Original Article

TLC Solvent System Development, HPTLC And GC-MS Analysis Of Methanolic Extract Of Lepidium Sativum Linn Seeds

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Abstract

Introduction: Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since ancient times. Lepidium sativum Linn. (Brassicaceae) is annual herb regionally referred to as halon in India, however, usually referred to as garden cress. Phyto-components are the compound that occurred in plant naturally and play important role for biologically activity to prevent the many diseases.

Methods: In the present study, we developed TLC solvent system, HPTLC analysis and GC-MS analysis of methanolic extract of Lepidium sativum Linn. seeds. TLC solvent system developed for methanolic extract of Lepidium sativum Linn. seeds was chloroform: methanol (5 : 5). Same solvent system was used for HPTLC analysis of methanolic extract, which showed presence of various compounds in extract.

Results: HPTLC at 254 nm showed presence of 10 types of compounds, Rf values of these ranging from 0.03 to 0.95. HPTLC at 366 nm showed presence of 9 types of compounds, Rf values of these ranging from 0.03 to 0.95. The major compound found in GC_MS analysis of methanolic extract was 1-isocyano-3-methyl- Benzene (83.02%) with retention time 8.03 and lower percentage compound was 2,3-dihydro-4-(1-methylpropyl)- Furan, (S)- (0.03%) with 10.18 retention time.

Conclusion: The identified compounds having biological and pharmacological activity such as Antimicrobial, Antifungal, Anticancer, Antioxidant, Anxiolytic, Cholinesterase inhiditor, Antiproliferative, Antiinflammatory etc. Results showed that Lepidium sativum Linn. seeds will play important in treatment of many diseases.

KEYWORDS:- Chromatogram, Biological activities, Lepidium sativum Linn. seeds, Methanolic extract, Retention time.

INTRODUCTION

Herbal medicines as the major remedy in traditional system of medicine have been used in medical practices since ancient times. Plants are known in the pharmaceutical industry due to their broad spectrum of structural multiplicity and their large range of pharmacological activities.

Lepidium sativum Linn. (Brassicaceae) is an annual herb locally known as halon in india but commonly known as Garden cress. Lepidium sativum is a polymorphous species and its centre of origin is Eritre and Ethiopia. Garden cress is a fast-growing edible plant. Seeds, roots and leaves of Garden cress are of economic significance; but, the crop is primarily cultivated for seeds. It is a medicinally useful herb in India[1]. Garden cress seeds are brownish red in colour and oval in shape[2]. Seeds are small, oval-shaped pointed, and triangular at one end, smooth about 2 to 3 mm long and 1 to 1.5 mm wide, reddish brown, an arrow present on both surfaces, extending up to two-thirds

downwards, a slight wing-like extension present on both the edges of seed when soaked in water seed coat swells and gets covered with a transparent, colorless mucilage[3]. The potential therapeutic properties of Lepidium sativum Linn. are wide-ranging and include treatment and prevention of airways disorders, gastrointestinal treatment, menstruation cycle regulation, iron deficiency treatment, inflammation cardiovascular disease, diabetes. Other potential applications include immunomodulatory and memory inhancing activity[4]. The medicinal usefulness of a plant is due to the existence of some special substances like alkaloids, glycosides, resins, volatile oils, tannins and gums, flavonoids etc. The active principles usually remain concentrated in the storage organs of the plant[5].

Thin layer chromatography (TLC) is less time consuming, low cost, and can be performed with less complicated technique it has a wide application in pharmaceutical analysis. If performed precisely 32 amino acids can be separated by TLC. Also it has a wide application in identifying impurities in a compound. It can be used as a preliminary analytical method prior to HPLC. The concept of TLC is simple and samples usually require only minimal pretreatment. TLC can be used to monitor the progress of a reaction, identify compounds present in a given substance. TLC is also used to separate the identical compounds in a mixture. Many standard methods in industrial chemistry, environmental toxicology, food chemistry, water, inorganic and pesticide analysis, dye purity, cosmetics, plant materials, and herbal analysis rely upon TLC as the preferred approach [6].

High Performance Thin Layer Chromatography (HPTLC) is the most powerful advanced form of Thin Layer Chromatography (TLC) and consists of chromatographic layers of utmost separation efficiency and the application of sophisticated instrumentation for all steps in the procedure include accurate sample application, standardized reproducible chromatogram development and software controlled evaluation[7]. HPTLC is a concept that includes a widely standardized methodology based on scientific facts as well as the use of validated methods for qualitative and quantitative analysis[8]. HPTLC meets all quality requirements for today's analytical labs, to increase the resolution and to allow more accurate quantitative measurements[9].

Gas Chromatography (GC) and mass spectrometry (MS) provides a powerful tool for identifying the various compound presences in the sample. GC separate mixture in to individual components and the MS detects components or molecules on the basis of their charged ion and mass to charge ratio. GC-MS analysis is a breakthrough in the analysis of phytoconstituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1 mg[10].

The objective of the present study was TLC solvent system development, HPTLC and GC-MS analysis of methanolic extract of Lepidium sativum Linn. Seeds.

2. MATERIALS AND METHODS

2.1 Procurement of plant material and extraction

Garden cress seed (Lepidium sativum) was purchased from local market of Mandsaur, Madhya Pradesh, India and authenticated by Department of pharmacognosy at B R Nahata College of Pharmacy, Mandsaur. A voucher specimen no. (BRNCP/LS/012/2019/ Lepidium sativum seeds) was deposited in the herbarium of the institute. Successive solvent extraction method was used for extraction for Lepidium sativum seeds with different solvents like n-hexane, chloroform, ethyl acetate and methanol[11].

2.2 TLC solvent system development

TLC plates were prepared by slurry of silica gel G, slurry was poured on glass plates and activated by keeping them in hot air oven at 110-120° C for 30 minutes.

TLC solvent system was developed for methanolic extract by using various solvent systems:

1. Methanol : Acetic acid : Water
   8 : 1.5 : 0.5
2. Methanol : Acetic acid : Water
   7 : 2.5 : 0.5
3. Methanol : Acetic acid : Water
   5 : 2.5 : 2.5
4. Chloroform : Methanol
   5 : 5
5. Toluene : Chloroform : Acetone
   7 : 5 : 8
6. Chloroform : Methanol : Water

5 : 4 : 1

7. Chloroform : Methanol : Water
   6 : 3 : 1
8. Chloroform : Methanol : Water
   5 : 2 : 3
9. Chloroform : Methanol : Aceticacid : Water

   5 : 4 : 0.5 : 0.5

By using all above solvent system, proper resolution and spots were found in following solvent system:-

Chloroform : Methanol

5 : 5

2.3 HPTLC analysis

Instrumentation

A CAMAG HPTLC system equipped with LINOMAT 5 applicator fitted with 100 µl syringe, CAMAG TLC scanner, and winCATS software was used.

Chemicals and solvents

All the solvents used were of chromatography grade, and all the chemicals used were of analytical reagent grade.

Preparation of samples

Dried methanolic extract (3 g) of Lepidium sativum Linn. seeds was dissolved in 30 ml HPTLC grade methanol and filtered. This solution was used as a test solution for the HPTLC study

Chromatographic conditions

The HPTLC was performed on 7.0 × 10.0 cm precoated silica gel 60 F 254 HPTLC plate (E. MERCK KGaA). No pre-washing and modification of the plate were done. The sample solution was applied as bands to the plate by CAMAG Linomat applicator fitted with 100 µl syringe. The stable application rate was 150 nl/s. The sample loaded plate was kept in automatic development chamber with mobile phase—chloroform : methanol (5:5 v/v). Densitometric scanning was performed with CAMAG TLC scanner-4 equipped with winCATS software. The bands were visualized using CAMAG visualizer, and the images were captured in 254 nm (short UV) and 366 nm (long UV) wavelengths. When exposed to short-wave UV light of 254 nm, UV-active compounds will undergo fluorescence quenching and appear as dark spots on a bright background. Conversely, compounds that absorb 366 nm UV light will appear as bright spots on a dark background[12].

2.4 GC-MS analysis

Table I : Identified compound, area and retention time of peak of methanolic extract of Lepidium sativum Linn. seeds

Peak	Retention Time	Area	Area%	Name
1	3.04	25221152	0.27	Methyl glyoxal
2	3.73	290614244	3.11	Furan, 2,5-dimethyl
3	4.37	9717876	0.10	1-Methoxyethanimine, N-acetyl-
4	5.39	42462021	0.45	1,3,2-Dioxaphosphorinane-2-methanol,

5	6.68	7607526	0.08	3-Amino-2-oxazolidinone
6	7.29	28683324	0.31	Orcinol, monoacetate
7	7.62	18553525	0.20	5-Acetyl-4-methylthiazole
8	8.03	7767588370	83.02	Benzene, 1-isocyano-3-methyl-
9	8.47	21161217	0.23	Benzeneacetic acid, methyl ester
10	9.45	375852640	4.02	Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)-
11	10.18	2437847	0.03	Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)-
12	11.18	178364827	1.91	Benzene, (isothiocyanatomethyl)-
13	12.09	35980239	0.38	1,2,3,4,5-Cyclopentanepentol
14	13.70	144129902	1.54	2,3,5,6-Tetrafluoroanisole
15	15.24	16126454	0.17	Isosorbide Dinitrate
16	16.15	33375304	0.36	Citronellol epoxide (R or S)
17	16.39	27383121	0.29	à-D-Glucopyranoside
18	16.61	70760851	0.76	Tridecanoic acid, thiophen-2-ylmethylenehydrazide
19	17.41	4428218	0.05	Dianhydromannitol
20	17.61	15588611	0.17	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-
21	18.06	25503409	0.27	n-Hexadecanoic acid
22	19.29	141757648	1.52	4-(m-Methylbenzoylamino)benzoic acid,
23	19.77	25182992	0.27	E-6-Octadecen-1-ol acetate
24	19.97	9975685	0.11	Octadecanoic acid
25	20.25	4404337	0.05	d-Glucitol, 1-thio-nonyl
26			0.26	Hexanal, 2-methyl-

	24.00	24378183		
27	24.53	9484215	0.10	Diisooctyl phthalate

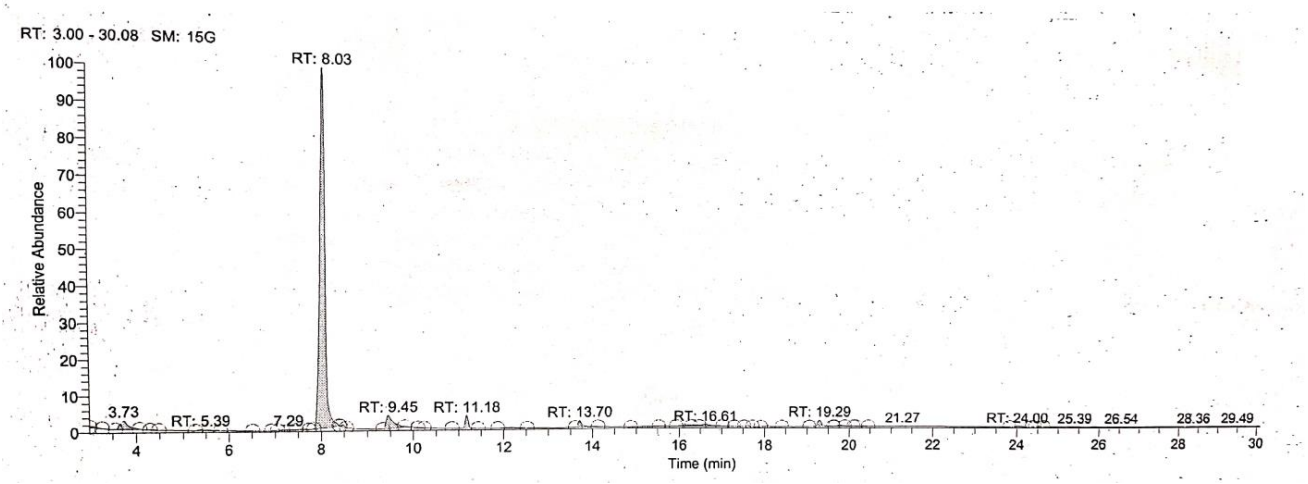
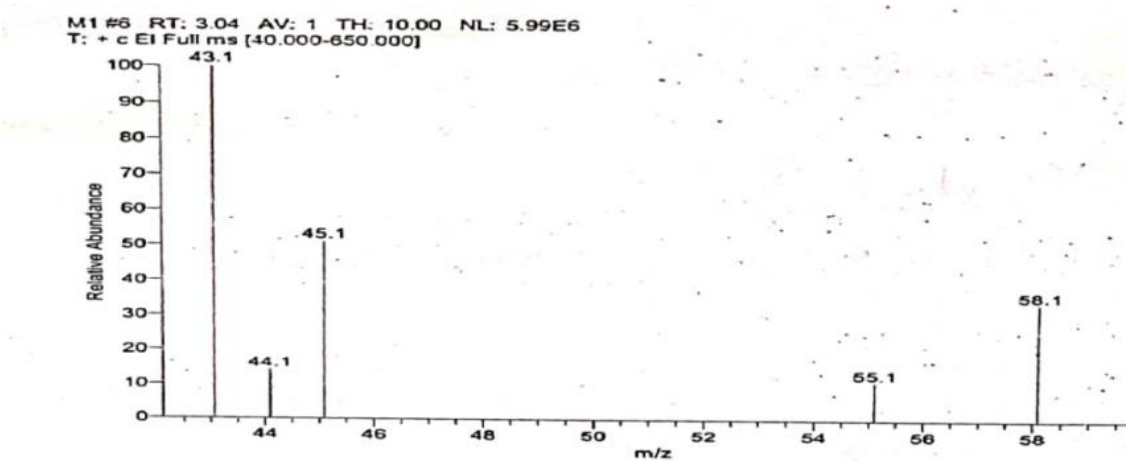
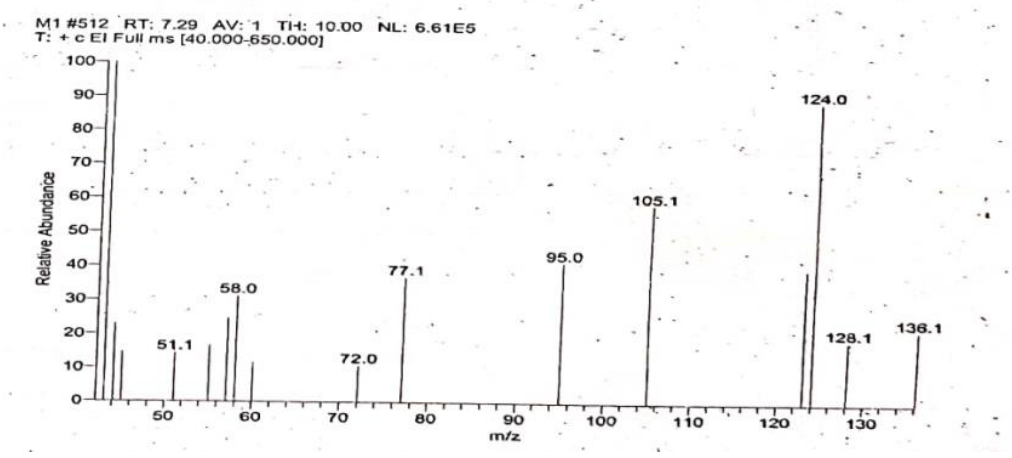


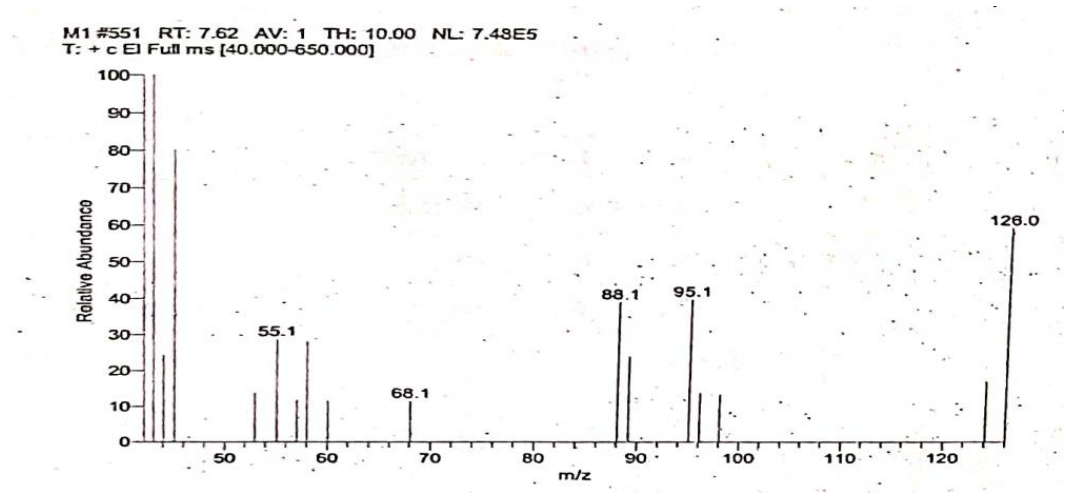
Figure 1: GC-MS chromatogram of methanolic extract of Lepidium sativum Linn. seeds



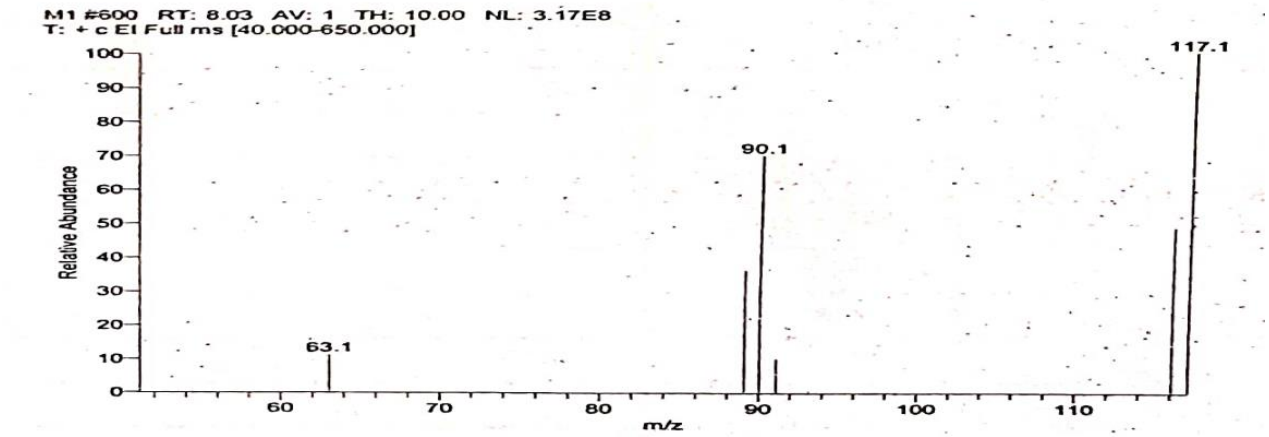
Methyl glyoxal



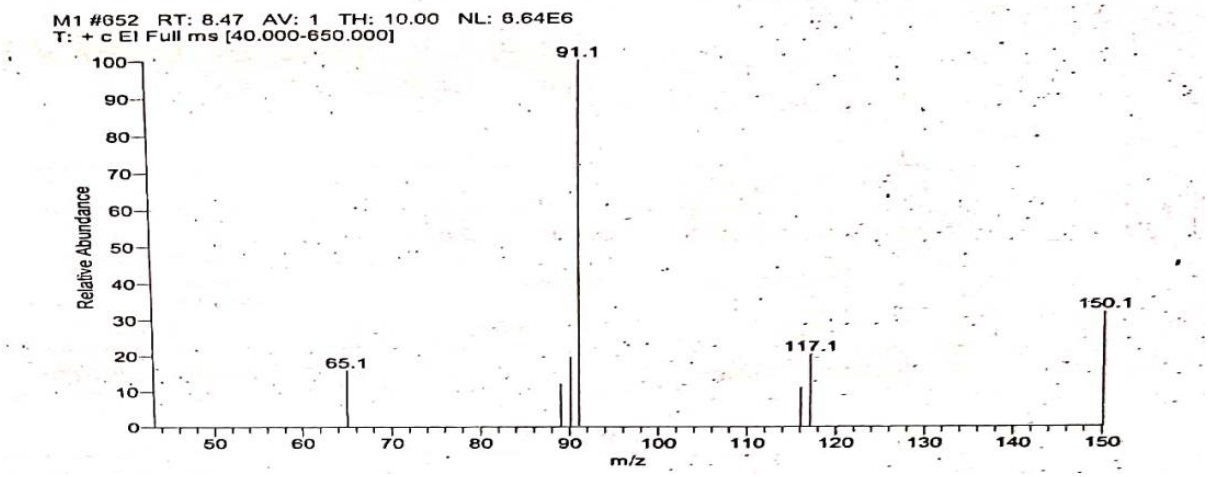
Orcinol, monoacetate



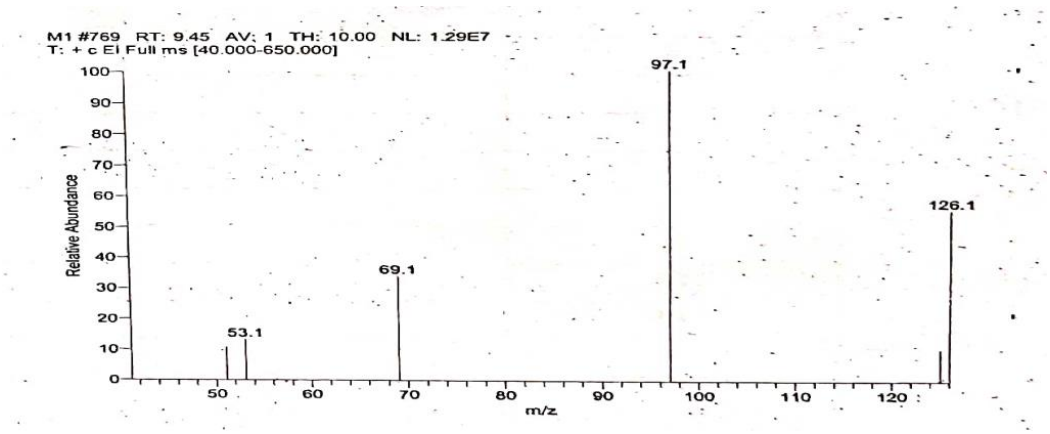
5-Acetyl-4-methylthiazole



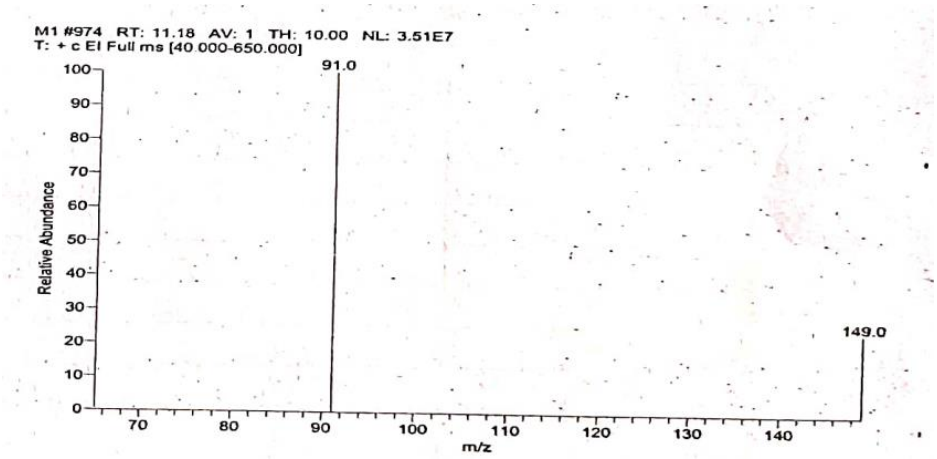
Benzene, 1-isocyano-3-methyl-



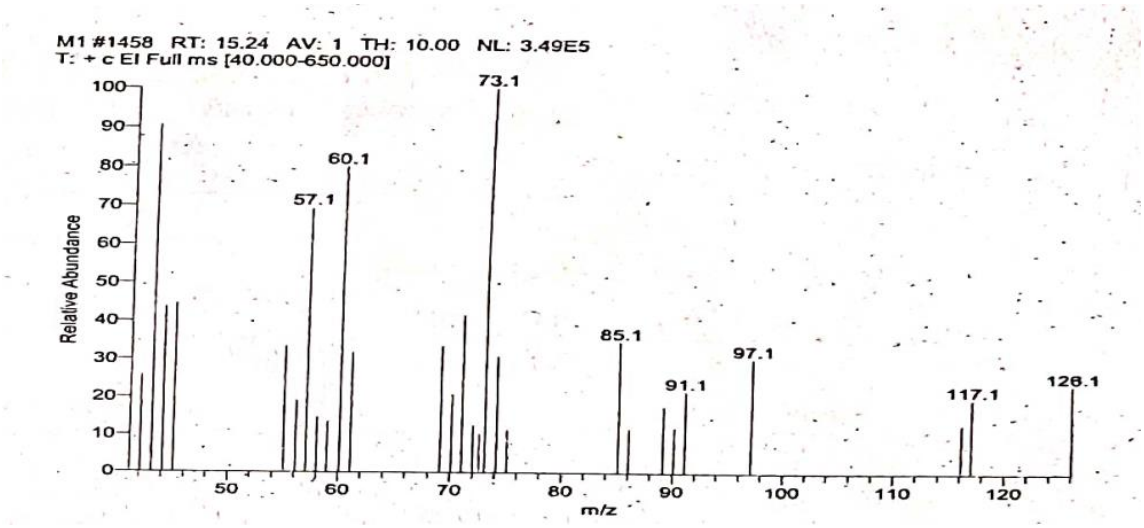
Benzeneacetic acid, methyl ester



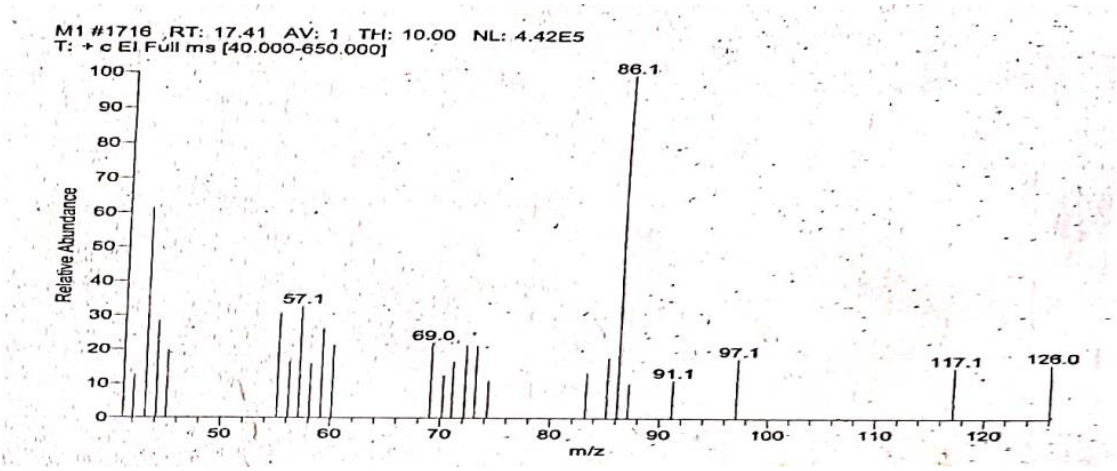
Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)



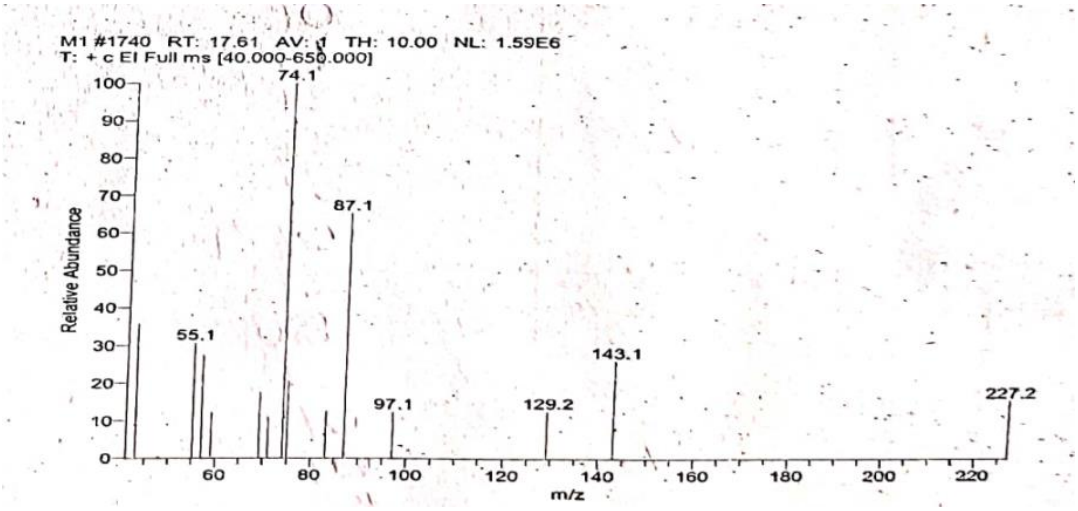
Benzene, (isothiocyanatomethyl)



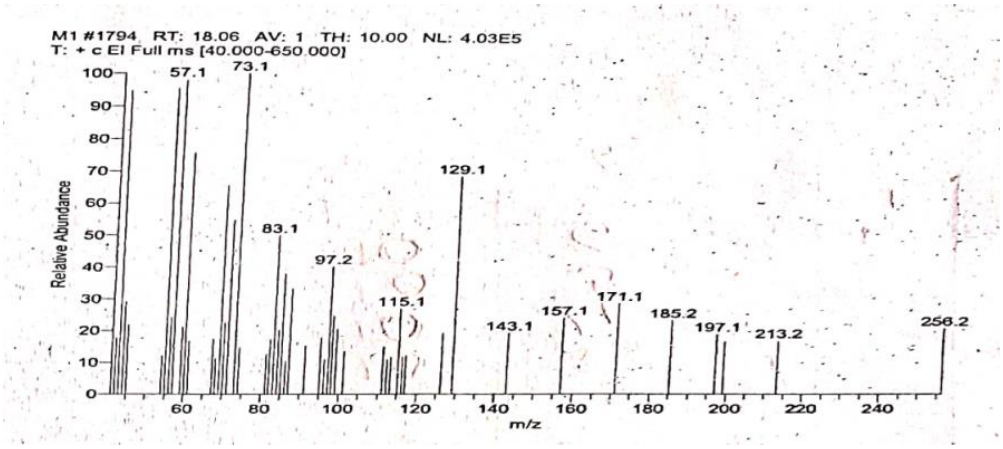
Isosorbide Dinitrate



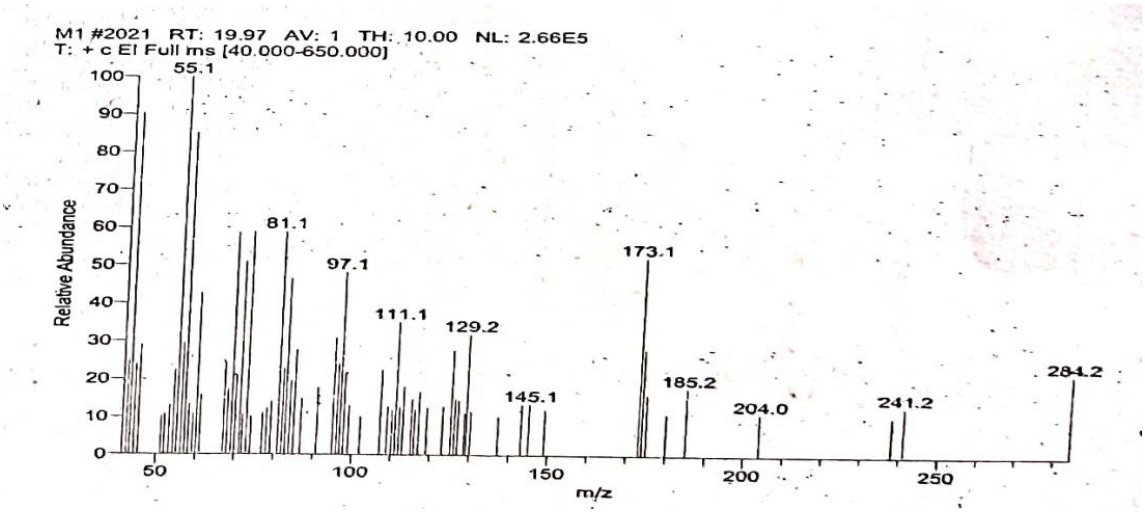
Dianhydromannitol



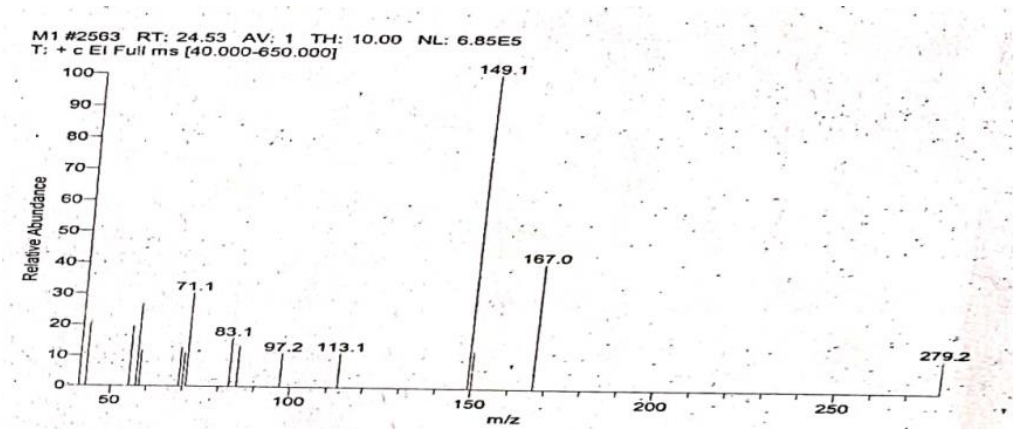
Tetradecanoic acid, 12-methyl-, methyl ester, (S)-



n-Hexadecanoic acid



Octadecanoic acid



Diisooctyl phthalate

Figure 2: Word mass spectrum of some compounds present in methanolic extract of Lepidium sativum Linn. seeds

3. RESULT AND DISSCUSSION

3.1 TLC analysis

Proper spots were found in following solvent system:-

Chloroform : Methanol

5 : 5

Figure 3: TLC of methanolic extract in chloroform: methanol (5 : 5)

Table II : Rf (Retention factor) value for methanolic extract TLC development

SPOT	SOLUTE DISTANCE (cm)	SOLVENT FRONT DISTANCE (cm)	Rf VALUE
First spot	1.5	6	0.25
Second spot	3.4	6	0.56
Third spot	4.5	6	0.75

Table II showed Rf values for all spots which were developed in chloroform: methanol (5 : 5) solvent system.

3.2 HPTLC analysis

The HPTLC analysis of methanolic extract of Lepidium sativum Linn. seeds revealed the presence of various phytochemicals as illustrated in the figures and tables below. The chromatograms (Figure 5) were obtained upon scanning at UV 254 nm and 366 nm, and peak tables were generated. The Rf values, peak height, peak area, and percent area of the unknown substances are depicted in the tables (Table: III and IV). HPTLC of methanolic extract at 254nm and 366nm shown in Figure 4.

Table III: Compounds shown at wavelength 254 nm in HPTLC of methanolic extract

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.03	6.7	-0.01	12.7	4.63	-0.00	1.4	114.7	1.67	unknown *
2	-0.00	3.8	0.02	20.7	7.53	0.03	20.4	275.4	4.00	unknown *
3	0.03	19.0	0.04	36.9	13.46	0.04	23.5	279.7	4.06	unknown *
4	0.06	34.5	0.09	53.7	19.58	0.13	22.8	1800.2	26.14	unknown *
5	0.17	22.4	0.22	47.7	17.39	0.24	40.7	1858.5	26.99	unknown *
6	0.38	28.5	0.39	33.7	12.30	0.46	12.3	1335.9	19.40	unknown *
7	0.57	6.5	0.59	17.5	6.38	0.63	1.8	431.1	6.26	unknown *
8	0.76	7.1	0.78	17.5	6.37	0.79	12.8	252.2	3.66	unknown *
9	0.81	13.3	0.83	19.7	7.19	0.84	5.1	346.0	5.02	unknown *
10	0.95	7.8	0.96	14.2	5.18	0.99	2.2	193.4	2.81	unknown *

HPTLC at 254 nm showed presence of 10 types of compounds, Rf values of these ranging from 0.03 to 0.95.

Table IV: Compounds shown at wavelength 366 nm in HPTLC of methanolic extract

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.03	1.4	0.04	23.0	12.20	0.05	11.3	197.7	4.05	unknown *
2	0.06	14.7	0.09	23.3	12.32	0.13	9.8	660.0	13.52	unknown *
3	0.17	2.4	0.22	28.8	15.23	0.22	26.3	625.6	12.81	unknown *
4	0.27	24.6	0.30	33.1	17.54	0.34	23.0	1386.4	28.39	unknown *
5	0.37	21.2	0.39	24.4	12.90	0.46	8.3	1129.1	23.12	unknown *
6	0.57	5.9	0.59	15.1	8.00	0.63	2.8	397.1	8.13	unknown *
7	0.71	2.3	0.74	11.9	6.32	0.76	6.3	245.2	5.02	unknown *
8	0.86	0.0	0.88	18.1	9.61	0.89	3.8	119.5	2.45	unknown *
9	0.95	6.9	0.96	11.1	5.88	0.98	0.3	122.7	2.51	unknown *

254 nm **366 nm**

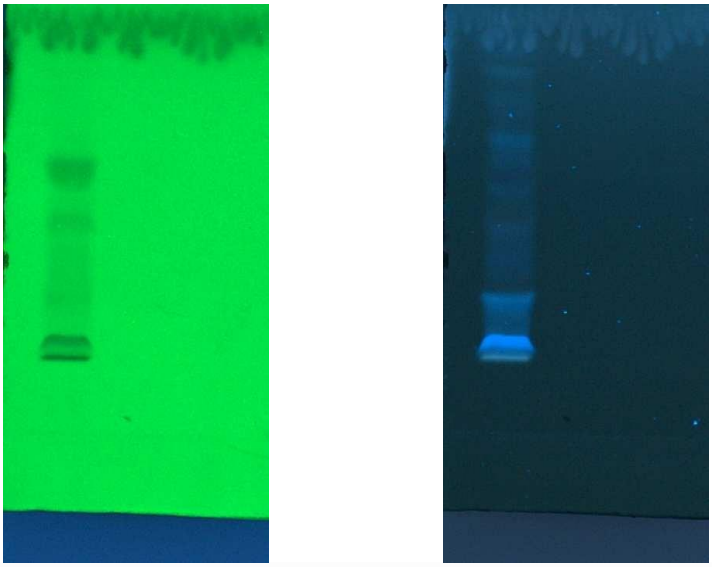
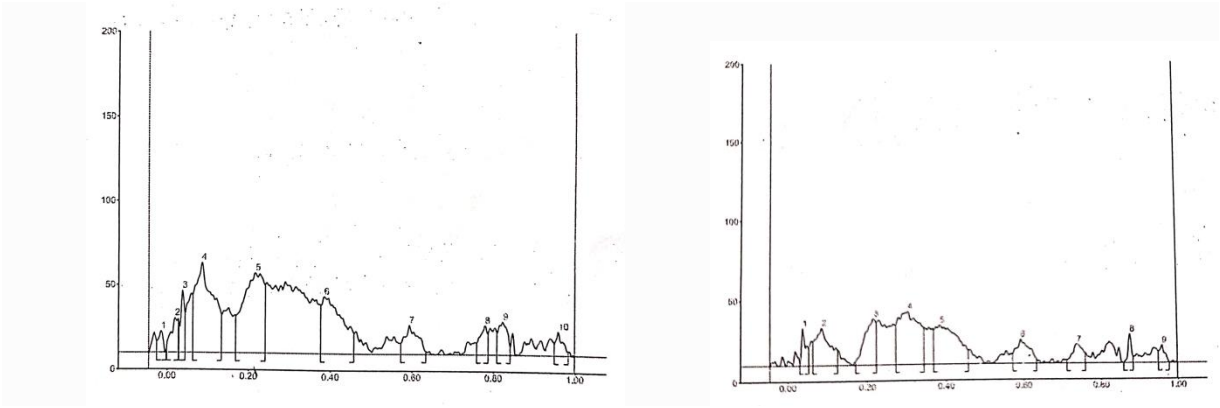


Figure 4 : HPTLC of methanolic extract at 254nm and 366nm

Chromatogram



254 nm **366 nm**

Figure 5 : HPTLC chromatogram of methanolic extract at 254nm and 366nm

HPTLC at 366 nm showed presence of 9 types of compounds, Rf values of these ranging from 0.03 to 0.95.

3.3 GC-MS analysis

GC-MS analysis of methanolic extract showed presence of many compounds, out of which many of compounds have biological activity which already reported in previous literatures summarized in table III and table IV.

Table V: Biological activity of identified compounds in methanolic extract of Lepidium sativum Linn. seeds

S. No.	Compound	Biological/Pharmacological activities	Reference
1.	Methyl glyoxal	Antibacterial	13
2.	Orcinol, monoacetate	Anxiolytic	14
3.	5-Acetyl-4-methylthiazole	Antimicrobial	15
4.	Benzene, 1-isocyano-2-methyl-	Antimicrobial	16

5.	Benzeneacetic acid, methyl ester	Antioxidant and immunity booster	17
6.	Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)-	Broad antifungal	18
7.	Benzene, (isothiocyanatomethyl)-	cholinesterase inhibiting, antioxidant, and anti-inflammatory activity	19
8.	Isosorbide Dinitrate	Vasodialator	20
9.	Dianhydromannitol	Anticancer	21
10.	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	Act as Lipid Anchor In Biomembranes, Anxiolytic	22
11.	n-Hexadecanoic acid	Antioxidant,Pesticide, Hypocholesterolemic Nematicide,Anti-Androgenic Flavor, Hemolytic, 5-Alpha Reductase Inhibitor	22
12.	Octadecanoic acid	antibacterial	23
13.	Diisooctyl phthalate	Antibacterial , Antifouling	24

GC-MS analysis of methanolic extract of Lepidium sativum Linn. seeds revealed the presence of 27 phytochemical compounds as depicted by 27 respective peaks for each compound in GC-MS chromatogram (Table I, Figure 1, 2). Some compound Benzene, 1-isocyano-3-methyl- (83.02%), Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)- (4.02%) Furan, 2,5-dimethyl (3.11%), and Benzene, (isothiocyanatomethyl) (1.91%) with retention time 8.03, 9.45, 3.73 and 11.18 respectively. Lower percentage compound were identified Diisooctyl phthalate (0.10%), 3-Amino-2-oxazolidinone (0.08), Dianhydromannitol (0.05) with retention time 24.53, 6.68 and 17.41 respectively. The compounds present were of different classes such as steroids, acids, phytosterols, alkaloids, ketones, ester, etc. Irrespective of the amount or concentration (high or low) in which these compounds were found to be present, almost all these compounds have been reported to possess some pharmacological or the other biological activity (Table V). For instance, Benzene, (isothiocyanatomethyl)- are known to possess cholinesterase inhibiting, antioxidant, and anti-inflammatory activity. Many phytochemical compounds identified such as, 5-Acetyl-4-methylthiazole, Benzene, 1-isocyano-2-methyl-, Methyl glyoxal , Furan, 2,3-dihydro-4-(1-methylpropyl)-, (S)-, Octadecanoic acid, Diisooctyl phthalate have been reported to be antimicrobial (antibacterial or antifungal) in nature. Benzeneacetic acid, methyl ester and n-Hexadecanoic acid are significantly important phytochemical compound, also found to be present in the extract and is known to have antioxidant activity. Dianhydromannitol possess anticancerous properties. Tetradecanoic acid, 12-methyl-, methyl ester, (S)-and Orcinol, monoacetate are two other biologically active compounds, which possess anxiolytic activity. Isosorbide dinitrate has been reported to be utilized in vasodilator therapy of heart failure.

4. CONCLUSION

TLC solvent system developed for methanolic extract of Lepidium sativum Linn. seeds was chloroform: methanol (5 : 5). Same solvent system was used for HPTLC analysis of methanolic extract, which showed presence of various compounds in extract. HPTLC at 254 nm showed presence of 10 types of compounds, Rf values of these ranging from 0.03 to 0.95. HPTLC at 366 nm showed presence of 9 types of compounds, Rf values of these ranging from 0.03 to 0.95. From the results obtained from GC-MS analysis of methanolic extract of Lepidium sativum Linn. seeds, it can be concluded that the plant exhibits several biological and pharmaceutical properties which provide an insight to the medical value of Lepidium sativum Linn. plant which can be further evaluated to optimize how the plant may be utilized to explore its medicinal potential.

5. ACKNOWLEDGEMENT

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