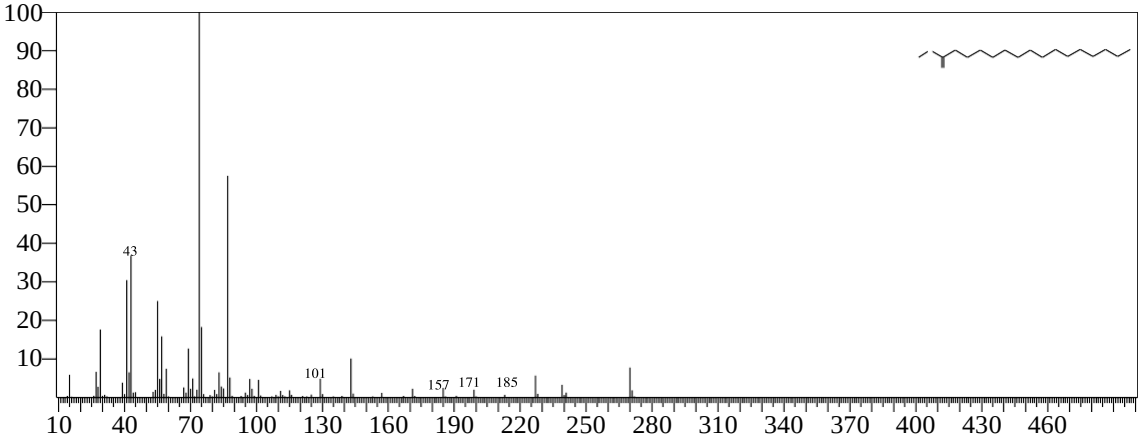

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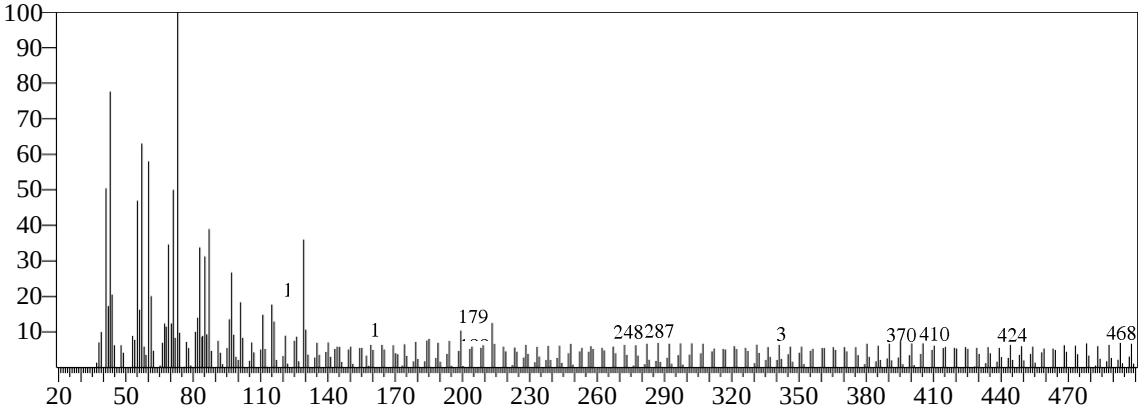

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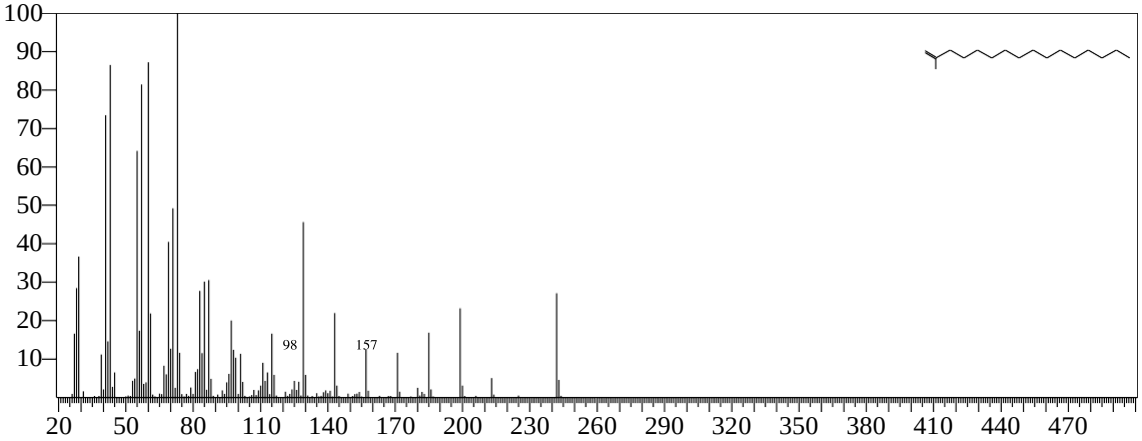

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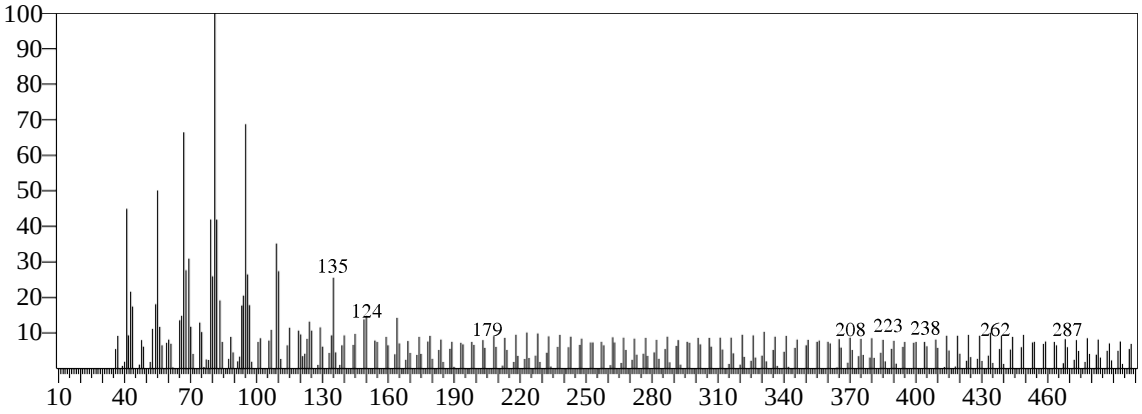

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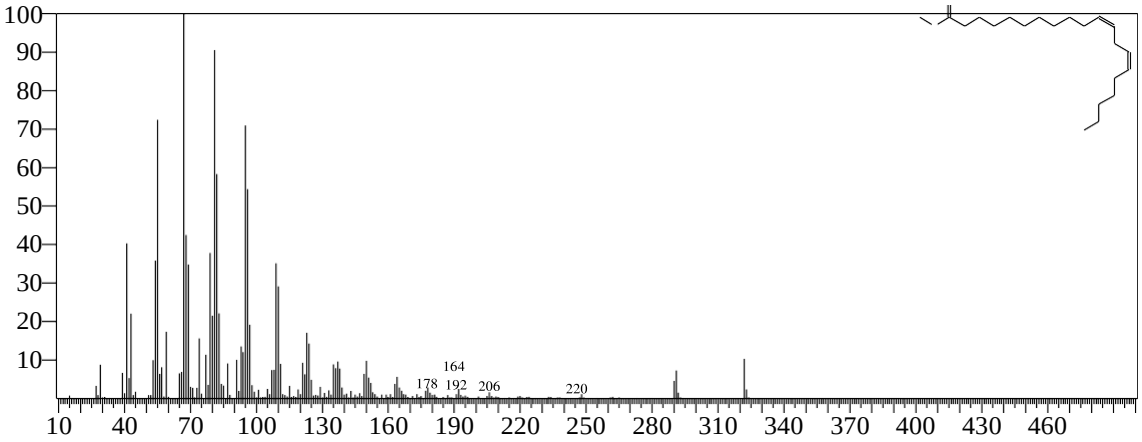

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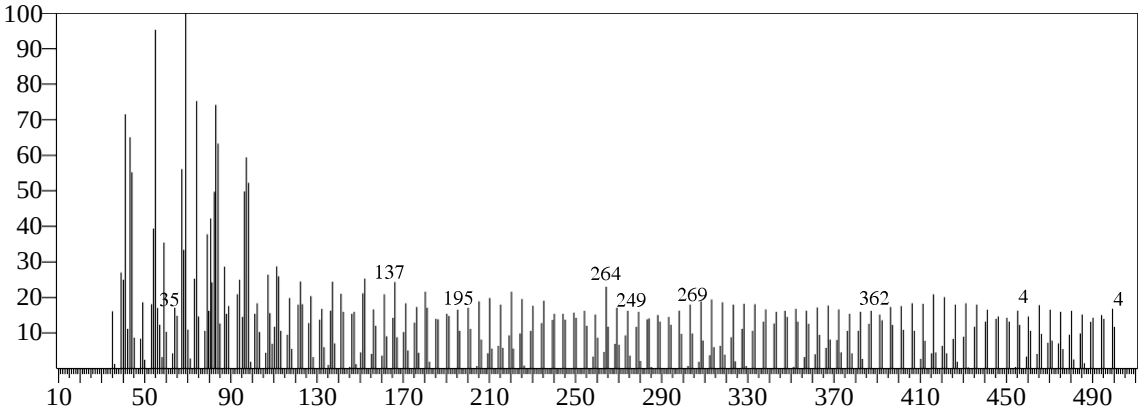

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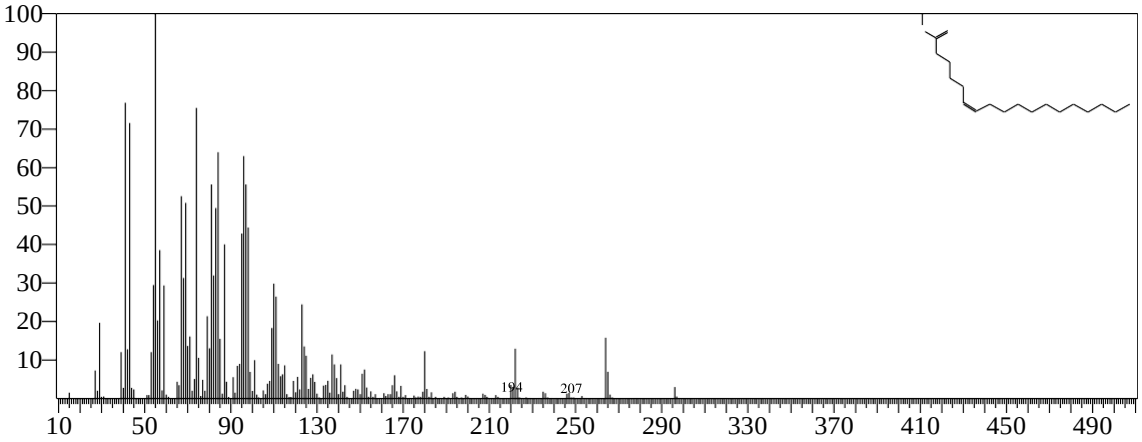

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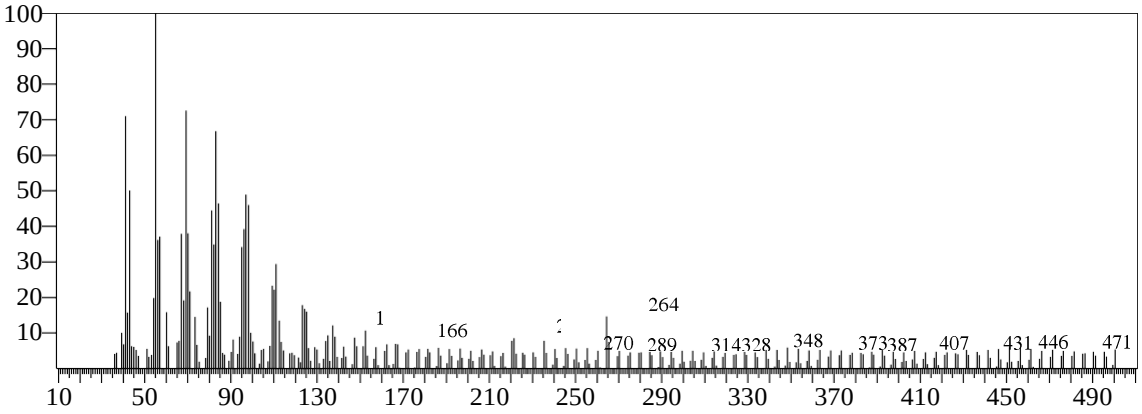

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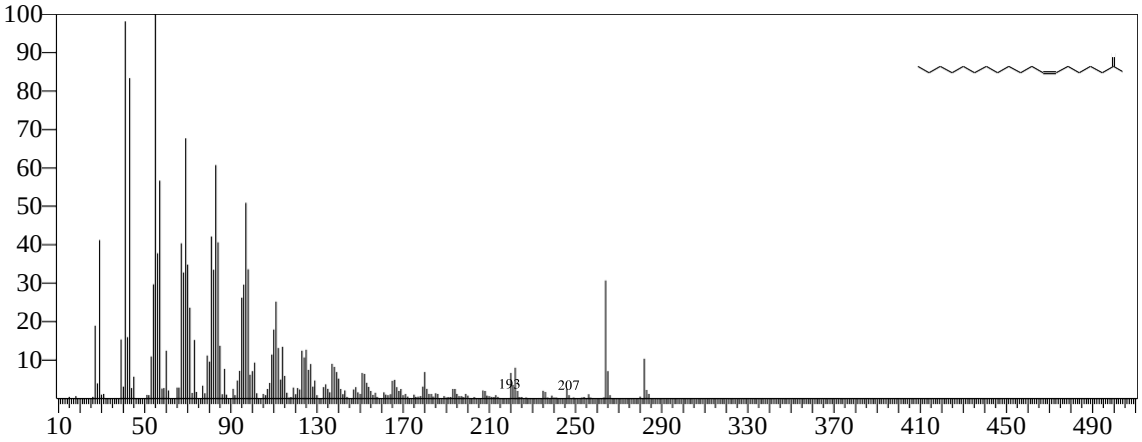

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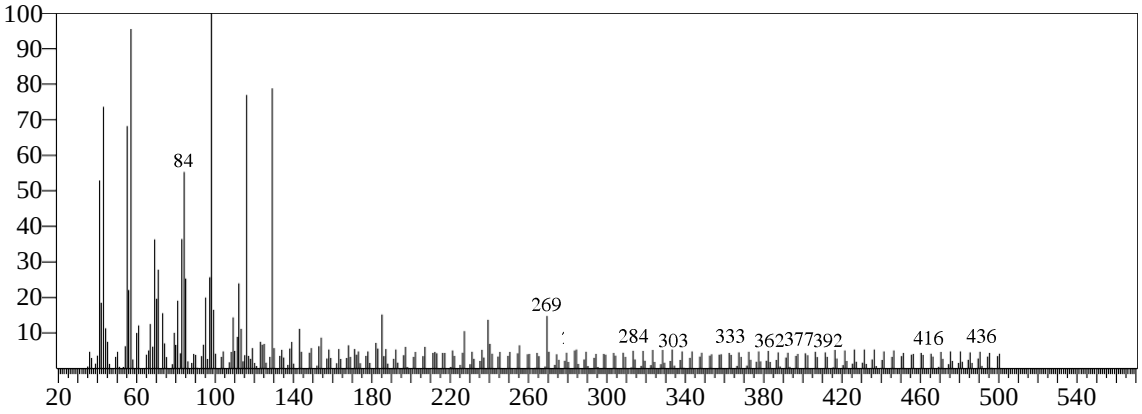

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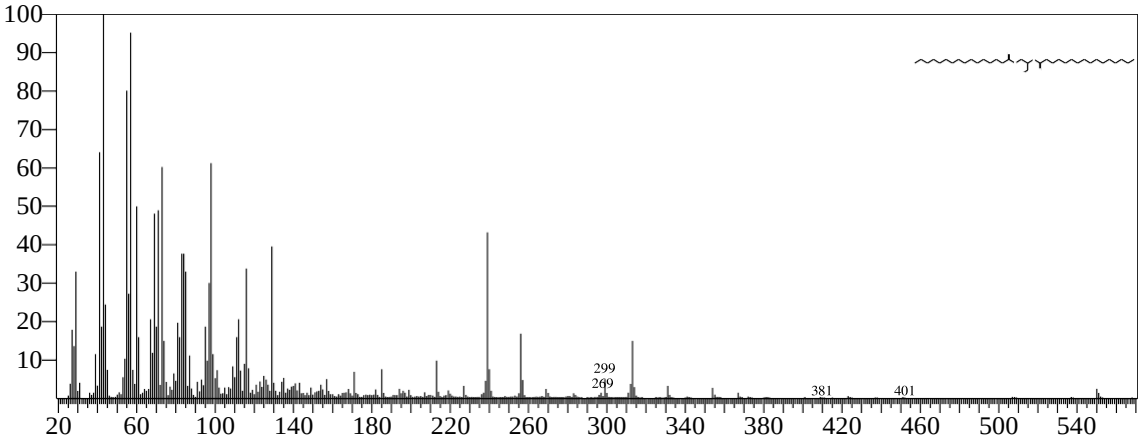

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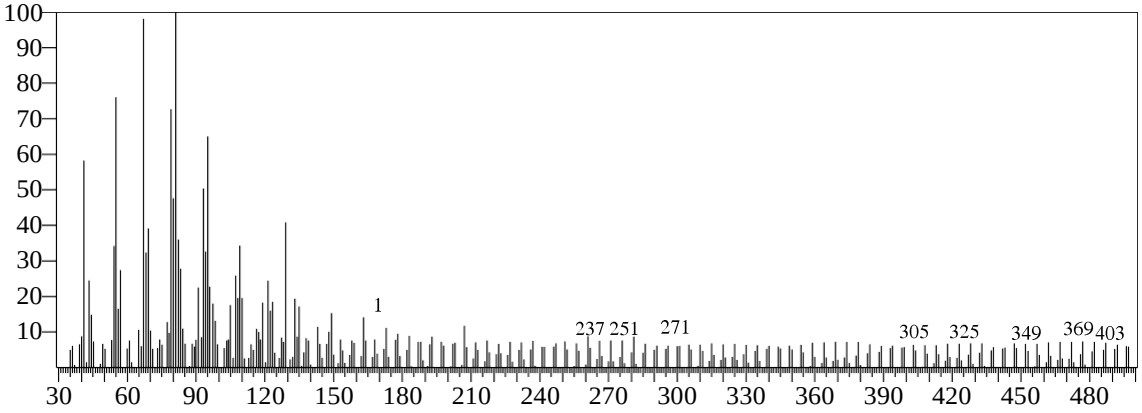

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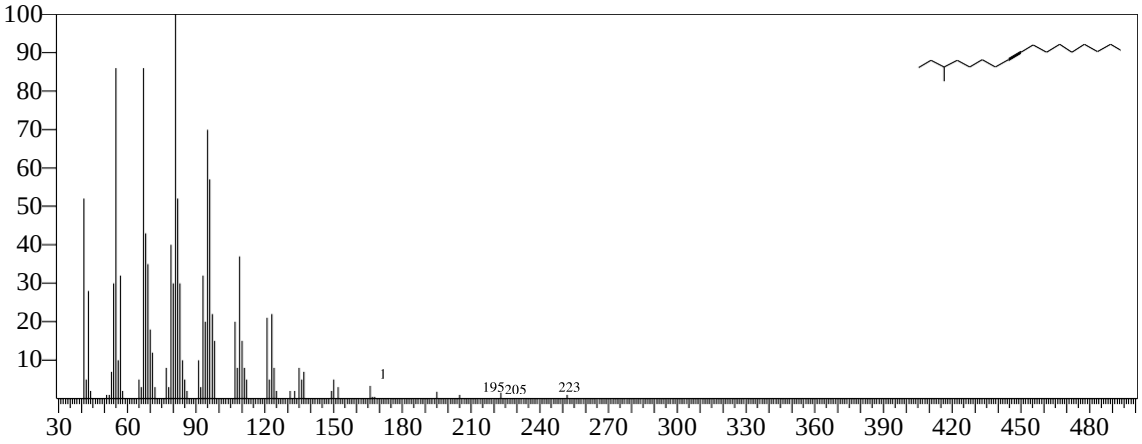
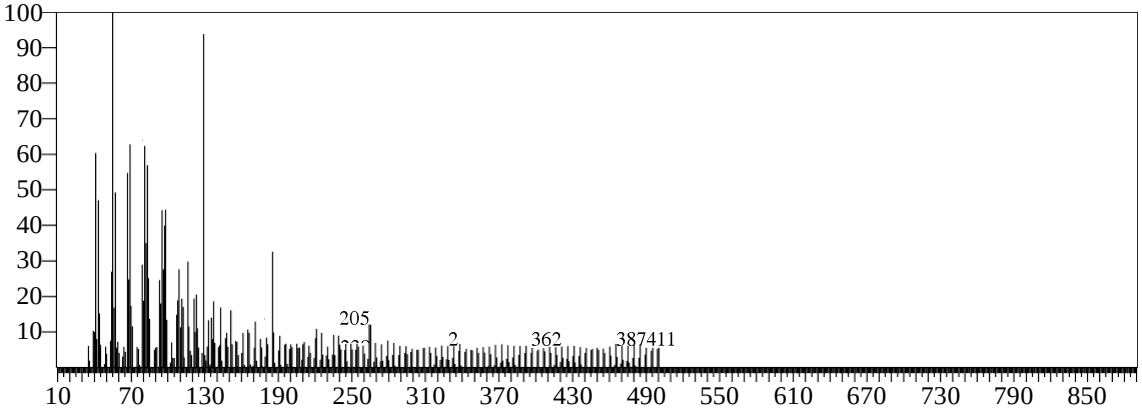
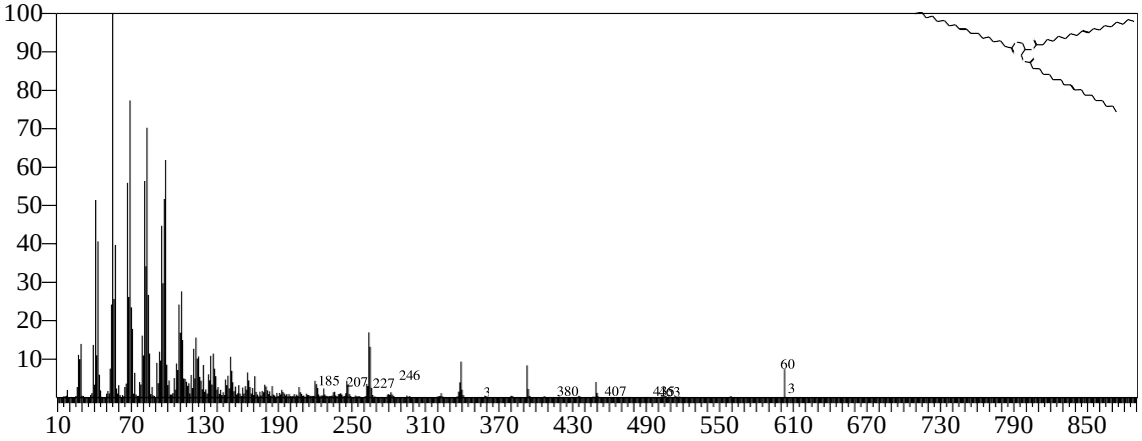
SI:82 Formula:C17H32O CAS:64566-18-3 MolWeight:252 RetIndex:1907
CompName:(R)-(-)-14-Methyl-8-hexadecyn-1-ol $$ 14-Methyl-8-hexadecyn-1-ol # $$



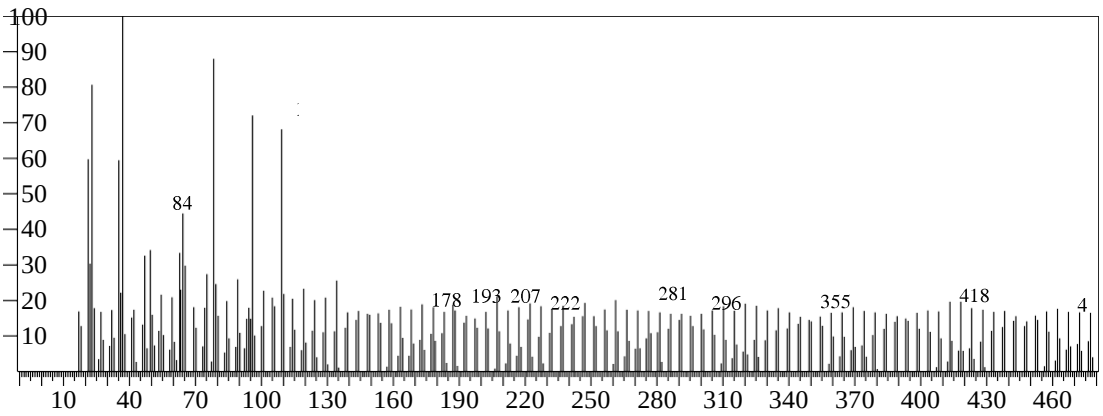
<< Target >>
Line#:8 R.Time:19.760(Scan#:3353) MassPeaks:284
RawMode:Single 19.760(3353) BasePeak:55.05(5339)
BG Mode:None Group 1 - Event 1 Scan



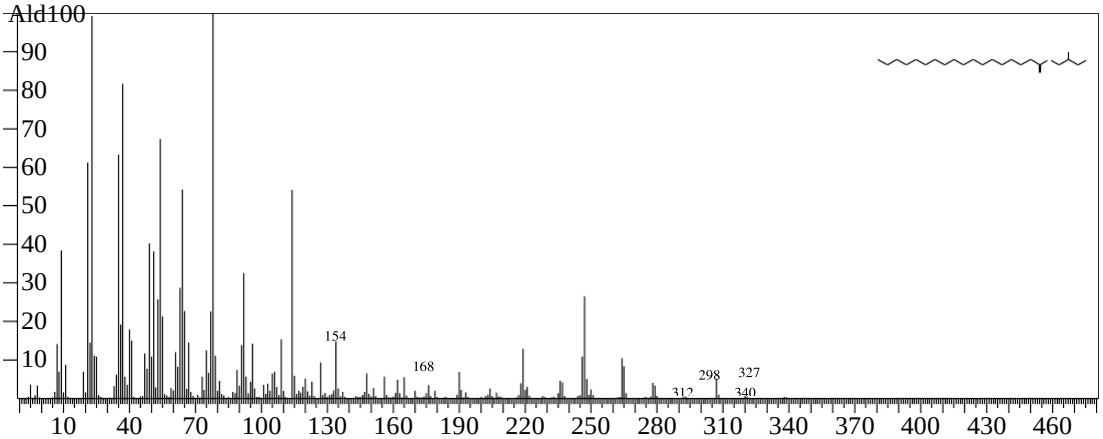
Hit#:1 Entry:212763 Library:NIST11.lib
SI:85 Formula:C57H104O6 CAS:537-39-3 MolWeight:884 RetIndex:6149
CompName:9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- $$ 2,3-Bis[(9E)-9-octadecenoyloxy]propyl



<< Target >>
Line#:9 R.Time:19.975(Scan#:3396)
MassPeaks:256 RawMode:Single 19.975(3396)
BasePeak:57.00(1860) BG Mode:None Group 1 - Event 1 Scan



Hit#:1 Entry:160782 Library:NIST11.lib
SI:59 Formula:C21H42O4 CAS:123-94-4 MolWeight:358 RetIndex:2681
CompName:Octadecanoic acid, 2,3-dihydroxypropyl ester $$ Stearin, 1-mono- $$ .alpha.-Monostearin $$ Ald100



Table(2): Determination of element result by ASS

Element	Flame actual Conc.
Na	0.0209
Cr	0.3330
Cd	0.0012
Ca	3.2550
Pb	0.1005

Table (3): Biological results for Anti-oxidant activity by DPPH:

NO	sample code	%RSA _+ SD (DPPH)
1	GS	50+_0.02
Standard	propyle gallate	91+_0.01

The sample were expressed in terms of diameter of the inhibiton zone < 9 mm, inactive 9-12 mm, partially active 12-18 mm, active >18mm very active.

Table (4): Microbiological results by Cub-plate agar diffusion assay

Plant	solvent	concent	E.C	Ps.a	S.a	B.s	C.a
Okra	Dimethyl sulphoxide	100mg/ml	08- 10	07-08	07-07	08-08	00-00

E.c : Escherichia coli

Ps.a : Pseudomonas aeruginosa

S.a : Staphylococus aureus

B.s : Bacillus subtitles

C.a : Candida albieans

Chapter Four

DISCUSSION

4 –1 Discussion

The okra seeds studied are generally good sources of oil. Their oils' good quantity and quality could be a substitute for conventional cooking oils. They also have good physicochemical properties which are comparable to conventional oils that can use for industrial manufacture of soap and cosmetics, have a good Antioxidant effect
(50+_0.02%) and have partially active antimicrobial effect against E.coli,and is rich of some element Ca,Cr,Pb.Na and Cd.

They also have rich content of fatty acids (20.32%) Octadecenoic acid, (17.84) R-14-Methyle-8-hexadecyn-1,(17.04%) Hexadecenoic acid,(16.46%) 9Octadecenoic acid 1,2,3-propanetril ester,(8.61%) Pentadecanoic acid, and(5.33%) Hexadecenoic acid.

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Appendices