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, diarrhea, 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 isued as an antiasthmatic; anti-scorbutic; aperient; diuretic; galactogogue; 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), butyl glucosinolate in addition to the glucotropaeolin which was isolated also from the seeds. The glucosinolates were identified by (UV, MS). The individual corresponding isothiocyanates (aglucose) 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 30-40ºC till the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC 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. 1filter 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 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. 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 was 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 (RizwanAhamadet al., 2015).

**Gas Chromatography Mass Spectrum (GC-MS) Analysis:**

Bioactive compounds 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-Dodecanoic acid, (isothiocyanatomethyl) ester (1.30%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (1.14%) and STIGMAST-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%); STIGMAST-5-EN-3-OI, (3.BETA), (7.14%), Dichloroacetic acid, tridecyl alcohol (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%), Benzoc 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%), Hexacosylethapfluoro butyrate(1.52%), Ethyl tetra cosanoate (1.52%), Cyclohexyldimethylsiloxyl butane, 1-Heptacosanol, Cholesterol, 4-Campesten-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-hexosidesthat 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-Haqet 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 docosatetraenoic 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%), eruic acid (C22:1; 4.40%), stearic acid (C18:0; 3.34%), and arachidic acid (C20:0; 3.23%). L. sativium 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-eicosatetraenoate (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-piperidiny1)-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 (Akash et al., 2017). The most effective ingredient present in LS is isothiocyanates, which is formed with glucosinolates (Kassie 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-y1)-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-Toopherol, Phthalic acid, decyl oct-3-y1 ester, Ergosta-5,22-dien-3-01,acetate, (3β,22E)-, Campesterol and Cholest-5-en-3-01,24-propylidene-(3β). (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

![Uv-vis spectra of ethanolic extract of Lepidium sativum](image-url)
Table: 2.5: Phytocomponents 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                | C₆H₇N            | 117              | 16.23       |
| 2.    | Benzene, (isothiocyanatomethyl)-              | 13.533               | C₆H₇NS           | 149              | 3.89        |
| 3.    | Benzoic acid, 2-(dimethylamino)ethyl ester    | 15.654               | C₁₁H₁₃NO₂        | 193              | 0.67        |
| 4.    | 3',5'-Dimethoxyacetophenone                   | 17.199               | C₁₀H₁₂O₃         | 180              | 7.93        |
| 5.    | n-Hexadecanoic acid                           | 23.446               | C₁₆H₃₂O₂         | 256              | 4.19        |
| 6.    | HEXADECANOIC ACID, ETHYL ESTER                | 23.801               | C₁₈H₃₆O₃         | 284              | 1.87        |
| 7.    | cis-9-Hexadecenal                             | 25.885               | C₁₆H₃₀O          | 238              | 8.97        |
| No. | Compound Name                                      | Molecular Formula | Mass (m/z) | Retention Time (s) | Comments |
|-----|---------------------------------------------------|-------------------|------------|--------------------|----------|
| 8.  | ETHYL OCTADECADIENOATE # (9Z,12Z)-9,12-Octa-     | C₂₀H₃₆O₂          | 26.038     | 308                | 0.83     |
| 9.  | ETHYL OCTADECADIENOATE # (9Z,12Z)-9,12-Octa-     | C₂₀H₃₆O₂          | 26.124     | 308                | 4.96     |
| 10. | Octadecanoic acid, ethyl ester                   | C₂₀H₄₀O₂          | 26.460     | 312                | 0.40     |
| 11. | 3-Cyclopentylpropionic acid, 2-dimethylaminooxy  | C₁₂H₂₃NO₂         | 27.585     | 213                | 0.44     |
| 12. | Glycidyloleate                                    | C₁₀H₁₆O₃          | 27.738     | 312                | 0.30     |
| 13. | (E)-9-Octadecenoic acid ethyl ester              | C₁₈H₃₄O₂          | 28.588     | 282                | 0.95     |
| 14. | Eicosanoic acid, ethyl ester                     | C₂₂H₄₄O₂          | 28.871     | 340                | 0.26     |
| 15. | 3-Cyclopentylpropionic acid, 2-dimethylaminooxy  | C₁₂H₂₃NO₂         | 29.467     | 213                | 0.36     |
| 16. | Fumaric acid, 2-dimethylaminooxyethyl nonyl ester| C₁₇H₃₃O₄          | 29.522     | 313                | 1.30     |
| 17. | Glycidyloleate                                    | C₁₈H₃₃C₃O        | 29.672     | 298                | 0.70     |
| 18. | O-ETHYL S-2-DIMETHYLAMINOETHYL ETHYLPHOS         | C₆H₂₀NO₂PS        | 29.893     | 225                | 14.37    |
| 19. | Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester | C₁₀H₁₈O₄         | 30.038     | 330                | 1.14     |
| 20. | Erucic acid                                       | C₂₂H₄₂O₂          | 30.462     | 338                | 0.55     |
| 21. | Oleoyl chloride                                   | C₁₈H₃₃C₃O        | 31.627     | 300                | 12.50    |
| 22. | (Z)-18-Octadec-9-enolide                         | C₁₈H₃₂O₂          | 32.145     | 280                | 0.46     |
| 23. | .gamma.-Tocopherol                               | C₂₅H₄₉O₂          | 36.128     | 416                | 4.01     |
| 24. | Cholesterol                                       | C₂₅H₴₆O          | 37.473     | 386                | 0.67     |
| 25. | ERGOST-5-EN-3-OL, (3.BETA.,24R)-                 | C₂₈H₄₈O        | 39.763     | 400                | 2.23     |
| 26. | Stigmasterol                                      | C₂₉H₄₈O          | 40.380     | 412                | 0.85     |
| 27. | .gamma.-Sitosterol                                | C₂₉H₅₀O          | 42.028     | 414                | 7.39     |
| 28. | STIGMASTA-5,24(28)-DIEN-3-OL, (3.BETA.)-         | C₂₀H₄₈O          | 42.454     | 412                | 1.59     |

Structure Of Compounds Isolated In *Lepidium Sativum* Seed Extract

![Fig 2.5.1: Mass Spectrum of Benzyl nitrile](image1)

![Fig 2.5.2: Mass Spectrum of Benzene, (isothiocyanatomethyl)](image2)
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                | \( \text{C}_{10}\text{H}_{15}\text{O} \) | 154              | 0.32        |
| 2.    | Linalool                                          | 7.650                | \( \text{C}_{10}\text{H}_{14}\text{O} \) | 154              | 0.17        |
| 3.    | Benzyl isocyanate                                 | 8.187                | \( \text{C}_{8}\text{H}_{7}\text{NO} \) | 133              | 0.36        |
| 4.    | Benzyl nitrile                                    | 8.581                | \( \text{C}_{8}\text{H}_{7}\text{N} \) | 117              | 1.32        |
| 5.    | Benzoic acid                                      | 9.486                | \( \text{C}_{7}\text{H}_{6}\text{O}_{2} \) | 122              | 1.47        |
| 6.    | 2,6-Octadienal, 3,7-dimethyl- , (Z)               | 10.852               | \( \text{C}_{10}\text{H}_{16}\text{O} \) | 152              | 0.23        |
| 7.    | Carvone                                           | 11.021               | \( \text{C}_{10}\text{H}_{14}\text{O} \) | 150              | 0.12        |
| 8.    | Linalyl acetate                                   | 11.063               | \( \text{C}_{12}\text{H}_{20}\text{O}_{2} \) | 196              | 0.11        |
| 9.    | Geraniol                                          | 11.122               | \( \text{C}_{10}\text{H}_{16}\text{O} \) | 154              | 0.54        |
| 10.   | Citral                                            | 11.488               | \( \text{C}_{10}\text{H}_{16}\text{O} \) | 152              | 0.28        |
| 11.   | 3-CYCLOHEXENE-1-METHANOL, . \( \text{ALPHA...ALPHA...} \) | 13.153               | \( \text{C}_{12}\text{H}_{20}\text{O}_{2} \) | 196              | 0.29        |
| No. | Compound                                      |分子式 | Molecular Weight | Density |
|-----|----------------------------------------------|--------|------------------|---------|
| 12  | Eugenol                                      | C10H15O | 164              | 0.76    |
| 13  | Geranyl acetate                              | C16H20O2 | 196              | 0.17    |
| 14  | Methylgenyl                                  | C11H10O2 | 178              | 0.30    |
| 15  | 1H-Cyclopentyle[1]azulene, 1a,2,3,4,5,6,7b-octahydro-1,1,4-| C14H24 | 204              | 0.16    |
| 16  | Caryophyllene                                | C14H24 | 204              | 0.84    |
| 17  | Phénon, 2,4-Bis(1,1-dimethylethyl)-           | C14H19O | 206              | 0.17    |
| 18  | Dodecanoic acid                              | C12H24O2 | 200              | 1.01    |
| 19  | Ethyl PentaDecanoate                         | C15H30O2 | 270              | 0.29    |
| 20  | Tetradecanoic acid                           | C14H28O2 | 228              | 0.71    |
| 21  | TETRADECANOIC ACID, ETHYL ESTER              | C16H32O2 | 256              | 0.41    |
| 22  | PENTADECANOIC ACID                           | C15H30O2 | 242              | 0.29    |
| 23  | Neophytadiene                                | C20H38  | 278              | 0.96    |
| 24  | 2-PentaDecane, 6.10.14-trimethyl-            | C18H30O2 | 268              | 0.56    |
| 25  | Ethyl PentaDecanoate                         | C15H30O2 | 270              | 0.23    |
| 26  | Pentadecanoic acid                           | C15H30O2 | 242              | 0.69    |
| 27  | 3.7,11,15-Tetramethyl-2-hexadecen-1-ol       | C20H36O  | 296              | 0.32    |
| 28  | Pentadecanoic acid, ethyl ester              | C15H30O2 | 270              | 0.38    |
| 29  | Hexadecanoic acid, methyl ester              | C15H30O2 | 270              | 0.22    |
| 30  | 9-Hexadecanoic acid                          | C16H34O2 | 254              | 0.37    |
| 31  | n-Hexadecanoic acid                          | C16H32O2 | 256              | 3.15    |
| 32  | Ethyl 9-hexadecenoate                        | C16H32O2 | 282              | 0.38    |
| 33  | HEXADECANOIC ACID, ETHYL ESTER               | C16H32O2 | 284              | 7.50    |
| 34  | (E)-9-Octadecenoic acid ethyl ester          | C20H38O2 | 310              | 0.28    |
| 35  | Heptadecanoic acid, ethyl ester              | C20H38O2 | 298              | 0.33    |
| 36  | 8,11,14-Eicosatrienoic acid, methyl ester    | C22H44O2 | 320              | 0.22    |
| 37  | 2-Hexadecen-1-OL, 3,7,11,15-Tetramethyl-     | C23H40O2 | 296              | 0.59    |
| 38  | 9,12-Octadecadienyl chloride, (Z,Z)-trans,trans-9,12-Octadecadienoic acid, propyl ester | C20H36O2 | 298             | 4.51    |
| 39  | Dichloroacetic acid, tridec-2-ynyl ester     | C16H24C12O2 | 306             | 6.70    |
| 40  | (E)-9-Octadecenoic acid ethyl ester          | C20H38O2 | 310              | 1.98    |
| 41  | OCTADECANOIC ACID, ETHYL ESTER               | C20H38O2 | 312              | 1.50    |
| 42  | ETHYL (9Z,12Z)-9,12-Octadecadienoate #       | C20H38O2 | 308              | 0.45    |
| 43  | (R)-(-)-14-Methyl-8-hexadecyn-1-ol           | C16H25O2 | 252              | 0.18    |
| 44  | 9-Octadecenal, (Z)-                         | C20H38O2 | 266              | 0.22    |
| 45  | 1,5-Pent-2-ene-3-Methyl-5-(2,6-dimethylhept     | C15H26O2 | 238              | 0.82    |
| 46  | 4,8,12,16-Tetramethylheptadecan-4-olide      | C21H40O2 | 324              | 0.35    |
| 47  | (E)-9-Octadecenoic acid ethyl ester          | C20H38O2 | 310              | 1.67    |
| 48  | HEPTADECANOIC ACID, ETHYL ESTER              | C20H38O2 | 298              | 0.70    |
| 49  | 1.3-Cyclopentadiene, 5-[3-(dimethylamino)propyl]- | C20H38O2 | 151              | 0.10    |
| 50  | 1.2-15,16-Diepoxyhexadecane                  | C20H38O2 | 254              | 0.48    |
| 51  | Behenic alcohol                              | C26H44O  | 326              | 1.36    |
| No. | Compound Description                                                                 | CAS No. | Molecular Formula | Molecular Weight | Percentage |
|-----|------------------------------------------------------------------------------------|---------|------------------|------------------|------------|
| 54. | 1,2-BENZENEDICARBOXYLIC ACID                                                       | 30.171  | C₉H₁₀O₄          | 190             | 0.25       |
| 55. | (E)-9-Octadecenoic acid ethyl ester                                                | 30.465  | C₂₃H₄₀O₂         | 310             | 0.68       |
| 56. | Docosanoic acid, ethyl ester                                                       | 30.671  | C₂₃H₄₀O₂         | 368             | 0.51       |
| 57. | 1-Cyclohexyldimethylisiloxoxybutane                                                | 30.728  | C₁₄H₃₅OSi        | 214             | 0.48       |
| 58. | Fumaric acid, 2-dimethylaminoethyl octadecyl ester                                 | 31.270  | C₂₀H₃₈NO          | 439             | 0.09       |
| 59. | Hexacosylheptafluorobutyrate                                                       | 31.613  | C₁₆H₄₀F₇O₂       | 578             | 1.57       |
| 60. | (E)-9-Octadecenoic acid ethyl ester                                                | 32.317  | C₂₀H₃₈O₂         | 310             | 0.32       |
| 61. | Ethyl tetraacetoate                                                                 | 32.564  | C₂₀H₄₀O₂         | 396             | 1.52       |
| 62. | 1-Cyclohexyldimethylisiloxoxybutane                                                | 32.645  | C₁₄H₃₅OSi        | 214             | 1.25       |
| 63. | Squalene                                                                           | 32.784  | C₂₀H₄₀          | 410             | 0.20       |
| 64. | Benzenepropanoic acid, octadecyl ester                                             | 33.392  | C₂₇H₄₆O₂        | 402             | 0.33       |
| 65. | 1-Heptacosanol                                                                     | 33.844  | C₂₀H₄₀          | 396             | 1.18       |
| 66. | OCTADECANOIC ACID, ETHYL ESTER                                                     | 35.146  | C₂₀H₄₀O₂         | 312             | 0.88       |
| 67. | Stigmast-5,22-dien-3-ol, acetate, (3.beta.)-                                        | 36.558  | C₁₃H₃₀O₂         | 454             | 0.53       |
| 68. | Stigmast-5-en-3-ol, oleate                                                         | 36.936  | C₂₀H₄₀O₂         | 678             | 0.29       |
| 69. | 1-Heptacosanol                                                                     | 37.075  | C₂₀H₄₀O₂         | 396             | 0.65       |
| 70. | Cholesterol                                                                        | 37.474  | C₂₀H₄₀O₂         | 386             | 1.25       |
| 71. | ERGOST-5-EN-3-OL, (3.BETA.,24R)-                                                   | 39.753  | C₁₃H₃₀O₂         | 400             | 2.38       |
| 72. | Stigmasterol                                                                       | 40.394  | C₂₀H₄₀O₂         | 412             | 2.86       |
| 73. | STIGMAST-5-EN-3-OL, (3.BETA.)-                                                     | 42.018  | C₂₀H₄₀O₂         | 414             | 7.14       |
| 74. | 4-Campestene-3-one                                                                 | 43.145  | C₂₀H₄₀O₂         | 398             | 1.90       |
| 75. | 4,22-Stigmastadiene-3-one                                                         | 43.920  | C₂₀H₄₀O₂         | 410             | 1.40       |
| 76. | STIGMAST-5-EN-3-OL, (3.BETA.,24R)-                                                 | 46.035  | C₂₀H₄₀O₂         | 412             | 4.10       |
| 77. | Cholest-3-one, 4,4-dimethyl, (5.alpha.)-                                            | 48.559  | C₂₀H₄₀O₂         | 414             | 3.58       |
| 78. | CYCLODODECANONE, 2-(3-HYDROXYBUTYL)-2-NIT                                          | 49.648  | C₁₈H₂₉NO₄        | 299             | 2.35       |
| 79. | STIGMAST-5-EN-3-OL, (3.BETA.,24R)-                                                 | 52.559  | C₂₀H₄₀O₂         | 428             | 5.05       |

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

![Mass Spectrum of Eugenol](image1)

![Mass Spectrum of n-Hexadecanoic acid](image2)
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 Stigmast-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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