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IDENTIFICATION OF ORGANIC COMPOUNDS IN STANDARDIZED ETHANOLIC EXTRACT OF OLEA EUROPAEA (OLIVE LEAVES) USING GCMS

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ABSTRACT

Olive leaves grown in wild Mediterranean region and America contain higher concentrations of active ingredients such as Oleuropein and Astrul and Allecin. It is used as oil which contains 75% of Oleic acid. The degrees of toxicity of the olive leaves after administration was noticed almost virtually non-existent. The use of pure Oleuropein or in a crude extract has its own therapeutic value in treating diabetes, kills a broad range of bacteria, viruses, parasites, molds and also found it regulates the blood pressure level thereby reducing the possibility of stroke and cardiac arrest. In this study we want to diagnosis some active organic compounds that are present in olive leaf extract using UV spectroscopy, Gas Chromatography Mass Spectrometry, Infrared Spectroscopy FT-IR techniques. There are about 20 chemical compounds of olive leaf ethanolate extracts have been identified, including hydrocarbons about 61.48%, while Al oxygenic vehicles represent 35.34%.

KEYWORDS: Olive Leaves, Gas Chromatography- Mass Spectrometry, Infrared Spectroscopy FT-IR, U V Spectroscopy.

INTRODUCTION

Plant and its derivatives are used in diet not only for its flavor but also used as food preservatives to preserve the nature of foods with its original content for longer duration. Some effective compounds are added as additives for food preservation. In present days herbs and plants are used widely for multiple purposes that include nutritional, medicinal, flavor in beverages, colorants, preservatives, insect repellants, cosmetics and many other industrial uses.[1]

Olive trees are grown in the wild Mediterranean region and with similar climate zones in the America. The leaves can be collected throughout the year and the fruit in late summer. It is believed that wild tree leaves contain higher concentrations of active ingredients such as Oleuropein and Astrul and Allecin. It is used as oil which contains 75% of Oleic acid.[2]

Recent scientific studies prove olive leaves contain active ingredients such as the Ieropiseid and Oleuropeines are safer and effective as herbal remedies The degrees of toxicity of the olive leaf are almost virtually non-existent, as it was noticed in some people who have been administered 10gms daily The use of pure Oleuropein or in a crude extract has its own therapeutic value in treating diabetes. In addition to this it has the ability to kill a broad range of bacteria, viruses, parasites and molds.[3] In 1962 it is found that Oleuropein or the olive leaf extract regulates the blood pressure level thereby reducing the possibility of stroke and cardiac arrest. This result is striking spurred many scientists to search for the other therapeutic effects (Auliorobin) in the treatment of high blood pressure, heart disease and diabetes. A group of scientists from University of Milan pharmaceutical Sciences reported olive leaf can increase the amount of blood flow in the coronary arteries and regulates heartbeat as well to reduce spasms and intestinal disorders.[4]

The scientist from the University of Grenada in Spain found that olive extracts can cause smooth muscle relaxation in laboratory animals, which once again confirms the efficacy of the substance in the treatment of high blood pressure.[5] It protects coronary arties by preventing oxidation of lipoproteins thereby reducing triglycerides in blood. It also prevents the accumulation of platelets in the arteries. Other benefits of Oleuropein include the prevention of respiratory diseases, influenza, meningitis, pneumonia, tuberculosis, colds, gonorrhea, fever, sepsis, malaria, herpes Impetigo, inflammation of the ears, the symptoms of chronic stress and

inflammation of the liver after surgery, different types of allergies and diabetes children. It was believed that the more number of therapeutic values of olive oil were due to the presence of unsaturated fat acids, which represents most of the oil compounds. Later it was discovered the presence of other substances in trace quantities (phenols) were also involved.[6] The aim of our study is to diagnosis some active organic compounds that are present in olive leaf extract using UV spectroscopy, Gas Chromatography Mass Spectrometry, Infrared Spectroscopy FT-IR techniques.

MATERIALS AND METHODS

1) Local Olive Leaf
2) Ethanol (99.98% Hayman)
3) Soxhlet apparatus
4) Rotary Evaporator- (Ika Werke Rv06 -Ml)
5) Oven Binder
6) Sensitive Balance Kern. Als220
7) Shimadzu 2010
8) Spectra Lab Mb3000
9) UV-Vis Spectra Photometer – (1650pc Shimadzu)

Preparation of Olive leaves extract

Weigh 25gm of olive crushed leaves and add the solution of ethyl alcohol and distilled water (50: 50ml) as a solvent in the circular flask.

Using Soxhlet apparatus at a temperature of 40ºC for a period of 16 hours the extraction of olive leaves can be prepared and then filter the extracts from impurities. By rotary evaporator vaporize the extracts to the extent of the drought in 40ºC and dried in the electric furnace to obtain a dry extract.

UV Spectra analysis

Using UV - Vis Spectra Photometer – (1650pc Shimadzu) UV Spectral analyses were performed.

Gas Chromatography – Mass spectrometry analysis

Melt a small amount of olive leaves extract and finally inject them to a gas chromatography for determining the optimum conditions used for the separation process. Finally inject the extracts to a gas chromatography-mass spectrometry were optimum conditions for operation as follows:

Detector	**FID**
Col. Used	DP5. 25.050
Carrier Gas	N_2
Injector Temperature	300˚ C
Initial Temperature	100˚ C
Final Temperature	300˚ C
Initial Time	0 min
Final Time	10 min
Rate	7 ˚C / min
Volum injaction	1 µ
Total Flow Rate	81.4 ml / min
Pressure Across the Column	1.6 Kg / cm^2

RESULTS

UV spectrum of the extract

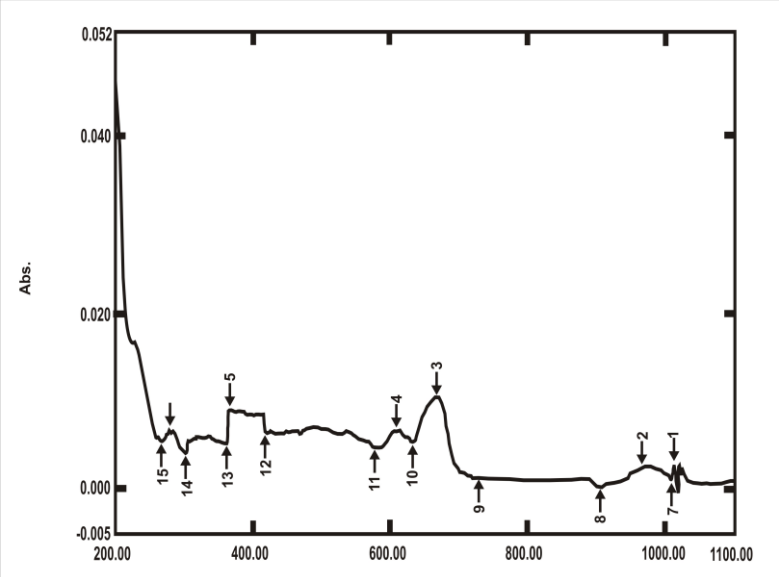


Fig.1: UV Spectrum of the Olive leaves extract

Infrared spectrum of the extract

Using FTIR the Infrared spectrum of the extracts is obtained.

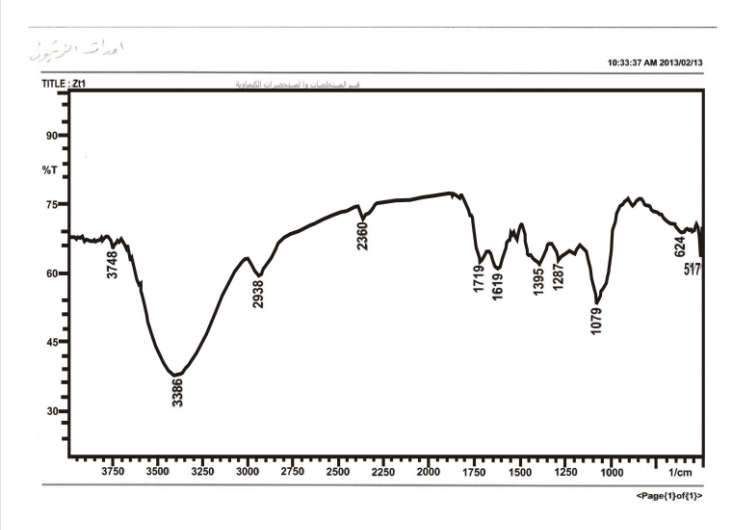
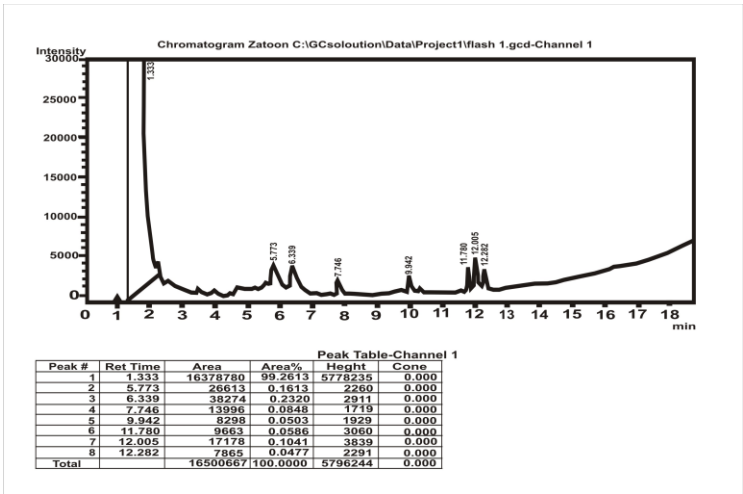


Fig. 2: Infrared Spectrum of the extract

Gas Chromatography-Mass Spectrometry - Chromatogram Abstract

The Gas Chromatography-Mass Spectrometry of the extracts were shown below:

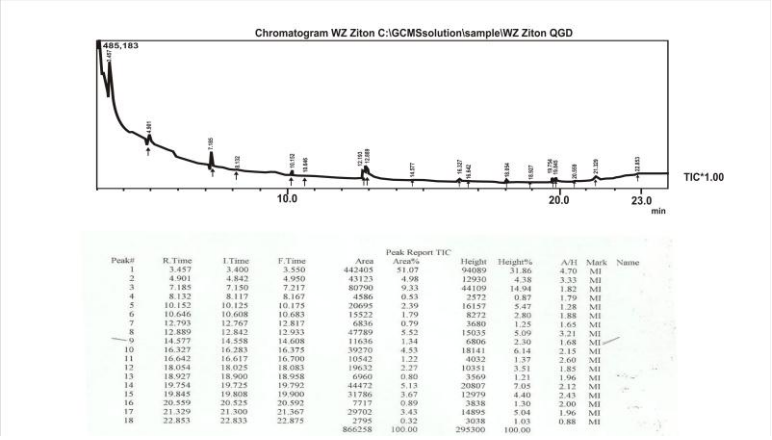


Peak Table-Channel 1

Peak #	Ret Time	Area	Area%	Heght	Cone
1	1.333	16378780	99.2613	5778235	0.000
2	5.773	26613	0.1613	2260	0.000
3	6.339	38274	0.2320	2911	0.000
4	7.746	13996	0.0848	1719	0.000
5	9.942	8298	0.0503	1929	0.000
6	11.780	9663	0.0586	3060	0.000
7	12.005	17178	0.1041	3839	0.000
8	12.282	7865	0.0477	2291	0.000
Total		16500667	100.0000	5796244	0.000

Fig.3: Chromatogram Abstract of extracts

Gas Chromatography - Mass Spectrometry – Chromatogram



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	3.457	3.400	3.550	442405	51.07	94089	31.86	4.70	MI	
2	4.901	4.842	4.950	43123	4.98	12930	4.38	3.33	MI	
3	7.185	7.150	7.217	80790	9.33	44109	14.94	1.82	MI	
4	8.132	8.117	8.167	4586	0.53	2572	0.87	1.79	MI	
5	10.152	10.125	10.175	20695	2.39	16157	5.47	1.28	MI	
6	10.646	10.608	10.683	15522	1.79	8272	2.80	1.88	MI	
7	12.793	12.767	12.817	6836	0.79	3680	1.25	1.65	MI	
8	12.889	12.842	12.933	47789	5.52	15035	5.09	3.21	MI	
9	14.577	14.558	14.608	11636	1.34	6806	2.30	1.68	MI	
10	16.327	16.283	16.375	39270	4.53	18141	6.14	2.15	MI	
11	16.642	16.617	16.700	10542	1.22	4032	1.37	2.60	MI	
12	18.054	18.025	18.083	19632	2.27	10351	3.51	1.85	MI	
13	18.927	18.900	18.958	6960	0.80	3569	1.21	1.96	MI	
14	19.754	19.725	19.792	44472	5.13	20807	7.05	2.12	MI	
15	19.845	19.808	19.900	31786	3.67	12979	4.40	2.43	MI	
16	20.559	20.525	20.592	7717	0.89	3838	1.30	2.00	MI	
17	21.329	21.300	21.367	29702	3.43	14895	5.04	1.96	MI	
18	22.853	22.833	22.875	2795	0.32	3038	1.03	0.88	MI	
				866258	100.00	295300	100.00			

Fig. 4: Chromatogram gas chromatography device - mass spectrometry

Identification of 3-Isopropoxybanzaldehyde

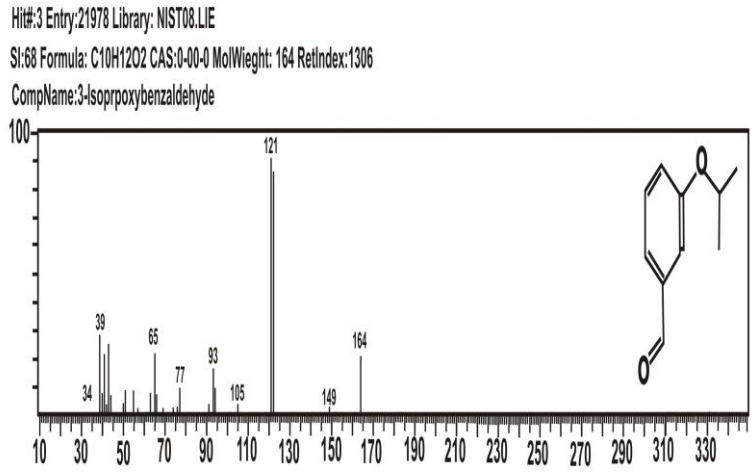


Fig.5: **Identification of 3-Isopropoxybanzaldehyde**

Identification of Mrkp2- Methoxy-5-propenyl Phenol

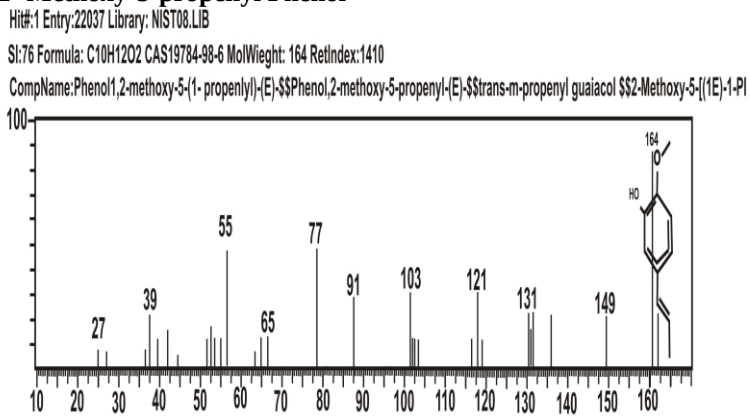


Fig.6: Identification of Mrkp2- Methoxy-5-propenyl Phenol

Identification of Methy ltartronic acid

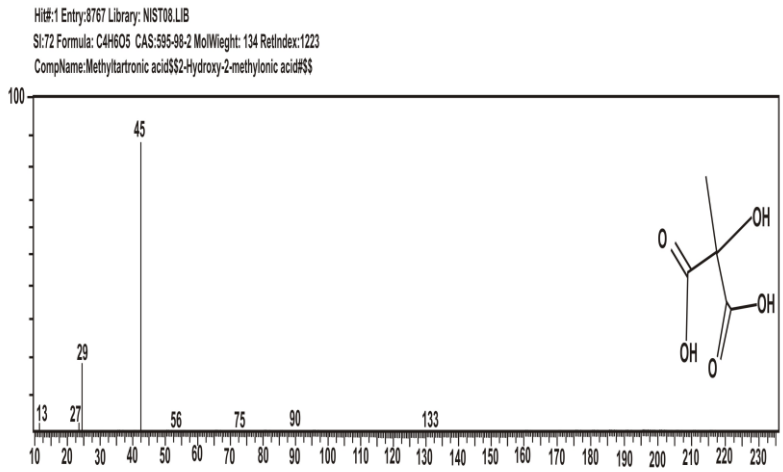


Fig.7: Identification of Methy ltartronic acid

Identification of Benzamide, N-benzoyl-N- (3propylthio-1, 2, 4-thiadiazol-5-yl)

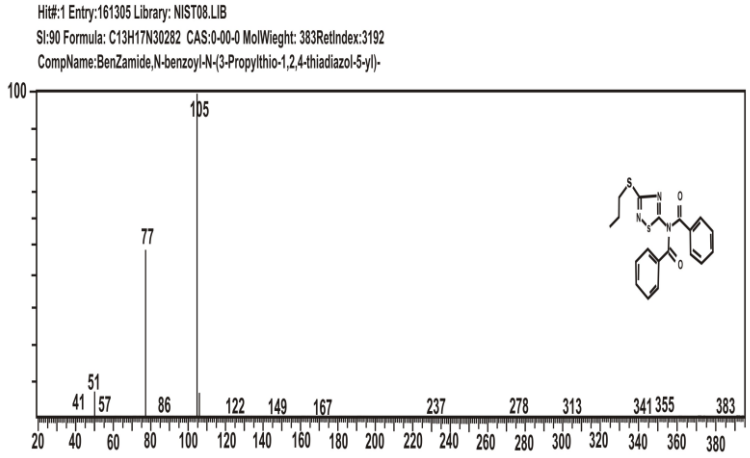


Fig.8: Identification of Benzamide, N-benzoyl-N- (3propylthio-1, 2, 4-thiadiazol-5-yl)

Identification of sulfurous acid compound, bis (1-methylethyl) ester

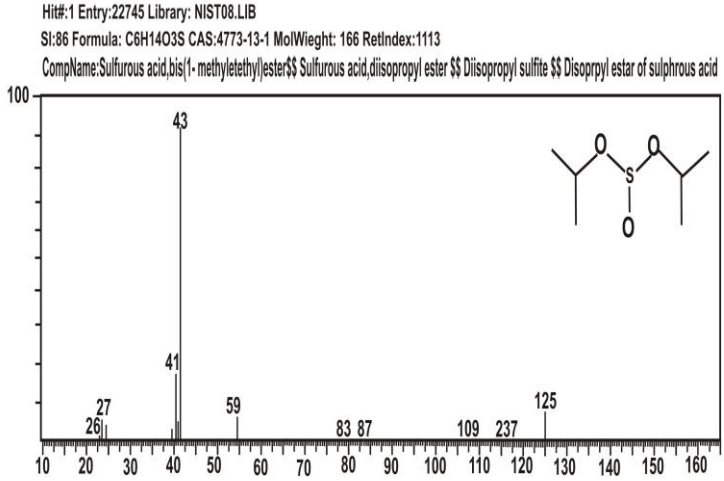


Fig. 9: Identification of sulfurous acid compound, bis (1-methylethyl) ester:

Identification of Nitrous acid, 3-methylbutyl ester

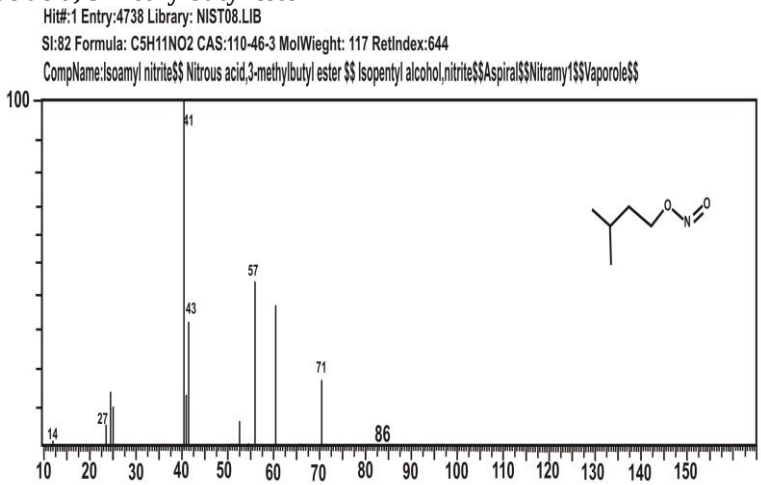


Fig. 10: Identification of Nitrous acid, 3-methylbutyl ester.

Identification of 2, 4’ Bisphenol A

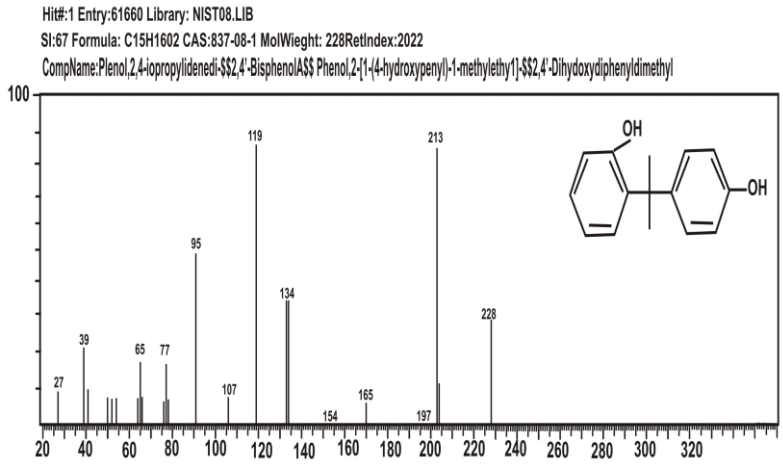


Fig. 11: **Identification of 2,4' Bisphenol A**

Identification of acetonitrile Propylsulfonyl composite

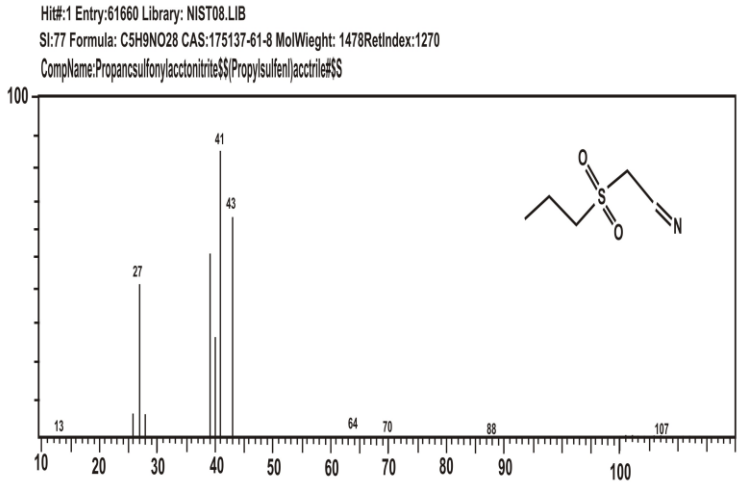


Fig. 12: Identification of acetonitrile Propylsulfonyl composite

Identification of 2, 2-dimethyl-3-hexanone

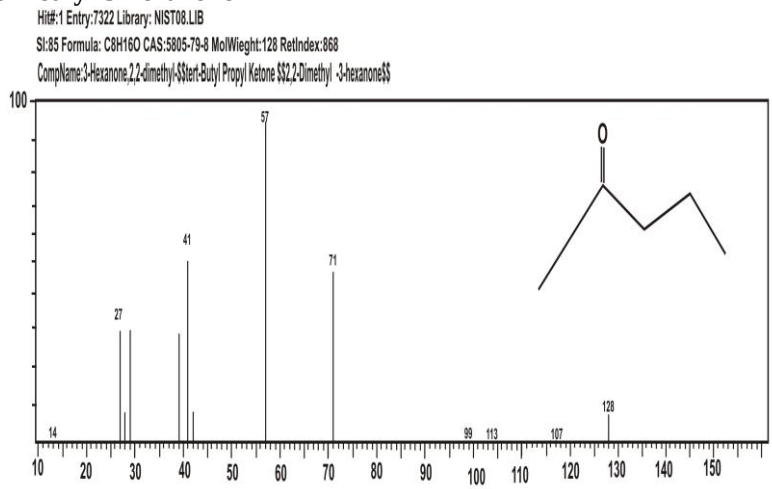


Fig. 13: Identification of 2, 2-dimethyl-3-hexanone

Identification of Propanoic acid / ethenyl ester

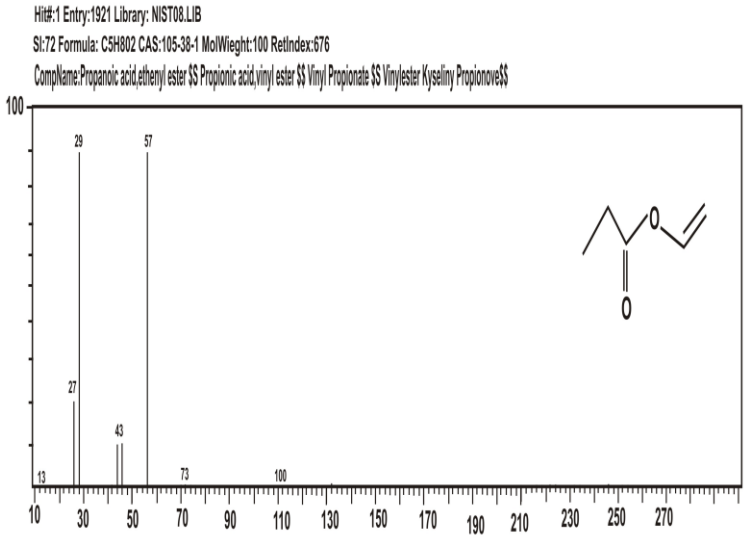


Fig.14: Identification of Propanoic acid/ethenyl ester.

Identification of Sulfurous acid / 2-ethylhexyl hexyl ester

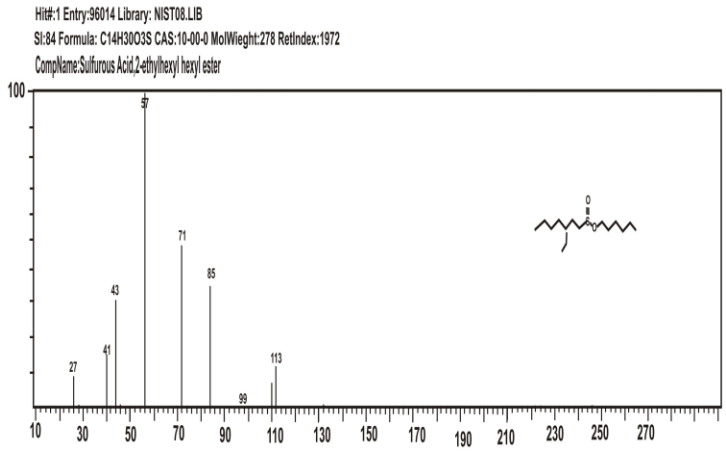


Fig.15: Identification of Sulfurous acid, 2-ethylhexyl hexyl ester.

Identification of 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester

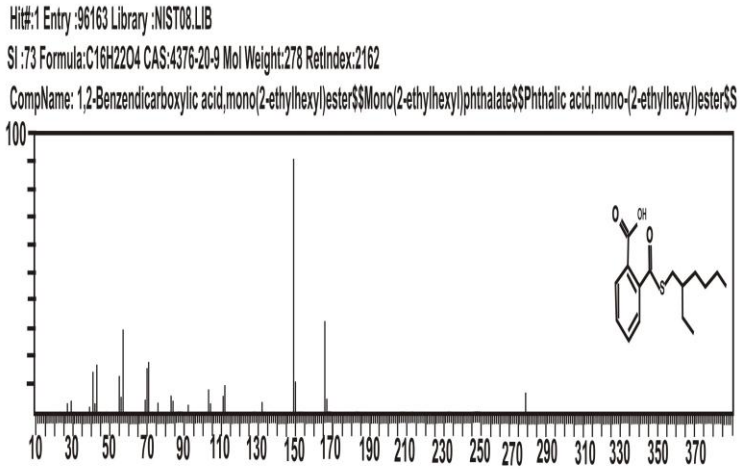


Fig.16: Identification of 1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester.

Identification of Borane / diethyl (decyloxy)

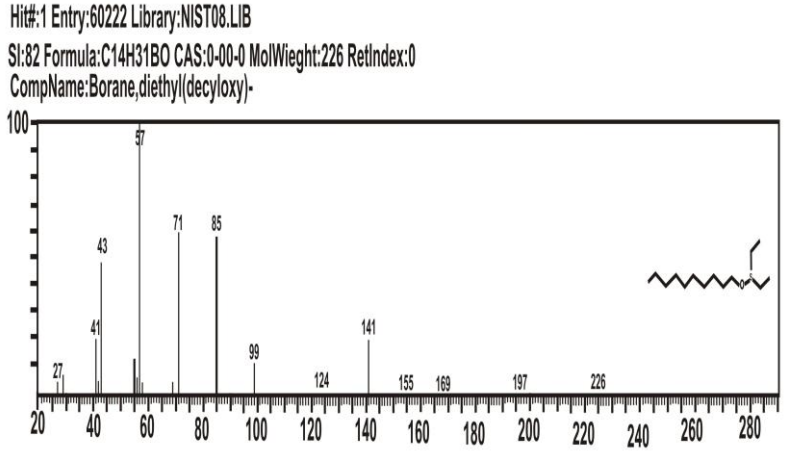


Fig.17: Identification of Borane, diethyl (decyloxy)

DISCUSSION

UV absorbance of Olive leaves extract was examined using spectrophotometer.

From Fig.1 at the wavelength 268 nm indicate the presence of compounds where transitions occur * π- π Conjugated From the Fig.2 the following observation in the Infrared spectrum were made. The appearance of peak in the region 1395 cm^{-1} shows that the compound contains bond N = O. The appearance of absorption at the top 1619 cm^{-1} shows the existence of bond C = O. The appearance of absorption at the top 2938 cm^{-1} indicates the presence of compounds containing groups H-C = O in aldehydes. As absorption at the top 3386cm^{-1} indicates the presence of O-H in alcoholic vehicles and phenolic in alcoholic extract.

From the Fig.3 the optimum separation conditions of the extracts are identified.

From the Fig.4 many peaks are clearly found shows that the extracts contain more compounds.

From Fig.5, the peak at the time of detention 4.9 min have a nice block at the height of the basis of M / Z = 121 which refers to the compound 3-Isopropoxybanzaldehyde which has antitumor activity.[9]

From Fig.6, the peak at the time of detention 7.18 min have a nice block at the height of the basis of M / Z = 164 which refers to the compound Mrkp2- Methoxy-5-propenyl Phenol which has Antioxidant Antimicrobial activity.[10]

From Fig.7, the The peak at the time of detention 8.13 min have a nice block at the height of the basis of M / Z = 45 which refers to the compound Methyl tartronic acid which helps in catalytic oxidation to form oxalic acid derivatives.[11]

From Fig.8, the peak at the time of detention 10.65 min have a nice block at the height of the basis of M / Z = 105 which refers to the compound Benzamide, N-benzoyl-N- (3propylthio-1, 2, 4-thiadiazol-5-yl) which are fungicides.[12]

From Fig.9, the peak at the time of detention 12.7 min have a nice block at the height of the basis of M / Z = 43 which refers to the compound sulfurous acid, bis (1-methylethyl) ester which are found to possess pharmaceutical antioxidants[13] and also acts as a good emulsifier.[14]

From Fig.10, the peak at the time of detention 14.5 min have a nice block at the height of the basis of M / Z = 57 which refers to the compound Nitrous acid and 3-methylbutyl ester. Nitrous acid has Analgesic and Euphoric effect.[15,16] 3-methylbutyl ester is commercially used as flavor.[17]

From Fig.11, the peak at the time of detention 16.32 min have a nice block at the height of the basis of M / Z = 213 which refers to the compound 2,4' Bisphenol A which has influencing effect in genital tract[18], Breast cells[19], Prostate cells[20], etc.

From Fig.12, the peak at the time of detention 64.16 min have a nice block at the height of the basis of M / Z = 41 which refers to the compound acetonitrile Propylsulfonyl composite has no significant beneficial role in humans.

From Fig.13, the peak at the time of detention 18.05 min have a nice block at the height of the basis of M / Z = 57 which refers to the compound 2, 2-dimethyl-3-hexanone has no significant beneficial role in humans.

From Fig.14, the peak at the time of detention 18.94 min have a nice block at the height of the basis of M / Z = 57 which refers to the compound Propanoic acid and ethenyl ester. Propanoic acid has influence in human metabolism.[21] and ethenyl ester helps in reducing triglycerides levels.[22]

From Fig.15, the peak at the time of detention 19.7 min have a nice block at the height of the basis of M / Z = 57 which refers to the compound Sulfurous acid, 2-ethylhexyl hexyl ester whose influence in human metabolism has not still reported.

From Fig.16, the peak at the time of detention 19.8 min have a nice block at the height of the basis of M / Z = 149 which refers to the compound 1,2-Benzenedicarboxylic acid mono (2-ethylhexyl) ester which has cytotoxic effects.[23]

From Fig.17, the peak at the time of detention 21.33 min have a nice block at the height of the basis of M / Z = 57 which refers to the compound Borane, diethyl (decyloxy) which biological benefits are not still reported.

CONCLUSION

Using Gas Chromatography/Mass Spectrometer (GC / MS), there are about 20 chemical compounds of olive leaf extracts have been identified, including hydrocarbons about 61.48%, while Al oxygenic vehicles represent 35.34%.

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