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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS OF *LEPIDIUM SATIVUM* USING UV-VIS AND GC-MS.

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Abstract

The present study was carried out to characterize the bioactive constituents present in seed and whole plant extracts of *Lepidium sativum* using UV-VIS and GC-MS. The crude extracts were scanned in the wavelength ranging from 200 to 800 nm by using Perkin Elmer spectrophotometer and the characteristic peaks were detected. For GC-MS analysis about 25g of powdered plant material was uniformly packed into a thimble and extracted with 150ml of ethanol as solvent using this plant extract was prepared. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl was employed (split ratio of 10:1); Injector temperature 80°C; Ion-source temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 250°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. The UV-VIS profile showed different peaks ranging from 280 and 290 nm with absorbance values of 0.26 and 3.98 respectively. The spectra for phenolic compounds (tannins) and flavonoids typically lie in the range of 230-290 nm. The results of the GC-MS analysis provide different peaks determining the presence of 28 phytochemical compounds in seed extract and the major phyto constituents were (Peak area 16.23%), o-ethyl S-2-Dimethylaminoethyl Ethylphos, (14.37%) Oleoyl chloride and (12.50%) cis-9-Hexadecenal (8.97%). Phytochemical compounds present in whole plant extract was 79 and the major phyto constituents were Eugenol (7.69 %); Hexadecanoic Acid, Ethyl Ester (7.50%) and Stigmast-5-EN-3-OL, (3.BETA.)- (7.14 %) were reported by GC-MS analysis. The results revealed the major compounds are fatty acid esters and alkaloids which showed antioxidant, antimicrobial and anticancer activities.

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Introduction:-

Herbal medicines are in great demand in both developed and the developing countries in primary healthcare because of their great efficacy and little or no side effects. In India, the indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. These traditional systems of medicine together with homoeopathy and folklore medicine continue to play a significant role largely in the health care system of the population (Yadav *et al.*, 2011.). The tribals and rural population of India are highly dependent on medicinal plant therapy for meeting their health care needs. This attracted the attention of several botanist and plant scientists of several medicinal plants and there was a spurt of scientific literature. (Cerutti, 1991).

Plants are the best sources for chemical ingredients or phytochemical agents for cure of different disease. Medicinal plants are an inexhaustible source of molecules with very different biological and pharmacological activities (Kshitij Chauhan *et al.*, 2012). *Lepidium sativum* Linn (Brassicaceae) commonly known as Asaliyo, is an erect, glabrous annual herb cultivated as a salad plant throughout India, Europe and United States. The seeds are used in chronic enlargement of liver and spleen, as carminative adjunct to purgatives, in skin diseases, dysentery, diarrhoea, asthma and in liver complaints (Shukla *et al.*, 2015).

Lepidium sativum, Family *Brassicaceae*, is a fast-growing, edible plant botanically related to watercress and mustard and known to share their peppery, tangy flavour and aroma (Prajapati *et al.*, 2014). In some regions, garden cress is known as garden pepper cress, pepper grass or pepperwort. Cress is one of the easiest vegetables to grow as it can grow just about anywhere. The plant is used as an antiasthmatic; anti-scorbutic; aperient; diuretic; galactagogue; poultice and stimulant (Cassidy, 2002).

The total glucosinolates of the seeds of *Lepidium sativum* revealed the presence of two glucosinolates, glucotropaeolin and gluconasturin. On the other hand, four glucosinolates were isolated from the fresh herb and were identified as 2-ethyl butyl glucosinolate, methyl glucosinolate (glucocapparin), butylglucosinolate in addition to the glucotropaeolin which was isolated also from the seeds. The glucosinolates were identified by (UV, MS). The individual corresponding isothiocyanates (aglucone) which was obtained by enzymatic hydrolysis of the individual glucosinolates were identified using GC / MS technique (Radwan *et al.*, 2007).

Materials and Methods:-

Collection of plant materials

Fresh materials of *Lepidium sativum* was grown in the laboratory of Sri Paramakalyani College, Alwarkurichi, Tamilnadu, India. The plant was dried in shade and was pulverized using mortar and pestle separately and stored in a closed vessel for further use.

Preparation of plant extracts - Solvent extraction

Crude plant extract was prepared by Soxhlet extraction method. About 25g of powdered plant material was uniformly packed into a thimble and extracted with 150ml of ethanol as solvent. The process of extraction was continued for 24h till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40°C till the solvent got evaporated. Dried extract was kept in refrigerator at 4°C for their future use in phytochemical analysis (Martins *et al.*, 2001).

Ultraviolet-visible spectroscopy analysis:

The extracts of *L. sativum* were examined under visible and UV light for proximate analysis. For UV-VIS spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the ethanol. The extracts were scanned in the wavelength ranging from 200-800 nm using Spectrophotometer and the characteristic peaks were detected. The peak values of the UV-VIS were recorded. Each and every analysis was repeated twice for the spectrum confirmation (AH and Aysel 2003).

Gas Chromatography Mass Spectrum (GC-MS) Analysis:

GC-MS analysis of the extract was performed using a Thermo GC –Trace ultra Ver: 5.0 system and Gas chromatograph interfaced to a Mass spectrometer (GC-MS) (Perkin-Elmer GC Clarus 500 system) equipped with TR 5 – MS capillary standard non-polar column (30mmX0.25mm 1D X 1 µMdf). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at

constant flow rate 1ml/min and an injection volume of 2 μ l was employed (split ratio of 10:1); Injector temperature 80°C; Ion-source temperature 250°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 250°C, ending with a 9min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da. Relative quantities of the chemical compounds present in each of the extracts of *L. sativum* was expressed as percentage based on peak area produced in the chromatogram. (Merlin *et al.*, 2001).

Results and Discussion:-

Uv-visible spectrographic analysis:

The UV-VIS profile of the plant extract was studied at a wavelength range of 200 to 800 nm. Two major peaks were recorded at 280 and 290 nm with absorbance values of 0.26 and 3.98 respectively. (Table 2.4 and Figure 2.2) The spectra for phenolic compounds (tannins) and flavonoids typically lie in the range of 230-290 nm. The result of UV-VIS spectroscopic analysis confirms the presence of tannins and flavonoids in the ethanolic extract of *L. sativum*.

The phenolic contents of methanolic extract of *Lepidium sativum* was determined by UV spectrophotometric method. The total content of phenolic compounds were found to be 46.0 mg GAE/100 g in methanolic extract of *Lepidium sativum*. The flavonoids content was determined by UV spectrophotometric method. The total content of flavonoids was found to be 4.28 mg QE/100 g in methanolic extract of *Lepidium sativum* respectively (RizwanAhamad *et al.*, 2015).

Gas Chromatography Mass Spectrum (GC-MS) Analysis:

Bioactives are chemical compounds often referred to as secondary metabolites. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula are presented in table 2.5 and figure 2.3 and 2.5.1. Eleven major bioactive compounds were identified in the ethanol seed extract of *Lepidium sativum*. GC-MS analysis of *Lepidium sativum* seed extracts revealed the existence of Benzyl nitrile (Peak area 16.23%), o-ethyl S-2-DIMETHYLAMINOETHYL ETHYLPHOS (14.37%) Oleoyl chloride (12.50%) cis-9-Hexadecenal (8.97%) 3',5'-Dimethoxyacetophenone (7.93%) gamma.-Sitosterol (7.39%) ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE (4.96%) n-Hexadecanoic acid (4.19 %) gamma.-Tocopherol (4.01%) Benzene, (isothiocyanatomethyl)- (3.89 %) and ERGOST-5-EN-3-OL, (3.BETA.,24R)- (2.23%) and also the minor compounds were HEXADECANOIC ACID, ETHYL ESTER (1.87%), Fumaric acid, 2-dimethylaminoethyl nonyl ester (1.30%), Hexadecanoic acid, 2-hydroxyl-1-(hydroxymethyl) ethyl ester (1.14%) and STIGMASTA -5-24(28)-DIEN-3-OL,(3.BETA)- respectively.

The bioactive compounds in ethanol extract of *Lepidium sativum* of whole plant by GC-MS report was reported in Table 2.6 and Figure 2.4 and 2.6.1. The chromatogram of GC-MS analysis showed the presence of 26 major constituents are m Eugenol (7.69 %); HEXADECANOIC ACID, ETHYL ESTER (7.50%); STIGMASTA-5-EN-3-OL, (3.BETA.)- (7.14 %), Dichloroacetic acid, tridec-2-ynyl ester (6.70 %). Stigmastane-3,6-dione, (5.alpha.)- (5.05 %) ,9,12-Octadecadienoyl chloride, (4.51%); gamma.-Sitostenone (4.10%) ,Cholestan-3-one, 4,4-dimethyl-, (5.alpha.) (3.58%)-, n-Hexadecanoic acid (3.15%); Stigmasterol (2.86 %); trans,trans-9,12-Octadecadienoic acid, propyl ester (2.54 %); ERGOST-5-EN-3-OL, (3.BETA.,24R)- 2.38% :CYCLODODECANONE,2-(HYDROXY BUTYL)-2-NIT (2.35%) and along with other minor constituents were Benzyl nitrite (1.32%), Benzoic acid (1.47%), Dodecanoic acid (1.01%), (E)-9-Octadecenoic acid ethyl ester (1.98%), OCTADECANOIC ACID, ETHYL ESTER (1.50%), (E)-9-Octadecenoic acid ethyl ester (1.67%), Behenic alcohol (1.35%), Hexacosylheptafluoro butyrate (1.52%), Ethyl tetra cosanoate (1.52%), Cyclohexyldimethylsilyloxy butane, 1-Heptacosanol, Cholesterol, 4-Campestene-3-one, 4,22-Stigmastadiene-3-one (1.40%) were reported by GC-S analysis. The results revealed the major compounds are fatty acid esters and alkaloids which showed antioxidant, antimicrobial and anticancer activities.

Lepidium sativum contained several flavonoids, including two quercetin-hexosides that shared [M-H] – at m/z 463, identified through the loss of sugar moieties (probably glucose and galactose units) and resultant ionization of quercetin at m/z 301. More phenolics have been identified in *L. sativum*. The chromatographic profile of some polyphenols identified in *L. sativum* is reported. At least three isomers of caffeoylquinic acid were identified in the seeds of *L. sativum* (Muhammad Zia-Ul-Haq *et al.*, 2012).

The GC-MS result of the *L. sativum* oil revealed that total 17 fatty acid methyl esters were determined in the esterified oil. The major component of *L. sativum* seed oil was docosatrienoic acid (C22:3; 47.66%) followed by linoleic acid (C18:2; 11.51%), eicosenoic acid (C20:1; 10.63%), palmitic acid (C16:0; 10.13%), arachidonic acid

(C20:4; 4.70%), erucic acid (C22:1; 4.40%), stearic acid (C18:0; 3.34%), and arachidic acid (C20:0; 3.23%). *L. sativum* seed oil was composed of total mono-unsaturated fatty acids (16.32%), poly-unsaturated fatty acids (65.35%), and saturated fatty acid (18.31%). This study was supported by Al-Jasass and Al-Jasser (2012) research works in which the percentages of total saturated and unsaturated fatty acids was reported as 16.76% and 83.24%, respectively (Solomon *et al.*, 2015).

GC-MS analysis determined the 15 compounds in total alkaloid extract of *Lepidium sativum* seeds. The compounds are methyl (Z)-5,11,14,17-icosatetraenoate (10.24%), guanosine (9.29%), dodecanamide, n-(2-hydroxyethyl) (7.48%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (7.13%), 1-(1-adamantyl)-3-(1-piperidinyl)-1-propanone (6%), hexadecanoic acid (5.33%), 3-butylindolizidine (4.80%), 9,12-octadecadienoic acid (Z,Z)-, 2-hydroxy-1-hydroxymethyl (4.79%), 3- methyl alpha.-d-glucopyranoside (1.81%), stigmast-5-en-3-ol, (3.beta.) (3.58%), soyasapogenol B (1.15%), stigmasterol (1.07%), fucosterol (3.29%), gamma.-tocopherol (5.04%) and squalene (3.44%). The results revealed the major compounds are fatty acid esters and alkaloids which showed antioxidant, antimicrobial, anticancer, antineuropathic, anti-inflammatory activities (Akashet *et al.*, 2017). The most effective ingredient present in LS is isothiocyanates, which is formed with glucosinolates (Kassiet *et al.*, 2002).

The Chromatogram GC-MS analysis of the methanol extract of *Lepidium sativum* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined to be Glycerin , Monoethanolamine , 1-Deoxy-d-mannitol , 1-Nitro-2-propanol , 2-Butanamine , (S)- , Furfural , Allylisothiocyanate , Paromomycin, 2-Hydroxy-2-(5-methylfuran-2-yl)1-phenylethanone, 3,6-Diazahomoadamantan-9-one Hydrazone, 2,3,4-Trimethoxycinnamic acid, 2-Naphthalenol, 2,3,4,4a,5,6,7-octahydro-1,4a-dimethyl-7-(2)-, cis-Vaccenic acid, 9-Octadecenamide , γ -Tocopherol , Phthalic acid , decyl oct-3-yl ester , Ergosta-5,22-dien-3-ol,acetate , (3 β ,22E)- , Campesterol and Cholest-5-en-3-ol ,24-propylidene-,(3 β). (Hussein *et al.*, 2017).

In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non-polar components and volatile essential oil, fatty acids, lipids and alkaloids.

The GC-MS chromatogram shows the peak area separation of the components. The above mentioned isolated compounds from the ethanol extract of *Lepidium sativum* seem to possess the reported biological activity and further study of these phytoconstituents may prove the medicinal importance in future.

Fig. 2.2:- Uv-vis spectra of ethanolic extract of *Lepidium sativum*

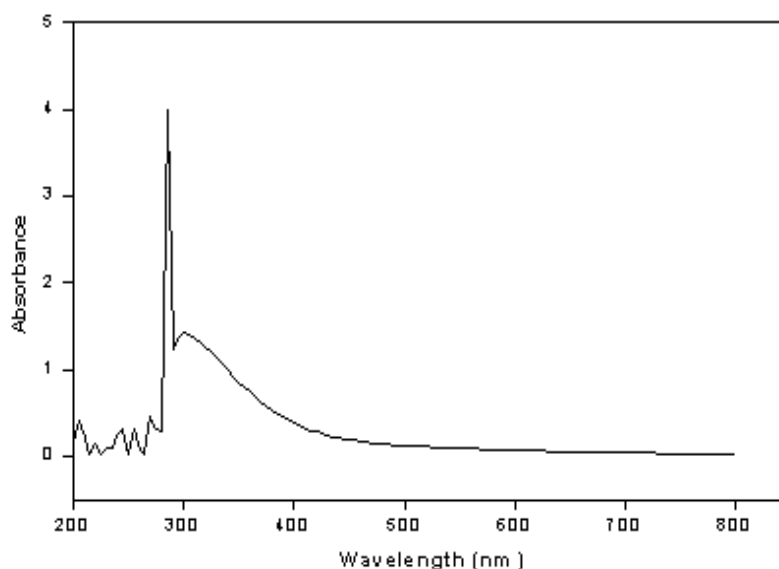
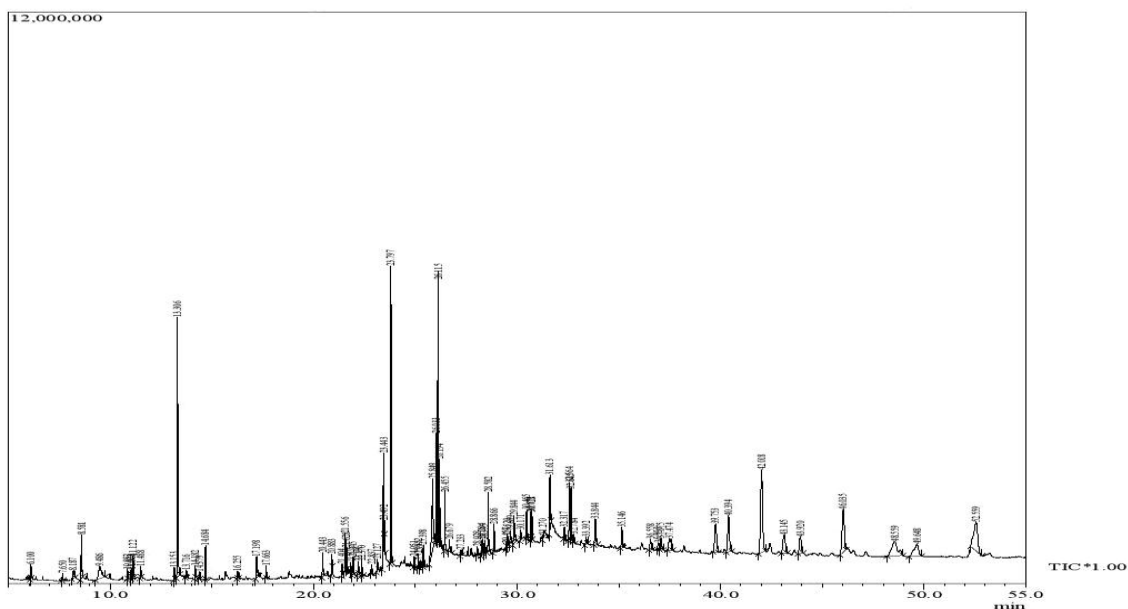
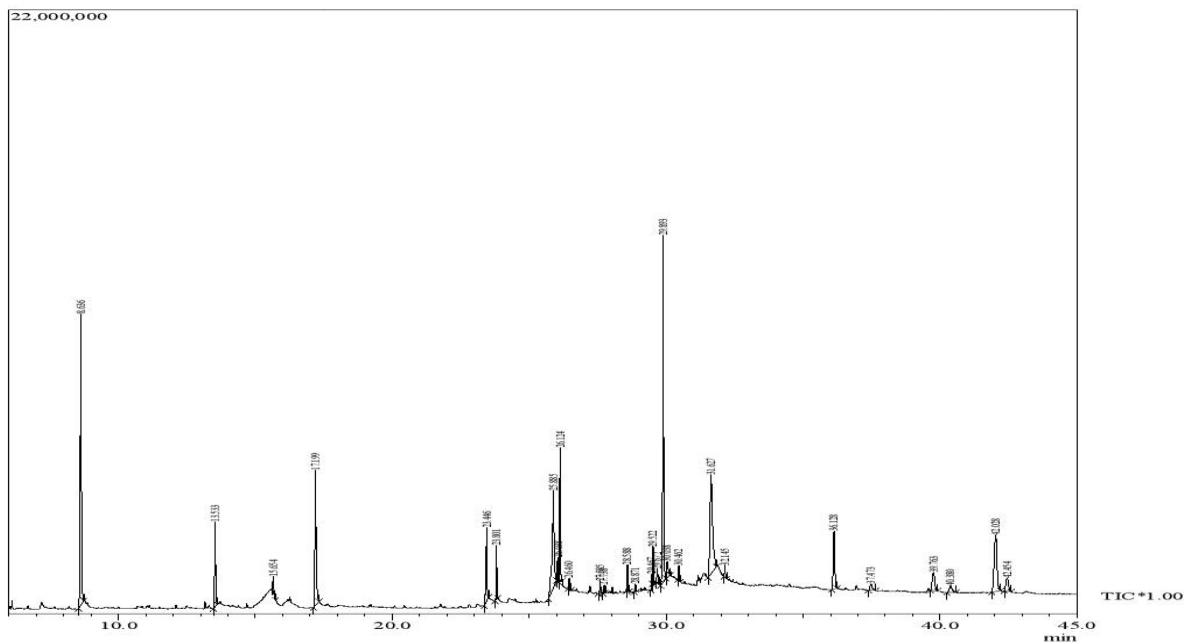


Fig. 2.3:-GC-MS Profile of Ethanol seed extract of *Lepidium sativum*Fig. 2.4:-GC-MS Profile of Ethanol whole plant extract of *Lepidium sativum*Table: 2.5:-Phytochemicals identified in the ethanol seed extracts of *Lepidium sativum* by GCMS analysis.

Sl.No	Name of compounds	Retention time(min)	Molecular formula	Molecular weight	Peak area %
1.	Benzyl nitrile	8.636	C ₈ H ₇ N	117	16.23
2.	Benzene, (isothiocyanatomethyl)-	13.533	C ₈ H ₇ NS	149	3.89
3.	Benzoic acid, 2-(dimethylamino)ethyl ester	15.654	C ₁₁ H ₁₅ NO ₂	193	0.67
4.	3',5'-Dimethoxyacetophenone	17.199	C ₁₀ H ₁₂ O ₃	180	7.93
5.	n-Hexadecanoic acid	23.446	C ₁₆ H ₃₂ O ₂	256	4.19
6.	HEXADECANOIC ACID, ETHYL ESTER	23.801	C ₁₈ H ₃₆ O ₂	284	1.87
7.	cis-9-Hexadecenal	25.885	C ₁₆ H ₃₀ O	238	8.97

8.	ETHYL OCTADECADIENOATE # (9Z,12Z)-9,12-	26.038	$C_{20}H_{36}O_2$	308	0.83
9.	ETHYL OCTADECADIENOATE # (9Z,12Z)-9,12-	26.124	$C_{20}H_{36}O_2$	308	4.96
10.	Octadecanoic acid, ethyl ester	26.460	$C_{20}H_{40}O_2$	312	0.40
11.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	27.585	$C_{12}H_{23}NO_2$	213	0.44
12.	Glycidylpalmitate	27.738	$C_{19}H_{36}O_3$	312	0.30
13.	(E)-9-Octadecenoic acid ethyl ester	28.588	$C_{18}H_{34}O_2$	282	0.95
14.	Eicosanoic acid, ethyl ester	28.871	$C_{22}H_{44}O_2$	340	0.26
15.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	29.467	$C_{12}H_{23}NO_2$	213	0.36
16.	Fumaric acid, 2-dimethylaminoethyl nonyl ester	29.522	$C_{17}H_{31}NO_4$	313	1.30
17.	Glycidyleolate	29.672	$C_{18}H_{31}C_1O$	298	0.70
18.	O-ETHYL S-2-DIMETHYLAMINOETHYL ETHYLPHOS	29.893	$C_8H_{20}NO_2PS$	225	14.37
19.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	30.038	$C_{19}H_{38}O_4$	330	1.14
20.	Erucic acid	30.462	$C_{22}H_{42}O_2$	338	0.55
21.	Oleoyl chloride	31.627	$C_{18}H_{33}ClO$	300	12.50
22.	(Z)-18-Octadec-9-enolide	32.145	$C_{18}H_{32}O_2$	280	0.46
23.	.gamma.-Tocopherol	36.128	$C_{28}H_{48}O_2$	416	4.01
24.	Cholesterol	37.473	$C_{27}H_{46}O$	386	0.67
25.	ERGOST-5-EN-3-OL, (3.BETA.,24R)-	39.763	$C_{28}H_{48}O$	400	2.23
26.	Stigmasterol	40.380	$C_{29}H_{48}O$	412	0.85
27.	.gamma.-Sitosterol	42.028	$C_{29}H_{50}O$	414	7.39
28.	STIGMASTA-5,24(28)-DIEN-3-OL, (3.BETA.)-	42.454	$C_{29}H_{48}O$	412	1.59

Structure Of Compounds Isolated In *Lepidium Sativum* Seed Extract

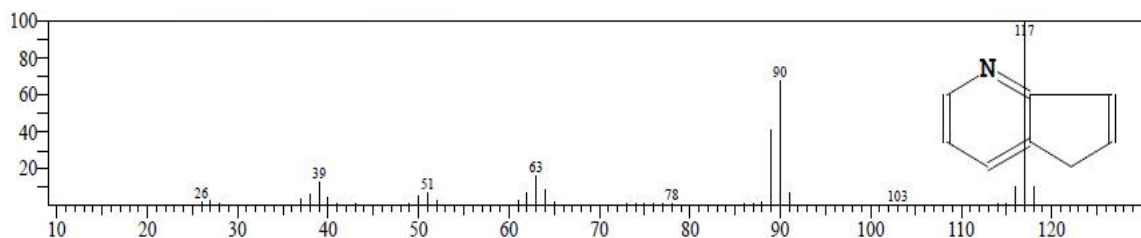


Fig 2.5.1:-Mass Spectrum of Benzyl nitrile

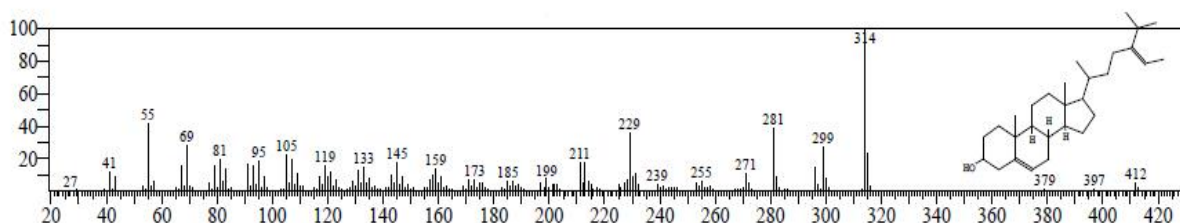


Fig 2.5.2:- Mass Spectrum of Benzene, (isothiocyanatomethyl)-

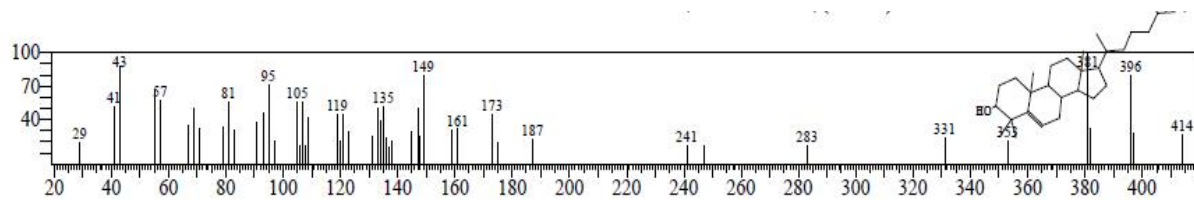


Fig 2.5.3:- Mass Spectrum of 3',5'-Dimethoxyacetophenone

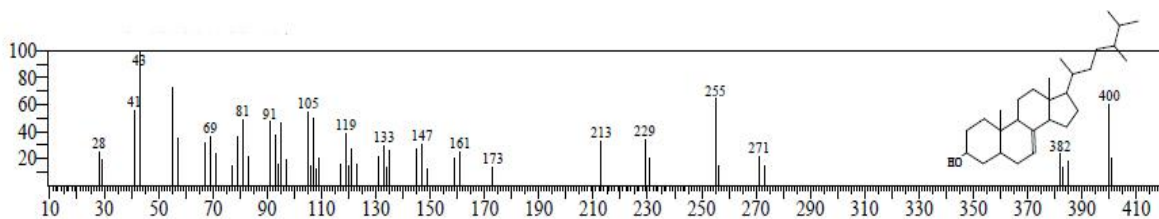


Fig 2.5.4:- Mass Spectrum of n-Hexadecanoic acid

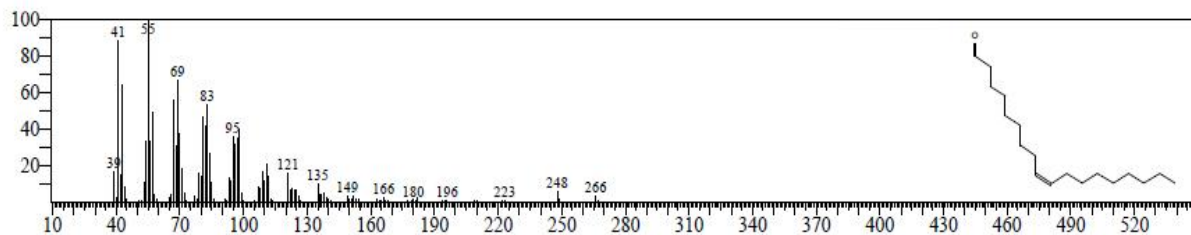


Fig 2.5.6:- Mass Spectrum of cis-9-Hexadecenal

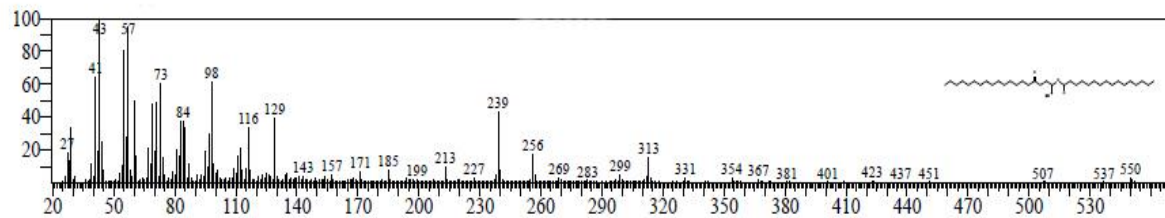


Fig 2.5.7:- Mass Spectrum of Ethyl (9Z,12Z)-9,12-Octadecadienoate #

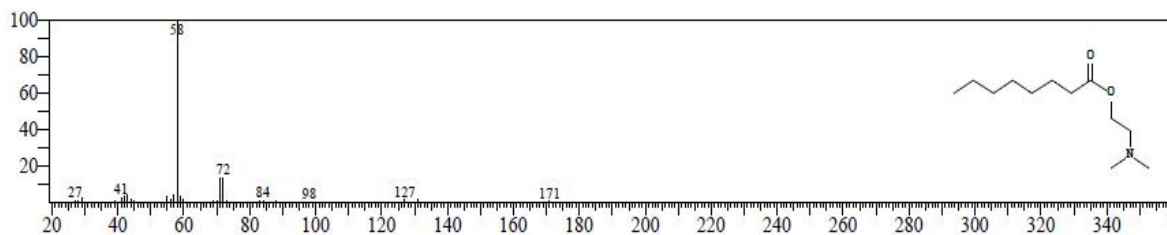


Fig 2.5.8:- Mass Spectrum of O-Ethyl S-2-Dimethylaminoethyl Ethylphos

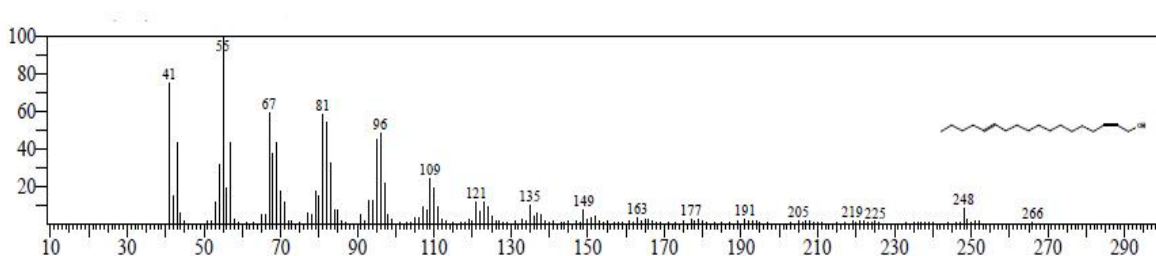


Fig 2.5.9:- Mass Spectrum of Oleoyl chloride

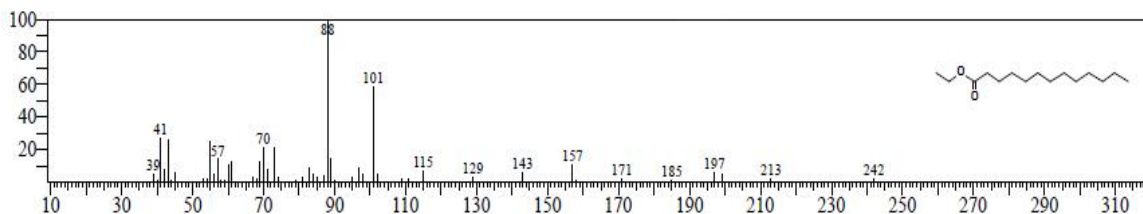


Fig 2.5.10:- Mass Spectrum of gamma-Tocopherol

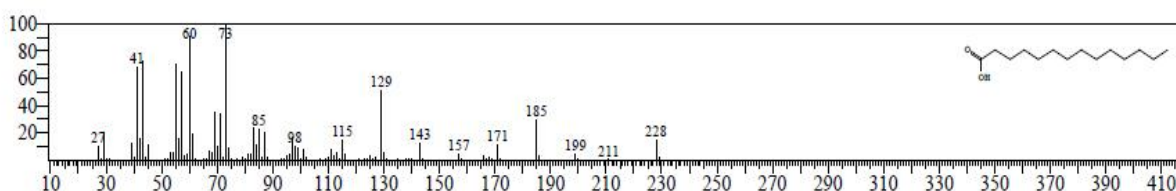


Fig 2.5.11:- Mass Spectrum of Ergost-5-EN-3-OL, (3.Beta.,24R)-

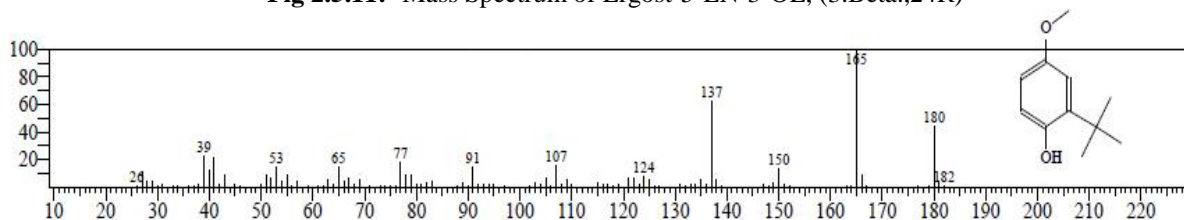


Fig 2.5.12:- Mass Spectrum of gamma-Sitosterol

Table 2.6:- Phytocomponents identified in the ethanol whole plant extracts of *Lepidium sativum* using GC-MS analysis

Sl.No	Name of compounds	Retention time (min)	Molecular formula	Molecular weight	Peak area %
1.	Eucalyptol	6.100	C ₁₀ H ₁₈ O	154	0.32
2.	Linalool	7.650	C ₁₀ H ₁₈ O	154	0.17
3.	Benzyl isocyanate	8.187	C ₈ H ₇ NO	133	0.36
4.	Benzyl nitrile	8.581	C ₈ H ₇ N	117	1.32
5.	Benzoic acid	9.486	C ₇ H ₆ O ₂	122	1.47
6.	2,6-Octadienal, 3,7-dimethyl-, (Z)-	10.852	C ₁₀ H ₁₆ O	152	0.23
7.	Carvone	11.021	C ₁₀ H ₁₄ O	150	0.12
8.	Linalyl acetate	11.063	C ₁₂ H ₂₀ O ₂	196	0.11
9.	Geraniol	11.122	C ₁₀ H ₁₈ O	154	0.54
10.	Citral	11.488	C ₁₀ H ₁₆ O	152	0.28
11.	3-CYCLOHEXENE-1-METHANOL, .ALPHA.,.ALPHA.,.4-	13.153	C ₁₂ H ₂₀ O ₂	196	0.29

12.	Eugenol	13.306	C₁₀H₁₂O₂	164	7.69
13.	Geranyl acetate	13.716	C ₁₂ H ₂₀ O ₂	196	0.17
14.	Methyleugenol	14.182	C ₁₁ H ₁₄ O ₂	178	0.30
15.	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octahydro-1,1,4	14.379	C ₁₅ H ₂₄	204	0.16
16.	Caryophyllene	14.684	C ₁₅ H ₂₄	204	0.84
17.	PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)-	16.255	C ₁₄ H ₂₂ O	206	0.17
18.	Dodecanoic acid	17.198	C ₁₂ H ₂₄ O ₂	200	1.01
19.	ETHYL PENTADECANOATE	17.663	C ₁₇ H ₃₄ O ₂	270	0.29
20.	Tetradecanoic acid	20.443	C ₁₄ H ₂₈ O ₂	228	0.71
21.	TETRADECANOIC ACID, ETHYL ESTER	20.883	C ₁₆ H ₃₂ O ₂	256	0.41
22.	PENTADECANOIC ACID	21.404	C ₁₅ H ₃₀ O ₂	242	0.29
23.	Neophytadiene	21.556	C ₂₀ H ₃₈	278	0.96
24.	2-Pentadecanone, 6,10,14-trimethyl-	21.628	C ₁₈ H ₃₆ O	268	0.56
25.	ETHYL PENTADECANOATE	21.825	C ₁₇ H ₃₄ O ₂	270	0.23
26.	Pentadecanoic acid	21.945	C ₁₅ H ₃₀ O ₂	242	0.69
27.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	22.191	C ₂₀ H ₄₀ O	296	0.32
28.	Pentadecanoic acid, ethyl ester	22.370	C ₁₇ H ₃₄ O ₂	270	0.38
29.	Hexadecanoic acid, methyl ester	22.837	C ₁₇ H ₃₄ O ₂	270	0.22
30.	9-HEXADECENOIC ACID	23.127	C ₁₆ H ₃₀ O ₂	254	0.37
31.	n-Hexadecanoic acid	23.443	C₁₆H₃₂O₂	256	3.15
32.	Ethyl 9-hexadecenoate	23.492	C ₁₈ H ₃₄ O ₂	282	0.38
33.	HEXADECANOIC ACID, ETHYL ESTER	23.797	C₁₈H₃₆O₂	284	7.50
34.	(E)-9-Octadecenoic acid ethyl ester	24.951	C ₂₀ H ₃₈ O ₂	310	0.28
35.	Heptadecanoic acid, ethyl ester	25.152	C ₁₉ H ₃₈ O ₂	298	0.33
36.	8,11,14-Eicosatrienoic acid, methyl ester	25.239	C ₂₁ H ₃₆ O ₂	320	0.22
37.	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R]-[R]	25.398	C ₂₀ H ₄₀ O	296	0.59
38.	9,12-Octadecadienoyl chloride, (Z,Z)-	25.849	C₁₈H₃₁ClO	298	4.51
39.	trans,trans-9,12-Octadecadienoic acid, propyl ester	26.033	C₂₁H₃₈O₂	322	2.54
40.	Dichloroacetic acid, tridec-2-ynyl ester	26.115	C₁₅H₂₄Cl₂O₂	306	6.70
41.	(E)-9-Octadecenoic acid ethyl ester	26.194	C ₂₀ H ₃₈ O ₂	310	1.98
42.	OCTADECANOIC ACID, ETHYL ESTER	26.455	C ₂₀ H ₄₀ O ₂	312	1.50
43.	ETHYL (9Z,12Z)-9,12-OCTADECADIENOATE #	26.679	C ₂₀ H ₃₆ O ₂	308	0.45
44.	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	27.233	C ₁₇ H ₃₂ O	252	0.18
45.	9-OCTADECENAL, (Z)-	28.028	C ₁₈ H ₃₄ O	266	0.22
46.	1,5-PENT-2-ENE-3-METHYL-5-(2,6-DIMETHYLHEPTH	28.284	C ₁₅ H ₂₆ O ₂	238	0.82
47.	4,8,12,16-Tetramethylheptadecan-4-olide	28.395	C ₂₁ H ₄₀ O ₂	324	0.35
48.	(E)-9-Octadecenoic acid ethyl ester	28.582	C ₂₀ H ₃₈ O ₂	310	1.67
49.	HEPTADECANOIC ACID, ETHYL ESTER	28.866	C ₁₉ H ₃₈ O ₂	298	0.70
50.	1,3-Cyclopentadiene, 5-[3-(dimethylamino)propyl]-	29.457	C ₁₀ H ₁₇ N	151	0.10
51.	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	29.520	C ₁₂ H ₂₃ NO ₂	213	0.29
52.	1,2-15,16-Diepoxyhexadecane	29.669	C ₁₆ H ₃₀ O ₂	254	0.48
53.	Behenic alcohol	29.844	C ₂₂ H ₄₆ O	326	1.36

54.	1,2-BENZENEDICARBOXYLIC ACID	30.171	C ₂₄ H ₃₈ O ₄	390	0.25
55.	(E)-9-Octadecenoic acid ethyl ester	30.465	C ₂₀ H ₃₈ O ₂	310	0.68
56.	Docosanoic acid, ethyl ester	30.671	C ₂₄ H ₄₈ O ₂	368	0.51
57.	1-Cyclohexyldimethylsilyloxybutane	30.728	C ₁₂ H ₂₆ OSi	214	0.48
58.	Fumaric acid, 2-dimethylaminoethyl octadecyl ester	31.270	C ₂₆ H ₄₉ NO ₄	439	0.09
59.	Hexacosylheptafluorobutyrate	31.613	C ₃₀ H ₅₃ F ₇ O ₂	578	1.57
60.	(E)-9-Octadecenoic acid ethyl ester	32.317	C ₂₀ H ₃₈ O ₂	310	0.32
61.	Ethyl tetracosanoate	32.564	C ₂₆ H ₅₂ O ₂	396	1.52
62.	1-Cyclohexyldimethylsilyloxybutane	32.645	C ₁₂ H ₂₆ OSi	214	1.25
63.	Squalene	32.784	C ₃₀ H ₅₀	410	0.20
64.	Benzenepropanoic acid, octadecyl ester	33.392	C ₂₇ H ₄₆ O ₂	402	0.33
65.	1-Heptacosanol	33.844	C ₂₇ H ₅₆ O	396	1.18
66.	OCTADECANOIC ACID, ETHYL ESTER	35.146	C ₂₀ H ₄₀ O ₂	312	0.88
67.	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-	36.558	C ₃₁ H ₅₀ O ₂	454	0.53
68.	Stigmast-5-en-3-ol, oleate	36.936	C ₄₇ H ₈₂ O ₂	678	0.29
69.	1-Heptacosanol	37.075	C ₂₇ H ₅₆ O	396	0.65
70.	Cholesterol	37.474	C ₂₇ H ₄₆ O	386	1.25
71.	ERGOST-5-EN-3-OL, (3.BETA.,24R)-	39.753	C₂₈H₄₈O	400	2.38
72.	Stigmasterol	40.394	C₂₉H₄₈O	412	2.86
73.	STIGMAST-5-EN-3-OL, (3.BETA.)-	42.018	C₂₉H₅₀O	414	7.14
74.	4-Campestene-3-one	43.145	C ₂₈ H ₄₆ O	398	1.90
75.	4,22-Stigmastadiene-3-one	43.920	C ₂₉ H ₄₆ O	410	1.40
76.	.gamma.-Sitostenone	46.035	C₂₉H₄₈O	412	4.10
77.	Cholestan-3-one, 4,4-dimethyl-, (5.alpha.)-	48.559	C₂₉H₅₀O	414	3.58
78.	CYCLODODECANONE, 2-(3-HYDROXYBUTYL)-2-NIT	49.648	C₁₆H₂₉NO₄	299	2.35
79.	Stigmastane-3,6-dione, (5.alpha.)-	52.559	C₂₉H₄₈O₂	428	5.05

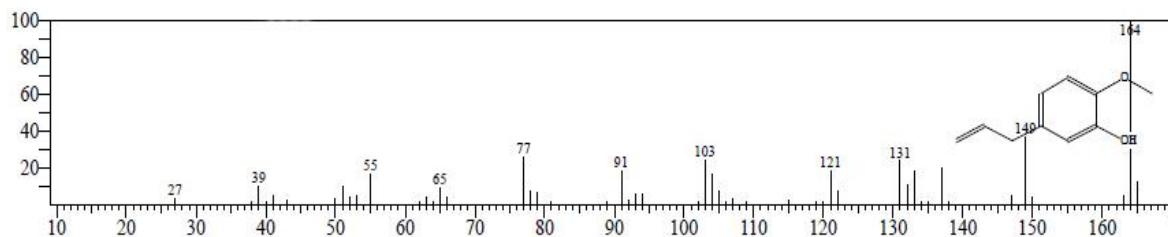
Figure :2.6. Structure Of Compounds Isolated In *Lepidium sativum* Whole Plant Extract

Fig 2.6.1:- Mass Spectrum of Eugenol

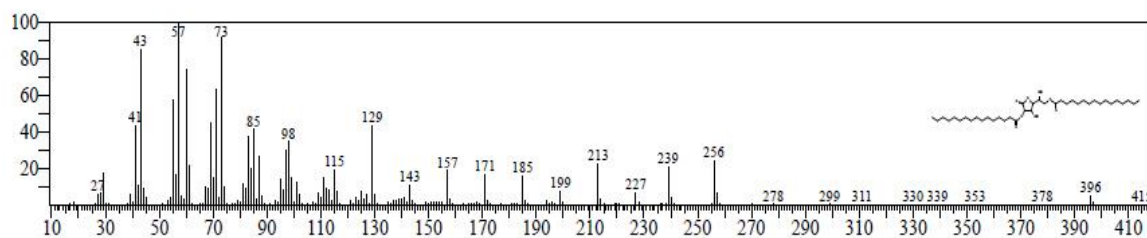


Fig 2.6.2:- Mass Spectrum of n-Hexadecanoic acid

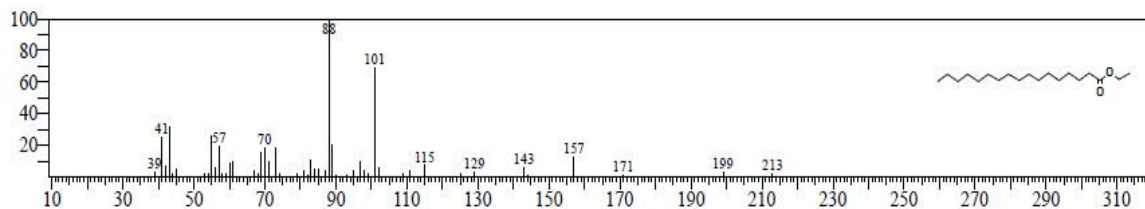


Fig 2.6.3:- Mass Spectrum of Hexadecanoic Acid, Ethyl Ester

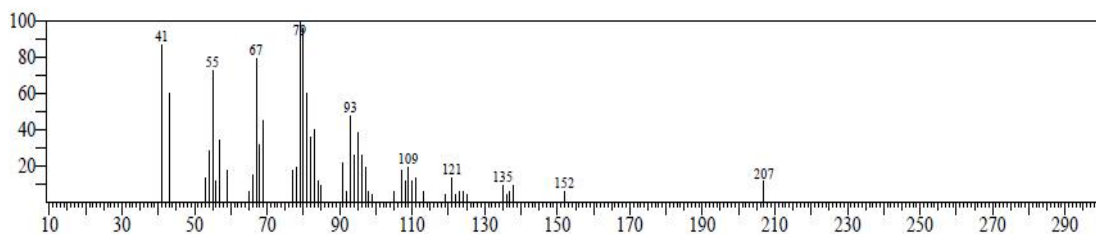


Fig 2.6.4:- Mass Spectrum of 9,12-Octadecadienoyl chloride, (Z,Z)-

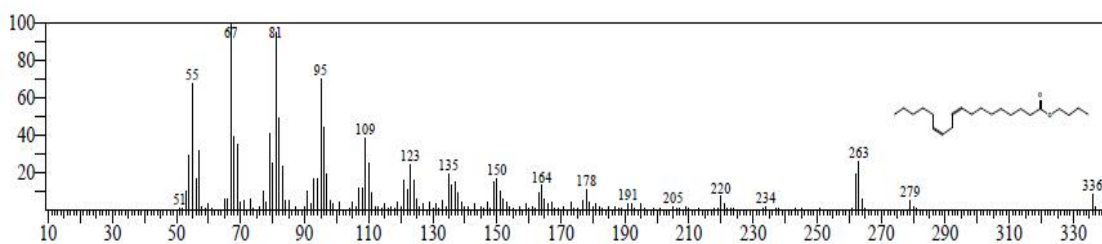


Fig 2.6.5 :- Mass Spectrum of trans,trans-9,12-Octadecadienoic acid, propyl ester

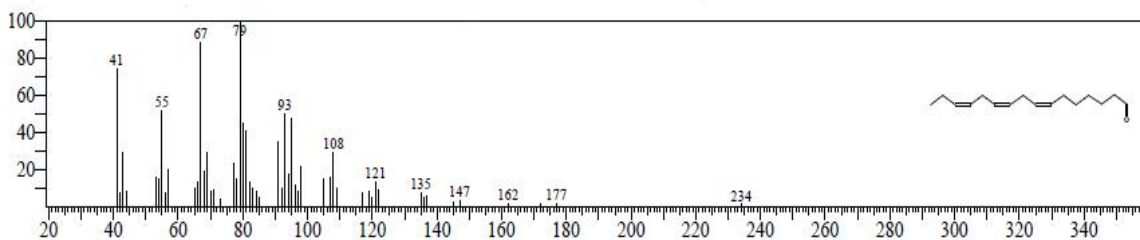


Fig 2.6.6:- Mass Spectrum of Dichloroacetic acid, tridec-2-ynyl ester

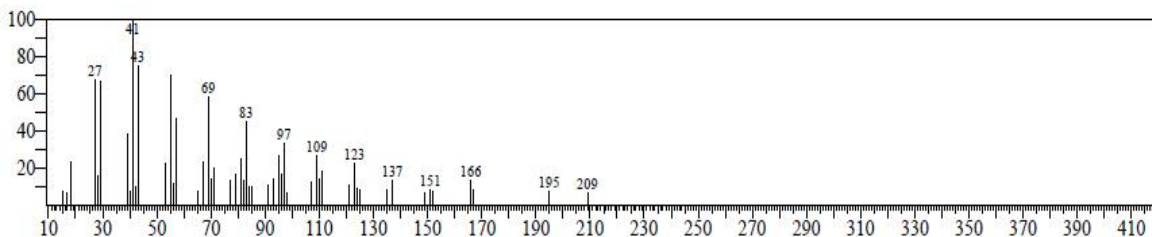


Fig 2.6.7:- Mass Spectrum of ERGOST-5-EN-3-OL, (3.BETA.,24R)-

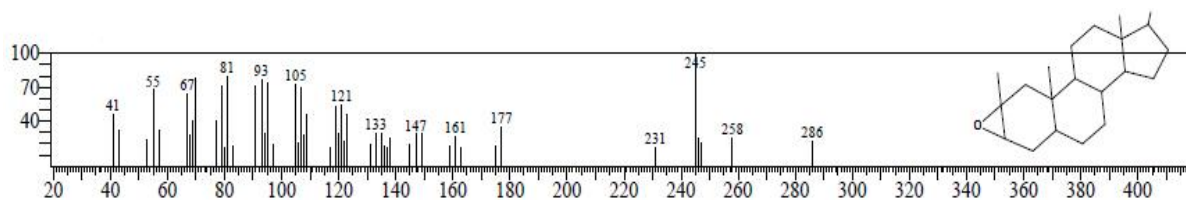


Fig 2.6.8:- Mass Spectrum of Stigmasterol

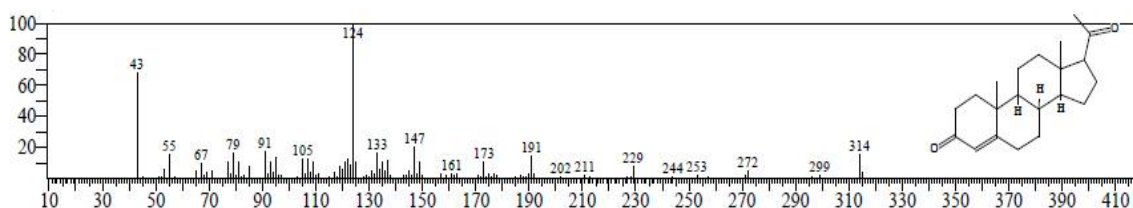


Fig 2.6.9:- Mass Spectrum of Stigmaster-5-EN-3-OL, (3.BETA.)-

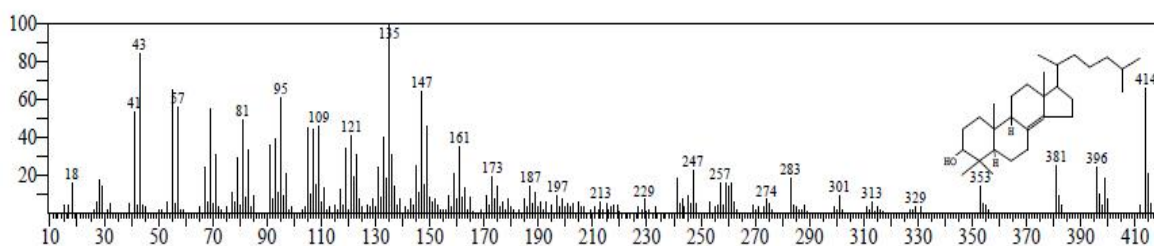


Fig 2.6.10:- Mass Spectrum of gamma.-Sitostenone

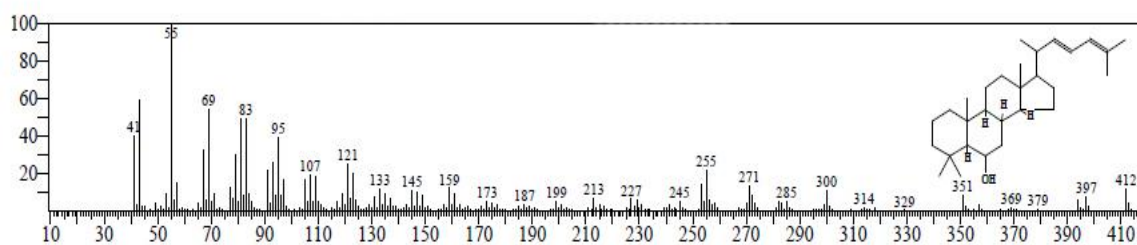


Fig 2.6.11:- Mass Spectrum of Cholestan-3-one, 4,4-dimethyl-, (5.alpha.)-

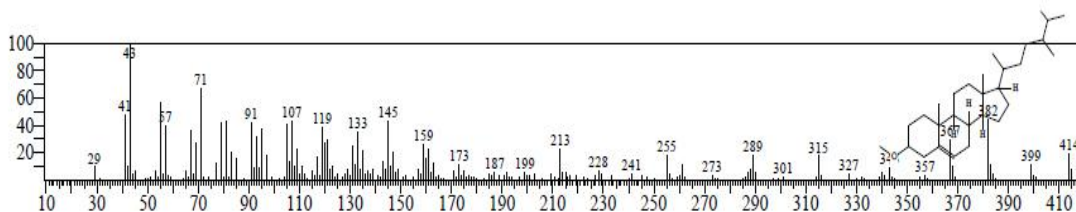


Fig 2.6.12 :- Mass Spectrum of Cyclododecanone, 2-(3-Hydroxybutyl)-2-NIT

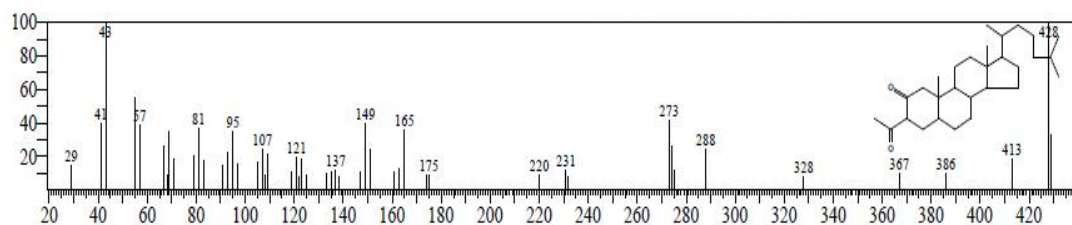


Fig 2.6.12 :- Mass Spectrum of Stigmastane-3,6-dione, (5.alpha.)-

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