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Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatography-mass spectrometric and fourier-transform infrared spectroscopy.

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ABSTRACT

The aims of present study were to determine five trace heavy metals and screen the antimicrobial activities of methanolic seed extract of *Lepidium sativum* against bacterial strains. Elements content in tested medicinal plant were 39±1.20 ppm, 6.5±0.17 ppm, 28.29±0.23 ppm, 0.46±0.03 ppm and 2.98±0.11 ppm for Fe, Zn, Mg, Co and Pb respectively. Plants were extracted with methanol and tested for their antimicrobial activity by disc diffusion method against eleven bacterial strains *Salmonella typhi*, *Streptococcus pneumonia, Pseudomonas eurogenosa, Staphylococcus epidermidis, Escherichia coli, Bacillus subtilis, Proteus mirabilis, Streptococcus pyogenes, Staphylococcus aureus, Streptococcus faecalis, Klebsiella pneumonia* and fourteen bacterial strains *Aspergillus niger, Aspergillus terreus, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae, Fusarium sp., Microsporum canis, Streptococcus faecalis, Mucor sp., Penicillium expansum, Trichoderma viride, Trichoderma horzianum and Trichophyton mentagrophytes*. Forty six bioactive phytochemical compounds were identified in the methanolic extract of *Lepidium sativum*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, MS Fragment-ions and Pharmacological actions. *Lepidium sativum* was highly active against *Aspergillus flavus* (7.01±0.11). Methanolic extract of bioactive compounds was assayed for in vitro antibacterial activity and the diameters of inhibition zones ranged from 6.03±0.27 to 0.04±0.01 mm for all treatments. It was concluded from this study that methanolic extract has prominent activities against most of the bacterial, yeast and fungal strains.

Keywords: GC/MS, Bioactive compounds, FT-IR, *Lepidium sativum*

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INTRODUCTION

Lepidium sativum L. (Family: Cruciferae) Chemically, the seeds of *L. sativum* contain essential oils,fatty oils, carbohydrate, protein, fatty acids, vitamins, flavonoidsand isothiocynates glycoside (Yadav et al., 2010). *Lepidium sativum* is an annual, herbaceous edible plant that is botanically related to mustard and watercress. *Lepidium sativum* plant is native to Egypt and South west Asia. Locally known as"El-Rshad" is a fast-growing, edible herb with tangy flavor andaroma (Ahsan et al., 1989). The plant is cultivated as culinary vegetable all over Asia. In traditional system of medicine various parts of this planthave been used for the treatment of jaundice, liver problems,spleen diseases, gastrointestinal disorders, menstrual problems,fracture, arthritis, and other inflammatory conditions (Al-Yahya et al., 1994; Al-Asmari et al., 2014; Al-Marzoqi et al., 2016; Sosa et al., 2016; Kadhim et al., 2016), anti-asthmatic(Paranjape and Mehta, 2006), antioxidant (Agarwal and Verma, 2011; Zia et al., 2012; Al-Jassaci et al., 2016) and antihypertensive (Maghrani et al., 2005). *Lepidium sativum* seeds are used in South Asia as traditional medicine to treat bronchitis, asthma and cough. It is considered aborfacient, diuretic, expectorant, aphrodisiac, antibacterial, gastrointestinal stimulant, gastro protective, laxative and stomadic (Patel et al., 2009). Yadav et al. (2010) studied the *in vitro* antioxidant potential of ethanolic extract of *L. sativum* seeds. Total polyphenol and total flavonoid content of extract were 4.46±0.14 mg GAE/gm and 3.57±1.2 mg QE/gm respectively. *Lepidium sativum* seeds are used in South Asia as traditional medicine to treat bronchitis, asthma and cough. It is considered aborfacient, diuretic, expectorant, aphrodisiac, antibacterial, gastrointestinal stimulant, gastro protective, laxative and stomadic (Patole et al., 1998). The potential of *L. sativum* germination as a bioindicator of PAH removal was investigated during phytoremediation of soil contaminated with PAH (Al-Asmari et al., 2014). The seeds of *L. sativum* (LSS) are also used to cure throat diseases, asthma, headache, uterine tumor, nasal polyps and breast cancer. Heavy metals include silver (Ag), cadmium (Cd), iron (Fe), copper (Cu), mercury (Hg), lead (Pb), zinc (Zn), arsenic (As), chromium (Cr), and platinum group elements. Copper and zinc are essential trace elements for living organism at low concentration (< 10 mg/L), however, they become toxic at high concentration (>10 mg/L) (Volesky, 1995). Most of these metal ions (Cd, Cu, Zn, Hg, As, Ag, Cr, and Fe) can be release from the industries are in simple cationic forms (Mustafa et al., 2004). This study was aimed to assess the bio-concentration of trace heavy metals (Fe, Zn, Mg, Co and pb in *L. sativum* plant and evaluation of antimicrobial activity.

MATERIALS AND METHODS

Plant material and extraction

Seeds of *L. sativum* were dried at room temperature for eleven days and when properly dried then powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. About fifteen grams of the plant sample powdered were soaked in 150 mL methanol individually. The filtrates (crude extracts) obtained were concentrated in rotary evaporator keeping the water bath at 55-60 C^{o}. The isolated extracts were re-suspended in a minimum required volume of corresponding solvents and placed on the water bath at 60 Cº to evaporate the extra solvents for the isolation of pure extracts (Altameme et al., 2015a; Jasim et al., 2015; Hameed et al., 2015a). Then all the extracts were preserved in separate containers at 5 Cº for further experimentations.

Elementary Analysis (trace heavy metals) of *L. sativum* samples:

Know weight of the *L. sativum* seed was mixed with few drops of Conc. trioxonitriate acid until a slurry is formed. Standard solutions were prepared by diluting the stock solution with 0.1 M nitric acid. Concentration of elements present were determined using atomic absorption spectrophotometer (WHO, 1998). Calibration curves were prepared using six concentrations, and the linear correlation coefficients had been determined (Altameme et al., 2015b; Hamza et al., 2015).

Gas chromatography – Mass Spectrum analysis

L. sativum GC–MS analysis were carried out in a GC system (Agilent 7890Aseries, USA). The flow rate of the carrier gas, helium (He) was set to beat 1 mL min−1, split ratio was 1:50. The injector temperature was adjusted at 250◦C, while the detector temperature was fixed to280◦C. The column temperature was kept at 40◦C for 1 min fol-lowed by linear programming to raise the temperature from 40◦to 120◦C (at 4 Cº min−1with 2 min hold time), 120 Cº to 170 Cº (at 6 Cº min−1with 1 min hold time) and 170 Cº to 200 Cº (at10◦C

min−1with 1 min hold time). The transfer line was heated at 280 Cº. Two microliter of FAME sample was injected for analysis. Mass spectra were acquired in scan mode (70 eV); in the range of 50–550 m/z. Identification of compounds interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA) (Altameme et al., 2015c; Hameed et al., 2015b; Hussein et al., 2016). The database consists of more than 62,000 patterns of known compounds. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library (Hussein et al., 2016; Shareef et al., 2016).

Collection of bacterial cultures

The samples were collected from the main laboratory of Hilla Hospital. The anti-bacterial activity was evaluated using Mueller-Hinton agar. The bacterial plates were incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was measured to evaluate the antimicrobial activity. Each test was performed twice and the average of the results was calculated. The extraction solvents were used as negative control (Hussein et al., 2016; Hameed et al., 2015c; Mohammed et al., 2016). The test pathogens were swabbed in Muller Hinton agar plates. 60µl of plant extract was loaded on the bored wells. The wells were bored in 0.5cm in diameter. The plates were incubated at 37C° for 24 hrs and examined. After the incubation the diameter of inhibition zones around the discs was measured (Kadhim et al., 2016; Mohammed et al., 2016).

Antifungal activities assay

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 50 µl of the samples solutions *L. sativum* was delivered into the wells. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation (Hadi et al., 2016; Hameed et al., 2016; Mohammed et al., 2016).

Statistical analysis

Results of the study were based on SPSS version 16.0 and Microsoft excel was used to evaluate the percentages of inhibitions and bactericidal activities

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic seeds extract of *L. sativum,* shown in Table 1. The GC-MS chromatogram of the 46 peaks of the compounds detected was shown in **Figure 1**. Chromatogram GC-MS analysis of the methanol extract of *L. sativum* showed the presence of forty six major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be Deoxyspergualin, 6-Oxa-bicyclo[3.1.0]hexan-3-one, **Figure 2**. The second peak indicated to be 2-Furancarboxaldehyde , 5-methyl, 9-Oxabicyclo[3.3.1]honane-2,6-diol **Figure 3**. The next peaks considered to be, Glycyl-dl-serine, 2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin, Methyl nicotinate, 4H-Pyran-4-one , 2,3-dihydro-3,5-dihydroxy-6-methyl-, Thiocyanic acid , octyl ester, Maltose, Benzofuran ,2,3-dihydro, 5-Hydroxymethylfurfural, 2-Methoxy-4-vinylphenol, Ascaridole epoxide, Phenol , 2-methoxy-5-(1-propenyl)-, (E), α-D-Glucopyranoside , O-α-D-Glucopyranosyl-(1.fwdarw)-ß-D-fruc, 2H-Indeno[1,2-b]furan-2-one,3,3a,4,5,6,7,8,8b-octahydro-8,8-dim, Limonen -6-ol , pivalate, (5ß)Pregnane -3,20ß-diol , 14α , 18α-[4-methyl-3-oxo-(1-oxa-4-azabul, Cinnamic acid , 4-hydroxy-3-methoxy-,{5-hydroxy-2-hydroxymethyl, 9-Oximino-2,7-diethoxyfluorene, Phorbol, Streptovitacin A, 4,25-Secoobscurinervan-4-ol,6,7-didehydro-22-ethyl-15,16-dimethy, Desulphosinigrin, d-Mannose, Methyl (1-O-retinyl-2,3,4-triacetyl-ß-D-glucopyran) urinate, Tetraacetyl-d-xylonic nitrile, Dasycarpidan -1-methanol , acetate (ester), Octadecanoic acid, 1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a,

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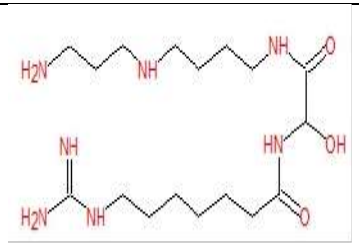
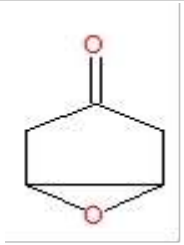
Oxiraneoctanoic acid 3-octyl -,cis, 9- Octadecenamide , (Z), Octadecanal, 2-bromo, Tributyl acetylcitrate, Pyrrolidine , 1-(1-oxo-7,10-hexadecadienyl), 8H-Azecino[5,4-b]indol-8-one , 5-ethylidene – 1,2,3,4,5,6,7,9-octahy, 16- Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one, 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one , 9-[[[2-(dimethylan, Pregn-5-ene -3,8,11,12,14,20 –hexol, (3ß ,11α ,12ß ,14ß,20R), y-Tocopherol, Vitamin E, 6,7-Epoxypregn-4-ene -9,11,18 triol-3,20-dione , 11,18 –diacetate, Stigmasterol, 9,19-Cyclolanostane -3,7-diol and Ergosta – 5,22,-dien-3-ol ,acetate , (3ß,22E) (**Figure 3-32**). The FTIR analysis of *L. sativum* seeds proved the presence of Alkenes, Alcohols, Ethers, Carboxlic acids, Esters, Alkanes, Alcohols and Phenols which shows major peaks at 765.74, 785.03, 790.81, 810.10, 810.10, 867.97, 1014.56, 1062.78, 1143.79, 1220.94, 1286.52, 1286.52, 1361.74, 1627.92, 1743.65, 2854.65, 2924.09 and 3298.28 (**Table 2; Figure 33)**, and the trace metal levels in the analyzed samples are shown in **Table 3**. In this study Fe content was (39±1.20 ppm) and higher content compare with (WHO, 2007). Iron is an essential element for plant growth and human life (Korfali et al., 2013). In *L. sativum* seeds zinc metal reach 6.5±0.17 ppm. Magnesium is essential metal and major biological compound in DNA and ATP (Ştef et al., 2010; Subramanian et al., 2012). Mg content was (28.29±0.23 ppm). Normal concentration of Cobalt metal was found in *L. sativum* (2.98±0.11). In our study, lead Pb content (2.98±0.11 ppm) was less than the WHO. Trace element (Cobalt) required for several biological actions in human body (Al-Dalain et al., 2012; Nicoleta et al., 2013; Sulaiman et al., 2013).

Table 1. Bioactive chemical compounds identified in methanolic extract of *Lepidium sativum*.

Serial No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragment-ions	Pharmacological actions
1.	Deoxyspergualin	3.287	387	387.295788		59,72,86,128,187,216,252	Anti-angiogenic action.
2.	6-Oxa-bicyclo[3.1.0]hexan-3-one	3.338	98	98.0367794		55,69,81,98	anti-HIV *activity*

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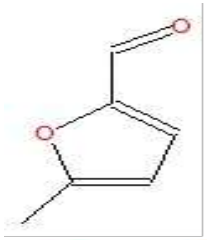
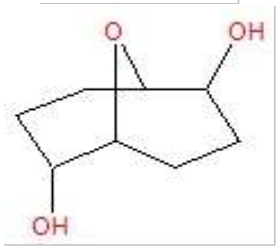
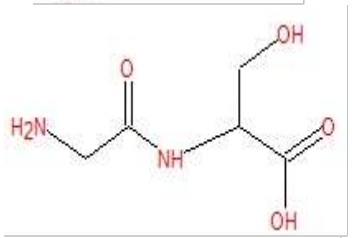
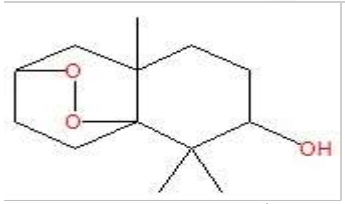
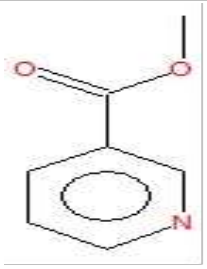
3.	2-Furancarboxaldehyde , 5-methyl-	3.642	110	110.0367794		53,81,95,110	antimicrobial activity
4.	9-Oxabicyclo[3.3.1]honane-2,6-diol	3.859	158	158.094295		57,68,79,84,97,112,122,140,158	Unknown
5.	Glycyl-dl-serine	4.603	162	162.064056		60,74,85,114,144	antagonistic effects
6.	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	4.929	226	226.156895		55,69,81,85,95,99,109,128,139,175,193,208	anti-tumour *effect*
7.	Methyl nicotinate	5.644	137	137.047678		51,78,106,137	anti-tumour activity

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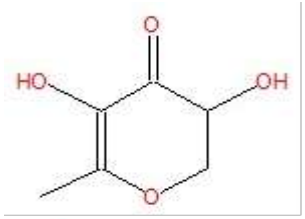
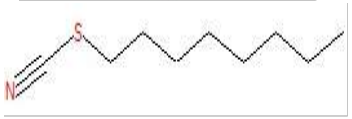
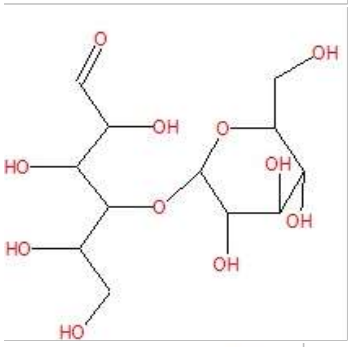
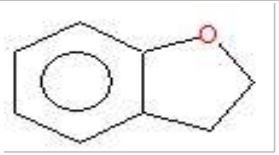
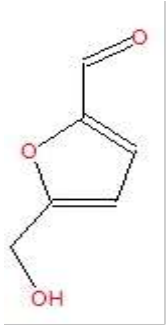
8.	4H-Pyran-4-one , 2,3-dihydro-3,5-dihydroxy-6-methyl	5.833	144	144.042258		55,72,85,101,115,126,144	anti-cancer activities
9.	Thiocyanic acid , octyl ester	6.028	171	171.1081705		55,69,83,101,115,124,144,156	antioxidant activity
10.	Maltose	6.520	342	342.11621		60,73,85,97,126,145,163,191,215	antioxidant, antidiabetic and anti-inflammatory activity
11.	Benzofuran ,2,3-dihydro-	6.686	120	120.0575147		51,63,77,86,91,105,120	anti-HIV, anticancer, antibacterial, and antifungal activities.
12.	5-Hydroxymethylfurfural	7.041	126	126.031694		53,69,81,84,97,109,126	anti-allergenics and anti-diabetics

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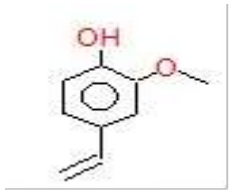
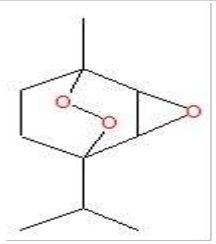
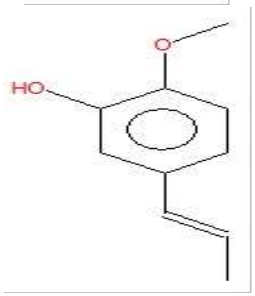
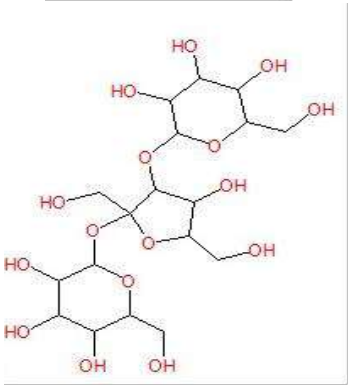
13.	2-Methoxy-4-vinylphenol	7.973	150	150.06808		51,63,77,89,107, 118,135,150	Antioxidant and anti-tumor.
14.	Ascaridole epoxide	8.168	184	184.109944		55,69,79,91,97,1 07,117,125,135, 150,168	anti-inflammatory
15.	Phenol , 2-methoxy-5-(1-propenyl)-, (E)-	8.442	164	164.08373		55,65,77,91,121, 131,149164	anti-plasmodial activity, cytotoxic effect, antipyretic, induce apoptosis
16.	α-D-Glucopyranoside , O-α-D-Glucopyranosyl-(1.fwdarw)-ß-D-fruc	8.769	504	504.169035		60,73,85,97,113, 126,145,187	anti-inflammatory, diuretic and tonic effects

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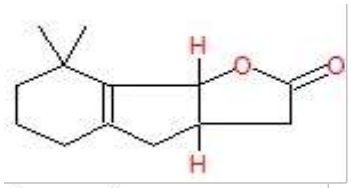
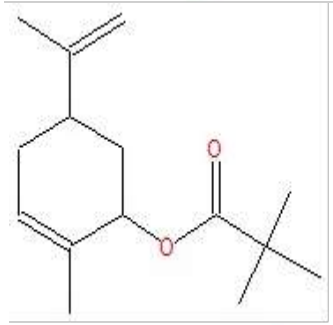
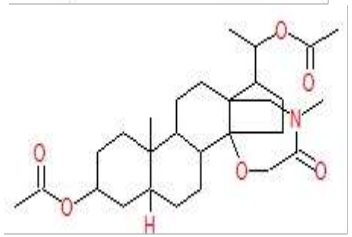
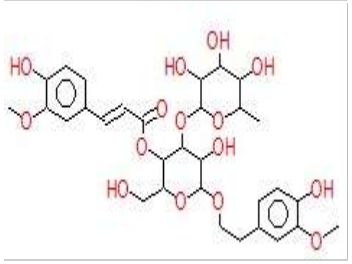
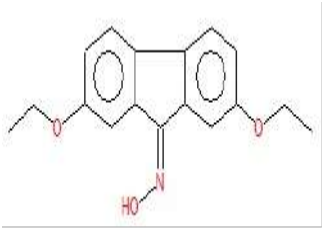
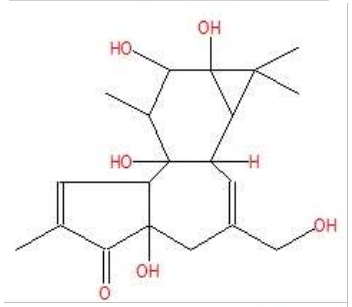
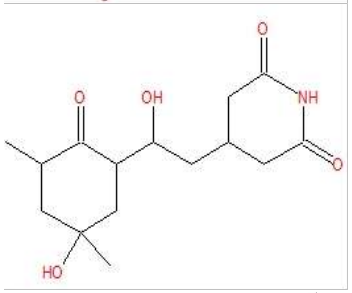
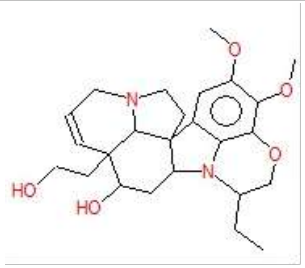
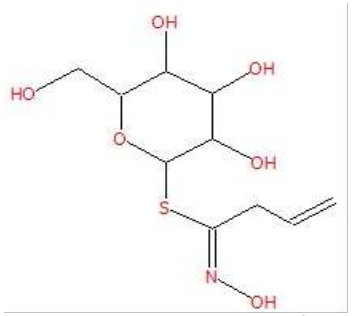
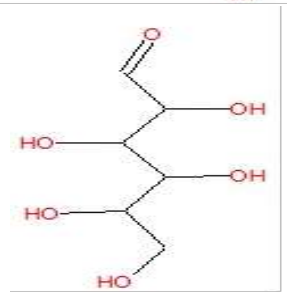
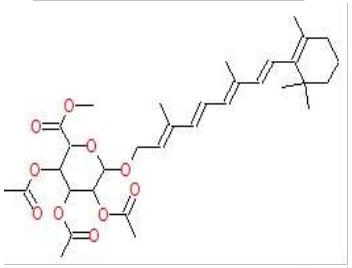
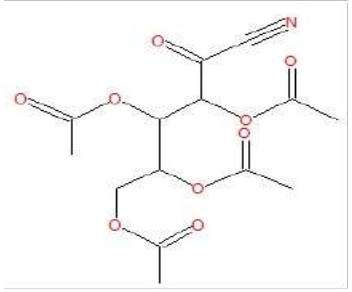
17.	2H-Indeno[1,2-b]furan-2-one,3,3a,4,5,6,7,8,8b-octahydro-8,8-dim	8.986	206	206.13068		55,67,79,91,107,119,131,145,173,191,206	Unknown
18.	Limonen -6-ol , pivalate	9.289	236	236.17763		57,93,107,119,134,185,236	anti-stress
19.	(5ß)Pregnane -3,20ß-diol , 14α , 18α-[4-methyl-3-oxo-(1-oxa-4-azabul	10.085	489	489.309038		57,73,133,161,177,267,328,360,399,459	New chemical compound
20.	Cinnamic acid , 4-hydroxy-3-methoxy-,{5-hydroxy-2-hydroxymethyl-	12.414	652	652.23672		55,69,77,91,105,121,137,151,168,215,268,296,330,354,386,418	anti-inflammatory effect

21.	9-Oximino-2,7-diethoxyfluorene	12.717	283	283.120844		58,69,81,94,149, 181,226,254,283	anti-pathogenic activity
22.	Phorbol	12.883	364	364.18859		55,69,83,91,109, 159,187,215,233 ,259,328	Antioxidant, anti-inflammatory and anticancer activities
23.	Streptovitacin A	12.963	297	297.157623		55,84,99,113,14 2,196,224,279	anti-diabetic activity
24.	4,25-Secoobscurinervan-4-ol,6,7-didehydro-22-ethyl-15,16-dimethy-	13.157	442	442.246773		107,138,176,191 ,221,244,260,41 3,427,442	anti-inflammatory and analgesic effects

25.	Desulphosinigrin	13.484	279	279.077658		60,73,85,103,127,145,163,213,262	anti-oxidant activity
26.	d-Mannose	15.789	180	180.063388		60,73,103,149	Anti-inflammatory effect
27.	Methyl (1-O-retinyl-2,3,4-triacetyl-ß-D-glucopyran) urinate	16.064	602	602.309097		57,73,111,155,171,220,268,285,317,346,386,420	Anti-Inflammatory
28.	Tetraacetyl-d-xylonic nitrile	16.253	343	343.090332		60,73,112,133,197,223,251,281	anti-oxidative activities

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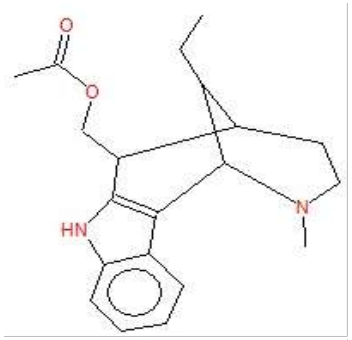
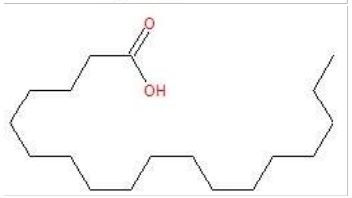
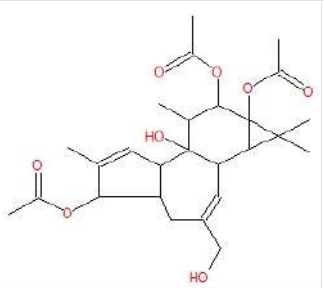
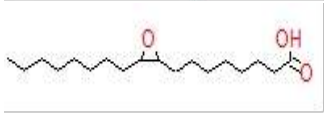
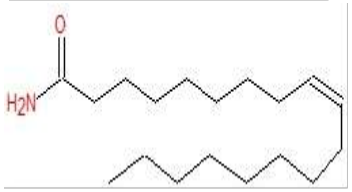
29.	Dasycarpidan -1-methanol , acetate (ester)	16.339	326	326.199429		60,69,83,97,111, 124,167,180,222 ,256,284,326	New chemical compound
30.	Octadecanoic acid	17.106	284	284.27153		60,73,83,97,115, 129,143,157,171 ,185,199,227,24 1,255,284	antiviral and anti-inflammatory activities
31.	1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a	16.722	476	476.241018		55,69,131,200,2 96,314,356,416, 476	Unknown
32.	Oxiraneoctanoic acid 3-octyl - ,cis-	17.323	298	298.250795		55,69,83,97,124, 155,185,200,280	anti-inflammatory
33.	9- Octadecenamide , (Z)-	17.294	281	281.271864		59,72,83,114,18 4,212,264,281	anti-inflammatory activity and antibacterial activity

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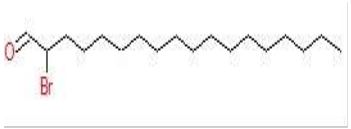
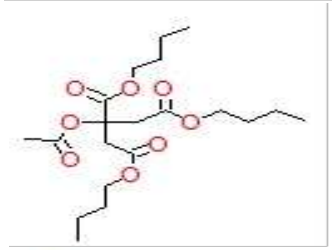
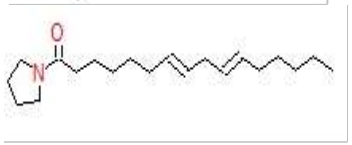
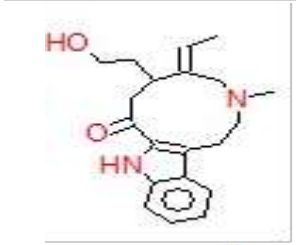
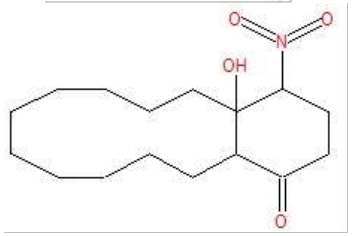
34.	Octadecanal, 2-bromo-	17.815	346	346.187128		57,83,95,124,224,267,296,346	nontoxic and efficient anti-microbial agents
35.	Tributyl acetylcitrate	17.952	402	402.225368		57,112,129,157,185,213,231,259,273329	Antibacterial and antitumor
36.	Pyrrolidine , 1-(1-oxo-7,10-hexadecadienyl)-	18.393	305	305.271864		55,70,79,98,113,126,154,168,194,208,248,276,305	anti-inflammatory effects
37.	8H-Azecino[5,4-b]indol-8-one , 5-ethylidene – 1,2,3,4,5,6,7,9-octahy	18.639	326	326.199429		55,69,81,95,144,168,196,210,240,281,308,326	New chemical compound
38.	16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one	18.971	297	297.194008		55,69,81,98,126,158,173,209,249,267,297	antibacterial activity against *Pseudomonas* aerogenosa

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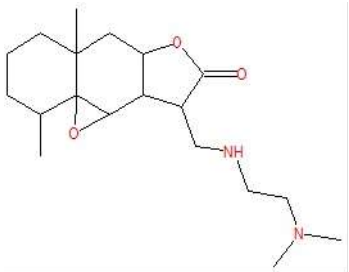
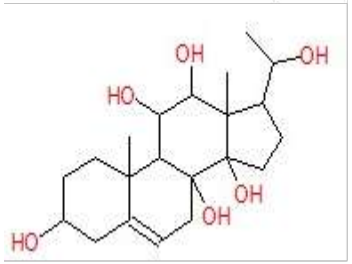
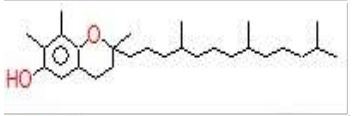
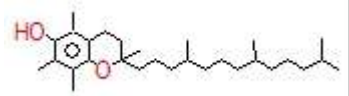
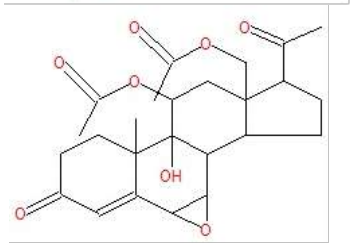
39.	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one , 9-[[[2-(dimethylan	19.514	336	336.241293		58,81,109,149,173,204,233,278,336	pharmacological activities like anti-inflammatory and analgesic
40.	Pregn-5-ene -3,8,11,12,14,20 –hexol, (3ß ,11α ,12ß ,14ß,20R)-	19.738	382	382.235538		55,67,81,97,120,138,191,209,227,244,284,364,382	New chemical compound
41.	y-Tocopherol	25.191	416	416.36543		57.107,151,191,246,288,330,372,416	Anti-inflammatory properties
42.	Vitamin E	26.427	430	430.38108		57,71,91,121,165,205,246,275,303,344,372,430	antioxidant, and anti-inflammatory activities
43.	6,7-Epoxypregn-4-ene -9,11,18 triol-3,20-dione , 11,18 –diacetate	26.867	460	460.209719		55,79,152,207,251,297,358,400,460	New chemical compound

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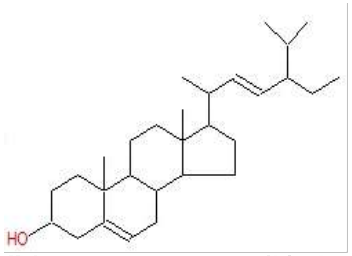
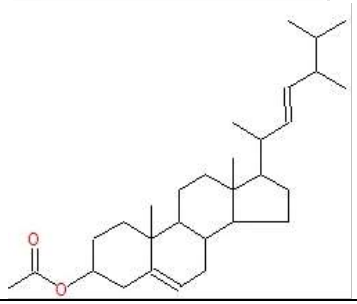
44.	Stigmasterol	28.870	412	412.370516		55,69,83,133,213,255,300,351,369,412	antimicrobial and antioxidative activities
45.	9,19-Cyclolanostane -3,7-diol	29.551	444	444.39673		57,69,95,149,201,219,261,295,313,339,393,411	New chemical compound
46.	Ergosta – 5,22,-dien-3-ol ,acetate , (3ß,22E)-	29.825	440	440.36543		55,67,91,105,145,213,255,281,327,365,380	anti-tumor *activity* and immunomodulatory activity

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Table 2. FT-IR peak values of *L. sativum*

No.	Peak (Wave number cm-	Intensity	Bond	Functional group assignment	Group frequency
1.	765.74	67.196	C-H	Alkenes	675-995
2.	785.03	67.381	C-H	Alkenes	675-995
3.	790.81	67.306	C-F stretch	Alkenes	675-995
4.	810.10	68.129	C-O	Alkenes	675-995
5.	867.97	70.579	C-O	Alkenes	675-995
6.	1014.56	47.791	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
7.	1062.78	52.573	NO2	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
8.	1143.79	67.873	C-H	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
9.	1220.94	79.171	-	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
10.	1286.52	79.761	-	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
11.	1361.74	79.175	-	Unknown	-
12.	1454.33	82.336	-	Unknown	-
13.	1627.92	82.070	-	Unknown	-
14.	1743.65	88.702	-	Unknown	-
15.	2854.65	85.695	C-H	Alkanes	2850-2970
16.	2924.09	81.185	C-H	Alkanes	2850-2970
17.	3298.28	77.507	O-H	Hydrogen bonded Alcohols, Phenols	3200-3600

Table 3. Total *L. sativum* elementary content.

No.	Elements	Concentration range (ppm)
1.	Iron (Fe)	39±1.20
2.	Zinc (Zn)	6.5±0.17
3.	Magnesium (Mg)	28.29±0.23
4.	Cobalt (Co)	0.46±0.03
5.	Lead (Pb)	2.98±0.11

Table 4. Antifungal activity of *Lepidium sativum*.

Fungi and Yeast	Plant extract /Antibiotics			
	Plant	Amphotericin B	Fluconazol	Miconazole nitrate
Aspergillus niger	5.00±0.13 [a]	2.05±0.10	2.00±0.11	1.26±0.10
Aspergillus terreus	4.42±0.10	2.62±0.11	2.35±0.17	2.06±0.13
Aspergillus flavus	7.01±0.11	3.00±0.20	1.05±0.09	2.05±0.19
Aspergillus fumigatus	7.00±0.11	2.76±0.13	2.06±0.13	2.65±0.18
Candida albicans	6.99±0.14	2.65±0.11	1.19±0.09	0.09±0.11
Saccharomyces cerevisiae	7.00±0.17	2.09±0.09	1.86±0.15	0.04±0.01
Fusarium sp.	4.09±0.18	2.00±0.08	2.95±0.19	0.07±0.05
Microsporum canis	4.88±0.16	3.04±0.17	2.12±0.16	0.88±0.10
Streptococcus faecalis	3.67±0.19	3.96±0.15	3.29±0.22	1.97±0.18
Mucor sp.	5.62±0.17	1.00±0.06	1.00±0.05	0.08±0.02
Penicillium expansum	4.72±0.10	3.08±0.14	3.04±0.28	2.00±0.19
Trichoderma viride	5.30±0.17	2.00±0.17	3.00±0.25	1.97±0.18
Trichoderma horzianum	5.00±0.11	2.05±0.16	2.09±0.17	2.06±0.13
Trichophyton mentagrophytes	3.24±0.19	1.55±0.12	0.95±0.02	0.35±0.08

[a] The values (average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract and 30 µg/mL of (Amphotericin B; Fluconazol and Miconazole nitrate).

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Table 5. Antibacterium activity of *Lepidium sativum*.

Bacteria	Plant extract /Antibiotics				
	Plant	Cefotoxime	Kanamycin	Rifambin	Streptomycin
Salmonella typhi	3.00±0.27 ª	0.94±0.05	1.87±0.15	0.52±0.11	0.91±0.12
Streptococcus pneumonia	2.77±0.14	1.31±0.17	1.03±0.12	1.62±0.17	1.00±0.13
Pseudomonas eurogenosa	4.60±0.28	1.06±0.10	1.07±0.10	0.57±0.10	0.97±0.09
Staphylococcus epidermidis	5.00±0.29	1.05±0.08	0.99±0.06	0.44±0.02	0.79±0.08
Escherichia coli	6.03±0.27	2.94±0.05	2.00±0.06	0.93±0.01	2.97±0.19
Bacillus subtilis	4.90±0.22	0.94±0.05	0.99±0.06	2.73±0.15	1.90±0.15
Proteus mirabilis	4.85±0.16	1.65±0.18	1.84±0.10	2.00±0.12	1.36±0.08
Streptococcus pyogenes	3.62±0.19	1.05±0.12	1.00±0.09	1.00±0.09	2.00±0.17
Staphylococcus aureus	4.70±0.29	1.65±0.18	0.76±0.01	0.46±0.08	1.00±0.13
Streptococcus faecalis	5.89±0.20	1.65±0.18	2.03±0.16	0.59±0.07	1.35±0.10
Klebsiella pneumonia	3.10±0.15	1.65±0.18	1.00±0.09	0.76±0.09	0.04±0.01

ª The values (average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract, 30 µg/mL of antibiotics (Streptomycin; Rifambin; Kanamycin; Cefotoxime and chloramphenicol).

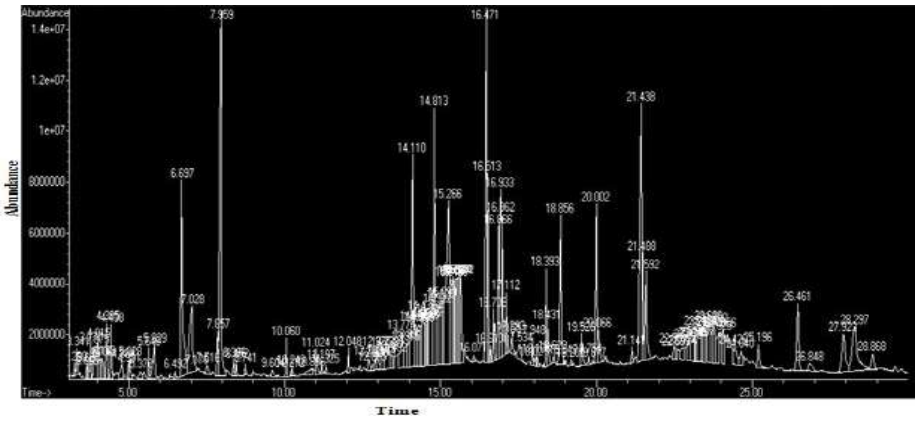

Figure 1. GC-MS chromatogram of methanolic seed extract of *Lepidium sativum.*

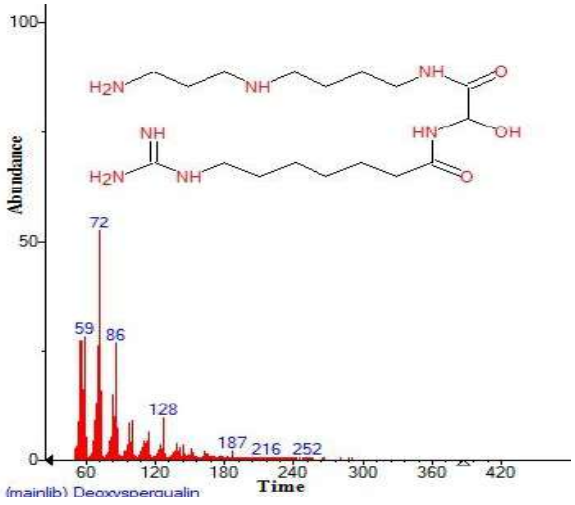

Figure 2. Structure of Deoxyspergualin present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

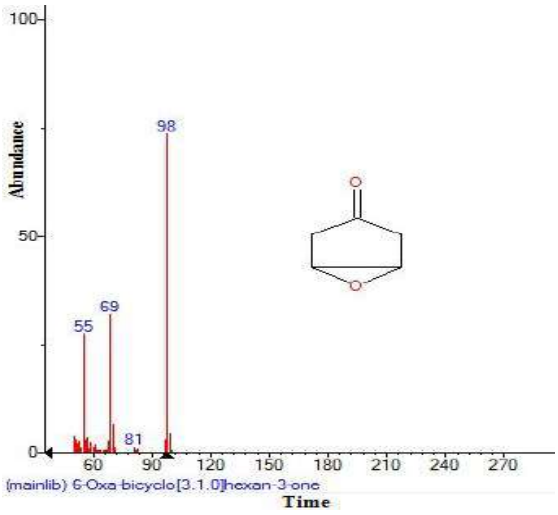

Figure 3. Structure of 6-Oxa-bicyclo[3.1.0]hexan-3-one present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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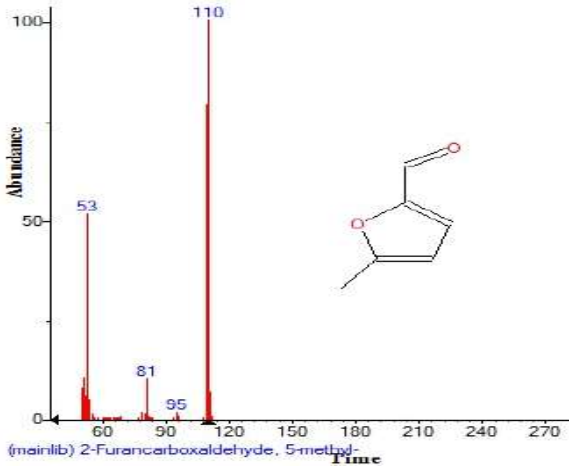

Figure4 . Structure of 2-Furancarboxaldehyde , 5-methyl present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

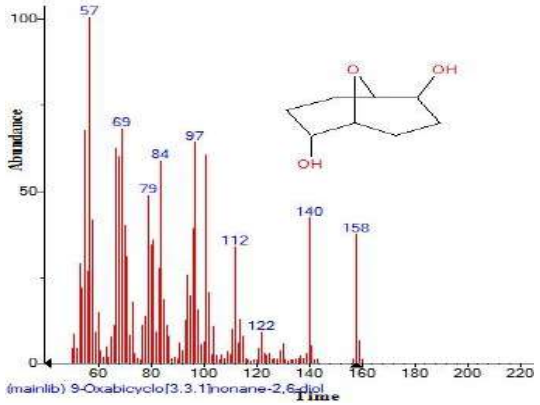

Figure 5. Structure of 9-Oxabicyclo[3.3.1]honane-2,6-diol present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

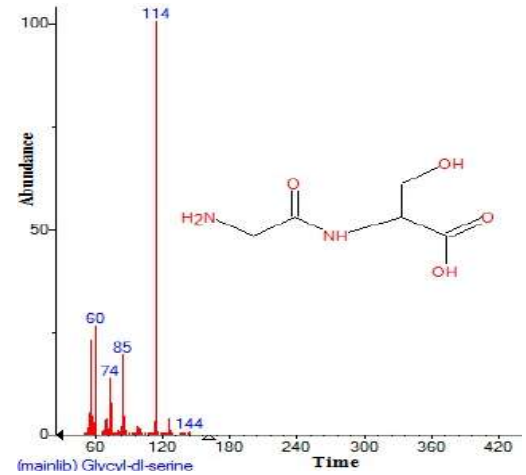

Figure 6. Structure of Glycyl-dl-serine present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

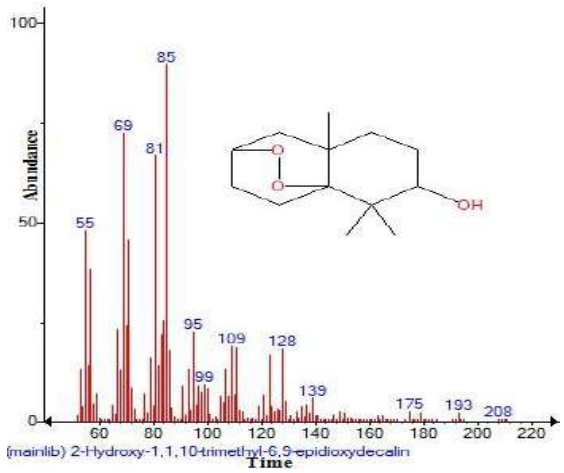

Figure 7. Structure of 2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

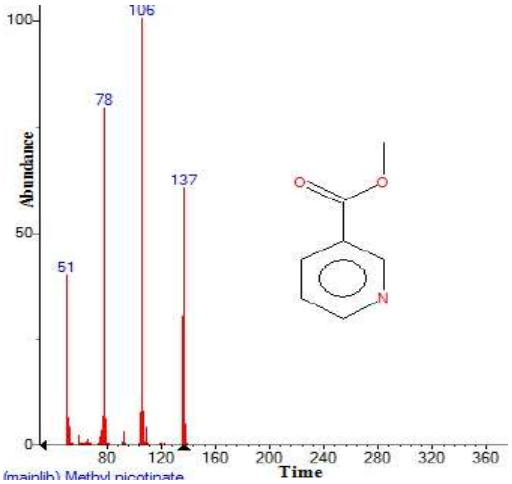

Figure 8. Structure of Methyl nicotinate present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

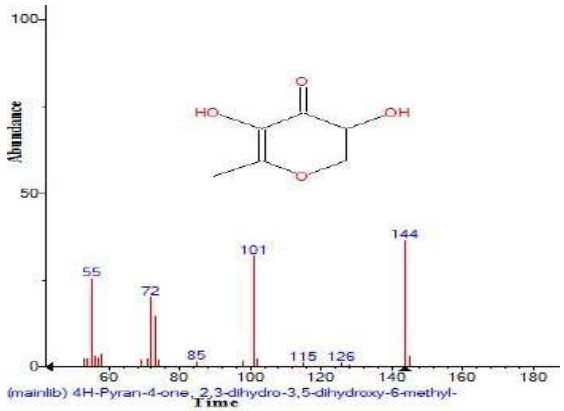

Figure 10. Structure of 4H-Pyran-4-one , 2,3-dihydro-3,5-dihydroxy-6-methyl present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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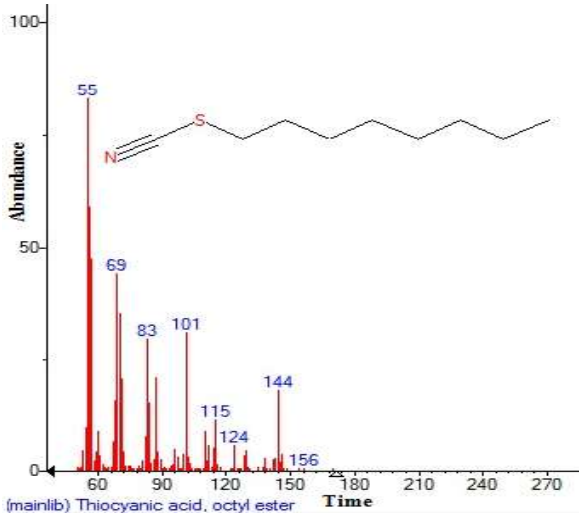

Figure 11. Structure of Thiocyanic acid , octyl ester present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

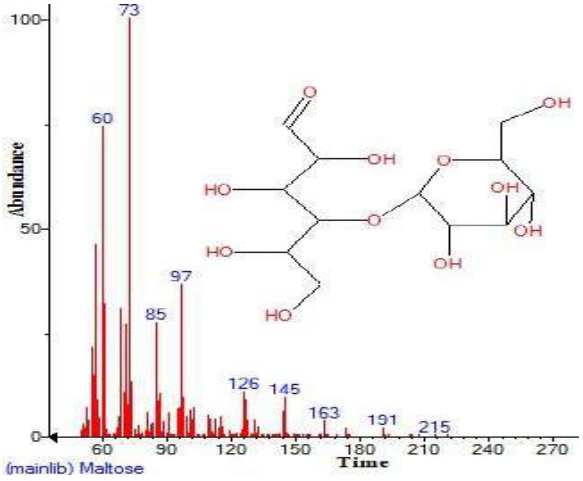

Figure 12. Structure of Maltose present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

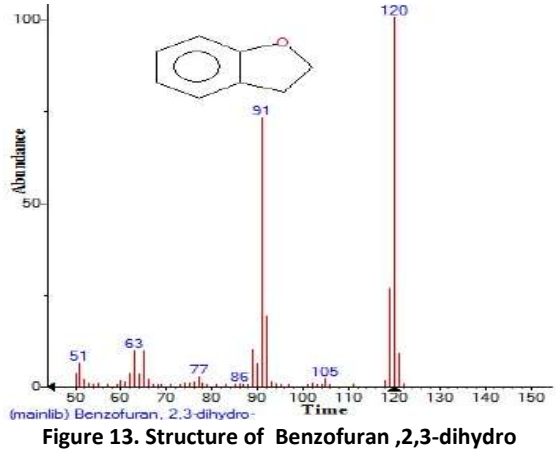

Figure 13. Structure of Benzofuran ,2,3-dihydro present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

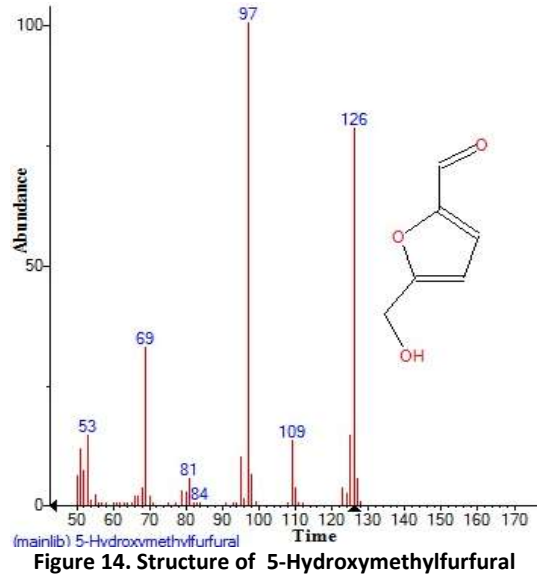

Figure 14. Structure of 5-Hydroxymethylfurfural present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

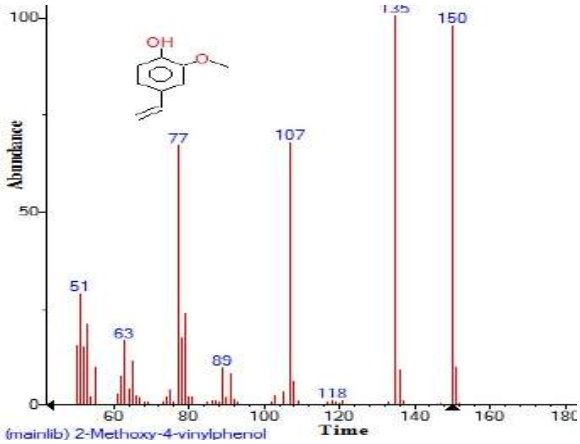

Figure 15. Structure of 2-Methoxy-4-vinylphenol present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

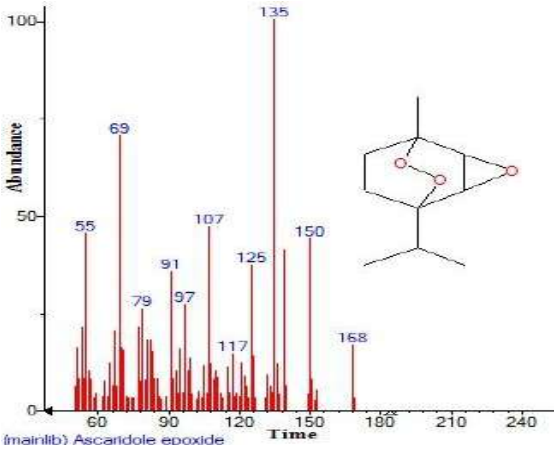

Figure 16. Structure of Ascaridole epoxide present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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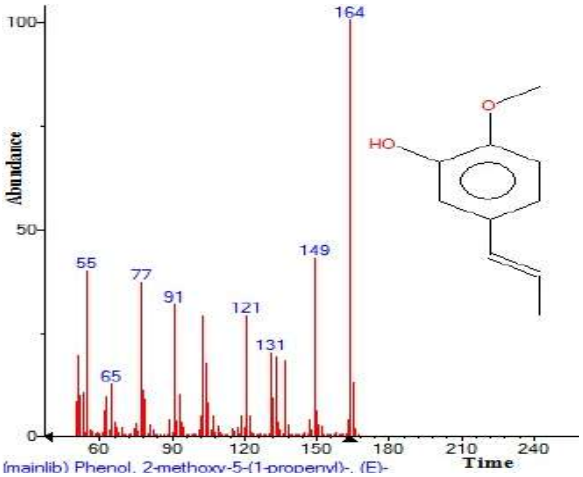

Figure 17. Structure of Phenol , 2-methoxy-5-(1-propenyl)-, (E) present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

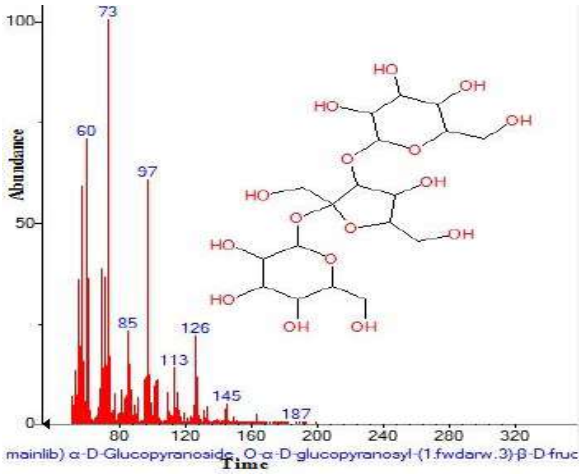

Figure 18. Structure of α-D-Glucopyranoside , O-α-D-Glucopyranosyl-(1.fwdarw)-ß-D-fruc present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

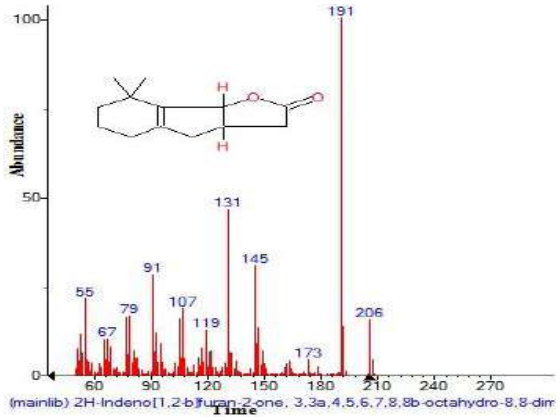

Figure 19. Structure of 2H-Indeno[1,2-b]furan-2-one,3,3a,4,5,6,7,8,8b-octahydro-8,8-dim present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

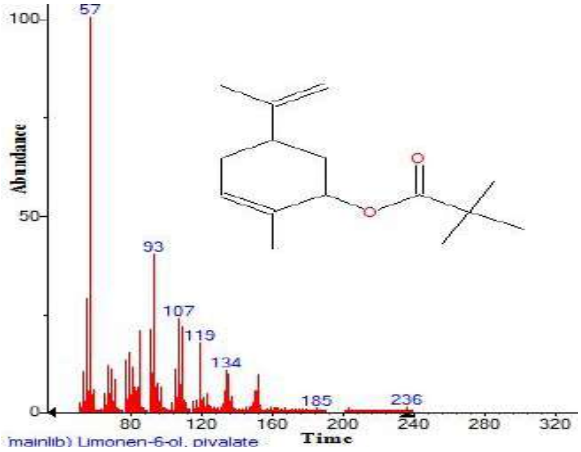

Figure 20. Structure of Limonen -6-ol , pivalate present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

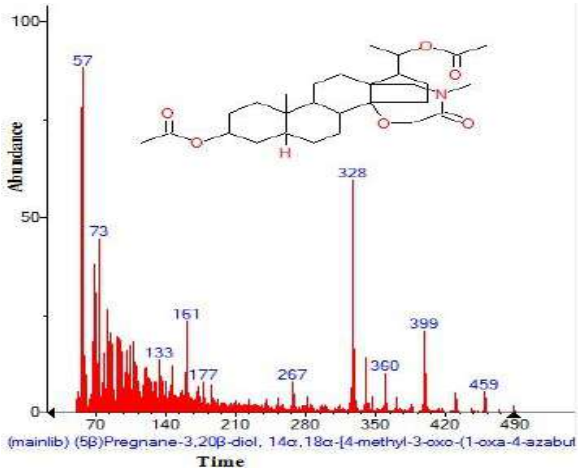

Figure 21. Structure of (5ß)Pregnane -3,20ß-diol , 14α , 18α-[4-methyl-3-oxo-(1-oxa-4-azabul present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

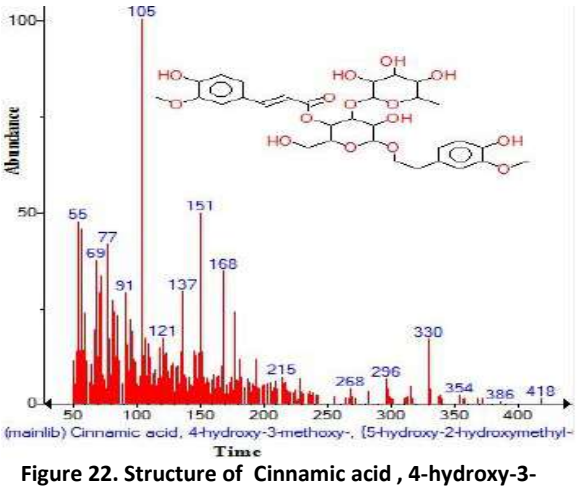

Figure 22. Structure of Cinnamic acid , 4-hydroxy-3-methoxy-,{5-hydroxy-2-hydroxymethyl present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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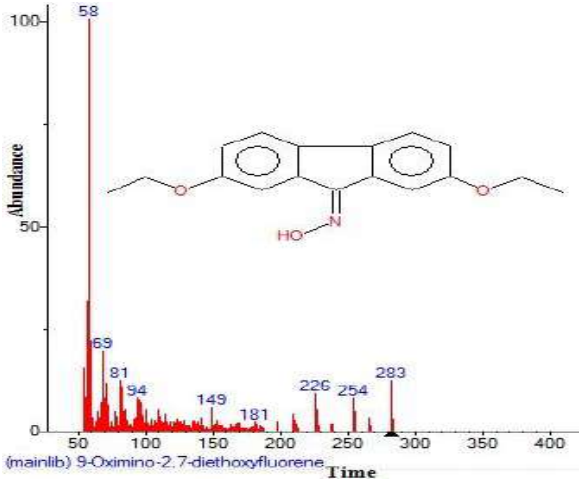

Figure 23. Structure of 9-Oximino-2,7-diethoxyfluorene present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

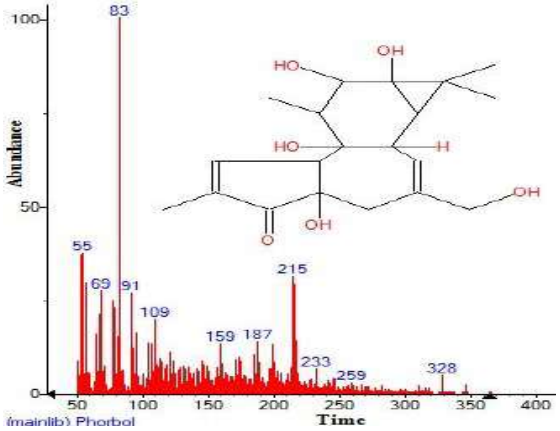

Figure 24. Structure of Phorbol present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

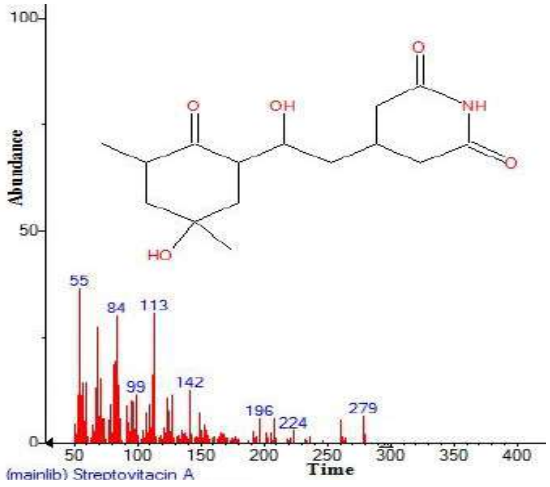

Figure 25. Structure of Streptovitacin A present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

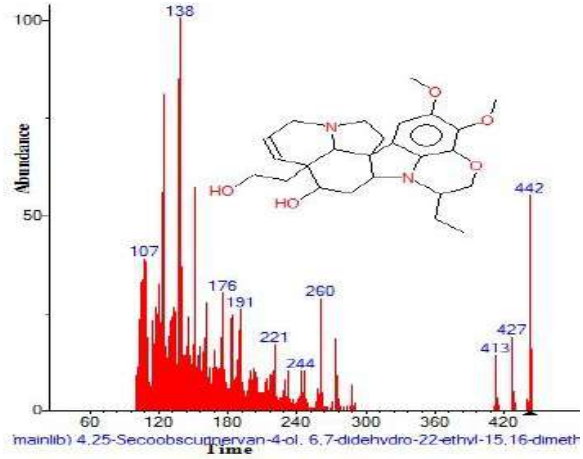

Figure 26. Structure of 4,25-Secoobscurinervan-4-ol,6,7-didehydro-22-ethyl-15,16-dimethy present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

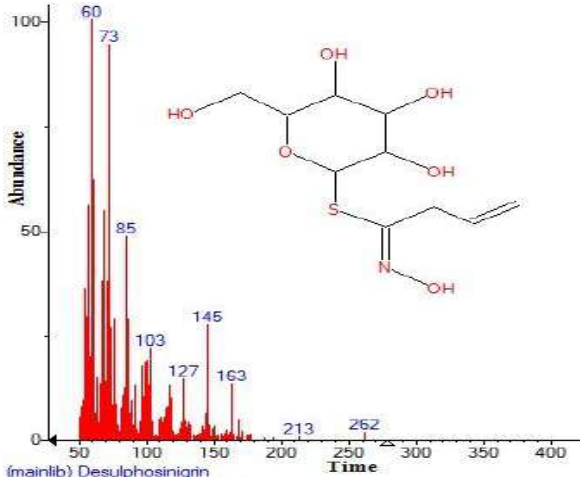

Figure 27. Structure of Desulphosinigrin present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

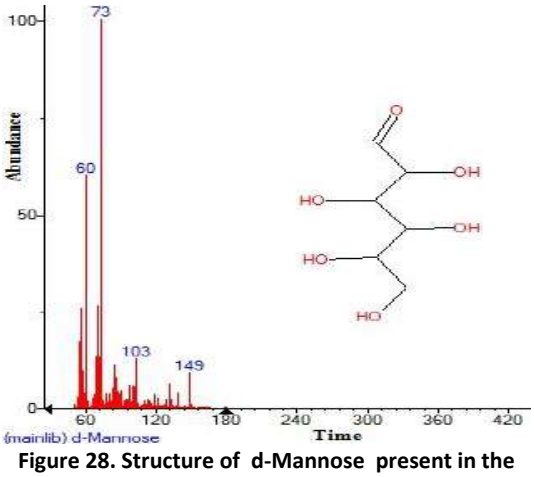

Figure 28. Structure of d-Mannose present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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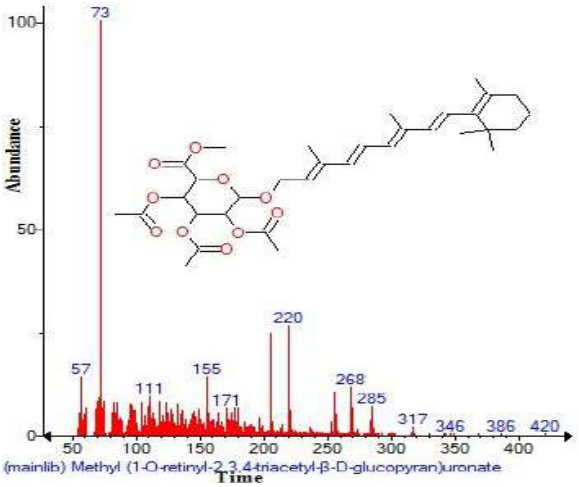

Figure 29. Structure of Methyl (1-O-retinyl-2,3,4-triacetyl-ß-D-glucopyran) urinate present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

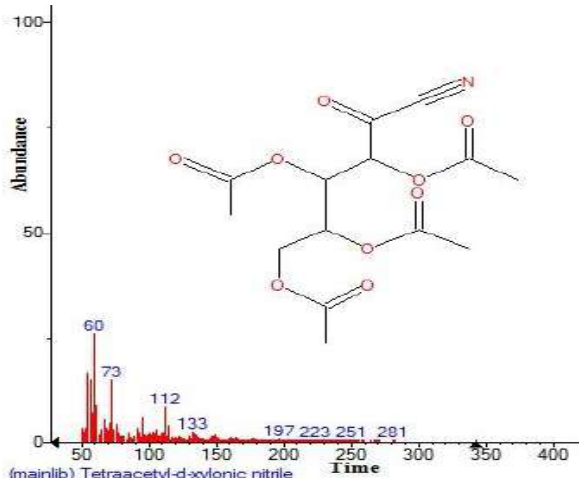

Figure 30. Structure of Tetraacetyl-d-xylonic nitrile present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

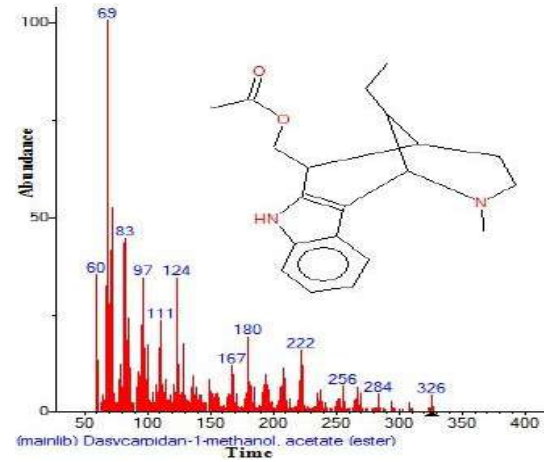

Figure 31. Structure of Dasycarpidan -1-methanol , acetate (ester) present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

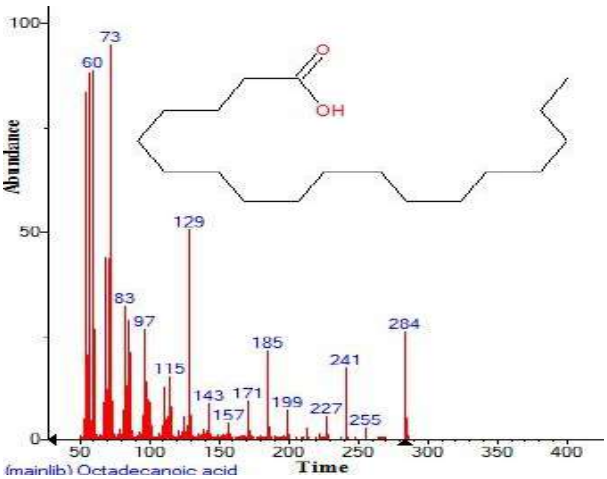

Figure 32. Structure of Octadecanoic acid present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

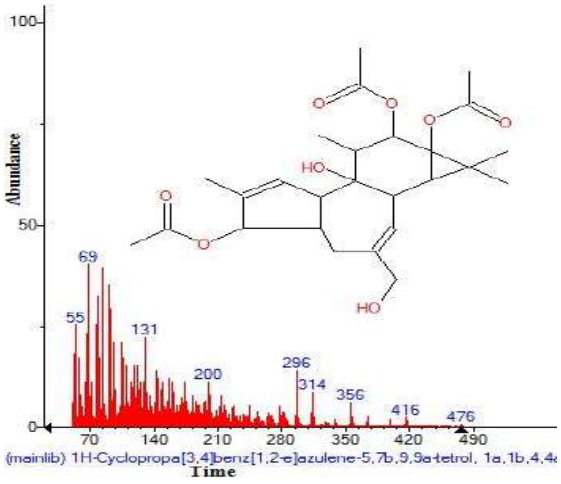

Figure 33. Structure of 1H-Cyclopropa[3,4]benz[1,2-e]azulene-5,7b,9,9a-tetrol,1a,1b,4,4a present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

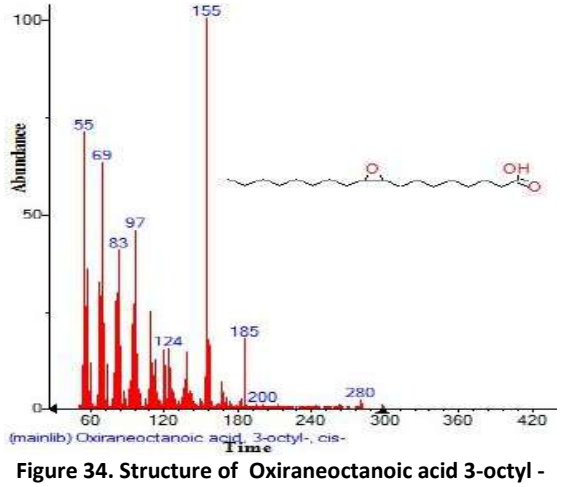

Figure 34. Structure of Oxiraneoctanoic acid 3-octyl - ,cis present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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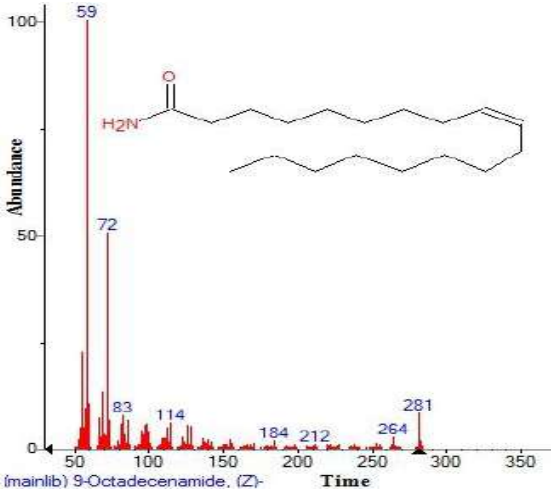

Figure35 . Structure of 9- Octadecenamide , (Z) present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

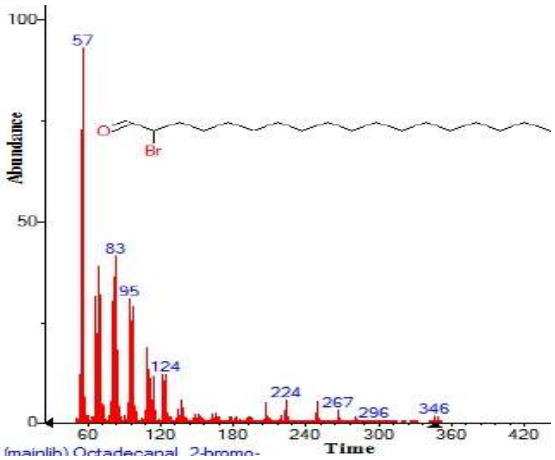

Figure 36. Structure of Octadecanal, 2-bromo present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

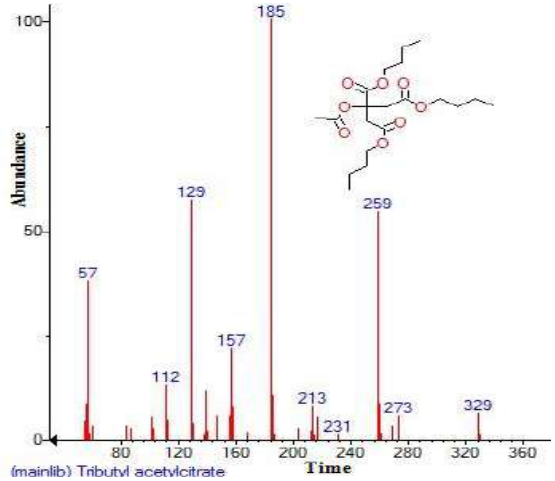

Figure 37. Structure of Tributyl acetylcitrate present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

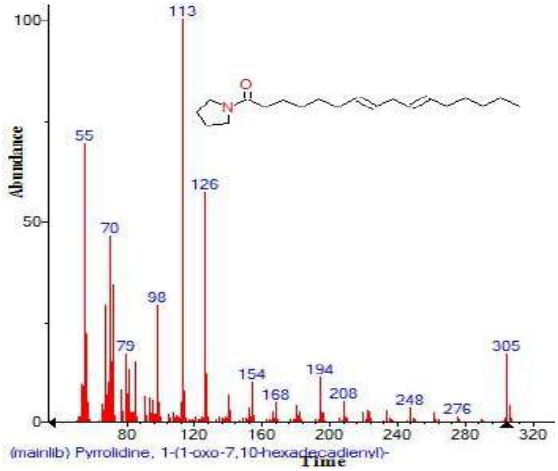

Figure 38. Structure of Pyrrolidine , 1-(1-oxo-7,10-hexadecadienyl) present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

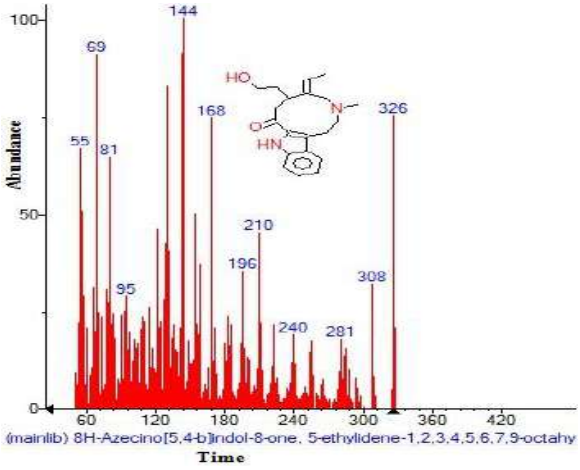

Figure39 . Structure of 8H-Azecino[5,4-b]indol-8-one , 5-ethylidene – 1,2,3,4,5,6,7,9-octahy present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

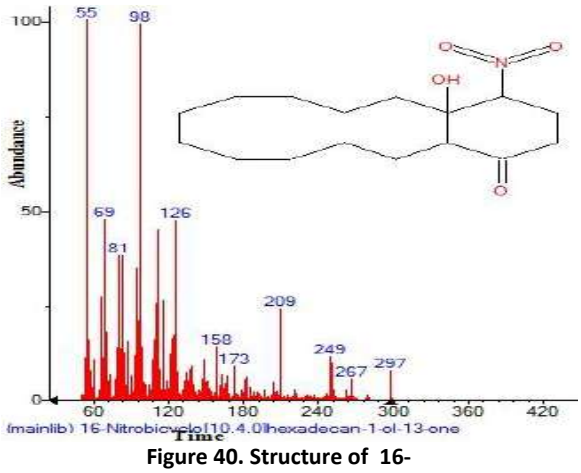

Figure 40. Structure of 16-Nitrobicyclo[10.4.0]hexadecane-1-ol-13-one present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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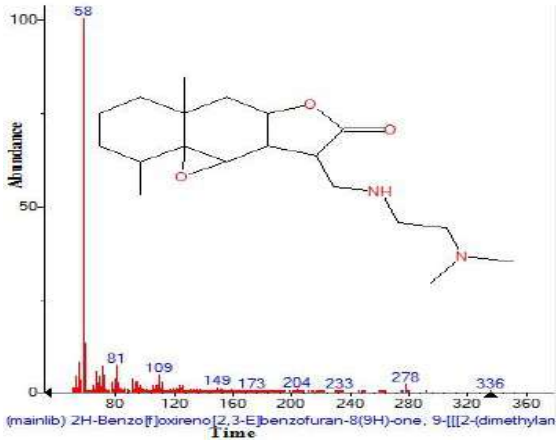

Figure 41. Structure of 2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)-one , 9-[[[2-(dimethylan present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

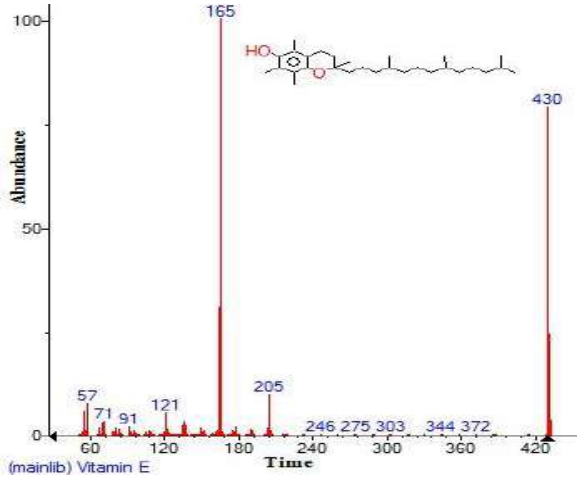

Figure 44. Structure of Vitamin E present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

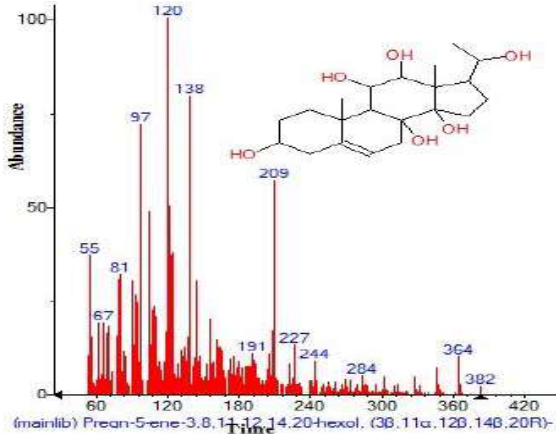

Figure 42. Structure of Pregn-5-ene -3,8,11,12,14,20 – hexol, (3ß ,11α ,12ß ,14ß,20R) present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

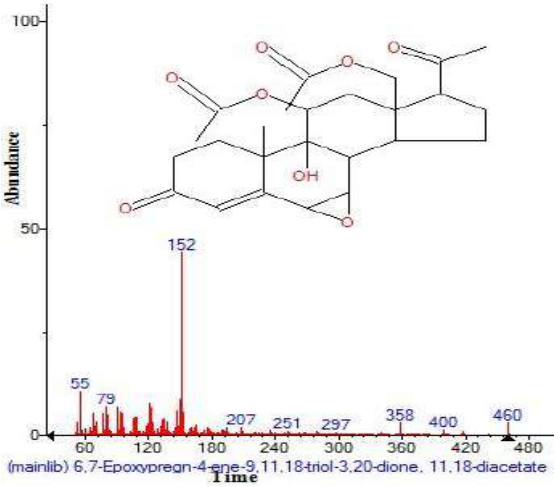

Figure 45. Structure of 6,7-Epoxypregn-4-ene -9,11,18 triol-3,20-dione , 11,18 –diacetate present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

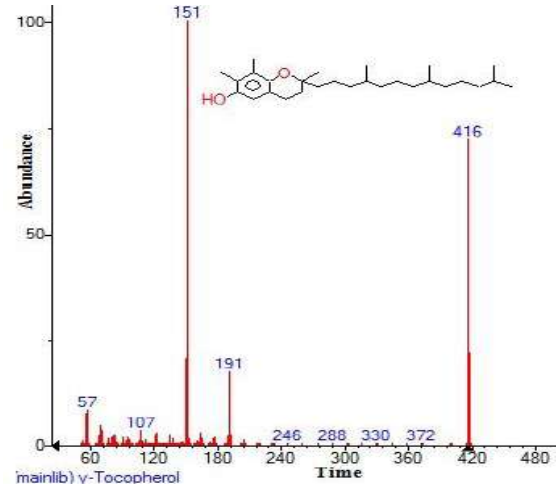

Figure 43. Structure of γ-Tocopherol present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

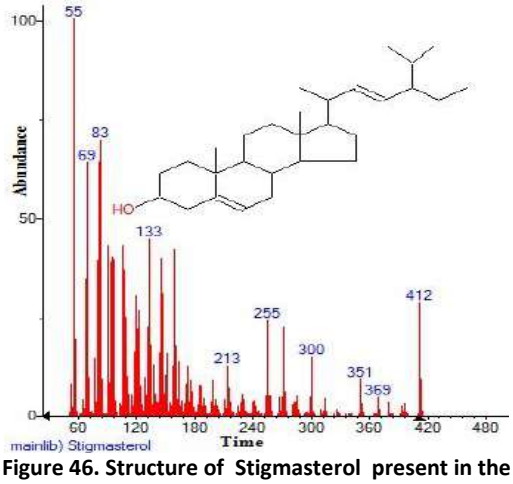

Figure 46. Structure of Stigmasterol present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

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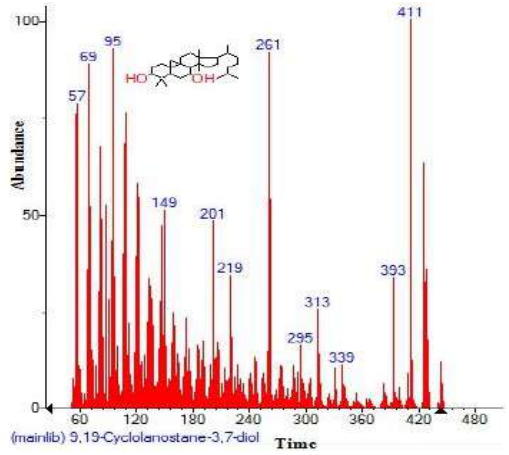

Figure 47. Structure of 9,19-Cyclolanostane -3,7-diol present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

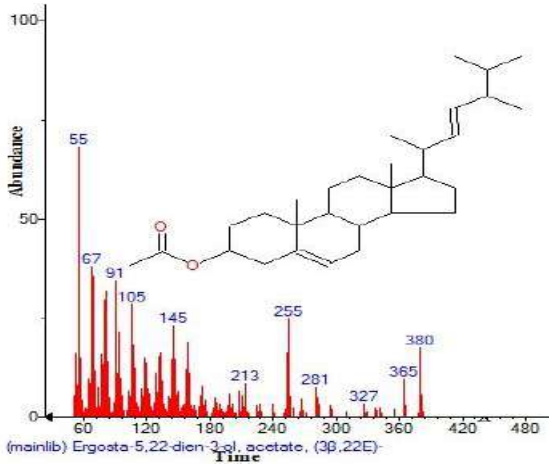

Figure 48. Structure of Ergosta – 5,22,-dien-3-ol ,acetate , (3ß,22E) present in the methanolic seeds extract of *Lepidium sativum* using GC-MS analysis.

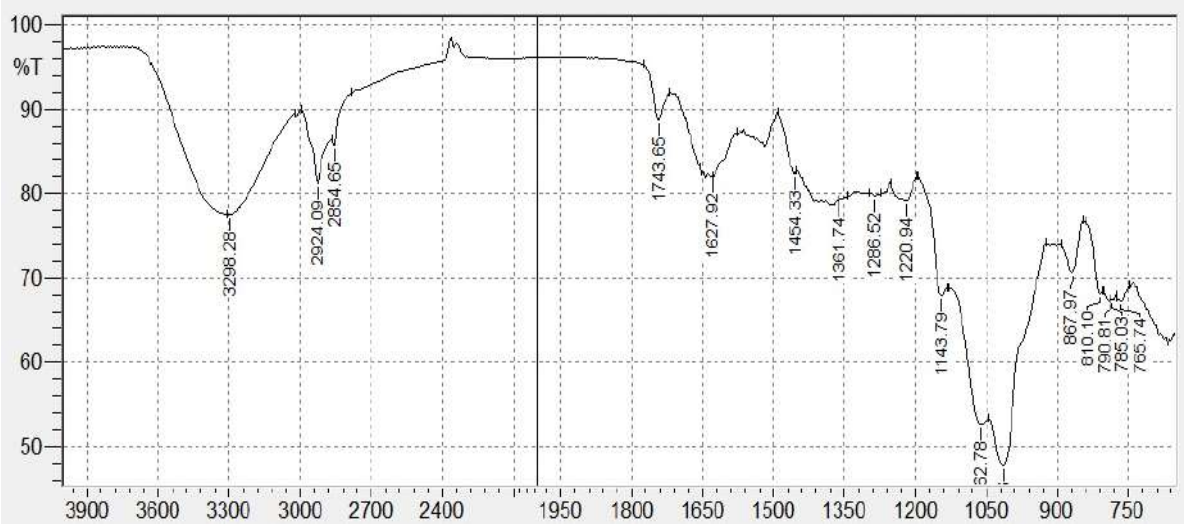

Figure 49. FT-IR profile of *Lepidium sativum*

The methanol extract of *L. sativum* was tested against bacterial and fungal strains. A volume of 75µl (1mg/1ml) of each extract was applied for antimicrobial activity evaluation through well diffusion method. Maximum zone formation was against *Staphylococcus epidermidis* (5.00±0.29). *L.*

sativum was very highly active against *Aspergillus flavus* (7.01±0.11). Results of antimicrobial activity are presented in **Table 4** and **Table 5**.

In comparison to the antibiotics used in this study, the plants extracts were far more active against the test bacterial strains. Raval et al., (2013) studied the antimicrobial activity of the petroleum ether, methanol and water extracts of *L. sativum* seeds against six pathogenic micro-organisms viz. *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and fungus *Candida albican*. The petroleum ether extract of *L. sativum* seeds in different concentrations (2.5, 5 and 10%) were found to be active antimicrobials against all the 6 pathogens with a strong antifungal activity at the concentration of 2.5 and 10%. Sharma et al. (2012) studied the antifungal activity of ethanolic extract of *Lepidium sativum* seeds against *Fusarium equiseta, Aspergillus flavus* and *Alternaria alternate,* by employing various concentrations of *Lepidium sativum* seed extracts (2-8%) in Potato Dextrose Agar (PDA). Traditional sweets for lactating mothers are prepared from the *Lepidium sativum* seeds. Liver inflammation also plays an important role in drug inducedacute hepatitis (Afaf et al., 2008; Lee et al., 2013; Raval et al., 2013). Lee et al., (2013) evaluated the free radical scavenging activity of total phenolic and flavonoid compounds extracted from *L. sativum* seeds. The methanolic extract was found to contain a noticeable amount of total phenols (8.651mgGAE/gm) and flavonoids (4.023 mg CAE/gm)

CONCLUSION

L. sativum seeds are rich source of essential amino acids, fibers and minerals. *L. sativum* is native plant of Iraq. In the present study determined that forty six phytoconstituents were identified from methanol seed extract of *L. sativum* by gas chromatogram and mass spectrometry (GC-MS) analysis. *L. sativum* seed can be used as a promising multipurpose medicinal source whereas further clinical trial is required to prove its efficacy.

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