An-Najah National University
Faculty of Graduate Studies

Characterization of Bioactive Phytochemicals from *Micromeria Fruticosa* Plant as an Approach to Develop Natural-based Drug Leads

By
Anwar Afeef Alhajeh

Supervisor
Prof. Mohammed Al-Nuri

Co- Supervisor
Dr. Ibrahim Abu-Reidah

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This thesis was defended successfully on 29/08/2019 and approved by:

Defense Committee Members	Signature
1- Prof. Mohammed AL-Nuri / Supervisor	.....
2- Dr. Ibrahim Abu-Reidah / Co-Supervisor	.....
3- Dr. Orwa Hosheh / External Examiner	.....
4- Dr. Mohammed Hawash / Internal Examiner	.....

Dedication

The author would like to express sincere appreciation to my family and friends special thanks to my advisor and co advisor and to everyone of AN-Najah National University Chemistry Department. Bunches of thanks to Professor Ghaleb Odwan and Dr. Ghadeer Omar of Biology Department for assisting me in doing the experimental part of biological activity of the plant extracts.

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Specially thanks for my parents, sisters (Manar and Inas), brothers (Obada, Ahmad and Abed-alrahman) and my aunt Ms. Afaf Al-hajeh who supported me during my study.

Thanks, are also due to my friends, particularly Ms. Nabaa Abu-Hafez and Ms. Zeinab Khaled, who always has been my best friend.

Finally, thanks to chemistry department at my University.

الإقرار

انا الموقع ادناه مقدم الرسالة التي تحمل العنوان:

توصيف المواد الكيميائية النباتية النشطة بيولوجيا من نبات الزعتمان (Micromeria fruticosa) كنهج لتطوير ادوية من مصدر طبيعي

Characterization of Bioactive Phytochemicals from *Micromeria Fruticosa* Plant as an Approach to Develop Natural-based Drug Leads

أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الاشارة اليه حيثما ورد، وأن هذه الرسالة ككل أو جزء منها لم يقدم من قبل لنيل أي درجة أو بحث علمي أو بحثي لدى أي مؤسسة تعليمية او بحثية أخرى

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree of qualification.

Students name:	**Anwar Afeef Alhajeh**	اسم الطالب:
Signature:	.....Anwar Afeef.....	التوقيع:
Date:	29/08/2019	التاريخ:

List of Contents

List of Figures

List of Tables

List of Abbreviations

R.T. : retention time (Min)

R.I. : retention index

M.F. : molecular formula

M.W. : molecular weight (g/mol)

Ref. : Reference

D.W. : distilled water

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Abstract

Four parts (flowers, leaves, stems, roots) from *Micromeria Fruticosa* plant used in traditional medicine in Palestine were tested for their biological activity against four types of bacteria and one type of fungi {S. sonnie (ATCC 25931), S. aureus (ATCC 25923), Escherichia coli (E. coli), MARSA, and Candida albicans (C. albicans) (ATCC 90028)} respectively. The plant was collected from Tulkarem region. The family name of plant is *Micromeria Fruticosa* (L) Druce ssp serpyllifolia (Lamiaceae) and is known as ashab a-shai in Arabic. The ethanolic, hexane and distilled water extracts of those parts of the plants were tested for their antioxidant activity through DPPH assay. The D.W. extract from leave has got the highest percentage inhibition (89%) at the concentration (100µg/ml).

Some constituents were detected from ethanolic and hexane plants extracts using GC-MS/MS spectrophotometer and separated by flash chromatography the most similar compound found in all part in plant is 9-OCTADECENAMIDE, (Z)-, Menthol and CYCLOHEXANONE,5-

METHYL-2-(1-METHYLETHYL)-, TRANS with different high, and area percentage.

Also, some elements were detected from distilled water plant extracts using ICPMS analysis, these elements are Fe, Zn, Sr with different percentage per extract part of plant.

Chapter one

Introduction

1.1 Traditional medicine

Thousands of years ago, herbs and plant products were used in folk medicine in treating a wide spectrum of ailments and diseases. Folk remedies are prepared as powders, poultices, ointments, baths, decoctions, infusions and teas. The interest in studying the biological effects of traditional medicinal plant or isolating their active components for treatment of illness has been increased all over the world and comprehensive screening programs have been established [1].

Currently, a great number of different medicinal plant products are available in markets including cosmetics and pharmaceuticals, which contain biologically active substances. Recently, some products of plant origins have shown biological activity without side effects. Such results have attracted the attention of many scientists and encouraged them to screen vast number of plants to test their biological activities [2].

Natural products have been a source of drugs and drug leads. In the past, it was extremely difficult, time consuming and invaluable efforts to build such library of purified natural products without applying improved technologies for separation, isolation and identification of such natural products. Natural products libraries have been established to preserve crude extracts, chromatographic fraction or semi -purified compounds. However,

the best result scan be obtained from fully identified pure natural products library since it provides scientists with the opportunity to handle the lead rapidly for further developed work [3]. *Micromeria Fruticosa* (L) Druc essp Serpyllifolia (Lamiaceae) is used widely in many Mediterranean regions for various inflammatory conditions and wound healers [4].

The name of the genus was derived from Greek words micro and meros (meaning small and part) because they were found as dwarf fragrant shrubs or perennial herbs which usually grow between rocks and in other dry open habitats. A number of Micromeria species are used in daily life for medicinal, insecticidal, herbicidal and culinary purposes [5].

Phenolics are plant metabolites with well-known protective action against various health diseases]. For instance, they possess numerous biological activities e.g. anti-inflammatory, anti-diabetic, antioxidant, cytotoxic and antitumor. *Micromeria Fruticosa* Druce (Wildflowers, White Micromeria) is used widely in many Mediterranean countries as herbal infusion for various inflammatory conditions and in wound healing. It is a member of genus Micromeria and is known as ashab a-shai in Arabic [6].

Micromeria Fruticosa

1.2 Main chemical constituents in medicinal plant

The plant extracts have shown that there are a large number of chemical compounds according to chemical analysis which have different functional groups are present in the plant [7]. Flavonoids and phenolic and their derivatives are found in most of plants. They are considered very important chemical groups for both human and plants [8]. They have many functions in plants. Flavonoids and phenolic acids also have antioxidative and anticarcinogenic effects [9]. Phenolic compounds present in plants as hydroxylated derivatives of benzoic and cinnamic acids [10]. They are important in the defense mechanisms of plants under different environmental stress conditions such as wounding, infection, and excessive light or UV irradiation [11].

There are two main groups of phenolic compounds that are listed below:

1. Hydroxybenzoic acids: Which they are derived from benzoic acid directly. Four acids are commonly present: syringic acid, hydroxyl benzoic acid, vanillic acid and protocatechuic acid as shown below in **Figure (1.1) [**12].

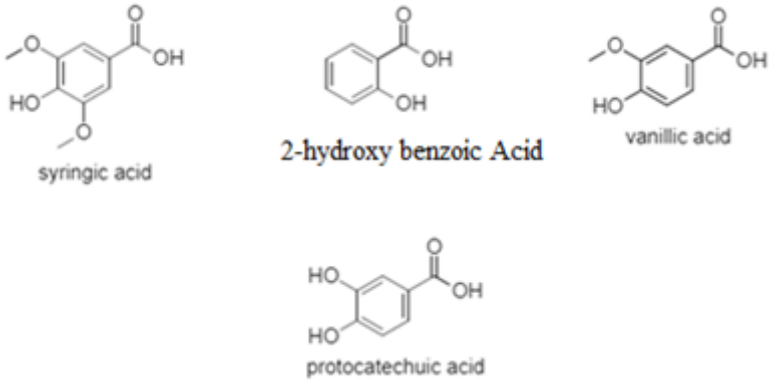

Figure (1.1): Common Hydroxybenzoic acids.

2. Hydroxycinnamic acids: The four common acids are distributed widely in plants are p-coumaric acid, sinapic acids, ferulic acid and caffeic acid shown in **Figure (1.2)** [12].

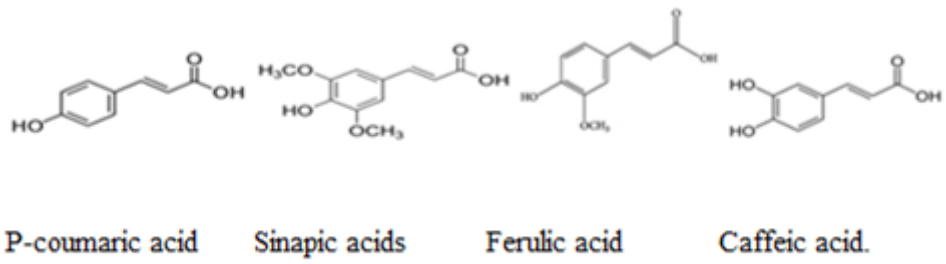

Figure (1.2): Structures of Hydroxycinnamic acids.

1.3 Methods of isolation and quantitative determination

1.3.1 Extraction

Extraction is the next step in the study of medicinal plants after collection of the plant. Several methods of extraction plant content. There

are many factors that affect the extract like, pH of the medium of extract, stability of the effective constituent, and biological activity of the constituents of the plants. And Solvent type either organic or aqueous.

Popular methods of extraction:

a. Decoction**:** It is traditional extraction methods, in which the extract is prepared by putting the part of plant in cold water, then boil for 15 minutes, after that the extract is filtered or decanted.

b. Soaking: The part of plant is soaked in organic or aqueous solvent for three days, and then the extract is decanted and dried [13].

1.3.2 Chromatographic types and techniques

A chromatographic technique was used for separation of colored pigments, and separation of mixtures that have high number of organic compounds. Chromatography avoids any reason for changing the structure of the contents in plant. So, it is being considered as a physical method of separation [14].

Several types of chromatographic techniques are: liquid chromatography (LC), thin layer chromatography (TLC), column chromatography, High-performance liquid chromatography (HPLC), and gas chromatography (GC) [15, 16].

1.4 Biological Activities

1.4.1 Biological Activity of Some Modified Compound

Biological activity refers to substances having effect on the living tissue or its ability to effect a change in a biological process.

The importance of biological processes refers to the description of functional relationships between biological activities and the chemical substances that express them [17].

1.4.2 Anti-microbial (antibacterial)

Microbes are tiny organisms seen by a microscope. These microbes are found in air, soil, rocks, plants, bodies and water. Microbial organisms include bacteria, fungi, viruses and protozoa. Some microbes cause disease and are called pathogens [18].

Antimicrobial drugs are synthesized to inhibit the microbe without any side effects on the patients [19].

1.4.3 Anti-microbial (antifungal activities)

Medicine of antifungal agent makes selectivity to reduce fungal pathogens. Unlike diseases of bacteria, diseases of fungi are more difficult to treat. Often oral and topical treatments are long term and may be partially successful in controlling the fungus. Fungal infections are the most spread on skin of all mycoses [20].

1.4.4 Anti-oxidants (free radical scavengers)

Anti-oxidant are called "free radical scavengers" are substances which delay or sometimes prevent types of cell damage by blocking the activity and reactions of free radicals from causing the damage or by giving hydrogen atoms. Free radical is very reactive species which have an odd number of electrons. Some of damages may cause cancer. In biological systems reactive species like "reactive oxygen species" (ROS). "Reactive nitrogen species" (RNS) are example of reactive species plays a dual role as both deleterious and beneficial species [21, 22, 23]. Free radicals are two types. The first is synthesized naturally in the body. And other type introduced through external sources to our bodies. The sun and tobacco smoke can be considere source of free radical. Also, the body needs external sources of antioxidants sources like fruits and vegetables [24]. Free radical with high potential gives high reactivity which harms the cell. It is created when a molecule or atom either loses or gains an electron [25].

1.5 The Aim of the study

The main objectives of this study are the followings:

1- Identification and characterization of bioactive phytochemical compound from *Micromeria Fruticosa* plant.

2- Evaluate the biological activity (antifungal and antibacterial activities) and anti-oxidant for *Micromeria Fruticosa* plant extract of different part of it in Palestine.

Chapter Two

Experimental Part

All chemical were purchased from Sigma-Aldrich Chemical Company and used without further purifications

The antibacterial activity of the extracts were determined against the following microorganisms; Escherichia coli, Shigella, MRSA, S. aureus. On the other hand, the antifungal activity test (*Yeast*) was done against Candida.

2.1 Collection of plant

The leaves, stems, flowers and roots of the plant were obtained during April 2019 from the City of Tulkarm Mountains.

The parts were placed in ventilated room temperature in a shaded area away from direct sunlight until is completely dry.

2.2 Extraction

The dry leaves, stem, flower, and roots were grinded partially and soaked in hexane for 5 days at room temperature.

The extract was obtained by suction filtration. The solvent were removed by evaporation (rotatory evaporation) to get the extract at 35^{o}C and then each part of the plant was soaked in ethanol, the filtered and

finally were soaked in water for 5 days and made the filtrate for each solvent. Water was removed by freeze drying for 3 days.

After the removal of each solvent by evaporation, the extracts were subjected to biological and chemical analysis.

Chapter Three

GC-MS/MS Analysis

3.1 Sample preparation for GC-ANALYSIS

A 2 mg of Organic plant extracts (ethanol and hexane extracts) were dissolved in 2ml of acetonitrile.

3.2 GC-MS/MS techniques

Gas chromatography-mass spectrometry (GC-MS) is an important technique for qualitative and quantitative analysis for plant extract. It is fast and sensitive, provides a high peak capacity and allows determination of thermally stable and volatile compounds [26].

GC-MS used for the separation of a mixture that contains a large number of organic compounds. Chromatography is a physical method that helps to avoid any reaction that may change the structure of the original compounds in the plant [27].

3.3 GC-MS/MS Apparatus and Conditions

The Clarus 500GC MS used in the analysis employed a fused silica column packed with Elite-1 (100% dimethyl poly siloxane, 30 m × 0.25 mm ID × 0.25µm df) and the components were separated using Helium as carrier gas at a constant flow of 1.1ml/min. The µL sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin

Elmer) with the aid of the Turbo mass 5.1 software. During the 62.5minute GC extraction process, the oven was maintained at a temperature of 50^{o}C with 5 minutes holding. The injector temperature was set at 250^{o}C (mass analyzer). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200^{0}C; Source temperature: 200^{0}C). Mass spectra were taken at 4 min; a scan interval of 0.2 s and fragments from 50 to 500 Da.

Figure (3.1): GC-MS/MS system.

3.4 Results and Discussion for GC-ANALYSIS

The analysis was carried out using GC-MS system. The compounds were identified from ethanol and hexane flower extract of *Micromeria Fruticosa* plant as shown in Figure 3.2, 3.3 respectively. The retention time (RT), percentage peak of the bioactive compounds and biological study are presented in Table 3.2 and Table 3.3.

There are similar compounds in the hexane and ethanol flower extract and with different percentage and these compounds are shown in Table 3.1 and Figure 3.5, 3.7, 3.9. The presence of these compounds in the flower makes this part of the plant to be so effective biologically and antioxidants activities.

Table (3.1): The similar compounds of hexane and ethanol flower extract from GC-MS/MS analysis

Similar Compounds Name	Molecular formula	%high peak (flower ethanol extract)	%high peak (flower hexane extract)
CYCLOHEXANONE, 5-METHYL-2-(1-METHYLETHYL)-,TRANS	$C_{10}H_{18}O$	35.86411%	5.75458%
MENTHOL	$C_{10}H_{20}O$	15.77273%	51.48503%
9-OCTADECENAMIDE, (Z)-	$C_{18}H_{35}NO$	9.635816%	3.250345%

Table (3.2): The compound isolated from GC-MS/MS analysis of flower ethanol extract

Extract Type	R.T (min.)	R.I	Possible Compound Name	High	Area	%High	%Area	M. F	M.W (g/mol)	Biological Study	Ref
Flower Ethanol	18.44	796	CYCLOHEXANO, 5-METHYL-2-(1-METHYLETHYL), TRANS	4773218	69317544	35.86411	30.04882	$C_{10}H_{18}O$.	154.2493.	antimicrobial activity, potential antibiofilm, antitumor activities	[35]
	19.204	866	MENTHOL	2099221	48961404	15.77273	21.22453	$C_{10}H_{20}O$	156.27	Antibacterial ,antifungal ,antipruritic, anticancer, cooling effects and toxicity, Antioxidant.	[28]
	20.825	816	PULEGONE	4218914	73340120	31.69928	31.79259	$C_{10}H_{16}O$	152.2334	Antimicrobial , Antioxidant, anti-inflammatory, anti-ulcer, insecticidal properties.	[29]
	25.122	614	4,6-DECADIENE	96688	1979632	0.726476	0.858161	$C_{10}H_{18}$	138.25	Antioxidant, antiasthmatics, antibacterial, for sexual and urinary system disorders, and also used for enhancing fertility, bioactive.	[35, 36]
			CARYOPHYLLENE	497747	955086	3.739878	4.14025	$C_{15}H_2$	204.3	anti-inflammatory, antibiotic, antioxidant, anticarcinogenic, local anaesthetic activities, anticancer activity. antinociceptive, neuro protective, anxiolytic, anti depressent, antialcoholism, anti pyritics, anti-microbial, anti-carcinogenic, anti dermatitic, allergenic, aldose- reductase inhibitor, anti-acne, anti-asthmatic, anti-ulcer, anti proliferants, cyto protective, gastro protective, sedative, anti-spasmodic, flavour.	[30, 40]

	29.038	725	1,6CYCLODECADIENE, 1-METHYL-5-METHYLENE-8-(1-METHYL ETHYL)-,[S-(E-E)]-	168458	3320428	1.265728	1.43939	$C_{15}H_{24}$	204.36	antioxidant activity	[49]
	36.711	770	1,2-(1,2-cyclobutanediyl) bis-, trans	74891	1292453	0.562702	0.560272	$C_{16}H_{16}$	208.298	antitumor activity	[51]
	54.653	558	(2,3-DIPHENYLCYCLOPROPYL)METHYL PHENYL SULFOXIDE, TRANS-	97594	1697719	0.733283	0.735953	$C_{22}H_{20}OS$	332.461	anticandidal and antioxidant activity.	[50]
	59.015	559	9-OCTADECENAMI, (Z)-	1282448	21222900	9.635816	9.200025	$C_{18}H_{35}NO$	281.477	Anti-inflammatory activity, antibacterial activity and Antioxidant Activities.	[31]

R.T: retention time (Min), R.I: retention index, M.F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.

Table (3.3): The compound isolated from GC-MS/MS analysis of flower hexane extract

Extract Type	R.T (min.)	R.I	Possible Compound Name	High	Area	%High	%Area	M.F	M.W (g/mol)	Biological Study	Ref
Flower Hexane	18.144	849	CYCLOHEXANONE, 5-METHYL-2-(1METHYLETHYL), TRANS	4773218	69317544	5.75458	5.42315	$C_{10}H_{18}O$	154.249	antimicrobial activity, potential antibiofilm, antitumor activities.	[32]
	19.144	872	MENTHOL	42704920	593062592	51.48503	46.39909	$C_{10}H_{20}O$	156.27	Antibacterial, antifungal, antipruritic, anticancer, cooling effects & toxicity, Antioxidant.	[28]
	22.631	777	(-)-1R-8-HYDROXY-P-MENTH-4-EN-3-ONE	7168615	114501280	8.642478	8.958169	$C_{10}H_{16}O_2$	168.232	No activity was recorded	
	25.497	702	CYCLOHEXENE,4-PENTYL-1-(4-PROPYLCYCLOHEXYL)-	800384	17259096	0.964942	1.35029	$C_{20}H_{36}$	276.508	No activity was recorded	[33]
	29.734	722	ethyl 14-methyl-hexadecanoate	1176306	24944786	1.418154	1.95159	$C_{18}H_{36}O_2$	284	Antioxidant, Hypocholesterolemic Nematicide, Antiandrogenic, Flavor, Hemolytic.	[52]

	32.100	846	1-FORMYL-2,2-DIMETHYL-3-TRANS-(3-METHYL-BUT-2-ENYL) -6-METHYLIDENE-CYCLOHEXANE	1356589	22548572	1.635503	1.764119	$C_{15}H_{24}O$	220.356	Antifungal, Insecticidal and larvicidal, Agent.	[42]
	42.129	808	N-HEXADECANOIC ACID	3167735	46401524	3.819019	3.630288	$C_{16}H_{32}O_2$	256.4	Antioxidant, anticancer, food additive, anti-inflammation.	[37]
	42.879	890	ETHYL 13-METHYL-TETRADECANOATE	17076364	308004000	20.58725	24.09713	$C_{17}H_{34}O_2$	270.457	antioxi-dant, β-glucuronidase inhibitory and anticancer activities	[43]
	47.486	867	10-BROMODECANOIC ACID, ETHYL ESTER	1568285	31102566	1.890723	2.433353	$C_{12}H_{23}BrO_2$	279.214	antidiabetic activities	[44]
	51.722	841	METHYL 17-METHYL-OCTADECANOATE	457836	7681900	0.551967	0.601004	$C_{20}H_{40}O_2$	312.538	Saturated fatty acid, antiasthmatics, antibacterial agents, antioxidants, anticancer, also used for urinary or sexual disorders treatment.	[53]
	58.950	781	9-OCTADECENAMIDE, (Z)-	2696041	43353476	3.250345	3.39182	$C_{18}H_{35}NO$	281.477	Anti-inflammatory activity, Antibacterial activity and Antioxidant Activities.	[31]

***R.T: retention time (Min), R.I: retention index, M.F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.**

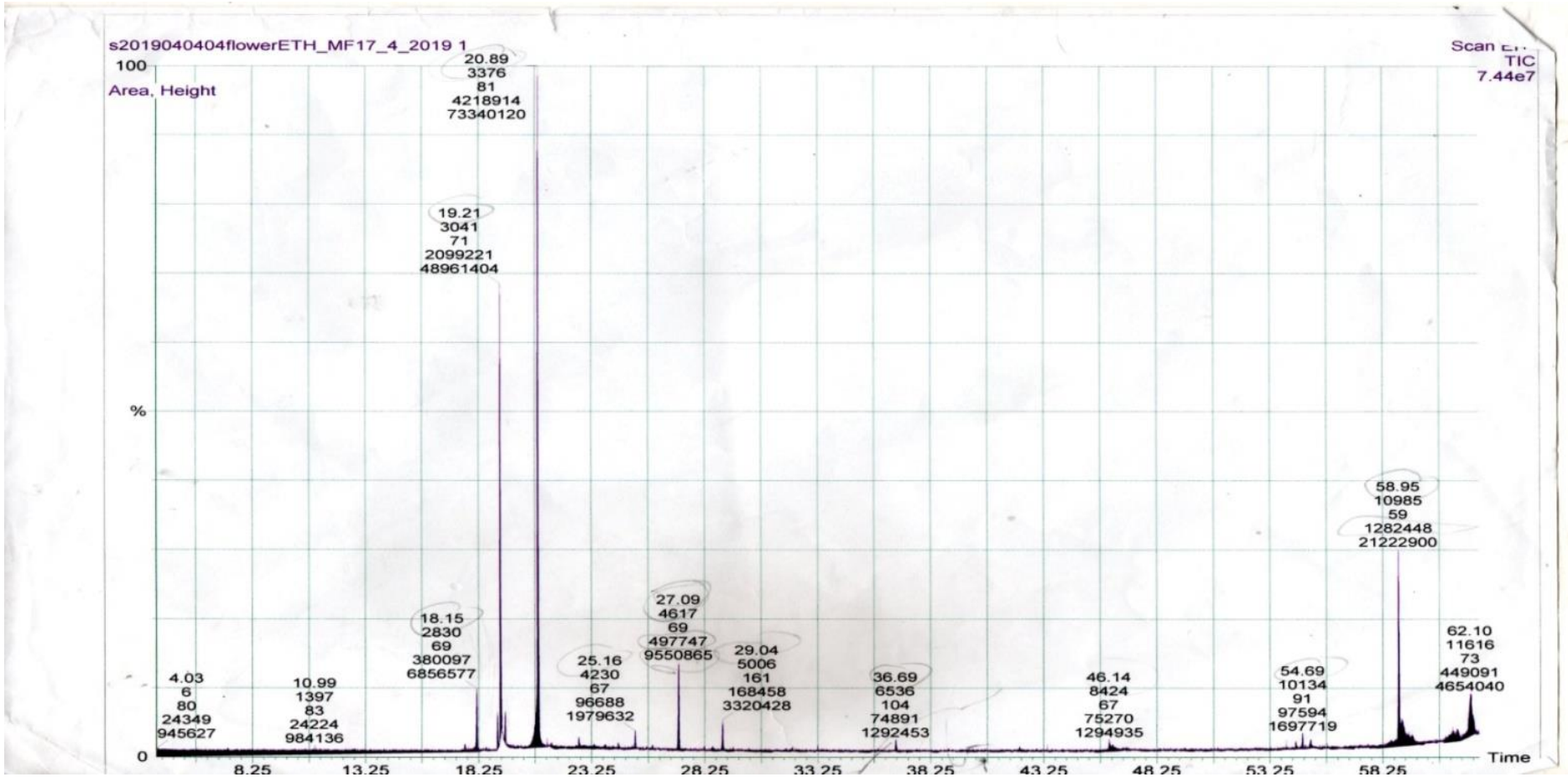

Fig. (3.2): Trace GC-MS Chromatogram of the ethanol flower extract of *Micromeria Fruticosa* plant.

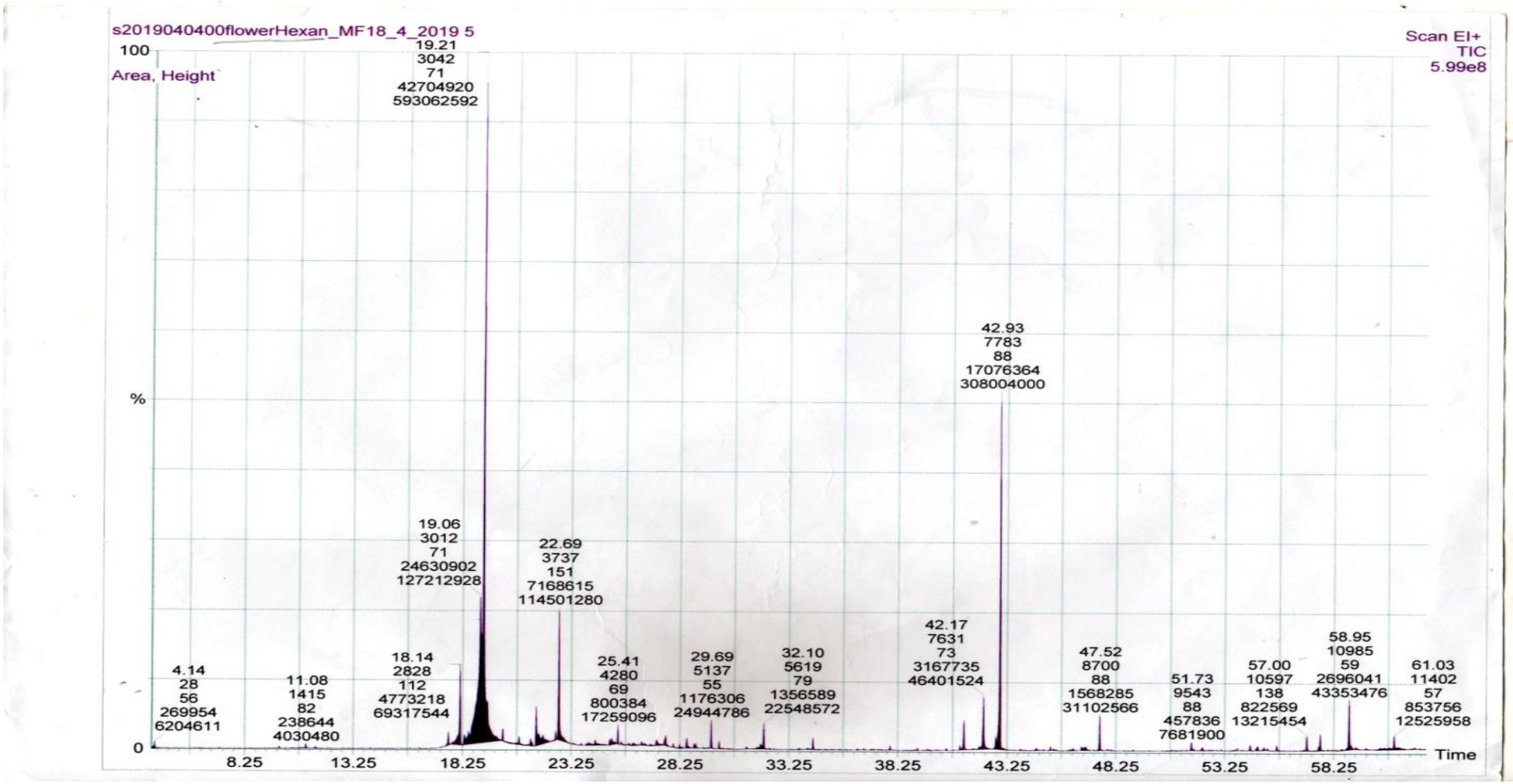

Fig. (3.3): Trace GC-MS Chromatogram of the hexane flower extract of Micromeria Fruticosa plant.

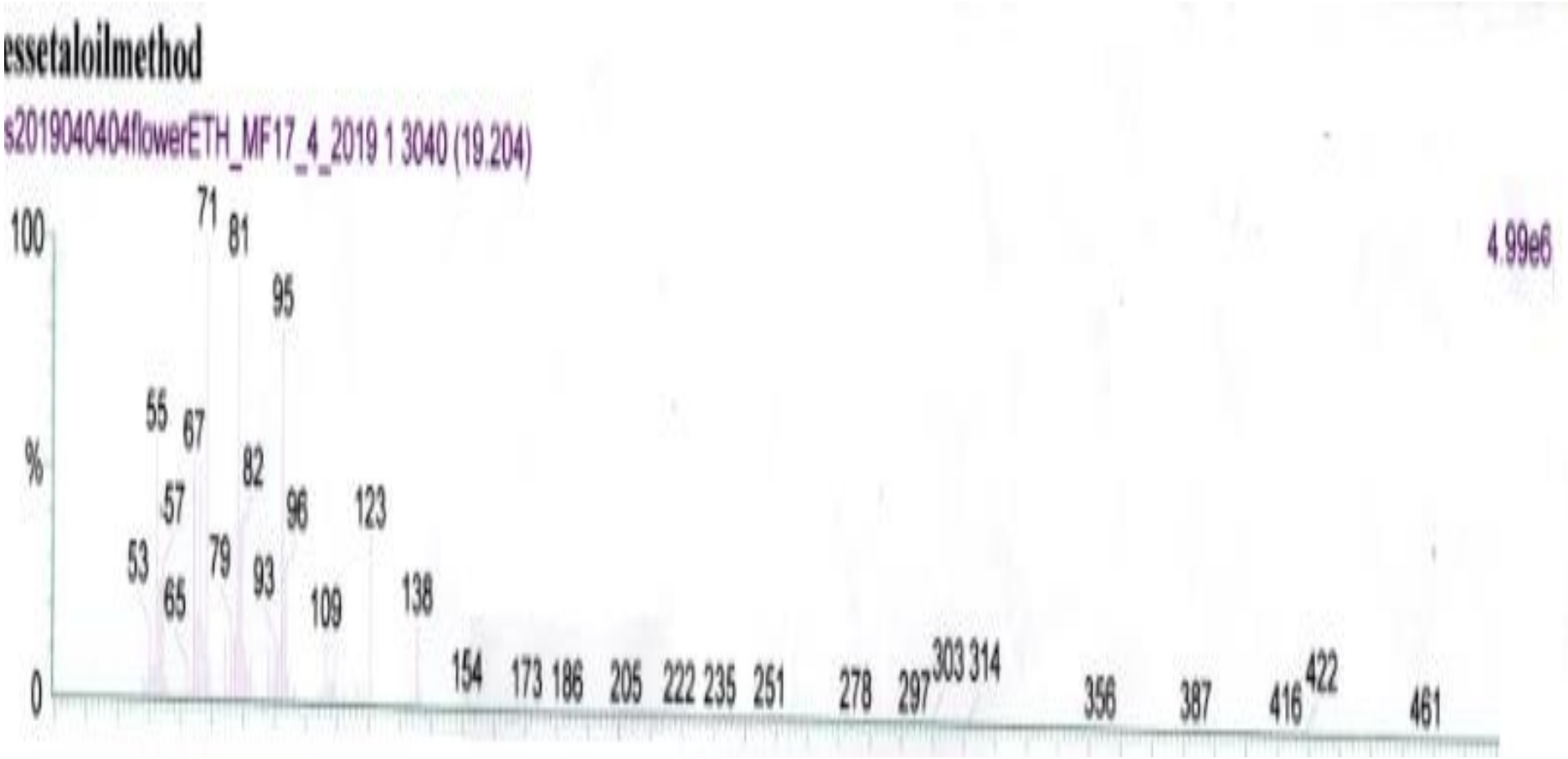

Fig. (3.4): Trace GC-MS Chromatogram spectrum of the ethanol flower extract of *Micromeria Fruticosa* plant at the R.T. (19.204) minute.

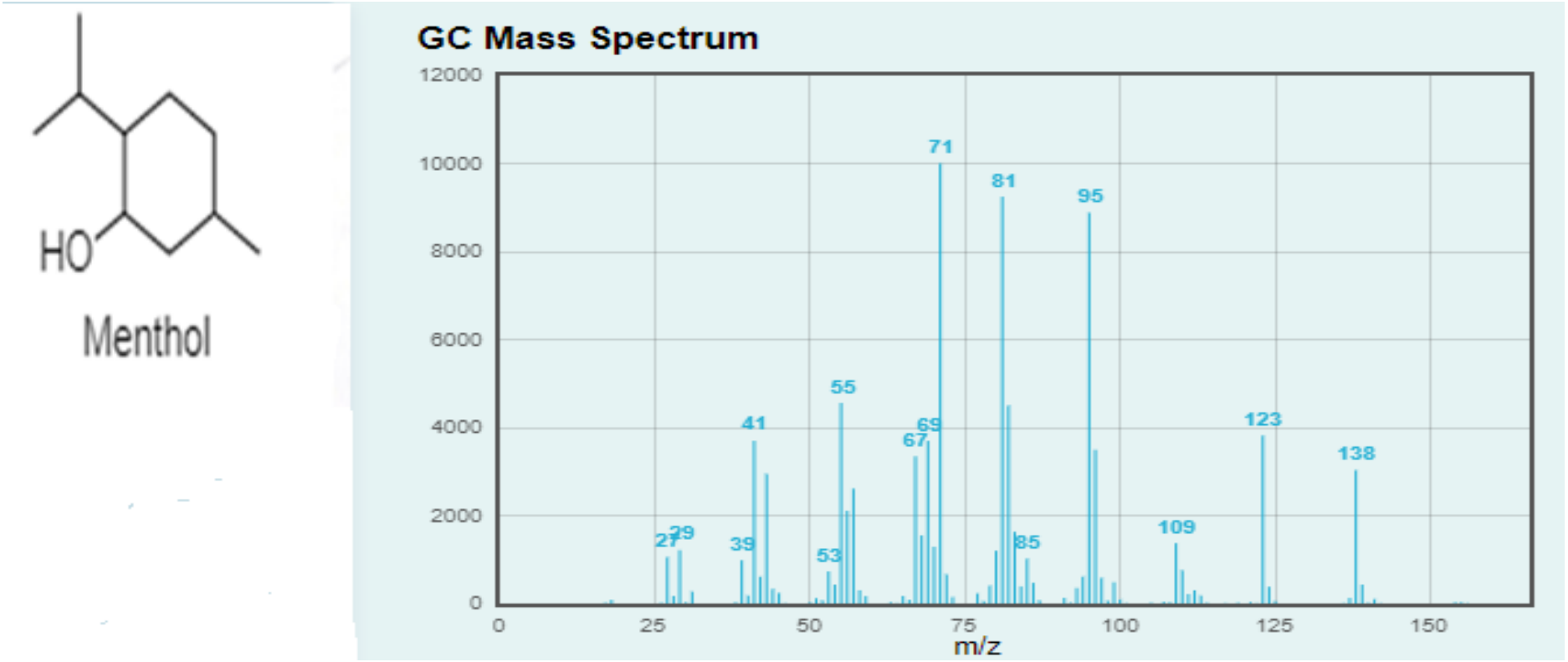

Fig. (3.5): Trace GC-MS Chromatogram of the menthol compound from ethanol flower extract of *Micromeria Fruticosa* plant.

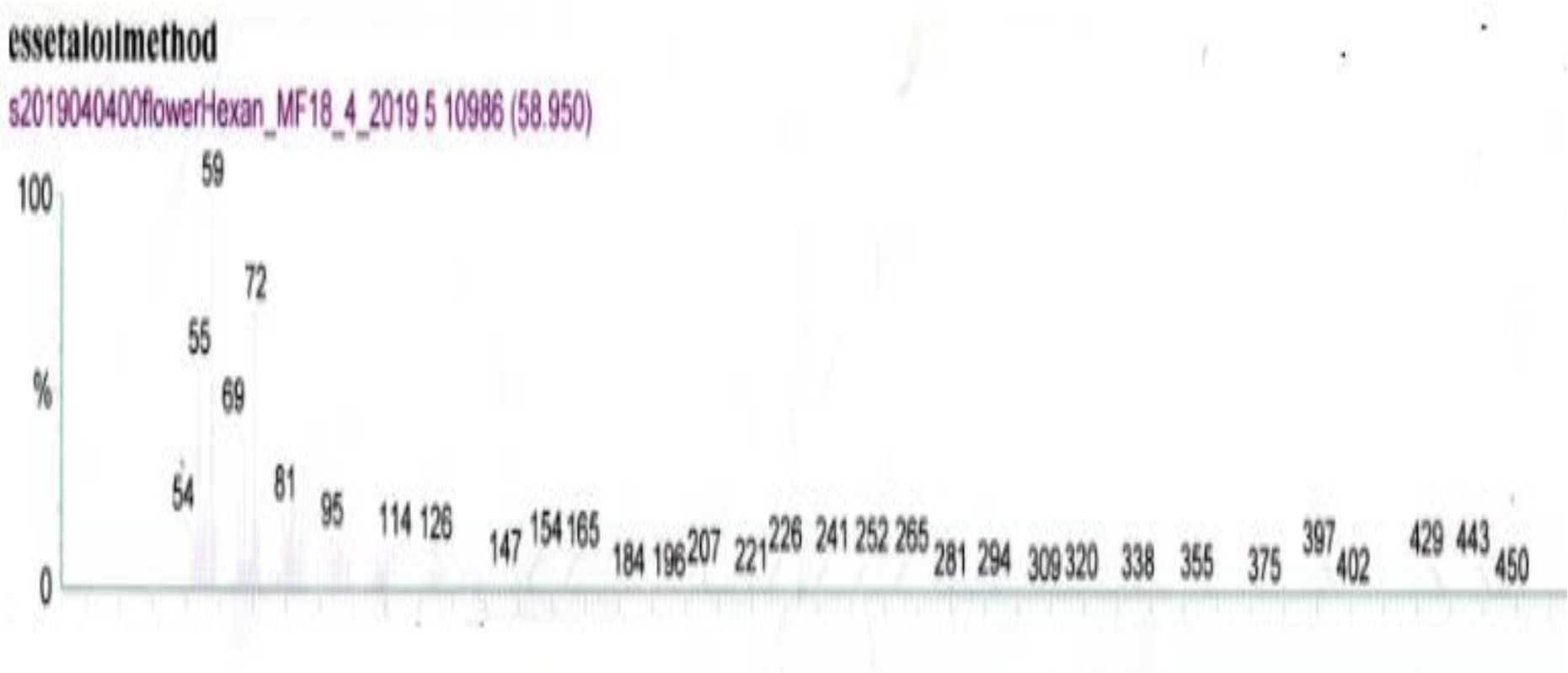

Fig. (3.6): Trace GC-MS Chromatogram spectrum of the hexane flower extract of *Micromeria Fruticosa* plant at the R.T. (58.950) minute.

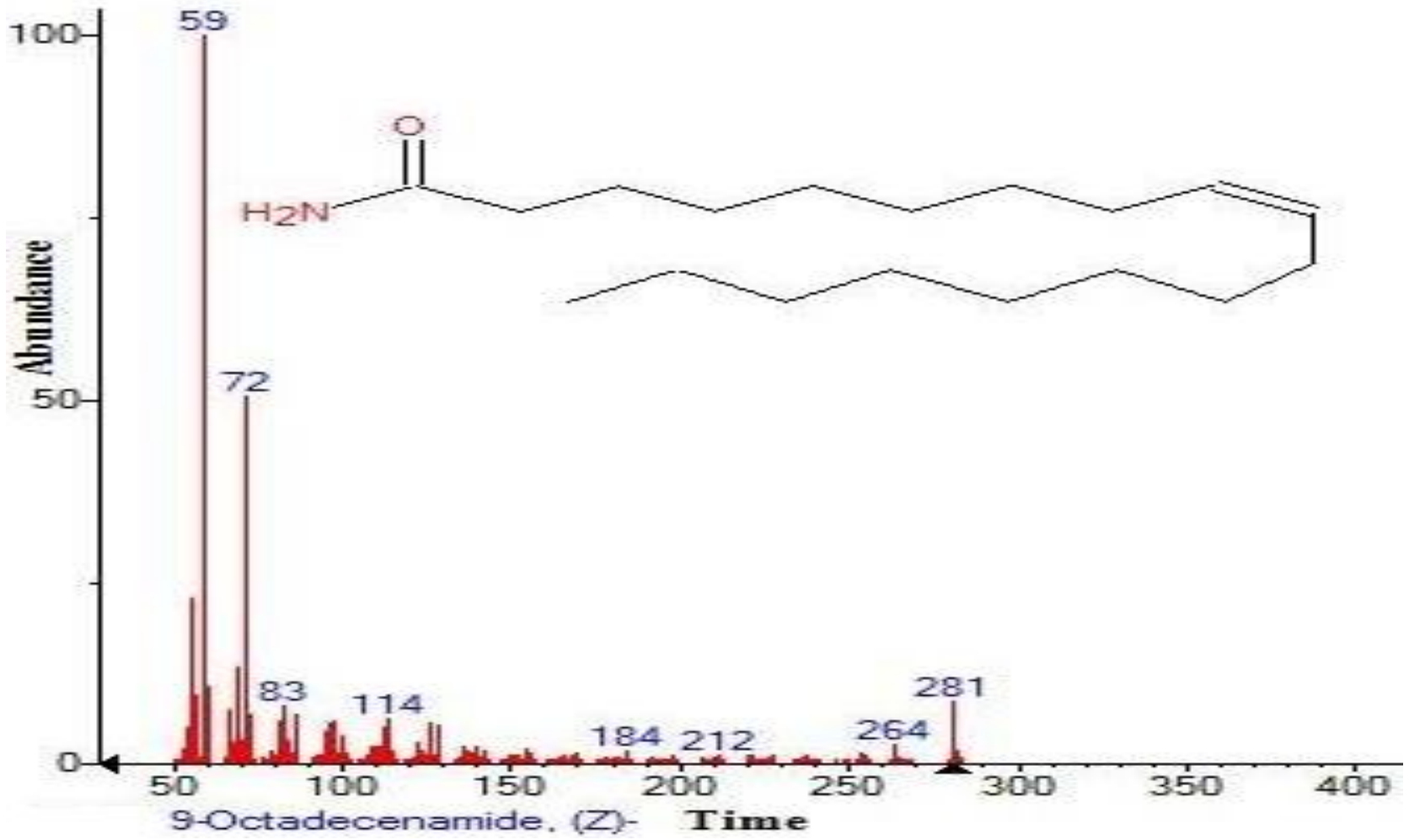

Fig. (3.7): Trace GC-MS Chromatogram of the 9-octadecenamide, (z) - compound from hexane flower extract of *Micromeria Fruticosa* plant.

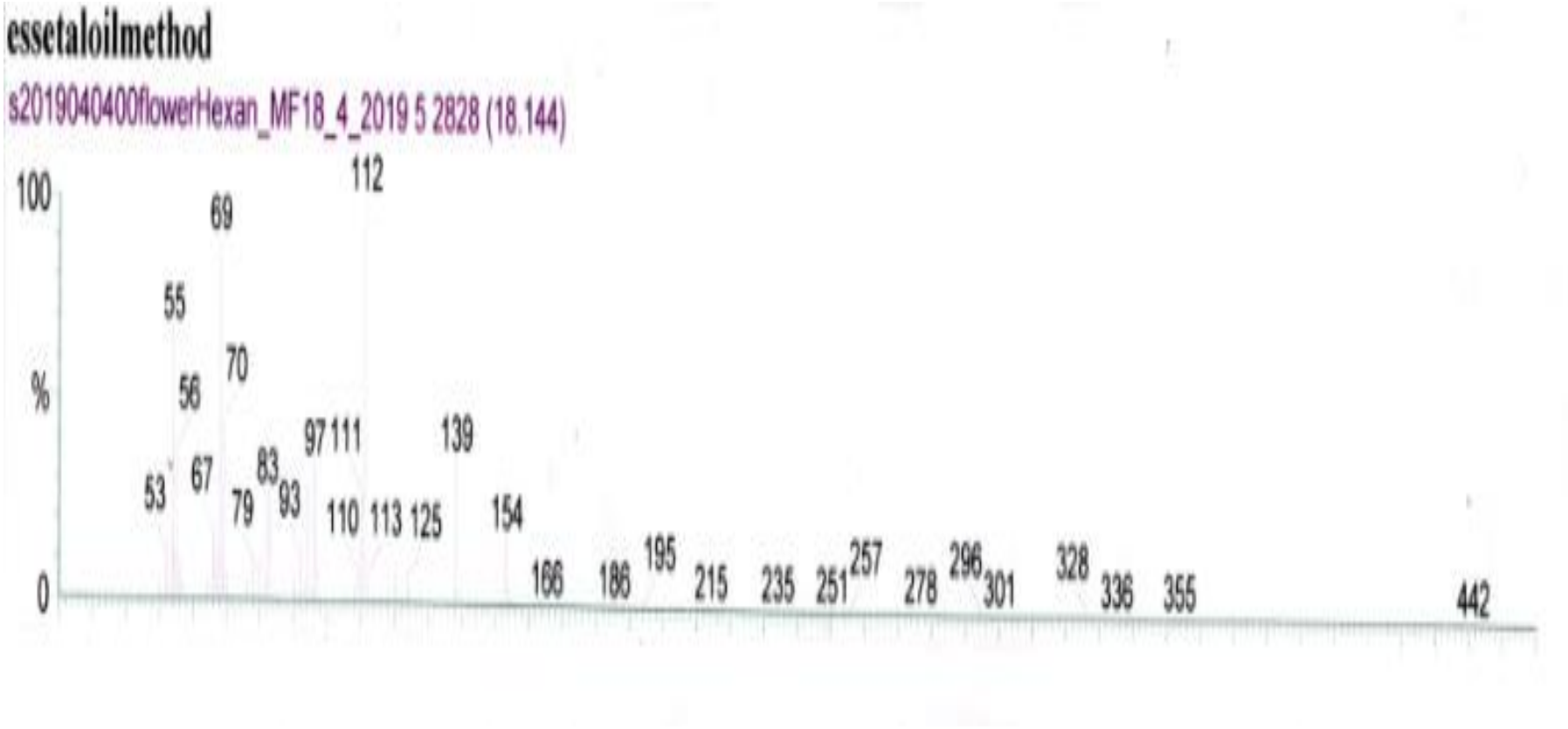

Fig. (3.8): Trace GC-MS Chromatogram spectrum of the hexane flower extract of *Micromeria Fruticosa* plant at the R.T. (18.144) minute.

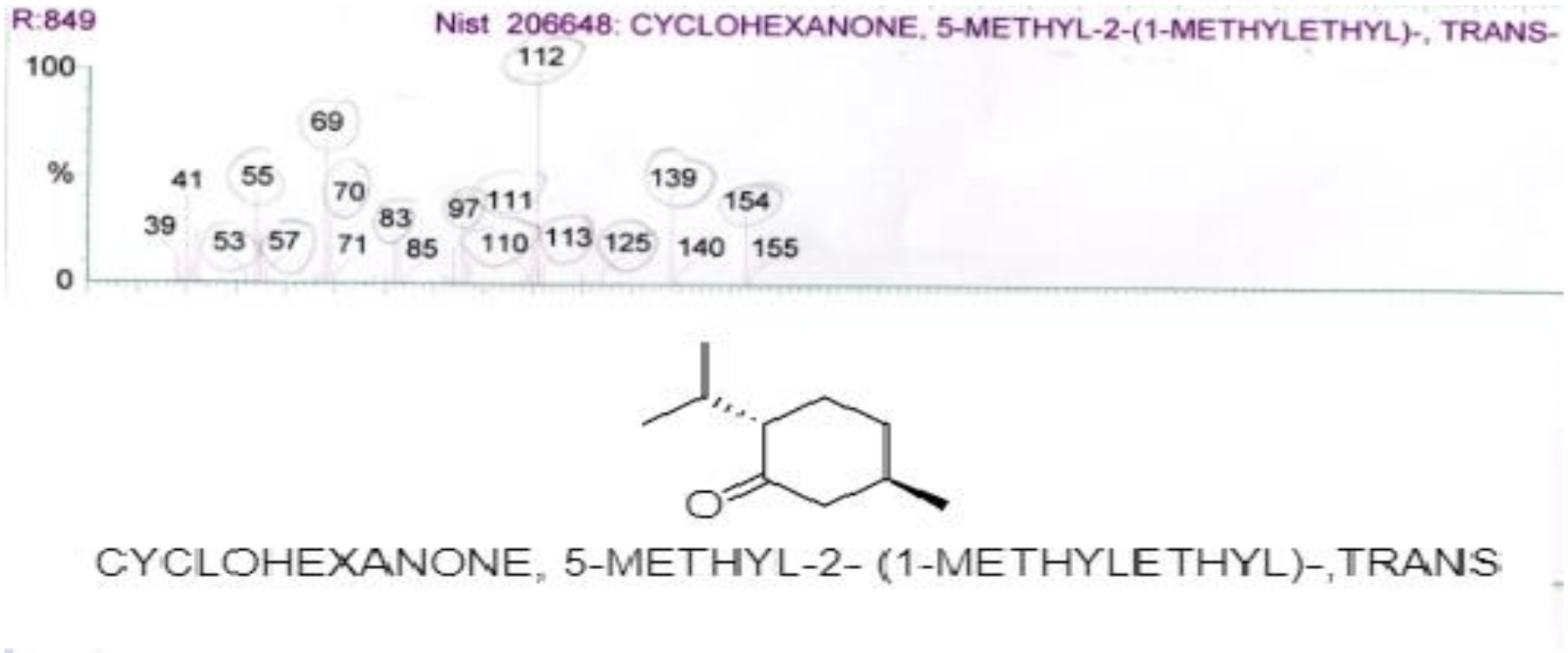

Fig. (3.9): Trace GC-MS Chromatogram of the CYCLOHEXANONE, 5-METHYL-2-(1-METHYLETHYL)-, TRANS compound from hexane flower extract of *Micromeria Fruticosa* plant.

The GC-MS chromatogram of hexane extract of leaves in Figure 3.11, ten compounds were identified. Some of the compounds identified were Menthol (21.2568%) and 9-OCTADECENAMIDE, (Z)- (34.34091%). The details of these bioactive compounds were given in Table 3.6.

The compounds were identified in ethanolic leave extract as shown in Table 3.5 and the GC-MS of this extract are shown in Figure 3.10.

There are similar compounds in the hexane and ethanol leave extract but with different percentage and these compounds are as shown in Table 3.4 and Figure 3.12, 3.13, 3.14. The presence of these compounds in the leave makes this part of the plant is highly effective when the biological and antioxidants activation have been examined.

Table (3.4): The similar compounds of hexane and ethanol leave extract from GC-MS/MS analysis.

Similar Compounds Name	Molecular formula	%high peak In leave ethanol extract	%high peak in leave hexane extract
CYCLOHEXANONE,5-METHYL -2-(1-METHYLETHYL)-, TRANS	$C_{10}H_{18}O$	13.33935%	7.407839%
PULEGONE-	$C_{10}H_{16}O$	63.71028	9.038732
CYCLOBUTANE,1,3-DIPHENYL-, TRANS	$C_{16}H_{16}$	1.112354	3.677446

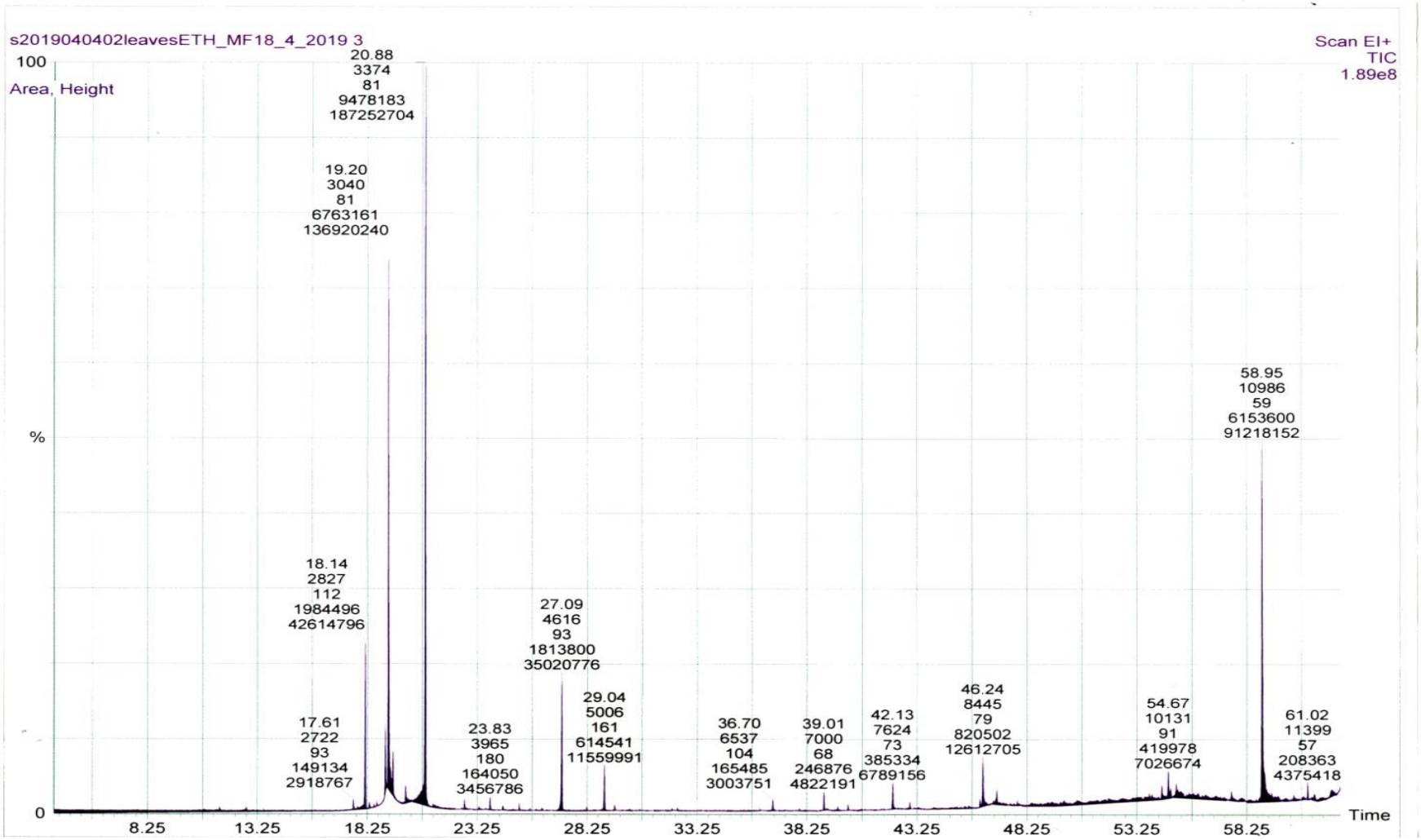

Fig. (3.10): Trace GC-MS Chromatogram of the ethanol leaves extract of *Micromeria Fruticosa* plant.

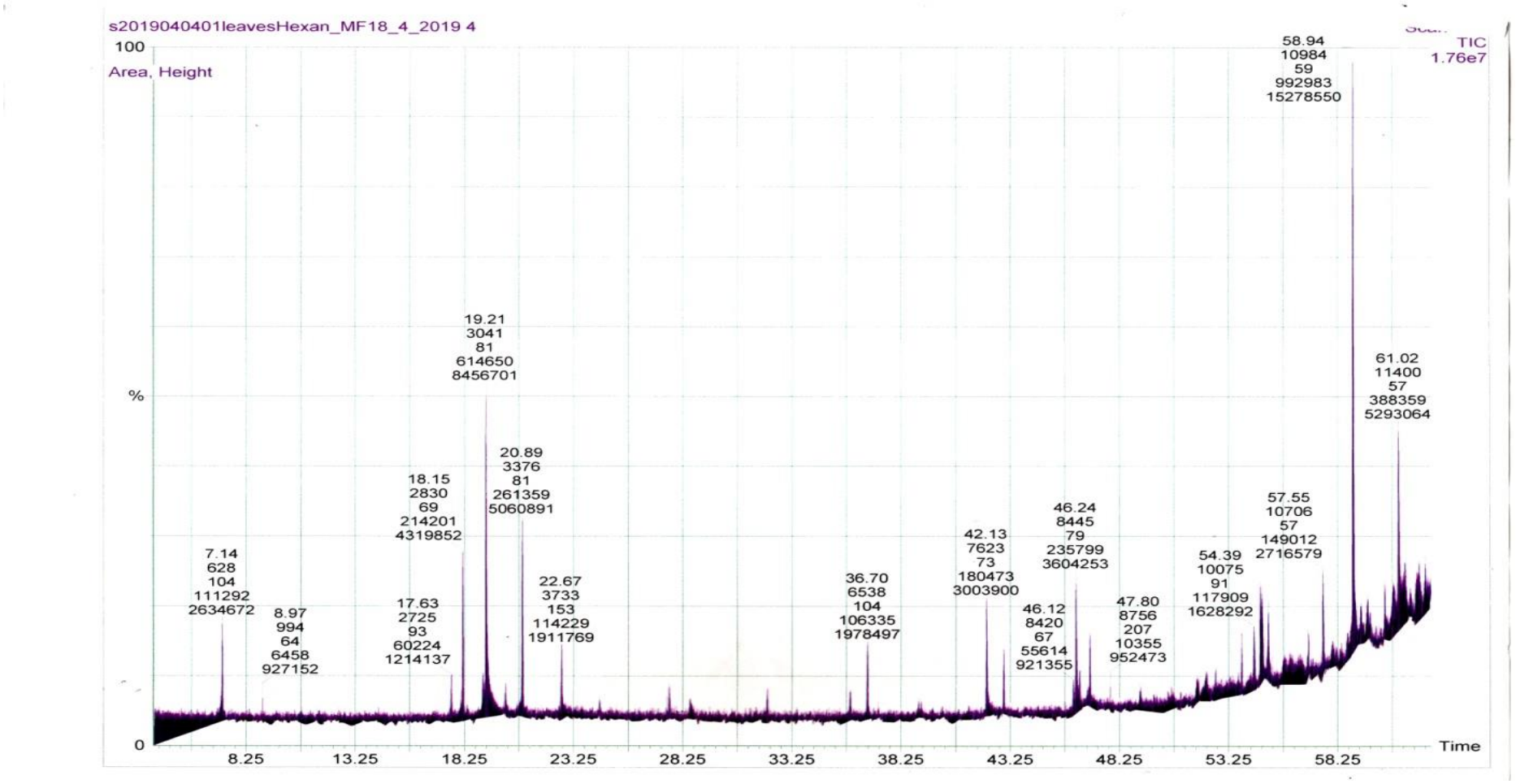

Fig. (3.11): Trace GC-MS Chromatogram of the hexane leaves extract of Micromeria Fruticosa plant.

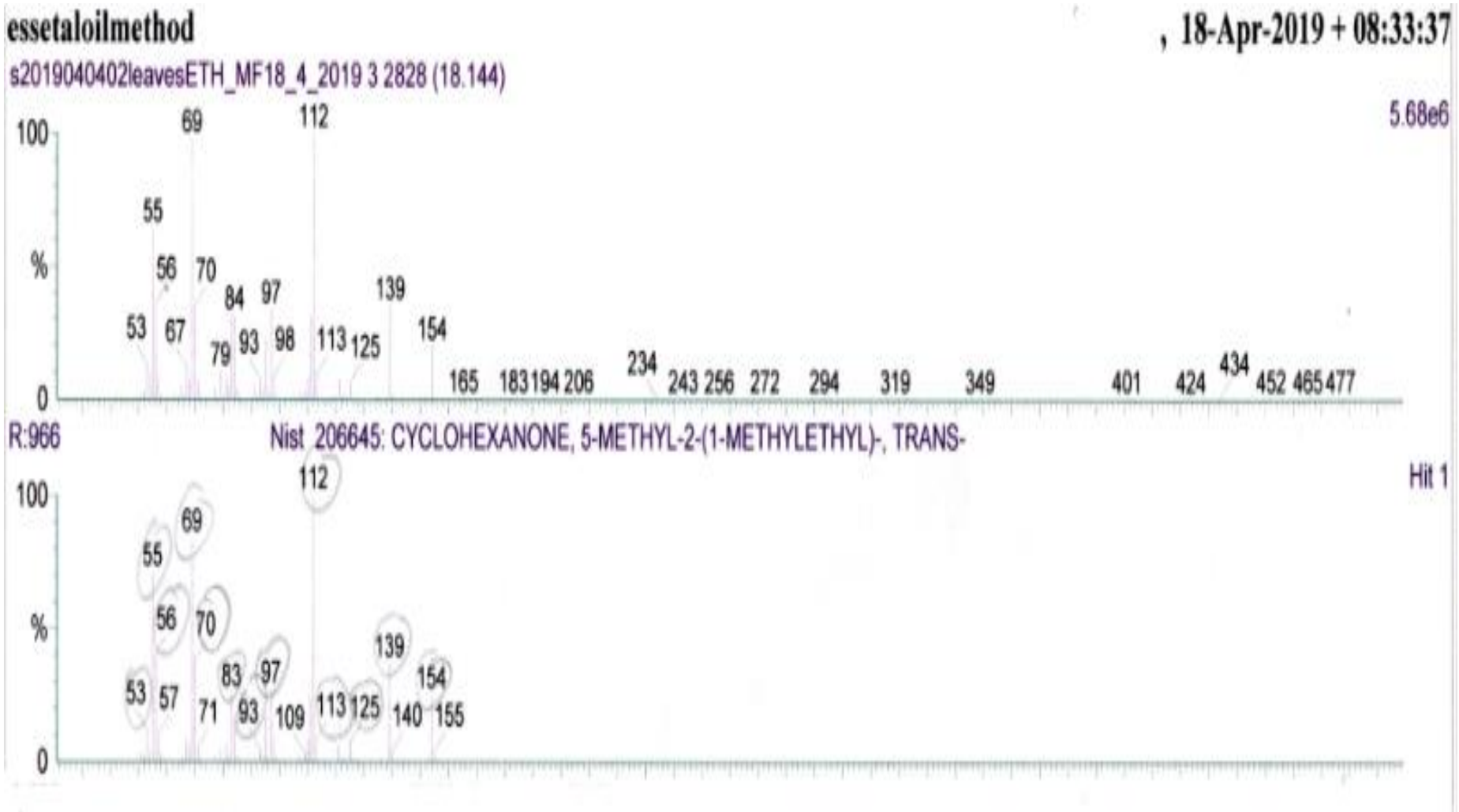

Fig. (3.12): Trace GC-MS Chromatogram spectrum of the ethanol leaves extract of Micromeria Fruticosa plant at the R.T. (18.144) minute and the fragment for CYCLOHEXANONE, 5-METHYL-2-(1-METHYLETHYL)-, TRANS.

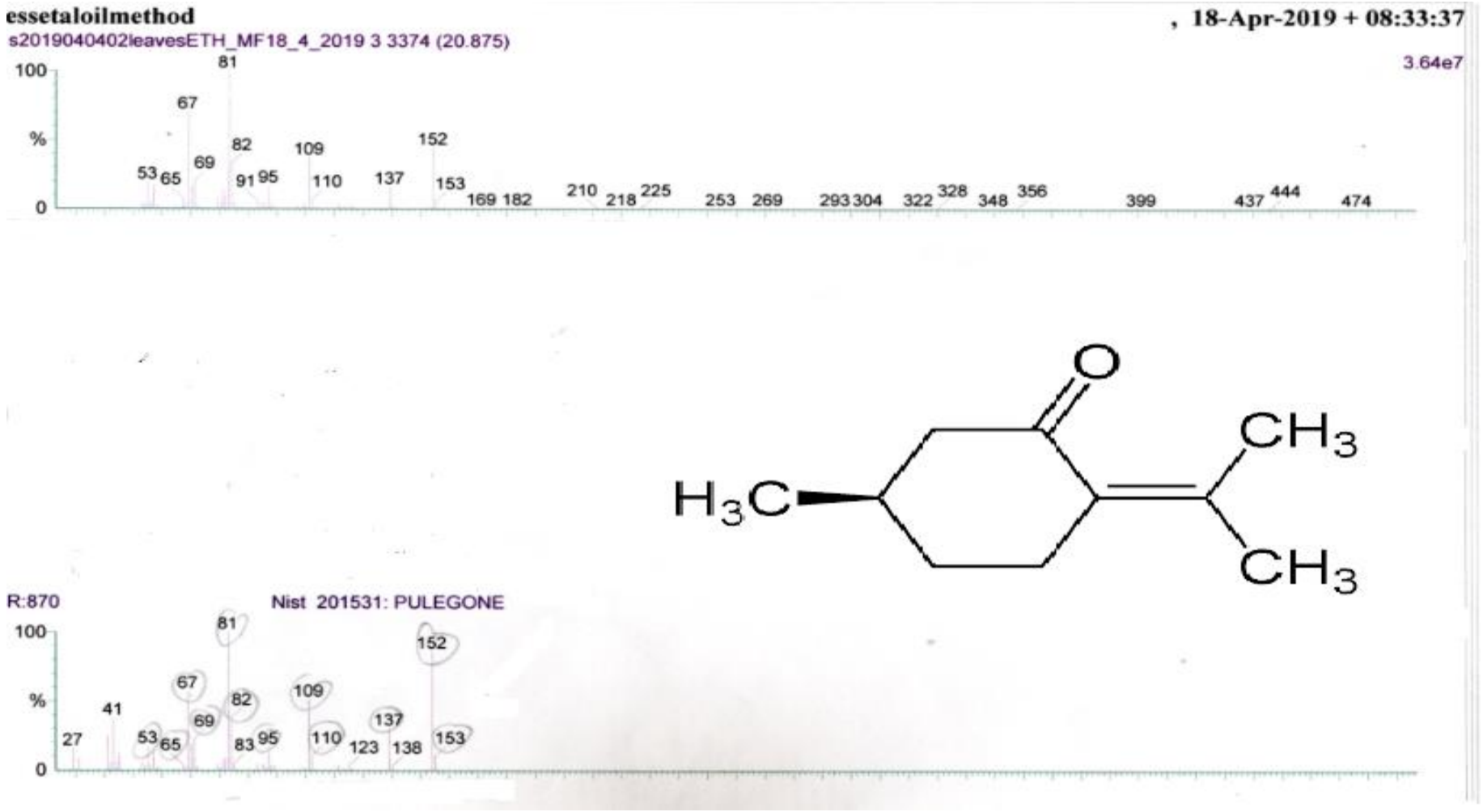

Fig. (3.13): Trace GC-MS Chromatogram spectrum of the ethanol leaves extract of Micromeria Fruticosa plant at the R.T. (20.875) minute and the fragment for Pulegone.

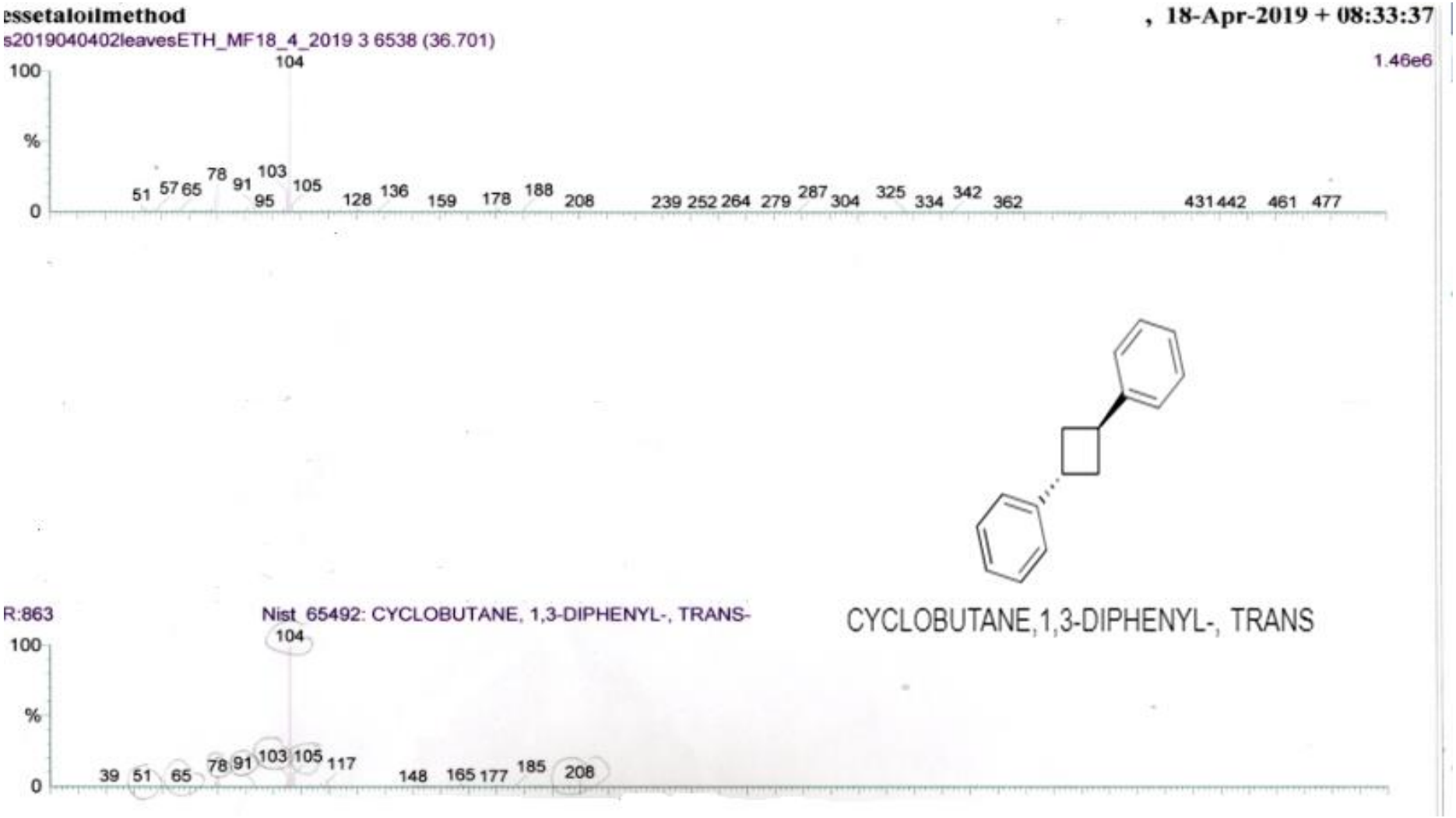

Fig. (3.14): Trace GC-MS Chromatogram spectrum of the ethanol leaves extract of Micromeria Fruticosa plant at the R.T. (36.701) minute and the fragment for Cyclobutane, 1, 3-Diphenyl-, Trans.

Table (3.5): The compound isolated from GC-MS/MS analysis of leave ethanol extract

Extract Type	R.T (min.)	R.I	Possible Compound Name	High	Area	%High	%Area	M.F	M.W (g/mol)	Biological Study	Ref.
Leave Ethanol	18.44	966	CYCLOHEXANONE, 5-METHYL-2-(1-METHYLETHYL), TRANS	1984496	42614796	13.33935	14.59087	$C_{10}H_{18}O$	154.249	antimicrobial activity, potential antibiofilm, antitumor activities	[32]
	20.875	870	PULEGONE	9478183	187252704	63.71028	64.11343	$C_{10}H_{16}O$	152.2334	Antimicrobial , Antioxidant, anti-inflammatory, anti-ulcer, insecticidal properties.	[29]
	27.088	905	CARYOPHLLENE	1813800	35020776	12.19197	11.99076	$C_{15}H_{24}$	204.36	anti-inflammatory, antibiotic, antioxidant, anticarcinogenic, local anaesthetic activities, anticancer activity. antinociceptive, neuro protective, anxiolytic, anti depressent, antialcoholism, anti pyritics, anti-microbial, anti-carcinogenic, anti dermatitic, allergenic, aldose- reductase inhibitor, anti-acne, anti-asthmatic, anti-ulcer, anti proliferants, cyto protective, gastro protective, sedative, anti-spasmodic, flavour.	[30, 40]

	29.033	**865**	**1H-CYCLOPENTA[1,3]CYCLOPROPA[1,2] BENZENE,OCTAHYDRO-7-METHYL-3-METHYLENE-4-(1-METHYLETHYL)-**	**614541**	**11559991**	**4.130811**	**3.958024**	**$C_{15}H_{24}$**	**204.3511**	**antibacterial activity.**	**[38]**
	36.701	**863**	**CYCLOBUTANE, 1,3-DIPHENYL-, TRANS**	**165485**	**3003751**	**1.112354**	**1.028454**	**$C_{16}H_{16}$**	**208.304**	**estrogen screening assay and estrogen reporter assay using estrogen-responsive human breast cancer cell line MCF-7.**	**[39]**
	46.260	**889**	**METHYL 8, 11, 14-HEPTADECATRIENOATE**	**820502**	**12612705**	**5.515236**	**4.318462**	**$C_{18}H_{30}O_2$**	**278.436**	**No activity was recorded**	

***R.T: retention time (Min), R.I: retention index, M.F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.**

Table (3.6): The compound isolated from GC-MS/MS analysis of leave hexane extract

Extract Type	R.T (min.)	R.I	Possible Compound Name	High	Area	%High	%Area	M.F	M.W (g/mol)	Biological Study	Ref.
Leave Hexane	7.140	898	STYRENE	111292	2634672	3.84887	5.55097	C_8H_8	104.15	No activity was recorded	
	17.619	717	CYCLOHEXENE, 1-METHYL-4-(1-METHYLETHYLIDENE)-	60224	1214137	2.082762	2.558059	$C_{10}H_{16}$	136.234	Antioxidant, antiasthmatics, antibacterial agents, for wound treatment, food, flavouring agent, also used for urinary and sexual disorder, bioactive.	[33, 34]
	18.144	920	CYCLOHEXANONE, 5-METHYL-2-(1-METHYLETHYL)-, TRANS	214201	4319852	7.407839	9.101472	$C_{10}H_{18}O.$	154.249	antimicrobial activity, potential antibiofilm, antitumor activities	[32]
	19.199	853	MENTHOL	614650	8456701	21.2568	17.81738	$C_{10}H_{20}O$	156.27	Antibacterial ,antifungal ,antipruritic, anticancer, cooling effects and toxicity, Antioxidant.	[28]
	20.880	823	PULEGONE	261359	5060891	9.038732	10.66276	$C_{10}H_{16}O$	152.2334	Antimicrobial, Antioxidant, anti-inflammatory, anti-ulcer, insecticidal properties.	[29]

	22.676	**701**	**(-)-1R-8-HYDROXY-P-MENTH-4-EN-3-ONE**	**114229**	**1911769**	**3.950449**	**4.027896**	**$C_{10}H_{16}O_2$**	**168.232**	**No activity was recorded**	
	36.691	**858**	**CYCLOBUTANE, 1,3-DIPHENYL-, TRANS**	**106335**	**1978497**	**3.677446**	**4.168484**	**$C_{16}H_{16}$**	**208.304**	**estrogen screening assay and estrogen reporter assay using estrogen-responsive human breast cancer cell line MCF-7.**	**[39]**
	42.108	**585**	**N-HEXADECANOIC ACID**	**180473**	**3003900**	**6.241404**	**6.3289**	**$C_{16}H_{32}O_2$**	**256.4**	**Antioxidant, anticancer, food additive, anti-inflammation.**	**[37]**
	46.215	**847**	**METHYL 2-HYDROXY-OCTADECA-9, 12 ,15-TRIENOATE**	**235799**	**3604253**	**8.154775**	**7.593781**	**$C_{19}H_{32}O_3$**	**308.462**	**No activity was recorded**	
	58.940	**771**	**9-OCTADECENAMIDE, (Z)-**	**992983**	**15278550**	**34.34091**	**32.19029**	**$C_{18}H_{35}NO$**	**281.477**	**Anti-inflammatory activity, antibacterial activity and Antioxidant Activities.**	**[31]**

***R.T: retention time (Min), R.I: retention index, M.F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.**

Table (3.7): The compound isolated from GC-MS/MS analysis of stem hexane and ethanol extract

Extract Type	R.T (min.)	R.I	Possible Compound Name	High	Area	%High	%Area	M.F	M.W (g/mol)	Biological Study	Ref.
Stem Hexane	19.199	880	MENTHOL	4641092	79568336	63.2489	66.6116	$C_{10}H_{20}O$	156.27	Antibacterial ,antifungal ,antipruritic, anticancer, cooling effects and toxicity, Antioxidant.	[28]
	54.753	887	HENTRIACONTANE	471329	5256410	6.423285	4.400468	$C_{31}H_{64}$	436.85	Antifugal against fungal spores germination, Antioxidant, antitumor activity and antibacterial.	[41]
	58.935	766	9-OCTADECENAMIDE, (Z)-	2225398	34626420	30.32779	28.98793	$C_{18}H_{35}NO$	281.477	Anti-inflammatory activity, antibacterial activity and Antioxidant Activities.	[31]
Stem Ethanol	19.214	864	MENTHOL	1372496	17921388	100	100	$C_{10}H_{20}O$	156.27	Antibacterial ,antifungal ,antipruritic, anticancer, cooling effects and toxicity, Antioxidant.	[28]

***R.T: retention time (Min), R.I: retention index, M.F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.**

The GC-MS chromatogram of ethanol and hexane stem extract as shown in Figure 3.15, 3.16. Three compounds were identified as shown in Table 3.7. The compound that had the highest percentage peak is Menthol (63.2489%, 100%) in stem hexane and ethanol extract respectively, and this compound has strong antioxidant, antibacterial activity when we were estimated.

The ethanolic extract had antifungal activity in [C. albicans (ATCC 90028)] as shown in chapter five.

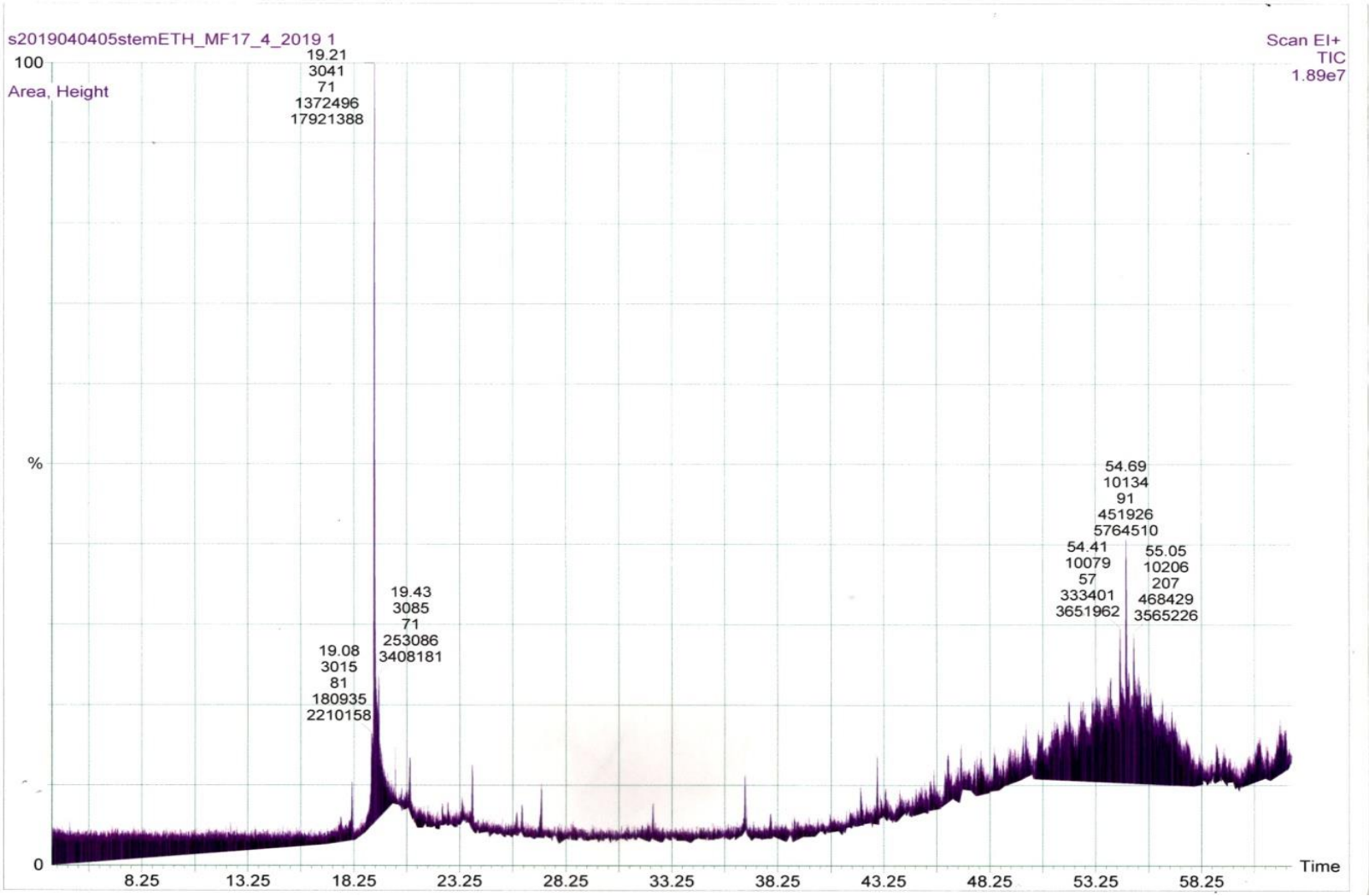

Fig. (3.15): Trace GC-MS Chromatogram of the ethanol stems extract of *Micromeria Fruticosa* plant.

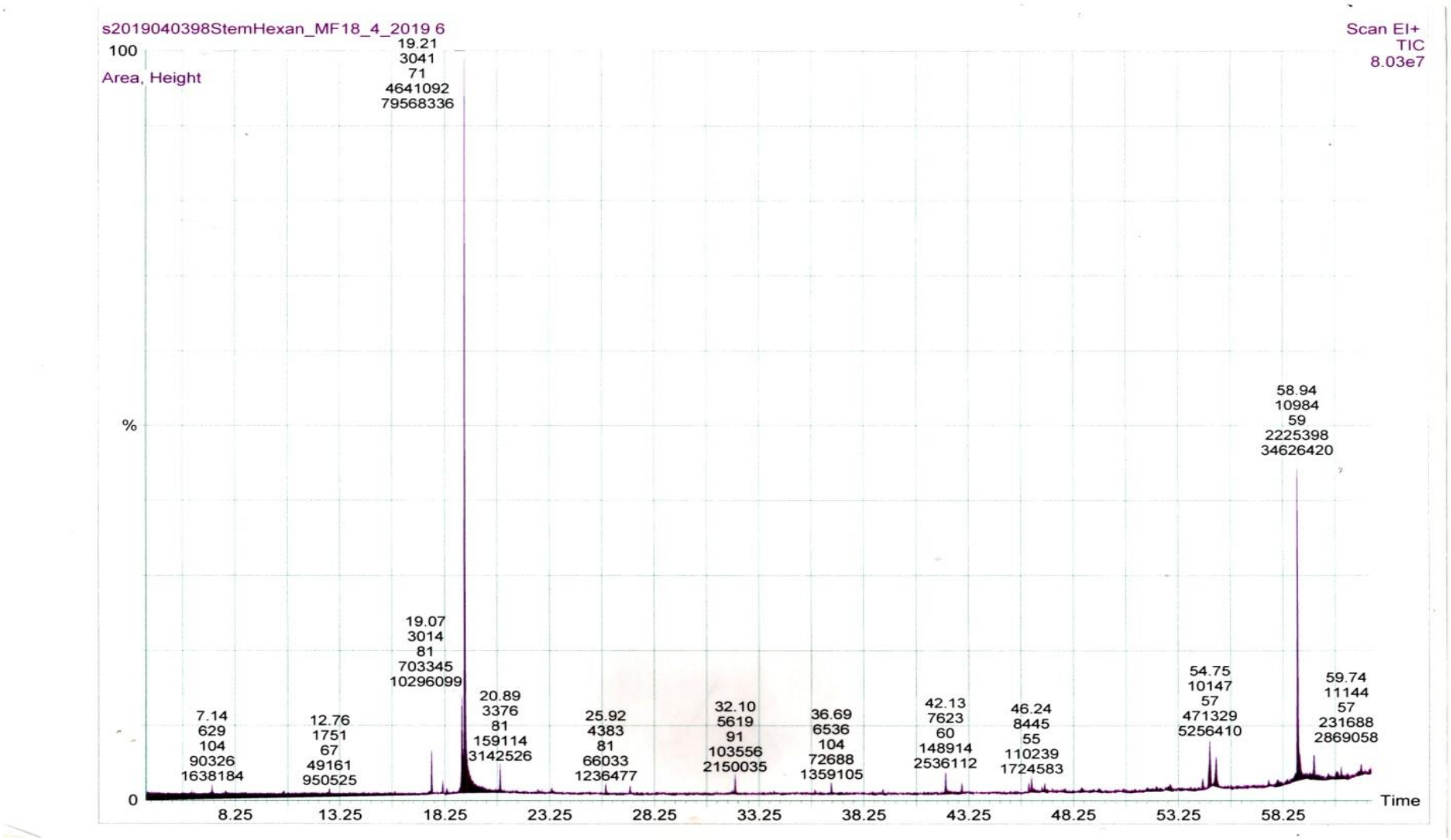

Fig.3.16. Trace GC-MS Chromatogram of the hexane stems extract of *Micromeria Fruticosa* plant.

Table (3.8): The compound isolated from GC-MS/MS analysis of roots ethanol extract

Extract Type	R.T (min.)	R.I	Possible Compound Name	High	Area	%High	%Area	M.F	M.W (g/mol)	Biological study	Ref.
Roots Ethanol	37.902	824	CIS-1-CHLORO-9-OCTADECENE	340164	6404544	3.5627	8.300122	$C_{18}H_{35}Cl$	286.923	Antibacterial activity.	[45]
	42.929	884	ETHYL 14-METHYL-HEXADECANOATE.	1139329	20698202	11.93303	26.82433	$C_{18}H_{36}O_2$	284.477	Antifungal, antitumor activity, Antibacterial.	[46]

***R.T: retention time (Min), R.I: retention index, M.F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.**

Table (3.9): The compound isolated from GC-MS/MS analysis of roots hexane extract

Extract Type	R.T (min.)	R.I	Possible Compound Name	High	Area	%High	%Area	M.F	M.W (g/mol)	Biological study	Ref
Roots Hexane	19.204	801	MENTHOL	307905	5175202	4.23009	3.60987	$C_{10}H_{20}O$	156.27	Antibacterial, antifungal ,antipruritic, anticancer, cooling effects and toxicity, Antioxidant.	[28]
	42.159	814	N-HEXADECANOIC ACID	682253	11813747	9.372994	8.24047	$C_{16}H_{32}O_2$	256.4	Antioxidant, anticancer, food additive, anti-inflammation.	[37]
	42.934	902	ETHYL 13-METHYL-TETRADECANOATE	3180246	68129408	43.69116	47.5224	$C_{17}H_{34}O_2$	270.457	antioxi-dant, β-glucuronidase inhibitory & anticancer activities	[43]
	54.433	823	1,2-BENZENEDICARBOXYLIC ACID, DIISOOCTYL ESTER.	486054	8963946	6.677554	6.25264	$C_{24}H_{38}O_4$	390.556	Antioxidant, Antimicrobial, Antifoulin.	[47, 48]
	58.965	750	9-OCTADECENAMIDE, (Z)-	1217126	20941278	16.72124	14.6072	$C_{18}H_{35}NO$	281.477	Anti-inflammatory activity, antibacterial activity and Antioxidant Activities.	[31]
	61.036	926	HENTRIACONTANE	1405339	28338940	19.30696	19.7673	$C_{31}H_{64}$	436.85	Antifugal against fungal spores germination, Antioxidant, antitumor activity and antibacterial.	[41]

***R.T: retention time (Min), R.I: retention index, M.F: molecular formula, M.W: molecular weight (g/mol), Ref: reference.**

The GC-MS chromatogram of root ethanol extract, three compounds were identified and are shown in Table 3.8 and Figure 3.17.

These compound DIDODECYL PHTHALATE (84.5%) and ETHYL 14-METHYL-HEXADECANOATE (11.9%) are shown in Figure 3.23, 3.24 respectively.

However root hexane extract, six compounds were identified as shown in Table 3.9 and Figure 3.18. That compound include ETHYL 13-METHYL-TETRADECANOATE (43.69%), HENTRIACONTANE (19.3%) as shown in Figure 3.22,

9-OCTADECENAMIDE, (Z)- (16.7%), N-HEXADECANOIC ACID (9.3%) as shown in Figure 3.21.

1,2-BENZENEDICARBOXYLIC ACID, DIISOOCTYL ESTER (6.6%) as shown in Figure 3.19, and MENTHOL (4.23%) as shown in Figure 3.20.

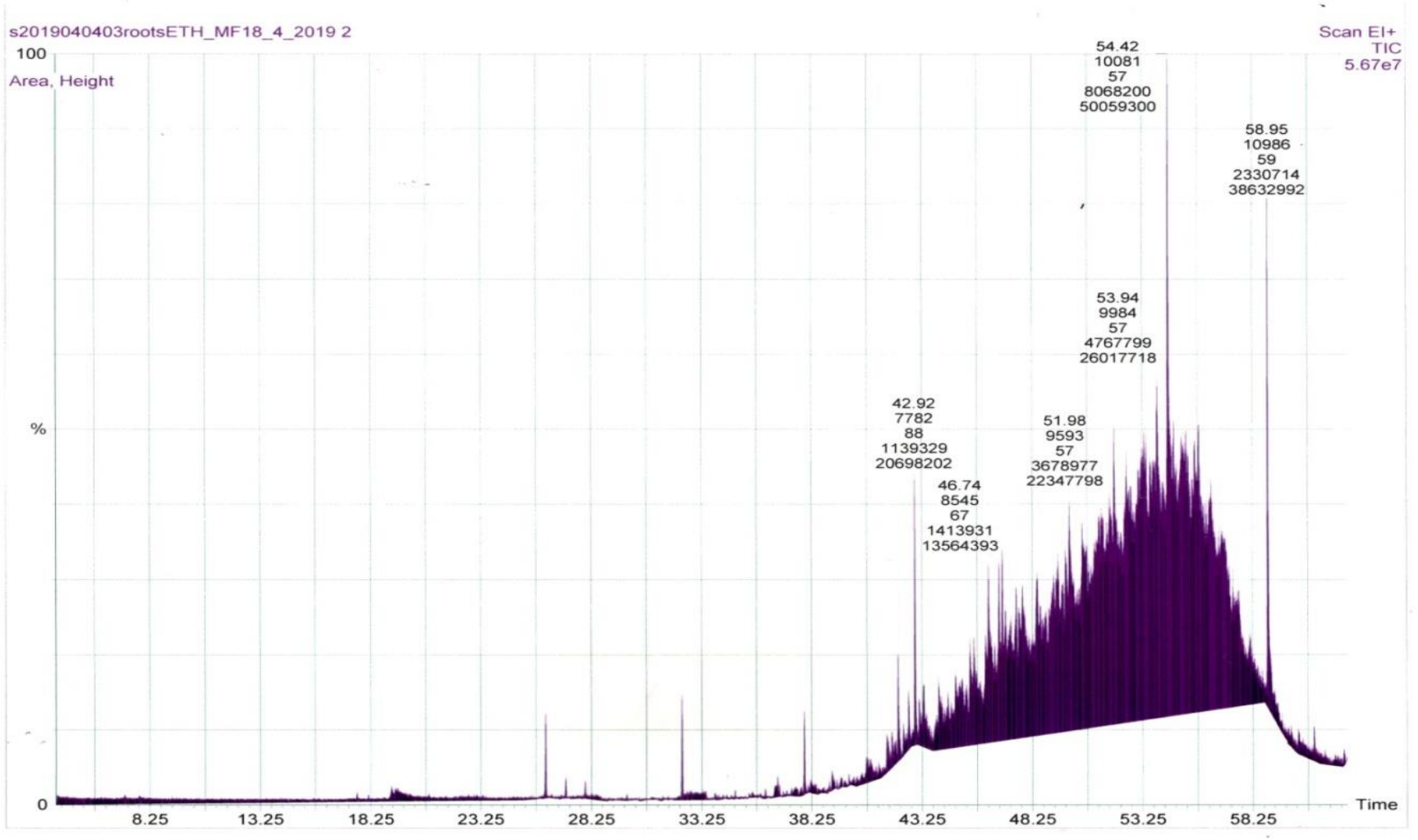

Fig. (3.17): Trace GC-MS Chromatogram of the ethanol root extract of *Micromeria Fruticosa* plant.

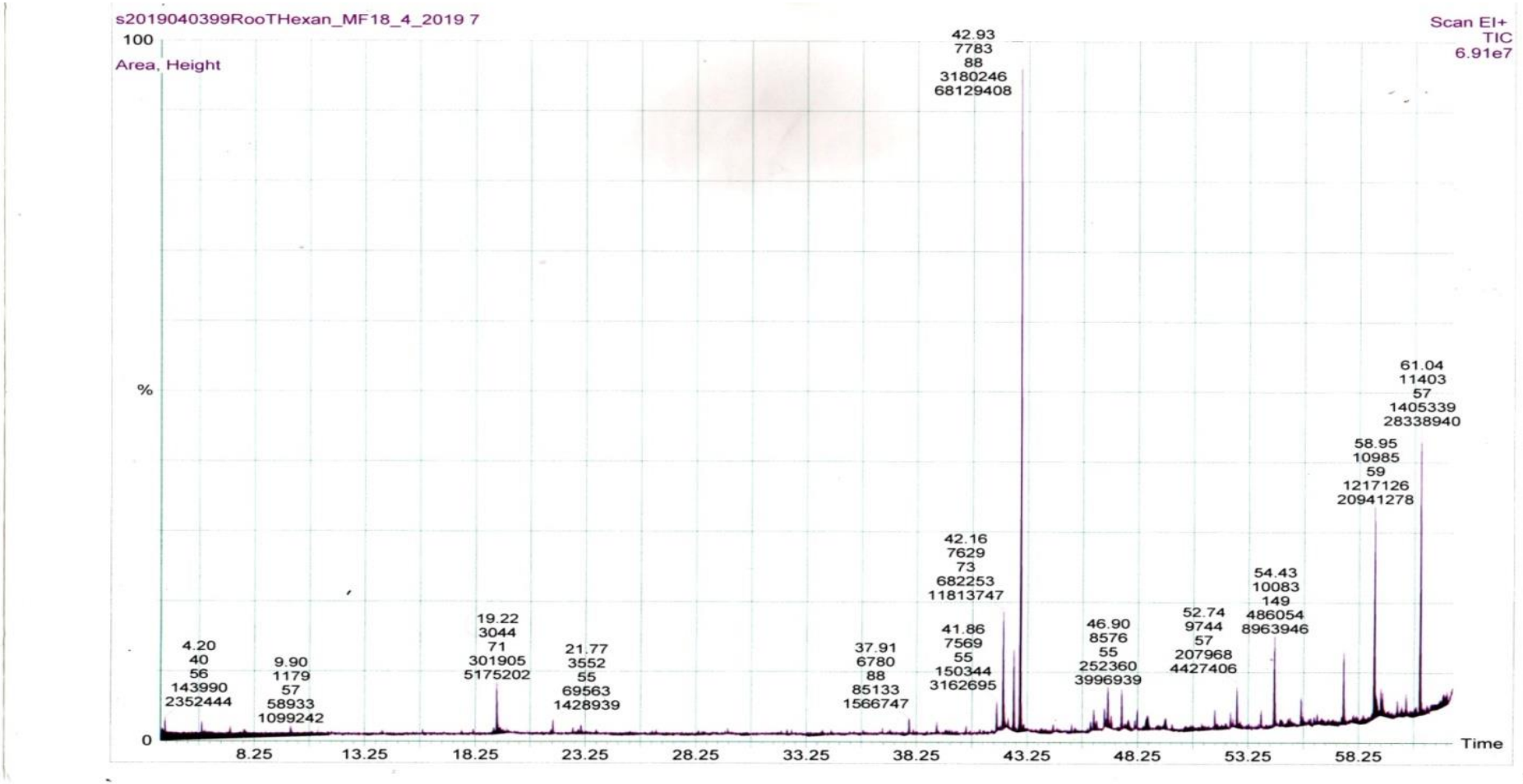

Fig. (3.18): Trace GC-MS Chromatogram of the hexane root extract of *Micromeria Fruticosa* plant.

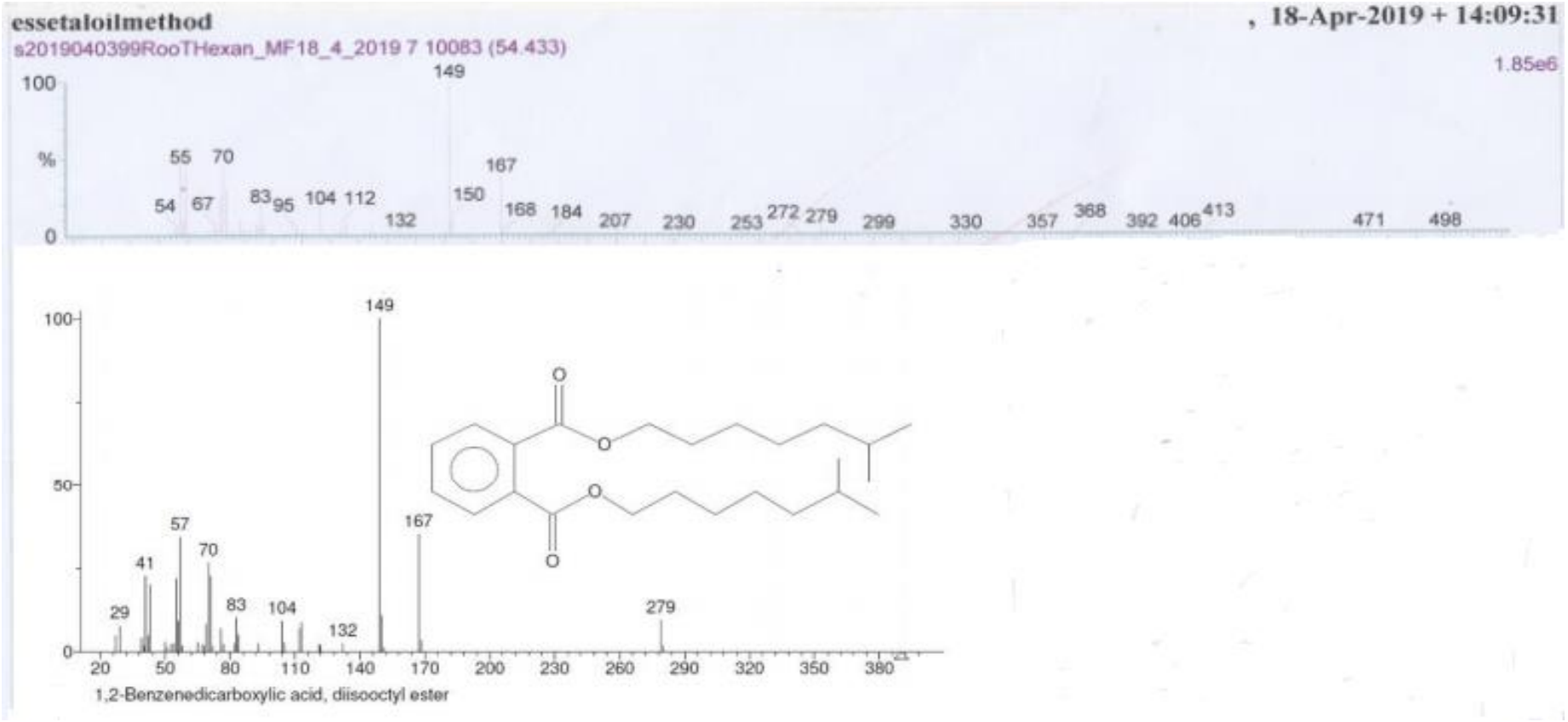

Fig. (3.19): Trace GC-MS Chromatogram spectrum of the hexane root extract of *Micromeria Fruticosa* plant at the R.T. (54.433) minute and the fragment for 1, 2-BENZENEDICARBOXYLIC ACID, DIISOOCTYL ESTER compound.

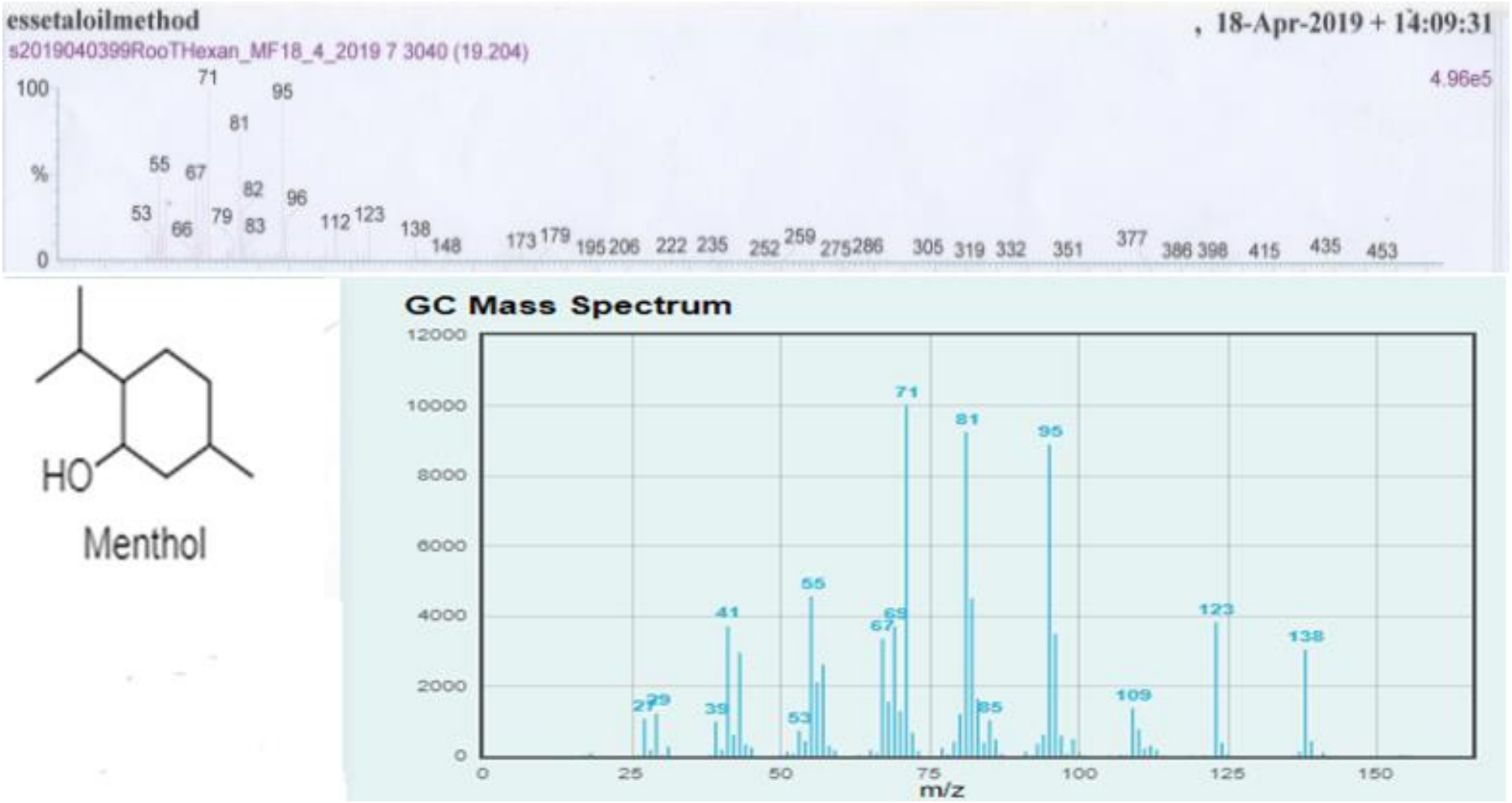

Fig. (3.20): Trace GC-MS Chromatogram spectrum of the hexane root extract of Micromeria Fruticosa plant at the R.T. (19.204) minute and the fragment for Menthol compound.

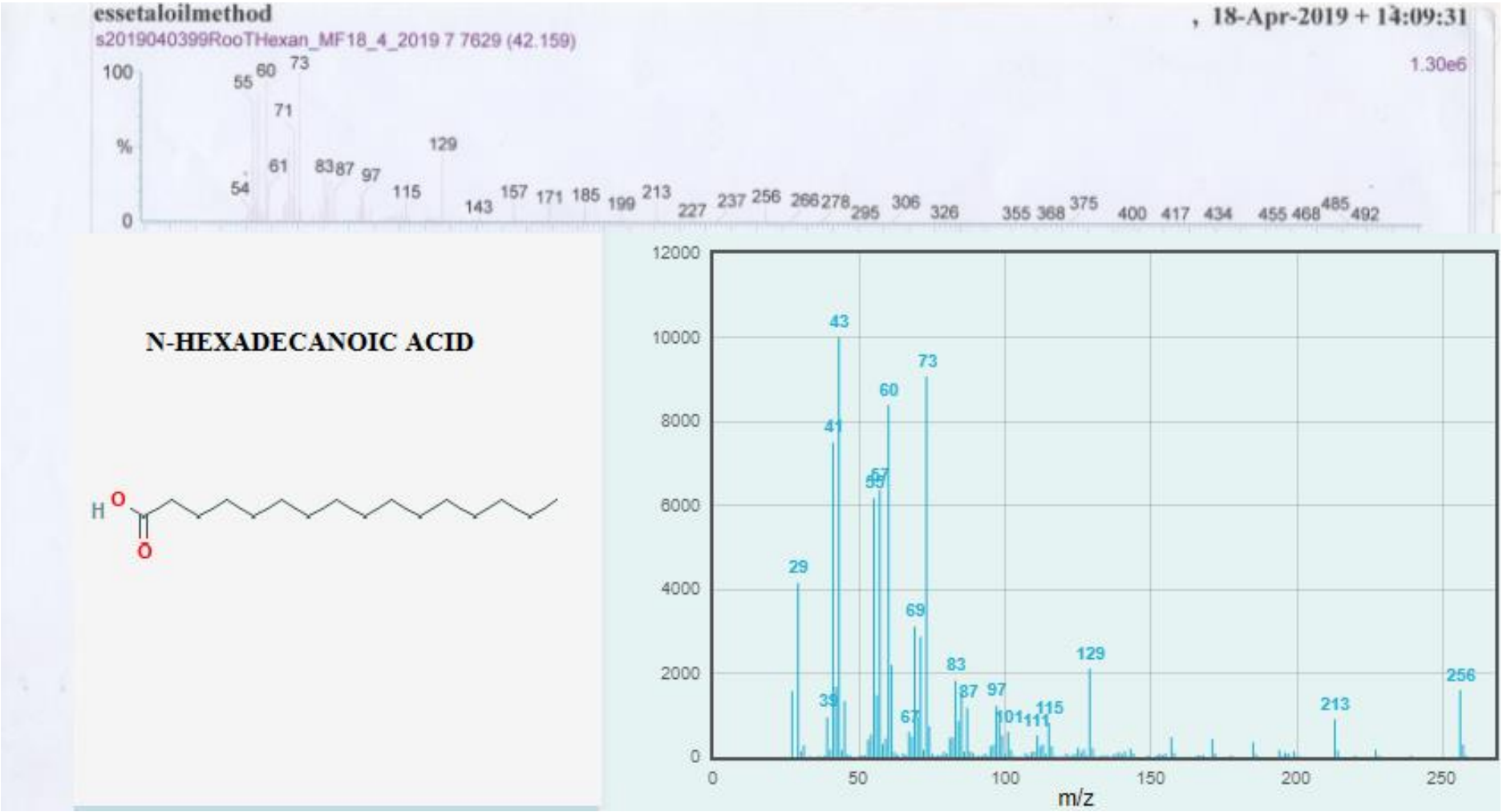

Fig. (3.21) Trace GC-MS Chromatogram spectrum of the hexane root extract of Micromeria Fruticosa plant at the R.T. (42.159) minute and the fragment for N-hexadecanoic acid compound.

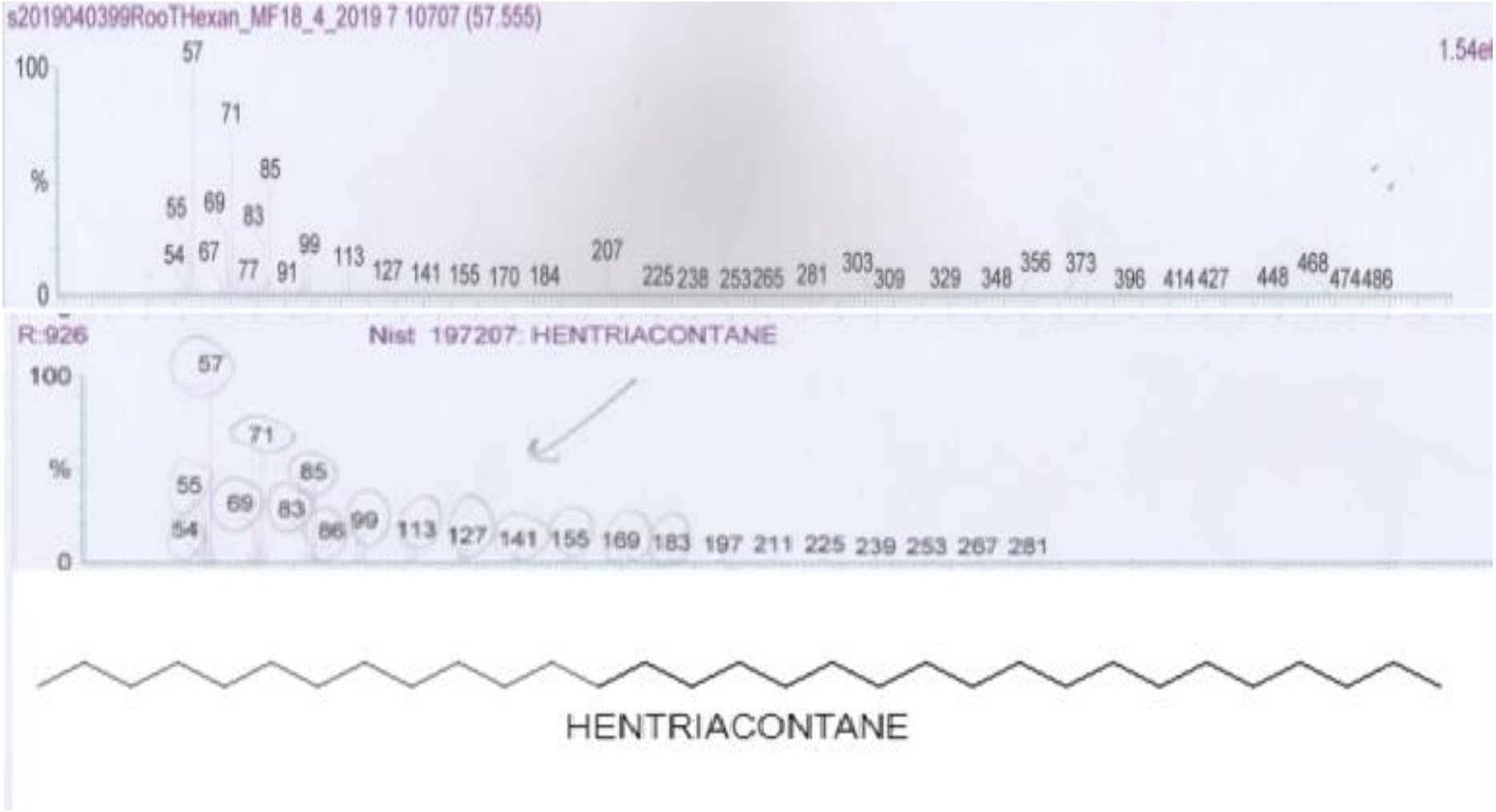

Fig. (3.22): Trace GC-MS Chromatogram spectrum of the hexane root extract of Micromeria Fruticosa plant at the R.T. (57.555) minute and the fragment for HENTRIACONTANE compound.

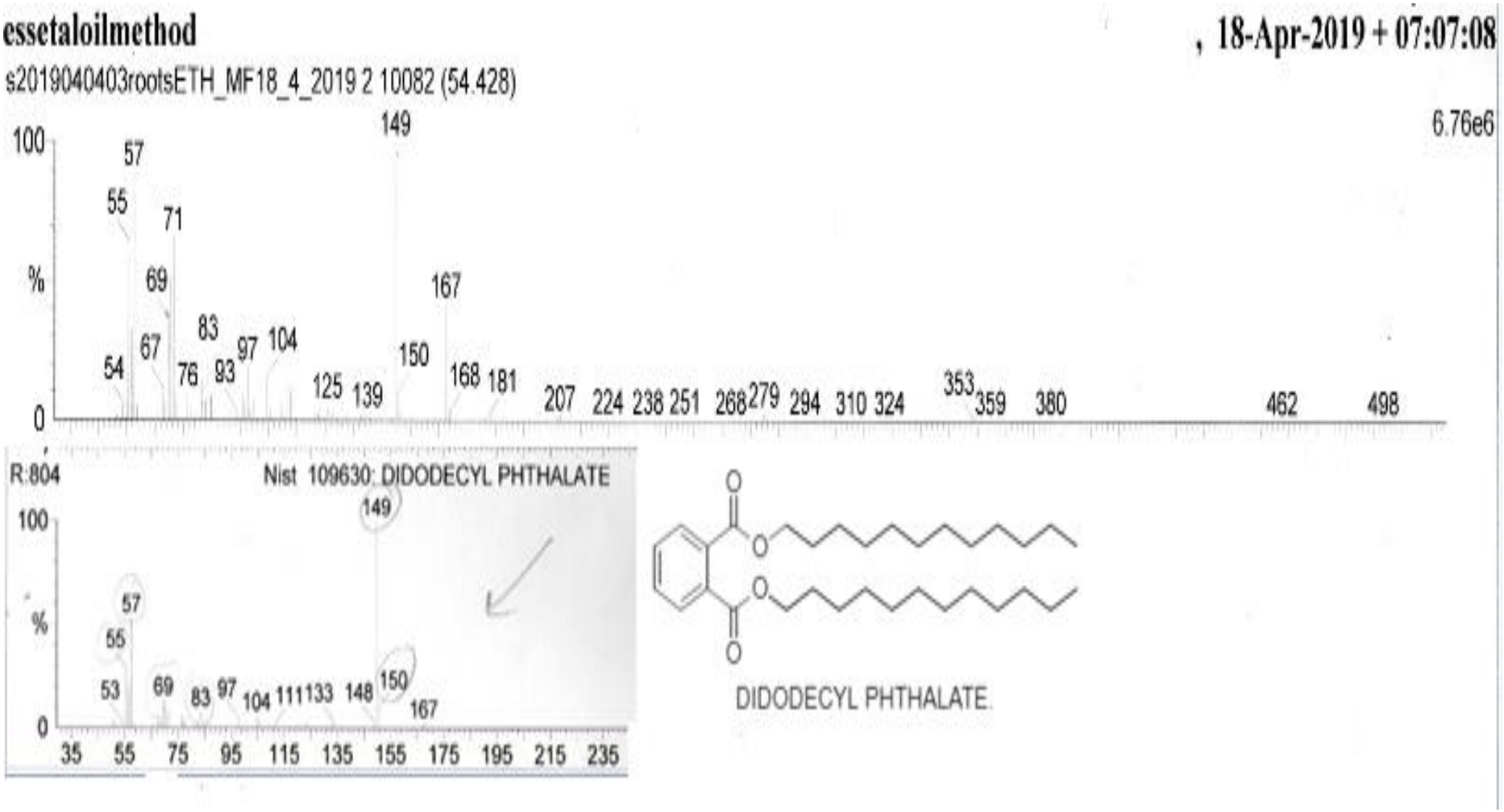

Fig. (3.23):Trace GC-MS Chromatogram spectrum of the ethanol root extract of Micromeria Fruticosa plant at the R.T. (54.428) minute and the fragment for DIDODECYL PHTHALATE compound.

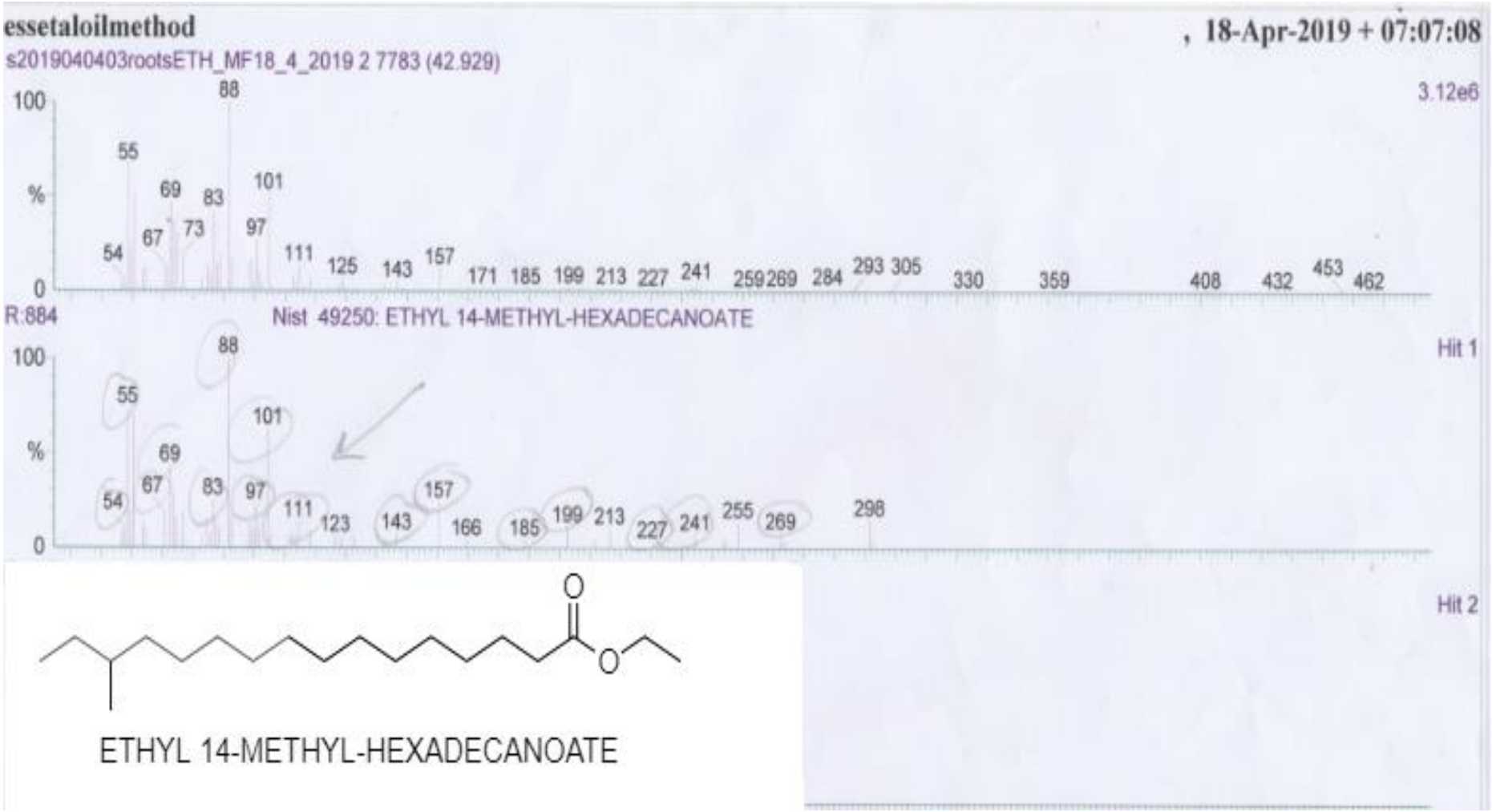

Fig. (3.24): Trace GC-MS Chromatogram spectrum of the ethanol root extract of Micromeria fruticosa plant at the R.T. (42.929) minute and the fragment for ETHYL 14-METHYL-HEXADECAOATE compound.

Chapter Four

ICPMS (Inductively coupled plasma mass spectrometry) Analysis

4.1 Sample preparation for ICPMS-Analysis

A 50 mg of aqueous plant extract was dissolved in 50 ml de-ionized distilled water.

4.2 ICPMS techniques

ICP-MS is used for quantification and qualification measurements of heavy metal in samples. Samples are decomposed to neutral elements in high-temperature argon plasma and analyzed based on their masse to charge ratios using a mass spectrometer system.

Elements can be analyzed at the parts-per-million (PPM) to parts-per-trillion (PPT) concentration levels. ICP-MS is also capable of monitoring isotopic specification for the ions of choice.

4.3 Results and Discussion for ICPMS ANALYSIS

The elements shown in Table 4.1. were present at high concentration in leaves aqueous extract. These elements were Fe, Zn, Mn, and Sr (346, 85.8, 81.7, and 67.7 ppb) respectively.

However, in stem aqueous extract, only Fe and Zn (165 and 77 ppb) had a high concentration in this part of the plant as shown in Table 4.2.

Table (4.1): The concentration of various elements from leave aqueous extract using ICPMS spectrometer.

Extract type	Analyte	Conc. (ppb)	Dilution factor
Leave D.W	Ag	0.286	286
	Al	33.514	33514
	Ba-1	21.494	21494
	Cd	0.067	67
	Cr	7.783	7783
	Co	0.344	344
	Cs	0.020	20
	Cu	8.969	8969
	Fe	346.751	346751
	Ga	0.174	174
	Li	1.305	1305
	Mn	81.700	81700
	Mo	1.245	1245
	Ni	10.784	10784
	Pb	1.066	1066
	Rb	13.949	13949
	Sr	67.704	67704
	V	0.181	181
	Zn	85.881	85881

Table (4.2): The concentration of various elements from stem aqueous extract using ICPMS spectrometer

Extract type	Analyte	Conc. (ppb)	Dilution factor
Stem D.W	Ag	0.043	43
	Al	17.991	17991
	Ba-1	14.663	14663
	Cd	0.257	257
	Cr	4.977	4977
	Co	0.194	194
	Cs	0.041	41
	Cu	6.882	6882
	Fe	165.348	165348
	Ga	0.115	115
	Li	1.006	1006
	Mn	23.311	23311
	Mo	0.607	607
	Ni	3.492	3492
	Pb	1.494	1494
	Rb	14.384	14384
	Sr	23.018	23018
	V	0.095	95
	Zn	77.144	77144

In Table 4.3 high concentration of elements from flower aqueous extract were Fe, Zn and Sr (238, 58, 38.9 ppb) respectively.

As shown in Table 4.4, Fe and Al (228 and 65 ppb) had the highest concentration in root aqueous extract of the plant.

Table (4.3): The concentration of various elements from flower aqueous extract using ICPMS spectrometer

Extract type	Analyte	Conc. (ppb)	Dilution factor
Flower D.W	Ag	0.145	145
	Al	27.264	27264
	Ba-1	15.640	15640
	Bi	0.021	21
	Cd	0.086	86
	Cr	5.891	5891
	Co	0.441	441
	Cs	0.062	62
	Cu	18.716	18716
	Fe	238.593	238593
	Ga	0.123	123
	Li	0.859	859
	Mn	43.494	43494
	Mo	0.305	305
	Ni	9.320	9320
	Pb	0.908	908
		Rb	
	Sr	38.965	38965
	V	0.091	91
	Zn	58.116	58116

***D.W: distilled water, Conc.: Concentration (PPb).**

Table (4.4): The concentration of various elements from root aqueous extract using ICPMS spectrometer

Extract type	Analyte	Conc. (ppb)	Dilution factor
Root D.W	Ag	0.204	204
	Al	65.201	65201
	Ba-1	19.218	19218
	Bi	0.021	21
	Cd	0.136	136
	Cr	6.972	6972
	Co	1.643	1643
	Cs	0.024	24
	Cu	11.910	11910
	Fe	228.406	228406
	Ga	0.174	174
	Li	0.496	496
	Mn	48.519	48519
	Mo	1.102	1102
	Ni	6.977	6977
	Pb	2.270	2270
	Rb	12.532	12532
	Sr	25.692	25692
	Continue to Table 4.4		
	V	1.170	1170
	Zn	25.926	25926

***D.W: distilled water, Conc.: Concentration (PPb).**

Chapter Five

Biological activities

5.1 Antimicrobial and antifungal activity tests

5.1.1 Preparation of samples for testing

Organic plant extracts (ethanol and hexane extracts) were dissolved in sterile 10% Dimethyl sulfoxide (DMSO) to obtain a concentration of 100 mg/ml, while aqueous extracts were dissolved in sterile distilled water to obtain a concentration of 100mg/ml. The dissolved extracts stored at 4°C for further assays.

5.1.2 Media and Solutions

5.1.2.1. Nutrient Broth

Nutrient broth (ACUMEDIA) was prepared according to manufacturer's instructions labeled on the bottle. 2.0 g of nutrient broth medium was dissolved in 250 ml de-ionized water. The broth was then distributed into tubes to have 5-10 ml each and plugged with cotton. The tubes were autoclaved at 121°C for 15 minutes, and allowed to cool to room temperature, then stored in refrigerate.

5.1.2.2. Mueller-Hinton Broth

Mueller-Hinton broth (Hi Media Laboratories) was prepared according to manufacturer's instructions labeled on the bottle. 5.25 g of

Mueller –Hinton Broth medium was dissolved in 250 ml de-ionized water. The broth was then distributed into tubes to have 5-10 ml each and plugged with cotton. The tubes were autoclaved at 121°C for 15 minutes, allowed to cool and then kept in refrigerator at 4-6°C.

5.1.2.3. Mueller Hinton Agar (MHA)

Mueller Hinton agar (BD) was prepared according to manufacturer's instructions labeled on the bottle. 19 g of Mueller –Hinton Agar medium was dissolved in 0.5 L de-ionized water. The solution allowed to boil for 1 minute, and then autoclaved at 121°C for 15 minutes. After that it was allowed to cool to about 55°C, and the media was poured into sterile Petri dishes to have (25-30) ml each, then the plates were left overnight at room temperature. The following morning the Petri dishes were turned upside down and kept in refrigerator at 4-6°C.

5.1.2.4. Sabouraud Dextrose Agar

Sabouraud dextrose agar (Hi Media Labratories) was prepared according to manufacturer's instructions labeled on the bottle. 16.25 g of Sabouraud Dextrose Agar medium was dissolved in 0.25 L de-ionized water. The solution allowed to boil for 1 minute, and then autoclaved at 121°C for 15 minutes. After that it was allowed to cool to about 55°C, and the medium was poured into sterile Petri dishes to have (25-30) ml each, then, the plates were left overnight at room temperature. The following

morning the Petri dishes were turned upside down and kept in refrigerator at 4-6°C.

5.1.2.5. Normal Saline (0.9% NaCl)

Normal saline solution (0.9% NaCl, MWt 58.44) was prepared by dissolving 2.25 g NaCl in 250 ml de-ionized water. The saline solution was then distributed into tubes to have 5-10 ml each and plugged with cotton. The tubes were autoclaved at 121°C for 15 min, allowed to cool and then kept in refrigerator at 4-6°C.

5.1.2.6. Preparation of McFarland turbidity standard No. 0.5

McFarland 0.5 turbidity standard was prepared by mixing 50 µl of a 1.175% (w/v) barium chloride dihydrate ($BaCl_2 \bullet 2H_2O$) solution and 9.95 ml of 1% (v/v) sulfuric acid. The tube which had the 0.5 McFarland standard was then sealed with parafilm to prevent evaporation and stored in the dark at room temperature. The 0.5 McFarland standard was vigorously mixed on a vortex mixer before use. As with the barium sulfate standards, a 0.5 McFarland standard is comparable to a bacterial suspension of 1-5 x 10^8 colony-forming units (CFU)/ml (Andrews, 2006) [54] or 0.5 McFarland standard is comparable to Candida albicans suspension of 1-5 x 10^6 yeast cells/mL (Branda and Kratz, 2006) [55].

Three to four colonies of each bacteria were transferred into tubes had 5.0-10 mL of sterile normal saline, the turbidity of the bacterial suspensions was adjusted to have similar turbidity of 0.5 McFarland

standard with bacterial suspension of about 1.5 x10^8 cfu/mL. Few colonies from C. albicans sub-cultured on Sabouraud Dextrose Agar were transferred into tube had 5.0-10 mL of sterile normal saline. The turbidity of the yeast C. albicans suspension of about 1-5 x 10^6 yeast cells/mL.

5.1.3. Test Microorganisms

Microorganisms used in this study represent pathogenic species commonly associated with infections. The microorganisms were stored in the Microbiology research laboratory at An-Najah National University, Nablus-Palestine. These microorganisms consisted of 2 Gram-positive strains *Staphylococcus aureus* (S. *aureus*) (ATCC 25923), clinical isolate of methicillin resistant *staphylococcus aureus* (MRSA), two Gram-negative strains, *Shigellasonnie* (*S. sonnie*) (ATCC 25931) and multidrug clinical *Escherichia coli* (*E. coli*) isolate and one yeast strain *Candida albicans* (*C. albicans*) (ATCC 90028). All the bacterial strains were subcultured on Mueller-Hinton agar while *C. albicans* was sub-cultured on Sabouraud Dextrose Agar.

5.1.4 Determination of Minimum Inhibitory concentration (MIC)

5.1.4.1Determination of Minimum Inhibitory concentration (MIC) against bacteria

Minimum Inhibitory concentration (MIC) of organic plant extracts (ethanol and hexane) and aqueous plant extracts was determined by the broth microdilution method in sterile 96- wells microtiter plates according

to standard method described previously by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2017). The organic plant extracts and aqueous plant extracts were dissolved in sterile 10% DMSO and sterile distilled water, respectively, to a final concentration 100mg/ml. Both extracts organic and water and 10% DMSO (negative control) were two-fold-serially diluted in nutrient broth in the wells of the plates in a final volume of 100µL. After that, a bacterial inoculum size of 10^5 CFU/ml was added to each well. Other negative control wells containing either 100µL nutrient broth only, or organic plant extracts (or aqueous plant extracts) and nutrient broths without bacteria were included in these experiments. Each plant extract was run in duplicate. The microtiter plates were then covered and incubated at 37°C for 24 hours. The MIC was considered as the lowest concentration of the plant extract which inhibited the bacterial growth.

5.1.4.2 Determination of Minimum Inhibitory concentration (MIC) against yeast

Minimum Inhibitory concentration (MIC) of organic plant extracts (ethanol and hexane extracts) and aqueous plant extracts was determined by the broth microdilution method in sterile 96- wells microtiter plates according to standard method described previously (CLSI, 2017) [56]. The organic plant extracts and aqueous plant extracts were dissolved in sterile 10% DMSO and sterile distilled water, respectively, to a final concentration 100mg/ml. Both extracts organic and water and 10% DMSO (negative control) were two-fold-serially diluted in Mueller-Hinton broth in

the wells of the plates in a final volume of 100µL. After that, a *C. albicans* inoculum size of0.5×10^5 to 2.5×10^5 CFU/ml was added to each well. Other negative control wells containing either 100µL Mueller-Hinton broth only, or organic plant extracts (or aqueous plant extracts) and Mueller-Hinton broth without bacteria were included in these experiments. Each plant extract was run in duplicate. The microtiter plates were then covered and incubated at 37°C for 48 h. The MIC was considered as the lowest concentration of the plant extract which inhibited the yeast growth.

5.2 Results and Discussion for biological activities

The antibacterial activities of the extracts obtained from the *Micromeria Fruticosa* under study by the broth microdilution method against different pathogens are shown in Table 5.1 and Figures 5.2, 5.3, 5.4, and 5.5.

Table (5.1): Minimum Inhibitory concentration values (µg/ml) for different *Micromeria Fruticosa* extract types against different pathogens

Microorganism	Type of extract											
	Leave extract			Flower extract			Stem extract			Root extract		
	W	E	H	W	E	H	W	E	H	W	E	H
	MIC (µg/ml)											
E. coli	12.5	0.78	100	12.5	12.5	50	0.78	25	100	12.5	100	100
***S.sonnie* (ATCC 25931)**	3.125	6.25	50	1.56	6.25	50	1.56	100	100	3.125	25	100
MRSA	6.25	12.5	50	3.125	6.25	25	1.56	100	100	6.25	25	100
***S. aureus* (ATCC 25923)**	3.125	6.25	50	12.5	3.125	50	1.56	25	100	6.25	6.25	100
***C. albicans* (ATCC 90028)**	100	3.125	50	100	50	25	100	25	100	100	25	100

*** W: aqueous extract, E: ethanol extract, H: hexane extract**

Results of the current study showed that aqueous leaf extract had the highest antimicrobial activity against *S. sonnie* (ATCC 25931) and *S. aureus* (ATCC 25923), while the ethanolic leaf extract had the highest antimicrobial and antifungal activity against *E. coli* and *C. albicans* (ATCC 90028). Minimum Inhibitory concentration of different leaf extract types against different pathogens are presented in Figure 5.1.

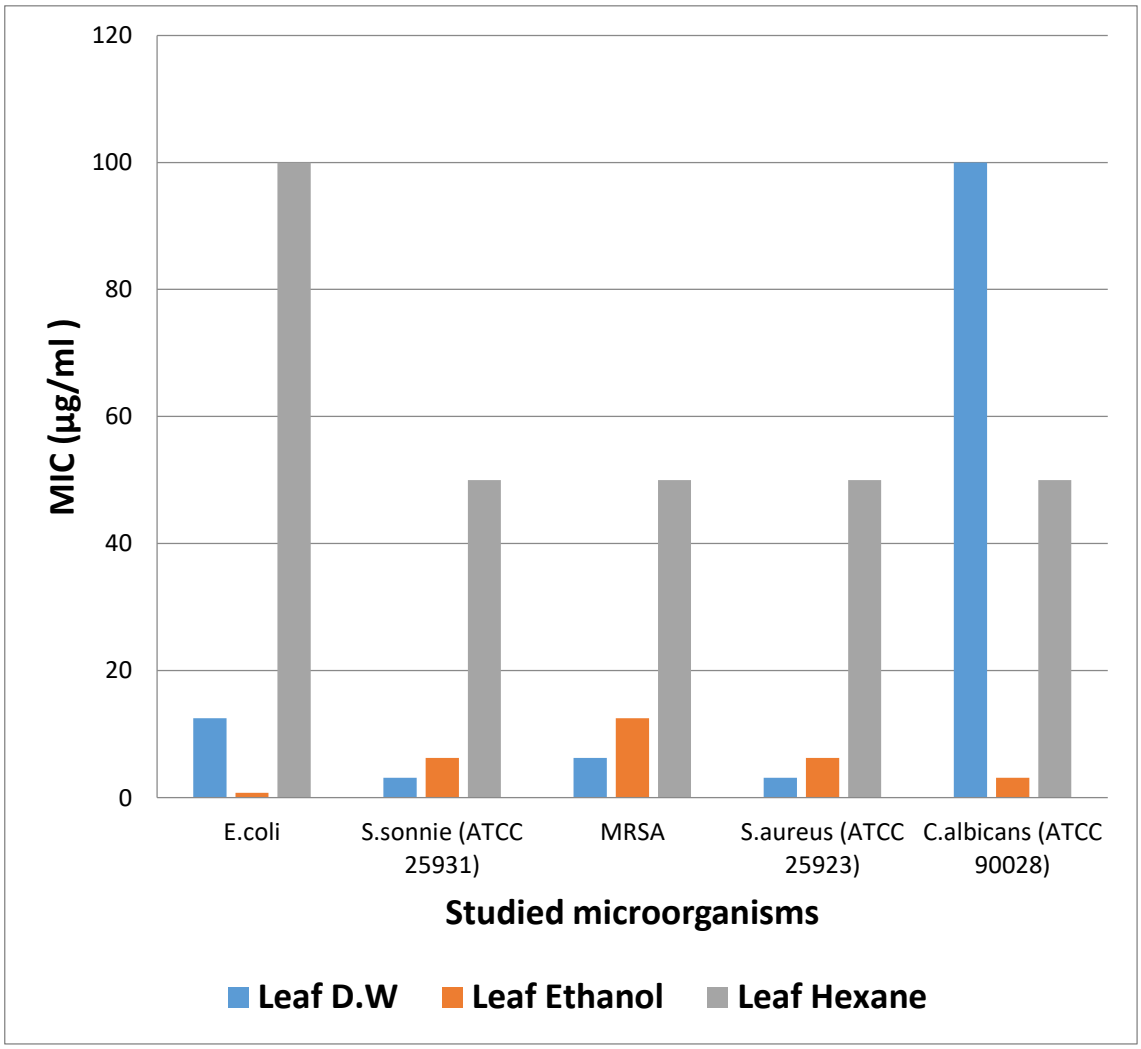

Figure (5.1): Minimum Inhibitory concentration (µg/ml) of different leave extract types against different pathogens.

Results of this study showed that aqueous flower extract had the highest antimicrobial activity against *S. sonnie* (ATCC 25931) and MRSA, while ethanolic flower extract had the highest antimicrobial activity against *S. aureus* (ATCC 25923). Hexane flower extract had the highest ativity against *C. albicans* (ATCC 90028) compared with aqueous and ethanolic flower extracts. Minimum Inhibitory concentration of different flower extract types against different pathogens are presented in Figure 5.2.

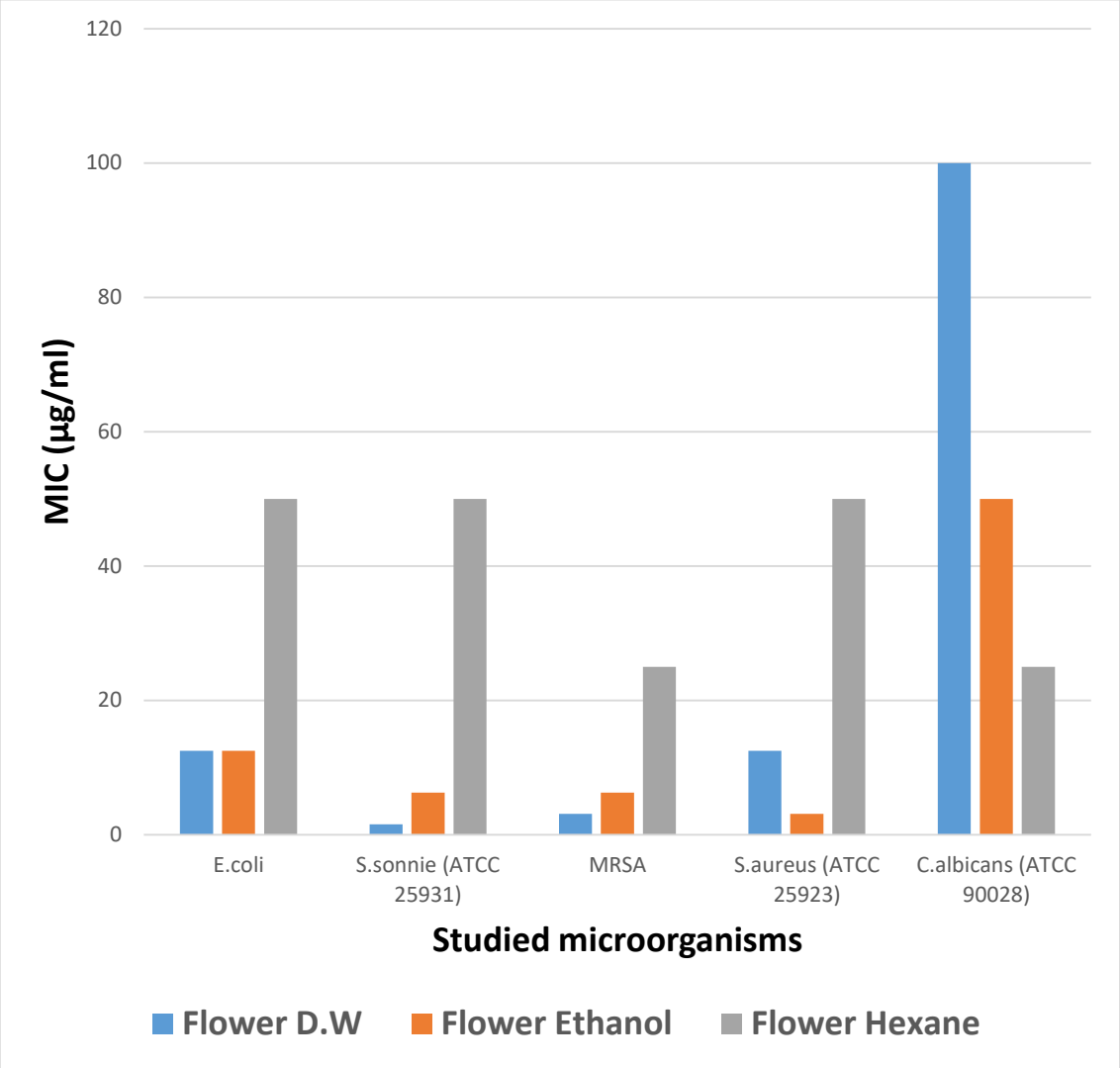

Figure (5.2): Minimum Inhibitory concentration (µg/ml) of different flower extract types against different pathogens.

Results of the current study showed that aqueous stem extract had the highest antimicrobial activity against both Gram-positive (*S. aureus* (ATCC 25923) and MRSA) and Gram-negative bacteria (*S. sonnie* (ATCC 25931) and *E. coli*). Hexane stem extract had the highest activity against *C. albicans* (ATCC 90028) compared with aqueous and ethanolic stem extracts. Minimum Inhibitory concentration of different stem extract types against different pathogens are presented in Figure 5.3.

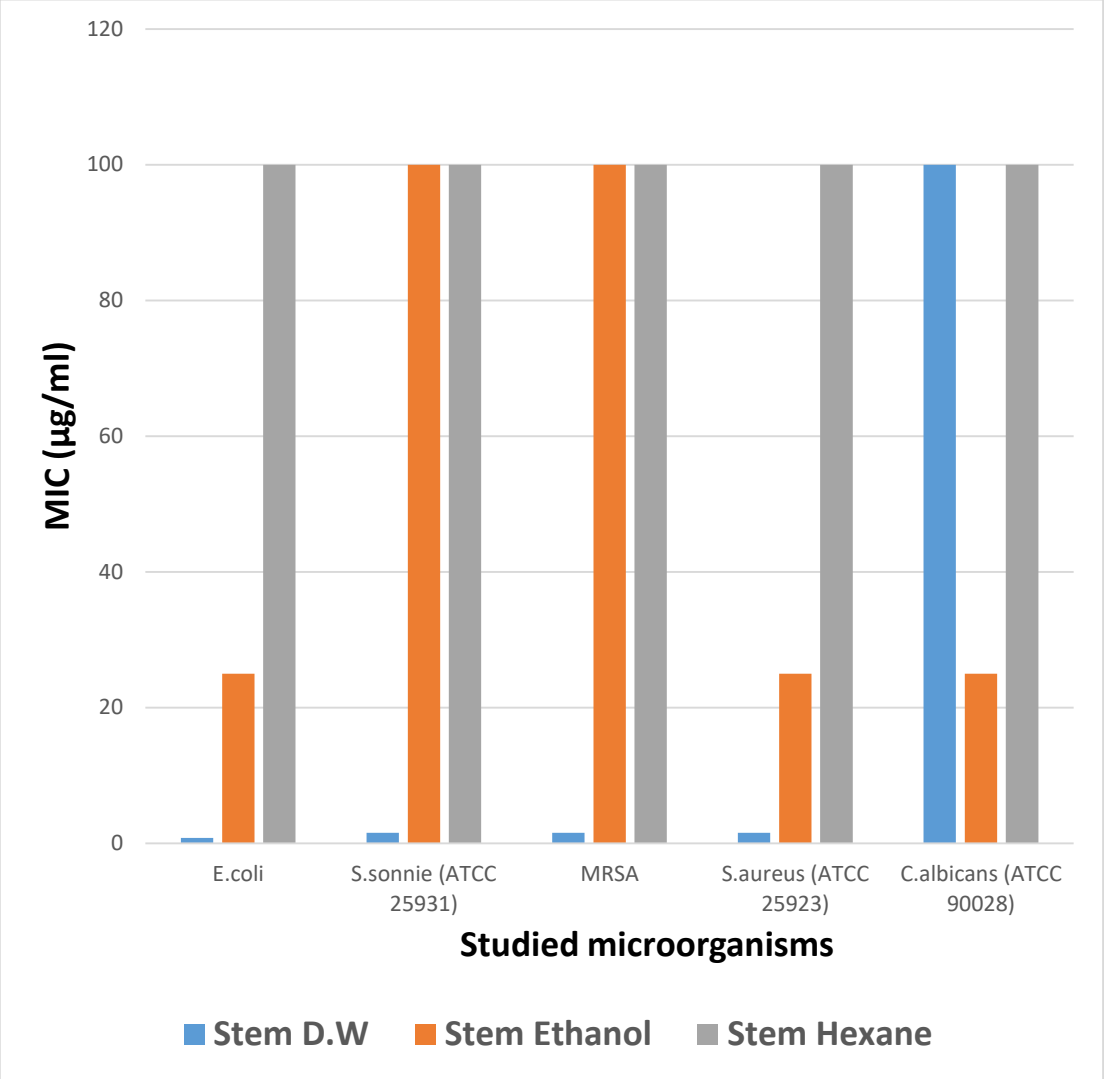

Figure (5.3): Minimum Inhibitory concentration (µg/ml) of different stem extract types against different pathogens.

Results of the current study showed that aqueous root extract had the highest antibacterial activity against both Gram-positive (*S. aureus* (ATCC 25923) and MRSA) and Gram-negative bacteria *S. sonnie* (ATCC 25931). Ethanol root extract had the highest activity against *C. albicans* (ATCC 90028) compared with aqueous and haxane root extracts. Minimum Inhibitory concentration of different root extract types against different pathogens are presented in Figure 5.4.

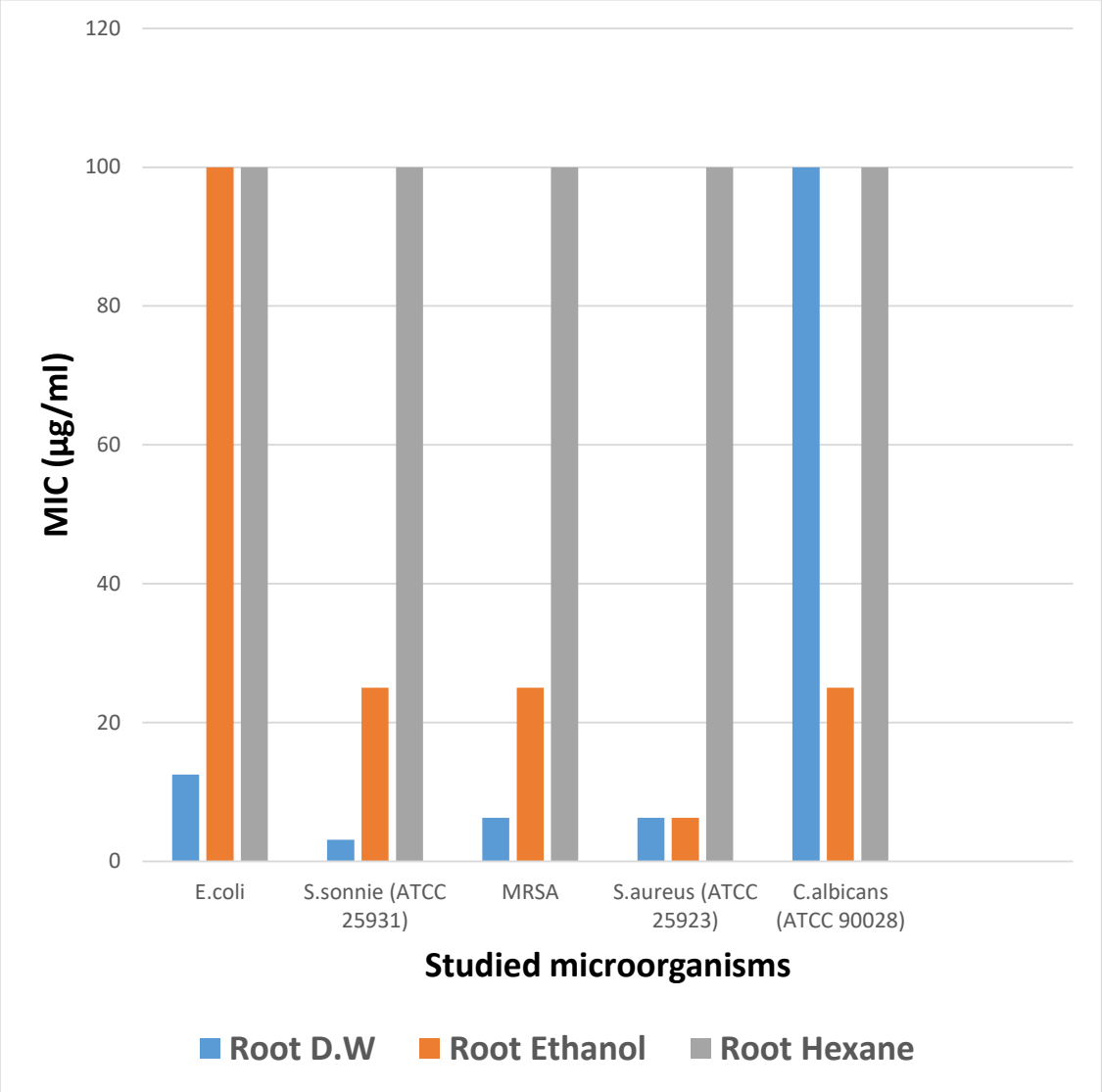

Figure (5.4): Minimum Inhibitory concentration (µg/ml) of different root extract types against different pathogens.

Chapter six

Anti-oxidant activity (free radical scavenging activity)

6.1. General procedure of anti-oxidant test for *Micromeria Fruticosa* plant extract.

The hydrogen atom or electron donation abilities of the corresponding compounds were measured from the bleaching of the purple-colored methanolic solution of DPPH (1, 1- Diphenyl -2-picryl-hydrazyl). This spectrophotometric assay uses the stable radical DPPH as a reagent.

A stock solution of a concentration of 1mg/ml in methanol was initially prepared for plant extract. Stock solutions were used to prepare working solutions with the following concentrations (2, 5, 10, 30, 50, 80, 100μg/ml) by using serial dilution in methanol.

A solution of DPPH was freshly prepared at a concentration of 0.002% w/v. Then, it was mixed with methanol along with each of the working concentration in ratio of 1:1:1.

The spectrophotometer was zeroed using methanol as a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated at room temperature in a dark cabinet for about 30 minutes. Then, their optical densities were determined by using the spectrophotometer at a wavelength of 517nm.

The percentage of antioxidant activity of plant extract were calculated the following formula:

DPPH inhibition activity (I %) = $(A_{blank}-A_{sample})/A_{blank}*100\%$.

6.2. Results and discussion

Values of %Inhibition for the *Micromeria Frtcosa* plant extract shown in Table 6.1. In D.W and Hexane extract the leaf had high %Inhibition at low concentration while root extract had minimum %Inhibition. But in ethanol extract the root had high %Inhibition at low concentration. As shown in Figures 6.1, 6.2 and 6.3.

Table 6.1: %Inhibition and IC50 for D.W, Ethanolic and Hexane extracts.

Conc.	%Inhibition for Different extracts											
Extract type	D.W from leaf	D.W from root	D.W from stem	D.W from flower	Hexane from leaf	Hexane from root	Hexane from stem	Hexane from flower	Ethanol from leaf	Ethanol from root	Ethanol from stem	Ethanol from flower
2	18.35	1.56	13.39	10.98	44.08	25.72	32.24	28.26	10.86	44.56	0	44.2
5	26.68	1.56	18.96	13.28	49.27	27.29	32.24	29.22	11.59	44.56	0	44.32
10	30.67	6.51	23.06	21.613	49.27	28.13	35.14	29.46	11.59	45.65	5.79	44.32
30	50.23	10.5	35.15	40.81	49.27	28.74	35.14	31.88	11.59	51.8	11.955	49.63
50	64.84	15.45	52.65	54.22	51.811	31.15	39.85	31.88	11.83	51.811	23.18	54.71
80	82.36	24.4	65.57	76.81	53.742	32.96	39.85	34.05	13.16	52	46.13	60.38
100	89.73	34.05	71.13	80.07	63.768	32.96	39.85	35.86	13.88	52.17	60.09	67.38

.* D.W: aqueous extract.

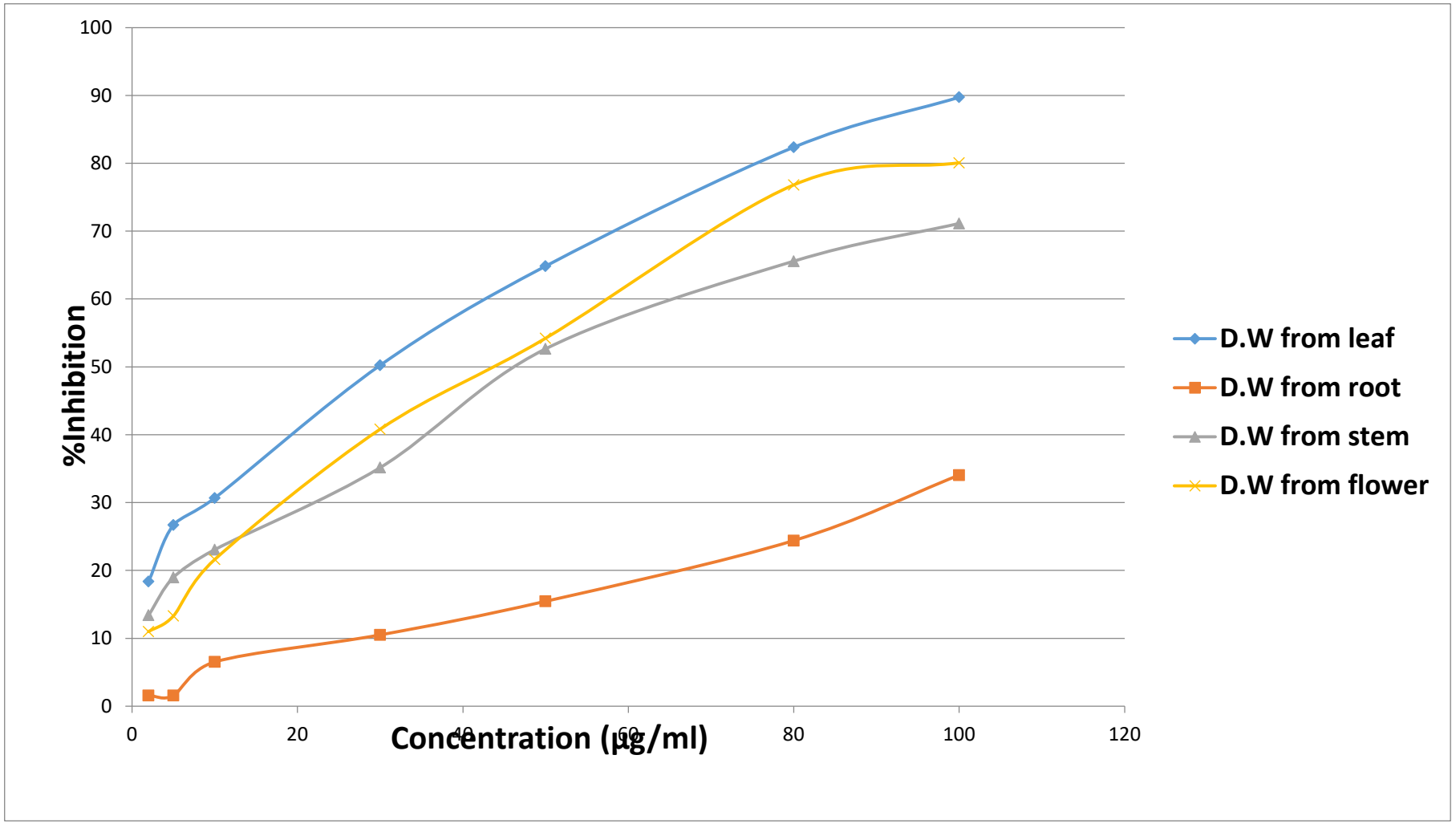

Figure (6.1): Anti-oxidant % Inhibition concentration (µg/ml) of different D.W extract types.

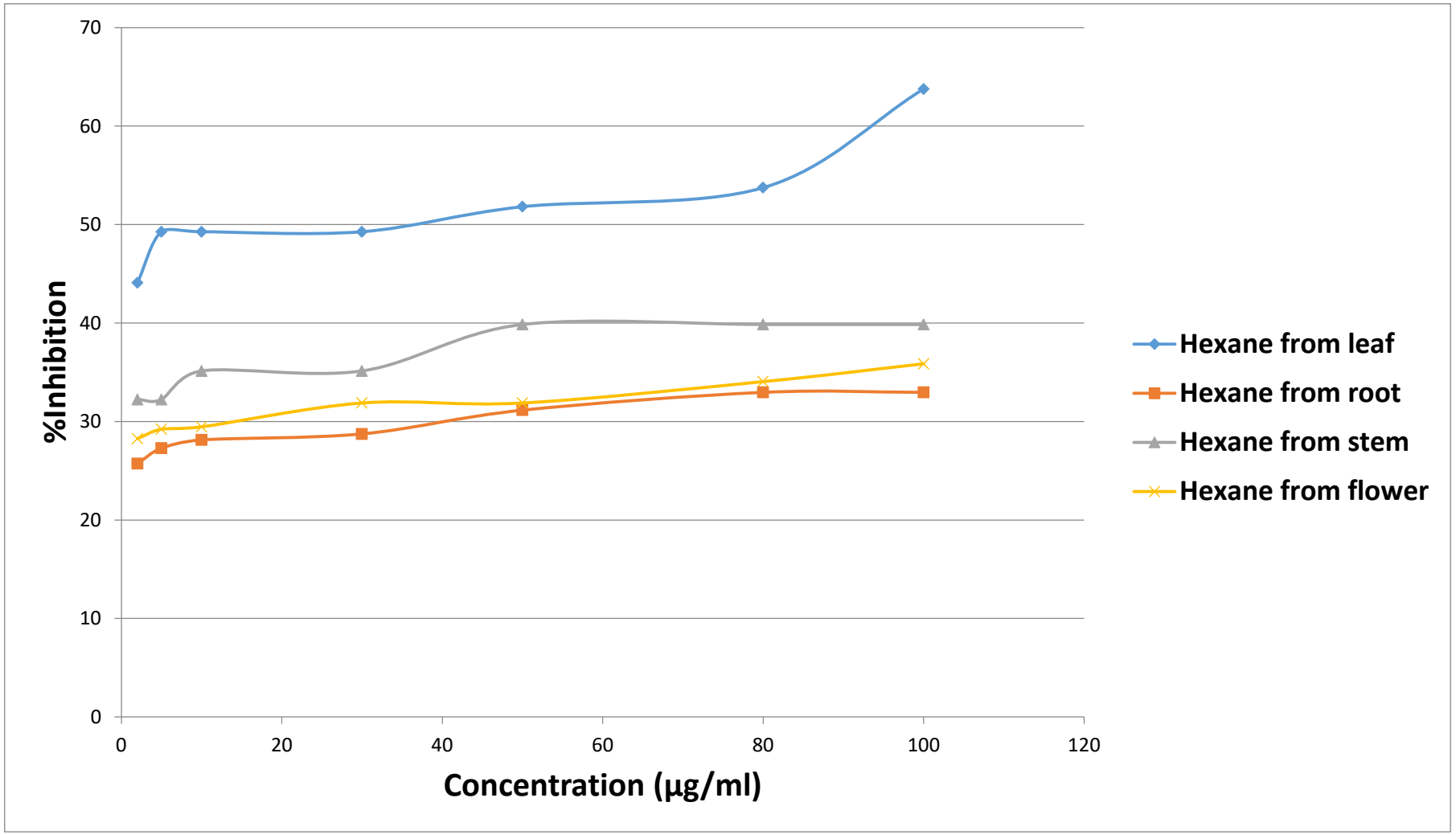

Figure (6.2): Anti-oxidant % Inhibition concentration (µg/ml) of different Hexane extract types.

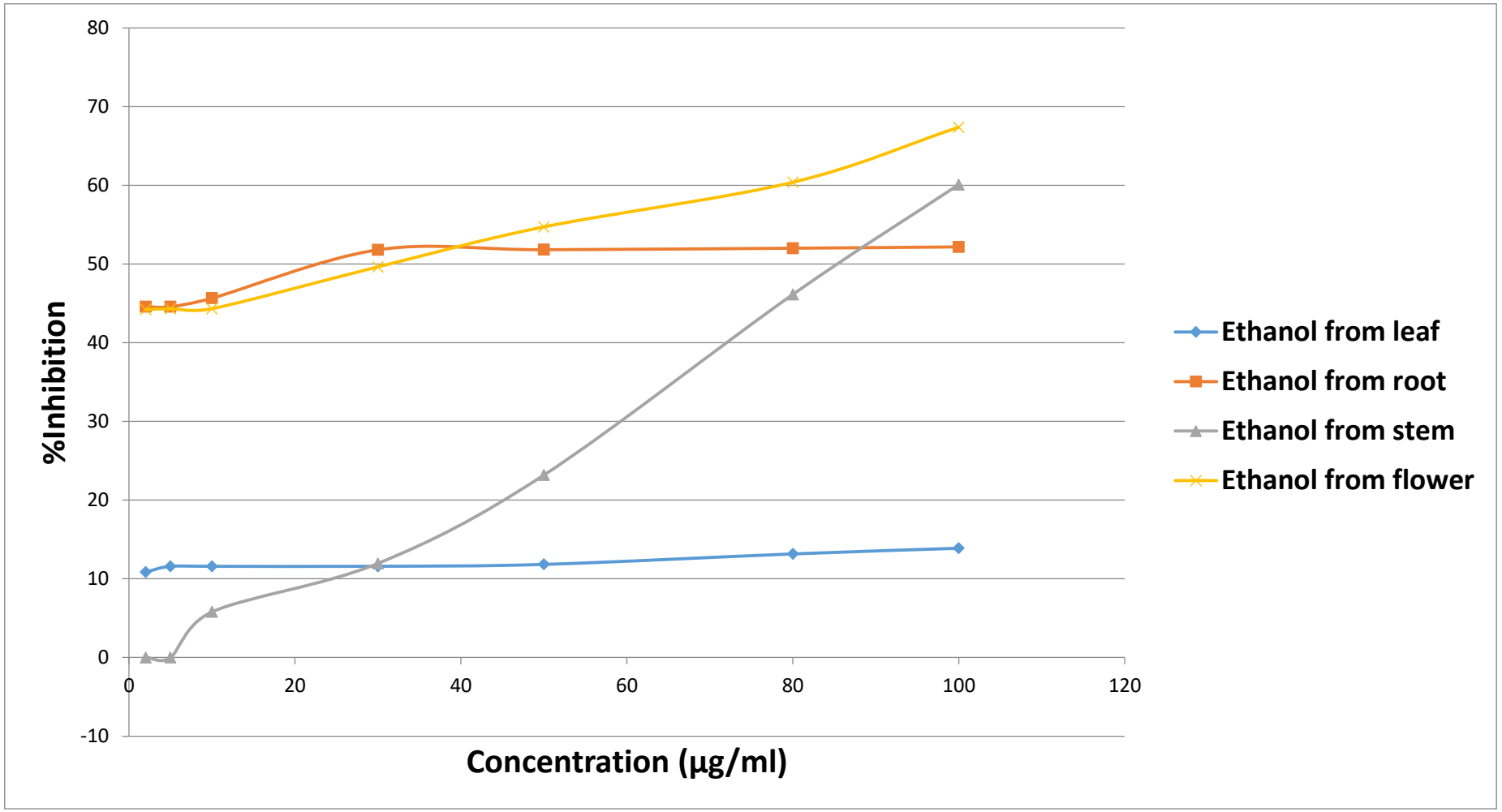

Figure (6.3): Anti-oxidant % Inhibition concentratio (µg/ml) of different Ethanol extract types.

Chapter Seven

Conclusion

Antifungal test:

Ethanolic leave extract showed complete inhibition against C.albicans (ATCC 90028) at 3.125 µg/ml.

Antibacterial test:

Ethanolic leave extract and aqueous stem extract showed complete inhibition against E.coli at 0.78 µg/ml.

Aqueous flower extract and aqueous stem extract showed complete inhibition against S.sonnie (ATCC 25931) at 1.56 µg/ml.

Aqueous stem extract showed complete inhibition against S.aureus (ATCC 25923) and MRSA at 1.56 µg/ml.

Antioxidant test:

In aqueous and hexane extracts the leaf showed good inhibition at low concentration 2µg/ml.

Ethanolic root extract is useful showed high inhibition 44.56% at 2µg/ml.

GC-MS/MS analysis:

Some constituents were detected from ethanolic and hexane plants extracts using GC-MS/MS spectrophotometer and separated by flash

chromatography the most similar compound found in all part in plant is 9-OCTADECENAMIDE, (Z)-, Menthol and CYCLOHEXANONE,5-METHYL-2-(1-METHYLETHYL)-, TRANS with different high, and area percentage. These compounds have strong anti-inflammatory, antibacterial, and antioxidant activities

ICPMS analysis:

Also, some elements were detected from distilled water plant extracts using ICPMS analysis, these elements are Fe, Zn, Sr with different percentage per extract part of plant.

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جامعة النجاح الوطنية
كلية الدراسات العليا

توصيف المواد الكيميائية النباتية النشطة بيولوجيا من نبات الزعتمان (Micromeria fruticosa) كنهج لتطوير ادوية من مصدر طبيعي

إعداد
أنوار عفيف عبد الرحمن الحجة

إشراف
أ. د محمد النوري
د. أبراهيم ابو ريدة

قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في الكيمياء بكلية الدراسات العليا في جامعة النجاح الوطنية، نابلس- فلسطين.

2019

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إعداد

أنوار عفيف عبد الرحمن الحجة

إشراف

أ. د محمد النوري

د. أبراهيم ابو ريدة

الملخص

يهدف هذا البحث الى فحص مستخلصات اربعة اجزاء (اوراق, زهرة, ساق, جذور) من نبتة الزعتمان التي تستخدم في الطب الشعبي في فلسطين لعلاج امراض مختلفة و اجراء بعض الفحوصات الحيوية عليها مثل أثر المستخلصات على أربعة أنواع من البكتيريا ونوع واحد من الفطريات بحث أكدت النتائج المخبرية على أن بعض المستخلصات لها أثر حيوي في بعض الفحوصات التي تمت.

تم جمع هذه النبتة من جبال مدينة طولكرم وتعرف هذه النيتة ايضا بأسم عشبة الشاي في هذه المناطق المستخلصات الناتجة من نقع هذه الاجزاء الاربعة من هذه النيتة بالايثانول والهكسان والماء المقطر تم فحصها ايضا لمدى أثرها في منع

بحيث كانت فعالية المستخلص الذي نقع في الماء المقطر في منع عملية التأكسد (DPPH assay) او وقف عملية التأكسد بأستخدام . تصل الى نسبة 89%.

أما عملية فصل المستخلصات العضوية والتعرف على المركبات الفعالة المكونة لكل جزء تم أستخدام جهاز الكروموتغرافي.

بحيث تم إيجاد مركبات فعالة متشابهة في مكونات النبتة الأربعة. (GC–MS/MS)

للتعرف على المعادن والاملاح الموجودة في المستخلصات المائية(ICPMS) وتم ايضا استخدام جهاز.