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Gas Chromatography–Mass Spectrometry Analysis of Melissa Officinal Leaves Extract and Evaluation of Its Biological Effects

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Abstract

The leaves of the Melissa officinalis plant were collected from the aqra region in northern Iraq Mosul Dam's medicinal plant development initiative is catalogued in the Directorate of Medicinal Plant Development, which is part of the Iraqi Ministry of Agriculture. The alcoholic extract of the leaves of the target plant was prepared using a soxlet device, and then the extract was analysis with GC-MS technology to find out the active compounds contained in the extract, and the analysis proved that it contains 18 compounds: (Butanoic acid, Propane, 2-Propen-1-ol, Ethylbenzene, Butane, 1-Butanol, Propanamide, p-Xylene, Silane, Ethanol, heptane, Triethoxysilanol, Propanoic acid, Verbenol, Hexadecanoic acid, Hexadecanoic acid, Benzenamine and Benzaldehyde). Then the alcoholic extract was used at concentrations of (200, 300, 400 and 500) µg/ml to study the antioxidant effectiveness on the free root DPPH and the experiment proved the ability of the extract to inhibit free radicals at all concentrations, the highest inhibition at a concentration of 500 µg/ml with an inhibition rate of 90.40% superior to the standard sample ascorbic acid, which inhibited the free Root reached 83.12% and the higher the concentration of the extract, the more inhibited free radicals. The study proved that the alcoholic extract of Melissa officinalis leaves has an effect on the vitality of Escherichia coli bacteria at various concentrations, including 50, 100 and 200 mg/mL. the highest inhibition of the alcoholic extract was at a concentration of 200 mg/mL with an inhibition diameter of 10 mm, followed by a concentration of 100 mg/mL with a diameter of 9 mm. this is consistent with the action of the extract as an antioxidant that the higher the concentration, the greater the inhibition.

Keywords: *Melissa officinalis, Antioxidant, Escherichia coli.*

1. Introduction

Melissa officinalis, is a tiny genus of fragrant plants that belongs to the Lamiaceae family. Melissa, the Greek word from which the name of this genus is derived, means "honey bee" because of the copious amounts of nectar secreted by its blossoms. (Bezenjani et al, 2014). The plant has a ribbed stem, ranging in height from 0.5 to 1.5 meters, and its green, heart-shaped leaves (Mousavi SM, 2019). The leaves are 2 to 8 cm long. The surface of the leaf is rough hairy, and the edge of the leaf is serrated The seeds of the plant are very small, about 1-1.5 mm long, oval in shape, and dark brown or black in color. seed weight 0.5 to 0.7 grams, (Rasmussen, 2016).

The plant grows naturally in sandy and arid regions, the plant is spread especially in the Mediterranean basin and Iran, as well as in central and western Europe and Asia, as well as its spread in North America and New Zealand (Shakeri et al, 2016). Chemical studies conducted on the components of this genus showed that they mainly contain terpenoids, flavonoids, tannins, phenolic acids and essential oils. (Abdel-Naime et al, 2016). The lemongrass plant is a well-known herb that has been used for a very long time to treat many diseases such as headaches, gastrointestinal diseases, and nervous diseases, and its medicinal use dates back more than 2000 years. The plant has been used in several ways, from a mild sedative and hypnotic drug to reduce heart rate, and is used in the treatment of colon, antibacterial, anti-inflammatory, antiviral, and a treatment for muscle spasms and an antioxidant (Queiroz et al, 2014).

One area in recent years has attracted a great deal of interest with potential therapeutic potential for controlling degenerative diseases associated with oxidative damage using antioxidants, and many different extracts have been identified as phytochemicals with very prominent activity as antioxidants. (Bhuiyan et al., 2009) Antioxidants work specifically in the cell of an organism to prevent free radical damage. Free radicals are chemical compounds with a non-double electron that makes them unstable compounds in the body and therefore react strongly to chemical attack in front of many bonds between substances that alter the target compound's properties. A healthy cell becomes unstable and triggers a cascade of damaging processes if free radicals are able to remove one electron from it. (Maysar, 2013). Damage in free radicals can lead to a chronic condition in the body that damages the DNA of cells, produces cancer cells, and oxidative stress is associated with aging and many diseases in humans such as cancer, diabetes, atherosclerosis, and neurological diseases such as Parkinson's, Alzheimer's, and stroke (Letelier et al,2008; Gulcin, 2020).

Plant extracts have an effect on the vitality of microorganisms, including Escherichia coli bacteria, belong to the Enterobacteriaceae family and they are Gram-negative bacilli (AL-Sa'ady and Al-Mawla, 2019). They are arranged singly or in pairs, and do not form spores. They have the ability to grow at temperatures of 45-44°C, and the optimum temperature for their growth is 3 °C. These bacteria are characterized by being fermenters of the sugar lactose. They consume glucose and the rest of the carbohydrates, producing acid and gas. They move by means of peripheral flagella. They are also facultatively anaerobic. These bacteria are considered part of the normal flora of the large intestine in humans and animals, but they are considered pathogenic when they leave their natural habitat or the immune status is disturbed. They are the most common species of the intestinal family in causing urinary tract infections. They also cause inflammation of the intestine and bile duct, diarrhea, burns, and meningitis in newborns, as well as their ability to cause wound infection.

2. Taxonomic position of the plant

Kingdom : Plantae

Division : Magnoliophyta
Class : Magnoliopsida
Order : Lamiales
Family : Lamiaceae
Genus: Melissa
Species : *M. officinalis*

3. Material and Methods

3.1. Plant collection

The plants were gathered in the northern Iraqi area of Aqrah and identified at the Medicinal Plants Development Centre in Mosul Dam, which is part of the Ministry of Agriculture there. The plant leaves were picked, clean and stored in paper bags away from moisture until they were ready to be used.

3.2. Preparation of plant extract using continuous soxhlet apparatus.

The leaves were first ground into a powder in an electric mill; next, 25 grams of the powder were added to the Soxhlet system batch; finally, 400 milliliters of ether were used to extract the oil from the leaves. The extraction process continued for 7 hours per day until the solvent became colorless, and then methanol with concentration of 70% was added Finally, the extract was concentrated by using Rotary vacuum evaporator (Al-Daody, 1998).

3.3. Analysis of alcoholic extract by GC-MS technique

The methanolic extract was analyzed using GC-MS technology in the Central Laboratory/ College of Applied Sciences/ Samarra University.

3.4. Investigation of the antioxidant activity of the isolated compounds

Diphenyl pecril hydrazine (DPPH) was dissolved in methanol at a concentration of 200 mM by mixing 7.9 mg into 100 ml. Concentrations of (200, 300, 400, and 500)µg/ml were used to make the plant extracts, whereas ascorbic acid was used as a reference. Each sample was treated with 1 mL of DPPH solution and left to incubate at room temperature in the dark for 30 minutes. before being read at a wavelength of 517 nm using a Japanese-made Shimadzo-UV-1800 dual-cell spectrophotometer, and then the following equation was applied for the purpose of identifying the inhibition ratios for free radicals (Sumathy *et al*, 2013; Sahu *et al*, 2013).

$$\% = (AbB - AbS)/AbB*100$$

AbB = absorbance of sample control
AbS = absorbance of the sample

3.5. *E. coli bacteria*:

E. coli bacteria were obtained from the laboratories of the Biology Department/College of Education for Pure Sciences/University of Mosul.

3.6. Preparation of alcoholic extract concentrations of the M. officinalis plant.

A concentration of 200 mg/ mL was prepared, the alcoholic extract was made in DMSO, from which the remaining concentrations of 100 and 50 mg/mL were taken, and then sterilized by pasteurization at a degree of 62 °C for 10-15 minutes (Sharif, 1998).

3.7. Sensitivity testing method (diffusion by etching):

The inhibitory effectiveness of the active ingredients in the growth of the bacteria under study was tested by the diffusion-etching sensitivity test, according to the method of Peres and Bazerque (1991). holes were made in the Muller-Hinton medium with a diameter of 5 mm and the bacterial suspension was prepared in the nutrient broth medium at the age of 14-16 hours. Then 0.1 cm^3 of the diluted bacterial suspension was transferred to the Muller-Hinton Acar medium, spread on the surface of the medium homogeneously using a cotton swab Cotton Swab, then incubated the petri dishes at a temperature of 37 M for 30 minutes to get impregnation. The pits were then filled with 50, 100, or 200 mg/ml of the active compounds of interest, and the petri dishes were incubated at 37 degrees Celsius for 24 hours. Finally, the inhibition zone was measured using a ruler inserted into the petri dish.

4. Results and discussion

4.1 Identification of the active compounds of the alcoholic extract by GC-MS

The analysis by GC-MS showed that the alcoholic extract of the *Melissa officinalis* plant contained a number of active compounds, which as shown in table (1) form (1-18):

- **a)** Butanoic acid: this compound has a 3.040 minute retention time and a probability of 86%. It is a short-chain saturated fatty acid present in the form of esters in vegetable oils and animal fats.
- **b)** Propane: this compound has a 3.285 minute retention time and a probability of 95%. It is an alkane, that is, it is an aliphatic hydrocarbon. It is derived through the distillation of petroleum, or during natural gas extraction processes.
- **c)** 2-Propen-1-ol: this compound has a 3.370 minute retention time and a probability of 76%. It is an allylic alcohol. It is an organic compound that exists under standard conditions in the form of a colorless liquid.
- **d)** Ethylbenzene: this compound has a 3.810 minute retention time and a probability of 88%, it is an organic compound that exists in the form of a colorless, flammable liquid with a benzene-like odor. This monocyclic aromatic hydrocarbon compound is of importance in the petrochemical industry as it is used in the production of styrene as an intermediate, which is the basic material for the manufacture of polystyrene, which is the common plastic.
- **e)** Butane: is an unbranched alkane with a retention time of 3.885 minutes and a probability of 90%. It is also a gas without smell or color, is highly combustible, and one of the gases is simple to liquefy.
- **f)** Butano compund: Having a retention time of 3.970 minutes and a 95% probability of being an organic alcoholic compound, it is a colourless liquid. Many foods and drinks contain this substance because it is produced naturally as a byproduct of ethanol fermentation from sugars or other carbohydrates.
- **g)** Propanamide: this compound has a 4.030 minute retention time and a probability of 81% is a monocarboxylic amide and a primary fatty amide. It is functionally related to propionic acid.
- **h)** P-xylene: this compound has a 4,200 minute retention time and a probability of 76%, is an aromatic hydrocarbon compound consisting of a benzene ring attached to two methyl groups and exists in three isomeric forms depending on the sites to which the methyl groups attach.
- **i)** Silane: this compound has a 4.565 minute retention time and a probability of 80%. It is an inorganic compound and exists as a colorless gas. The compound is the simplest of the silanes, and is structurally similar to methane
- **j)** Ethanol this compound has a 5.095 minute retention time and a probability of 77%. It is an organic chemical compound. It is a colorless, flammable liquid prepared from the fermentation of sugars. It is used in the perfume industry and in the production of alcoholic beverages. It is also used as a fuel in ethanol-powered mechanical engines.
- **k)** Heptane: this compound has a 5.365 minutes and a probability of 84% is a hydrocarbon. Heptane is also an alkane.
- **l)** Triethoxysilanol: this compound has a 5.610 minute retention time and a probability of 76%, it is a colorless organic

silicon compound used in hydrogenation reactions catalyzed by precious metals.

m) Propionic acid: this compound has a retention time of 6.125 minutes and a probability of 78%. It is a colorless, oily organic chemical compound. It belongs to the group of carboxylic acids, and is called propionic acid, and its salts are called propionate.

n) Verbenol: this compound has a 8.110 minute retention time and a probability of 76%, it is a group of monoterpene alcohols that belong to diisomers. These compounds are found in the form of active components of essential oils and insect pheromones.

o) Hexadecanoic acid: this compound has a 8.250 minute retention time and a probability of 83%, Palmitic acid is one of the most prevalent saturated fatty acids, which is a component of animal and plant cells. It is a major component of the oils extracted from palm.

p) Pentadecanoic acid: this compound has a 8.340 minute retention time and a probability of 92% is a carboxylic acid and is in the form of a white crystalline powder. Heptadecanoic acid belongs to the saturated fatty acids.

q) Benzenamine: this compound has a 8.905 minute retention time and a probability of 91%, and it is an organic compound. Aniline consists of a phenyl group (−C6H5) to an amino group (−NH2), and is the simplest aromatic amine.

r) Benzaldehyde, this compound has a 9.120 minute retention time and a probability of 93% is an organic

Table (1) Compounds identified by GC-MS technology of the alcoholic extract of *Melissa officinalis*.

Comp.Name	R.Time	Probability %
Butanoic acid	3.040	86%
Propane	3.285	95%
2-Propen-1-ol	3.370	76%
Ethylbenzene	3.810	88%
Butane	3.885	90%
1-Butanol	3.970	95%
Propanamide	4.030	81%
p-Xylene	4.200	76%
Silane	4.565	80%
Ethanol	5.095	77%
Heptane	5.365	84%
Triethoxysilanol	5.610	76%
Propanoic acid	6.125	78%
Verbenol	8.110	76%
Hexadecanoic acid	8.250	83%
Pentadecanoic acid	8.340	92%
Benzenamine	8.905	91%
Benzaldehyde	9.120	93%

compound consisting of a benzene ring and a substituted formyl group. It is the simplest and most useful industrial aromatic aldehyde. All active compounds identified by Gc-MS have an effect in inhibiting the biological activity of microorganisms because they work synergistically with each other (Rajalakshmi and Mohan, 2016). Figure (1) Butanoic acid,Figure (2) Propane, Figure (3) 2-Propen-1-ol, Figure (4) Ethylbenzene, Figure (5) Butane Figure (6) 1-Butanol, Figure (7) Propanamide, Figure (8) p-Xylene, Figure (9) Silane, Figure (10) Ethanol, Figure (11) heptane, Figure (12) Triethoxysilanol, Figure (13) Propanoic acid, Figure (14) Verbenol, Figure (15) Hexadecanoic acid Figure (16) Pentadecanoic acid, Figure (17) Benzenamine Figure (18) Benzaldehyde(see appendix)

4.2 Antioxidant activity of alcoholic extract of *Melissa officinalis*

Table 2 When using lemongrass as an antioxidant, it was found that the chemicals in the alcoholic extract were more effective in sniping the free radicals DPPH than the typical antioxidant, ascorbic acid. With focus 500 µg/ml, the extract had the highest snipping percentage (90.40%), which was significantly better than the standard sample's snipping percentage (83.12%), and at a concentration of 200 µg/ml, the extract had the lowest sniping effect of free radicals (67.40%), which was significantly better than the standard sample which showed 64.30.

It is clear from the table that the result of the process of capturing free radicals that is carried out by the alcoholic extract of the lemongrass plant reduces the risk of these radicals, as the hydrogen atom is given to the free radicals DPPH, which works to stabilize them, and the reason for the strong effectiveness that is due to the total active compounds for each sample, and what Each compound has hydroxyl groups, and this is consistent with Cosme *et al*. (2018), who indicated that the activity of antioxidants is mainly due to the redox properties, as well as the ability of antioxidants to donate electrons (hydrogen), and thus leads to inhibit the reaction to the free oxygen radical and also leads to stop the production of free radicals again. (Blainski *et al*., 2013 and Gulcin, 2020).

Table (2): Concentration and Percentage of Alcoholic Extract of *Melissa officinalis* and Standard Sample as Antioxidants

Concentration (µg/ml)	alcoholic extract	standard sample
200	67.40% f	64.30% h
300	71.88% e	66.79% g
400	75.89% c	73.5% d
500	90.40% a	83.12% b

4.3 The inhibitory effect of the alcoholic extract of the leaves of the *Melissa officinalis* plant on the vitality of *Escherichia coli* bacteria

The results of Table (3) indicated the effect of the alcoholic extract of the *Melissa officinalis* plant growing in the aqra region of northern Iraq which affected the vitality of *E. coli* bacteria in all concentrations, but the highest inhibition was at a concentration of 200 mg/mL with an inhibition diameter of 10 mm, followed by a concentration of 100 mg/mL with an inhibition diameter of 9 mm.The results are consistent with the antioxidant activity of the alcohol extract, which is a good indicator in the study of its viability on species of bacteria, including *E. coli*. Perhaps the reason for the inhibition is due to the ability of alcoholic solvents to extract the maximum possible number of active compounds that contribute to inhibit the vitality of bacteria and affect their function, and this was proved by the analysis with the GC-MS technique that detected the number of possible active compounds in the alcoholic extract of the leaves of the *Melissa officinalis* plant which reached to 18 compounds Table (1).

Table (3) inhibitory effect of alcoholic extract of leaves of the *Melissa officinalis* plant on the vitality of *E. coli* bacteria

Compound	alcoholic extract		
The concentration is mg/mL	50	100	200
Inhibition diameter (mm)	3	9	10

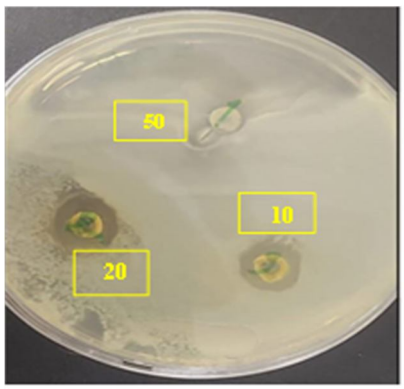

Photo (1): the effect of alcoholic extract on *E.coli*

Through the studies, the mechanism of the effect of some plant extracts on pathogenic bacterial strains is that they work to damage the bacterial membrane, Cell wall and decompose the bacterial cells, in addition to their effect on the permeability of the Cytoplasmic membrane, as in the *E. coli* bacteria (Jenie *et al*., 2008). As in another study, it was demonstrated that treating the bacteria under study with alcoholic extracts of the medicinal plants studied results in the lysis of the bacterial cells, in addition to the mechanism of action of the cell wall (Carson *et al*., 2002). Another study was found that using *Jatropha curcas* , oregano, and thyme plants to treat pathogenic bacteria *E. coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Staphylococcus aureus* works to inhibit the action of extracellular microbial enzymes necessary for the multiplication of microbes or to have a direct effect on microbial metabolism by inhibiting oxidative phosphorylation, as antibacterial compounds can have an antibacterial effect on pathogenic bacteria, and that the studied plant extracts have an effect due to the presence of their phenolic compounds individually or the synergism between them on different types of bacteria. Synergistic effects can be created if the components of the extract affect different targets or interact with each other to enhance solubility, and the bioavailability of one or more components of the extract leads to the destruction of cell membranes and walls, stopping the cell's reproductive machinery, and thus the cell is reduced and unable to grow and divide (Nefzi et al., 2022).

5. Conclusion

The results of the study showed that the alcoholic extract of the leaves of the *Melissa officinalis* plant contained a number of different active compounds through analysis using the GC-MS technique, in addition, the alcoholic extract was effective in its inhibitory effect on DPPH free radicals, as well as its effect on the vitality of *E. coli* bacteria.

Acknowledgement

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Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conficts of interest.

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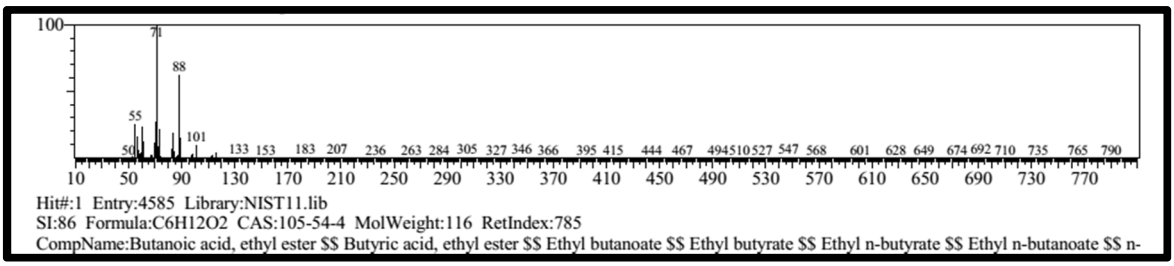

Figure (1) Butanoic acid

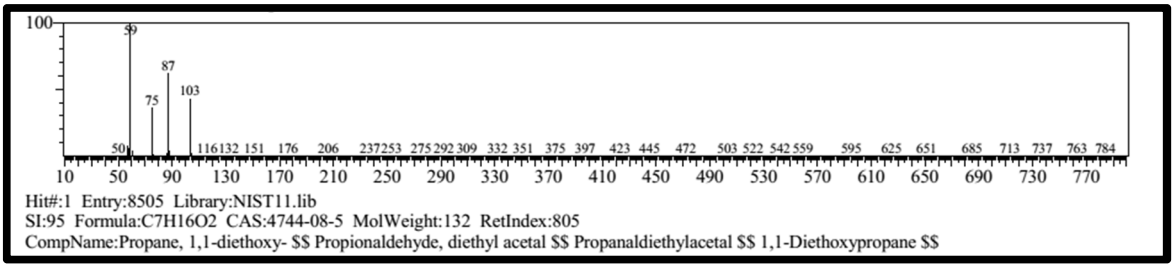

Figure (2) Propane

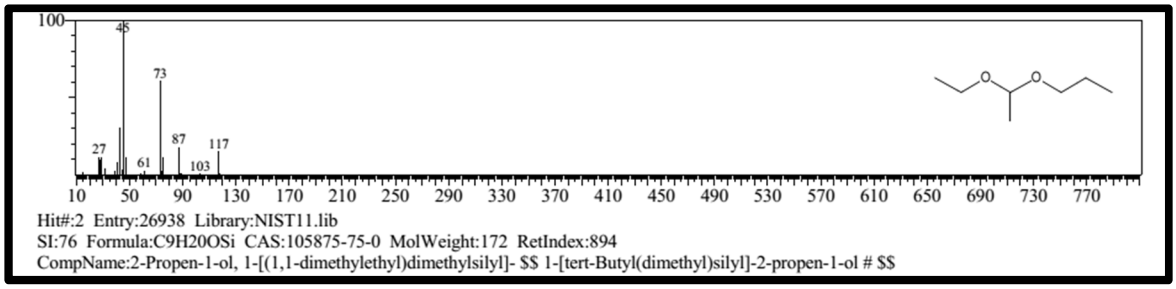

Figure (3) 2-Propen-1-ol

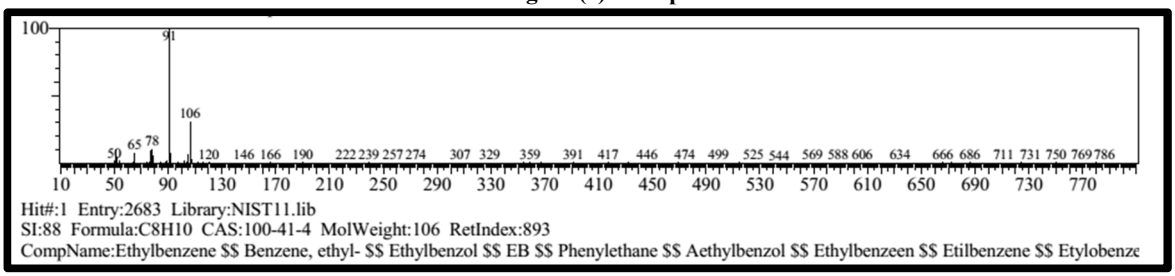

Figure (4) Ethylbenzene

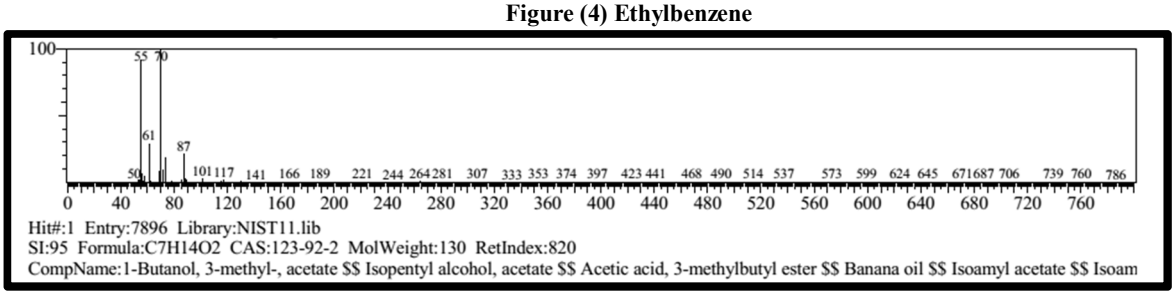

Figure (5) Butane

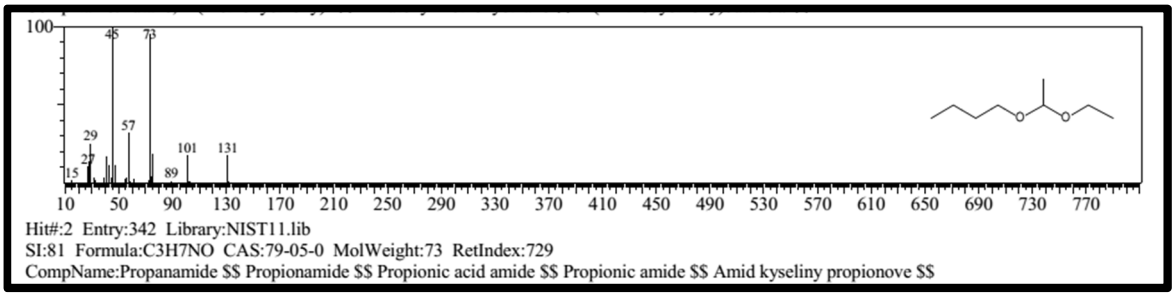

Figure (6) 1-Butanol

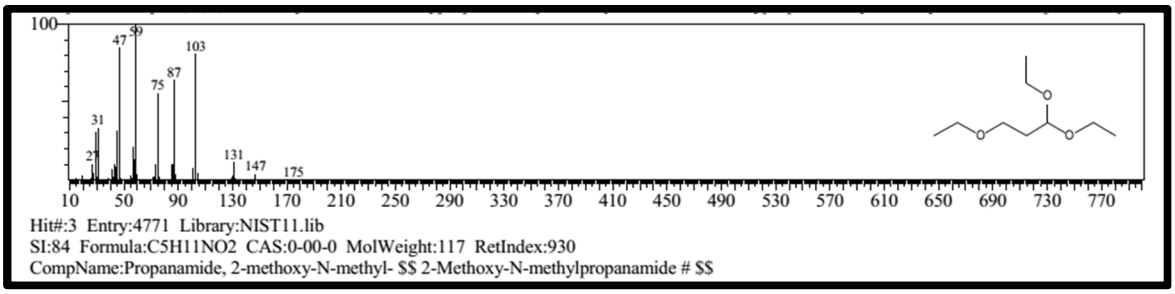

Figure (7) Propanamide

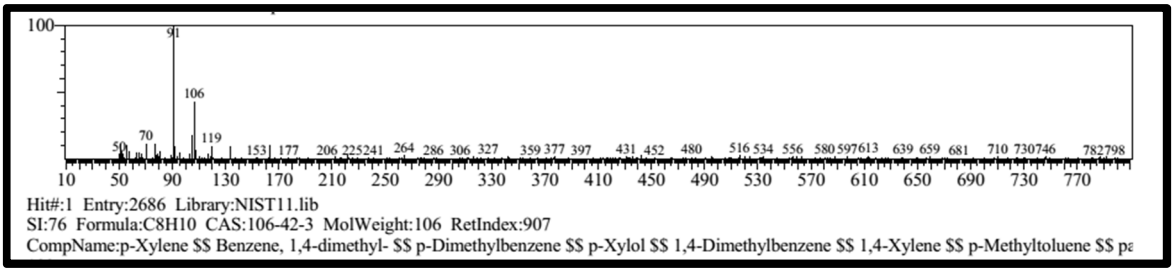

Figure (8) p-Xylene

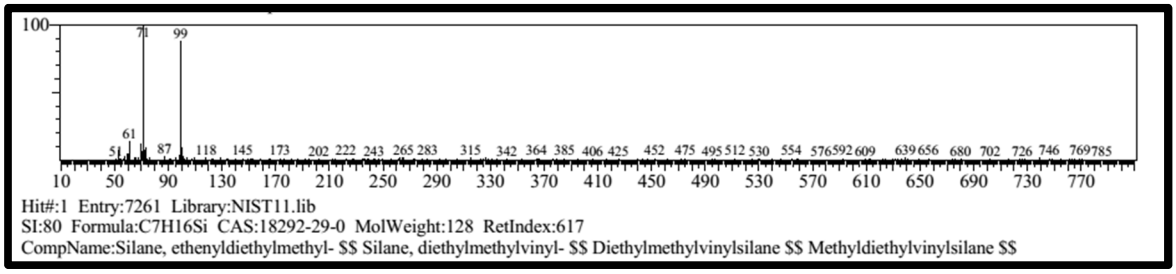

Figure (9) Silane

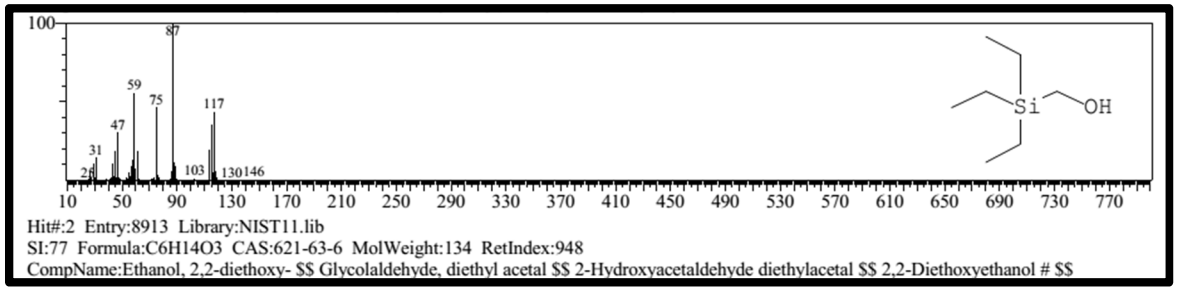

Figure (10) Ethanol

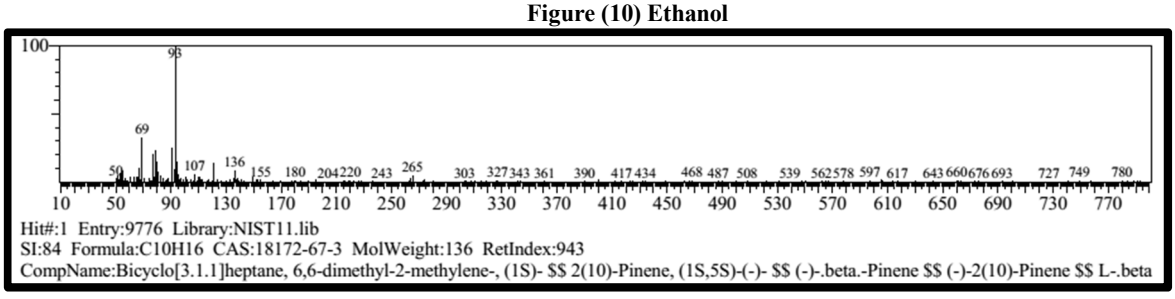

Figure (11) heptane

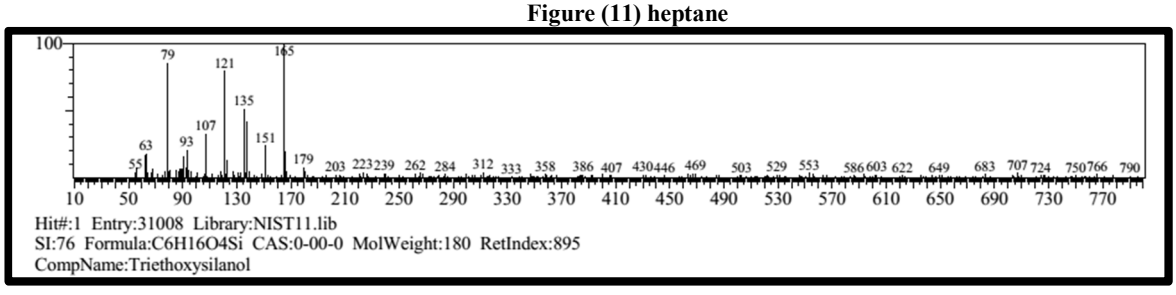

Figure (12) Triethoxysilanol

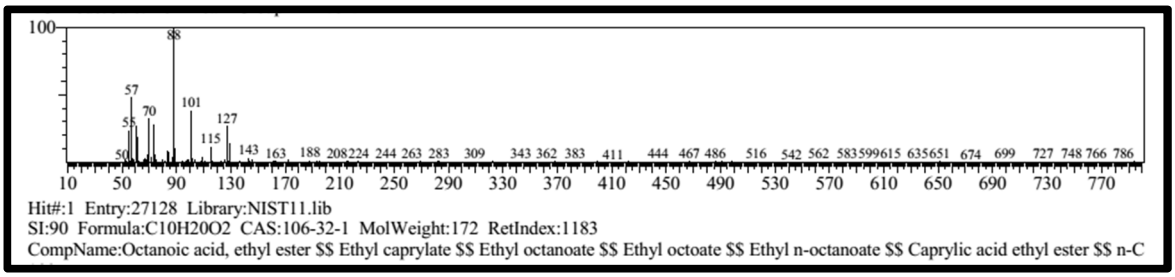

Figure (13) Propanoic acid

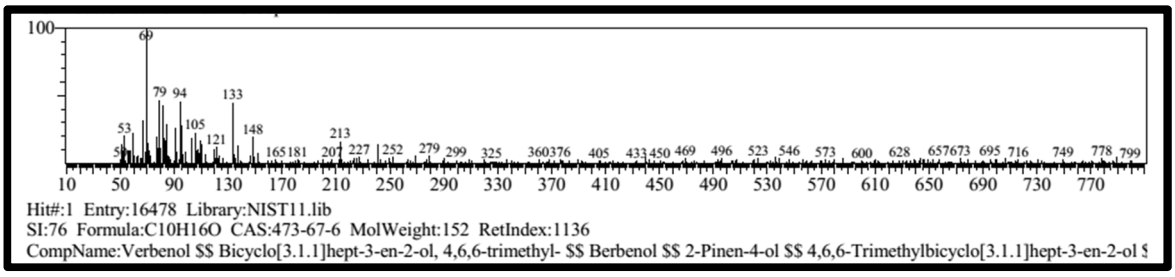

Figure (14) Verbenol

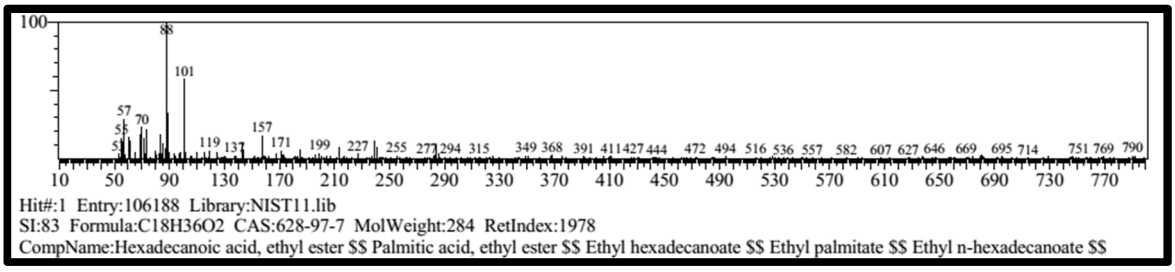

Figure (15) Hexadecanoic acid

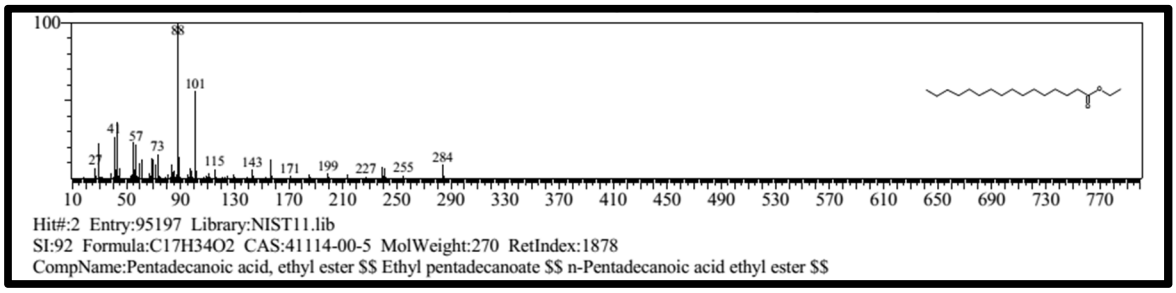

Figure (16) Pentadecanoic acid

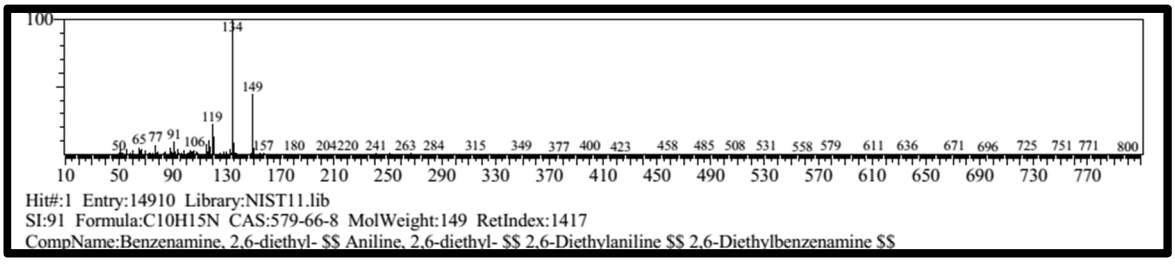

Figure (17) Benzenamine

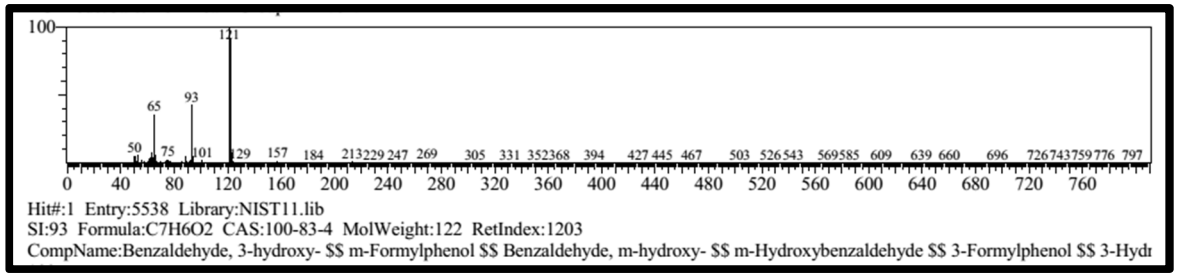

Figure (18) Benzaldehyde