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GC-MS Studies on Bioactive Food Plant-*Linum usitatissimum* L. Grown Through Recovery Drip System

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***Abstract*:** The aim of the current study is to monitor the phytochemical constituents in the *Linum usitatissimum* by GC MS analysis.The leaf, stem, root of hydroponically grown Flax *Linum usitatissimum* were extracted with methanol at room temperature for 8 h. Further subjected for evaluation using GC-MS.The phytochemical analysis revealed the presence of 20 compounds in leaf methanolic extract and the chromatogram showed peaks with individual compounds. The major constituents identified in the leaf methanolic of extract were Silane, Trimethyl-2-Propyne-, Cyano Methyl-Triiron Disulfide Octacarbonyl, Guanidino-L-proline, Cyclohexanecarboxylic acid, 2(3H)-Furanone, 5-methyl-, Ethane, 1-chloro-1-fluoro, 5,6-Dichlorohexene similarly in stem extract Carbonochloridic acid, Furfuryl glycidyl ether, 4-Amino-6-methyl-piperidin-2-one, Goitrin, Nerinine whereas in roots of Flax herbs seven compounds were shown and they are 2(3H)-Furanone, Ethane, 1-chloro-1-fluoro-, Silane, Trimethyl-2-Propyne, Guanidino-L-proline, 1,3,5-Triazine and many other compounds were identified as low level.The result of this study offers a platform of using *Linum usitatissimum*as herbal alternative for various diseases and it can be used as functional bioactive food.

***Keywords*:** *Linum usitatissimum,* GC-MS, Hydroponic unit, Phytochemicalstudies, Bioactive food

Flax (*Linum usitassimum*) belonging to family Lineaceae, itis not a new crop and native to West Asia and the Mediterranean. By virtue of the presence of physiologically active food components that may provide health benefits beyond basic nutrition, flaxseed is often grouped into one of several categories namely; "functional food" "bioactive food" and an "endocrine active food." Ancient centres of flax-growing are mountainous areas of India and China early as in the 4th or 5th millennium B.C. flax was cultivated for its fiber in Mesopotamia, Assyria and Egypt.Wild narrow-leaved flax and semi-cultured procumbent flaxes are grown in Transcaucasia. The seed contains approximately 40% lipids, 30% dietary fiber and 20 % protein. The chemical composition varies considerably among varieties and also depends on the environmental conditions in which the plant is grown. Cotyledons of seeds contain 25% of the lipids, and 35% of proteins. The endosperm contains only 23% of the lipids and 16% of protein.Other species of Linum shows many compounds at high concentrations of omega-3 fatty acids and lignans which exhibits goitrin, nerinine content. Soilles culture through recovery drip system automatically promotes nutrients, fresh water in the form of irrigation with modern fertigation technology. (Savvas and Gruda 2018).

MATERIAL AND METHODS

The study was laid out in hydroponic unit i.e., recovery drip system having a size 100 sq. ft with an anti-insect mesh, 15m wide, 15.0 m length and 4.70 m height. The pressurized irrigation system (on each shelf) was controlled with gate valves. The conductive and distribution pipes of the nutritive solution were made of black PVC tubing with 1cm diameter with one nebulizer at each 25 cm of distance, with a consumption of mineral nutrient enriched water at 3L hr^{-1} in each device. Seedlings of each plantlet was placed in each slot running with the nutritive solution containing Ca (300 ppm), Mg (400 ppm), NPK (260 ppm), boric acid (300 ppm), Zn (400 ppm) and S (300 ppm) andwere applied from the 5th up to the 12th day after sowing while the plants were growing and at the time of harvest. The nutrientsolution was maintained with pH 6.5, E.C 1.10 ds m^{-1} at 22°Cwith 70% humidity in a 500 L water tank circulated through PVC tubes. Later the growth was monitored with length of plant (LP), number of shoot lets (S) and number of days (d) (Fig. 1).

Gas Chromatography–Mass Spectrometry (Gc/Ms) Analysis

Plant parts of *Linum usitatissimum* were taken to run under soxhlet for the extraction in presence of methanolic solvent. GC/MS analysis was performed using a Perkin Elmer GC Claurus 500 system and Gas Chromatograph interfaced to a Mass Spectrometer (GC/MS) equipped with an Elite-1 fused silica capillary column (30 m × 0.25 mm ID. ×1 µMdf, composed of 100% Dimethyl poly siloxane). For

Table 1. Phytocomponents in methanolic extract of hydroponically grown flax leaf

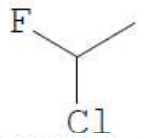
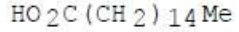
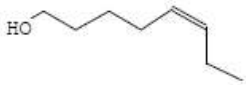
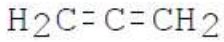
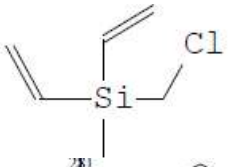
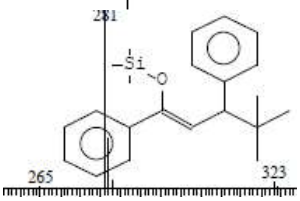
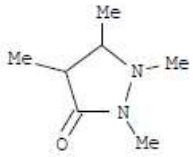
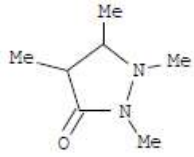
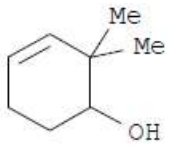
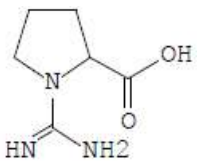
RT	Name of the compound	Molecular formula	MW	Peak area	Chemical structure
0.158	2(3H)-Furanone, 5-methyl-	C5H6O2	98	7.17	
1.166	Ethane, 1-chloro-1-fluoro- (CAS) 1-Chloro-1-fluoroethane 1-Chlorofluoroethane	C2H4ClF	82	7.98	
19.518	Hexadecanoic acid (CAS) Palmitic acid	C16 H32 O2	256	22.84	
21.668	5,6-Dichlorohexene	C6 H10 CL2	152	3.99	
29.100	1,2-Propadiene	C3H4	40	3.55	
30.335	2-methylene-7-oxabicyclo	C7 H10 O	110	4.09	
30.825	Silane, trimethyl-2-propyne-	C6 H12 SI	112	2.22	
31.168	Cyanomethyl-triiron disulfide octacarbonyl	C10 H3 FE3 N O8 S2	497	2.44	
31.397	3-Pyrazolidinone, 1,2,4,5-tetramethyl- (CAS) 1,1 N-N Dimethyltetrahydrapyrazol-3-One	C7 H14 N2 O	142	6.61	
31.492	2',5'-diamine, N,N'-dicyclohexyl	C30 H36 N2	424	4.13	
31.858	2,2-Dimethyl-3-cyclohexen-1-ol	C8 H14 O	126	3.27	
32.042	Guanidino-L-proline	C6H11N3O2	157	3.76	

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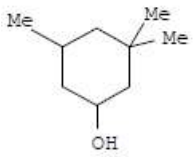
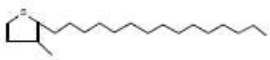
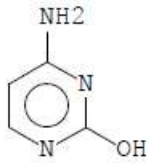
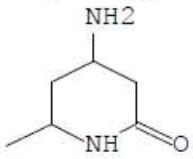
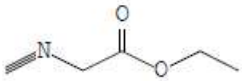
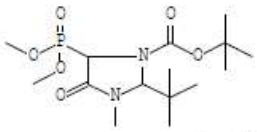
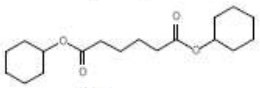
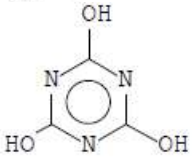
Table 1. Phytocomponents in methanolic extract of hydroponically grown flax leaf

RT	Name of the compound	Molecular formula	MW	Peak area	Chemical structure
32.217	Cyclohexanol, 3,3,5-trimethyl-	C9H18O	142	3.37	
32.383	Thiophene, 3-methyl-2-pentadecyl	C20H36S	308	2.71	
32.471	4-Amino-6-methyl-piperidin-2-one	C6H12N2O	128	4.10	
32.509	4-Amino-6-methyl-piperidin-2-one	C6H12N2O	128	3.90	
32.659	Acetic acid,2-cyano-, ethyl ester	C5H7NO2	113	4.77	
33.641	2-t-Butyl-5-(dimethoxy-phosphoryl)-3-methyl-4-oxoimidazolidine-1-carboxylic acid, t-butyl ester	C15H29N2O6P	364	4.23	
34.458	Cyclohexanecarboxylic Acid, Cyclohexyl Ester (Cas) Cyclohexyl Cyclohexanecarboxylate	C13 H22 O2	210	2.64	
34.542	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione	C3H3N3O3	129	2.23	

GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 ml/min. and an injection volume of 2 µl was employed (split ratio of 10:1). Injector temperature 250°C; Ion-source temperature 280°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 280°C, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative percentages were calculated.

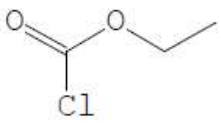
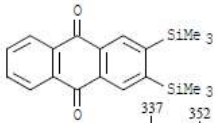
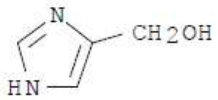
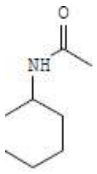
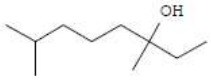
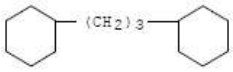
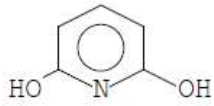
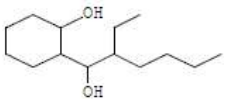
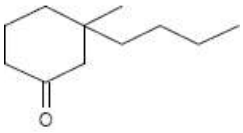
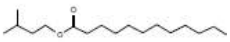
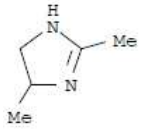
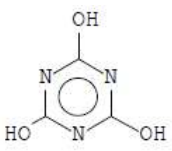
Characterization of compounds: Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Table 4 and Figure 1 showed the maximum length of shoot lets of Flax seeds grown in Hydroponics unit for 5 weeks of treatments. Table 5 showed the number of leaves of flax seeds grown in hydroponics for 5 weeks through one-way ANOVA statistical analysis was performed.

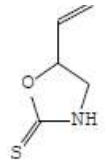
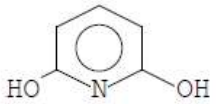
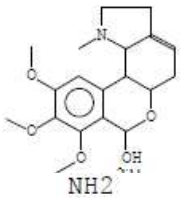
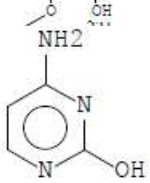
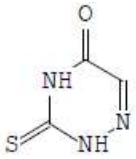
The studies on the active principles in the flax leaf methanolic extract by GC-MS analysis clearly showed the presence of twenty compounds with their retention time (RT). molecular formula, molecular weight (MW), and concentration (peak area (Table-1). The major components

Table 2. Phytocomponents in methanolic extract of hydroponically grown flax stem

RT	Name of the compound	Molecular formula	MW	Peak area	Chemical structure
1.166	Carbonochloridic acid, ethyl ester (CAS) Cathyl chloride	C3 H5 CL O2	108	4.94	
19.568	Tridecanoic acid (CAS) Tridecylic acid	C13 H26 O2	214	8.26	
30.826	Furfuryl glycidyl ether	C8H10O3	154	4.12	
31.348	4-Cyclohexene-1,2-diol	C6H10O2	114	4.77	
31.867	Tetrahydrolinalool	C10 H22 O	158	3.78	
32.725	Cyclohexane, 1,1'-(1,3-propanediyl)bis-	C15H28	208	10.43	
32.806	Isoamyl laurate	C17H34O2	270	3.43	
32.967	4-Amino-6-methyl-piperidin-2-one	C6H12N2O	128	7.00	
33.077	Cyclohexanol, 2-(2-ethyl-1-hydroxy-1-hexyl)-	C14H28O2	228	4.11	
33.167	1-Cyclohexanone, 3-butyl-3-methyl-	C11H20O	168	4.62	
33.242	3-methylbutyl decanoate	C15 H30 O2	242	3.80	
33.418	1H-Pyrrole (CAS) Pyrrole Azole Pyrrol Imidole Monopyrrole Divinylenimine 1-Aza-2,4-cyclopentadiene	C4 H5 N	67	4.40	
33.800	1H-Imidazole, 4,5-dihydro-2,4-dimethyl-	C5 H10 N2	98	5.76	
33.883	1,3,5-Triazine-2,4,6(1H,3H,5H)-trione	C3H3N3O3	129	3.89	

Cont...

Table 2. Phytocomponents in methanolic extract of hydroponically grown flax stem

RT	Name of the compound	Molecular formula	MW	Peak area	Chemical structure
34.025	Goitrin 2-Oxazolidinethione, 5-ethenyl-, (S)-	C5H7NOS	129	6.16	
34.208	3-oxocanone 1-oxacyclooctan-3-one	C7 H12 O2	128	4.95	
34.376	2,6-pyridinediol pyridine-2,6-diol	C5 H5 N O2	111	4.20	
34.508	Nerinine	C19H25NO5	347	4.17	
34.650	2(1H)-Pyrimidinone, 4-amino-	C4H5N3O	111	3.98	
34.908	3-Thio-1,2,4-triazin-3,5(2H,4H)-dione	C3H3N3OS	129	3.22	

present in *Linum usitatissimum* L. leaf methanolic extract areSilane, Trimethyl-2-Propyne- (2.22%), Cyano Methyl-Triiron Disulfide Octacarbonyl (2.44%), Cyclohexanecarboxylic acid (2.64%),Guanidino-L-proline (3.76%), 5,6-Dichlorohexene (3.99%), 2(3H)-Furanone, 5-methyl-(7.17%), Ethane, 1-chloro-1-fluoro (7.98%) and Hexadecanoic acid (CAS) is a Palmitic acid ester which possess antioxidant,heomolytic, anti-androgenic property. The GC-MS analysis of *P. stratiotes* leaves revealed the presence of 7 major compounds L-Glutamine (0.38%), 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.96%), Stigmasterol (2.57%), 9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z) (2.7%), n- Hexadecanoic acid (7.18%), Hexadecanoic acid, ethyl ester (13.29%), Hexadecanoic acid, Diisooctyl phthalate (53.84%), (Tulika Tyagi and Mala Agarwal 2017).Study of using coco peat as a medium in hydroponic system, while environmental and ecological concerns in the recent years suggested mitigating the use of peat because it may destroy endangered wetland ecosystems worldwide. Abul-Soud et al (2016). Asaduzzamana et al (2013).

The studies on the active principles in the flaxstem methanolic extract by GC-MS analysis clearly showed the presence of twenty compounds with their retention time (RT). Molecular formula, molecular weight (MW), and concentration peak area (Table 2). The major components present in *Linum usitatissimum* L. stem methanolic extract are3-Thio-1,2,4-triazin-3,5(2H,4H)-dione (3.22%). Isoamyl laurate (3.43%), Furfuryl glycidyl ether (4.12%), Nerinine (4.17%) Carbonochloridic acid (4.94%), Goitrin (6.16%) and 4-Amino-6-methyl-piperidin-2-one (7.00%) and Different phytochemicals have been found to possess a wide range of activities. The phytochemicals are known to have antimicrobial activity. Flavanoids show anti allergic, anti-inflammatory, anti-microbialand anti-cancer activity. Marinou et al (2013) reported that use of saw dust, coco soil & pumice can increase the growth abundantly for the cultivation. Glycoside, flavonoid, tannin and alkaloid have hypoglycemic activities.Number of multiple shoots was increased in *Mentha arvensis* grown to the length of 19-45cm with 15-37 numbers of shoots and Table top plant with 25-38 shoots after five weeks of nutritional treatment whenit was grown in cocopeat at different treatments (Nazneen Bobby et al 2021). Flax lignans are reported to have antioxidant property which presumably is the main reason of the anticancer activity. Secoisolariciresinol diglycoside existing bound form as a

Table 3. Phytocomponents in methanolic extract of hydroponically grown flax roots

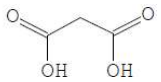
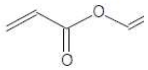
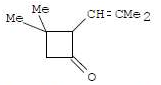
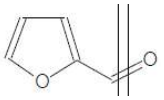
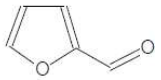
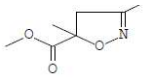
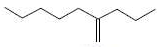
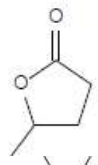
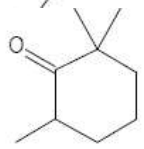
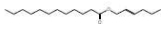
RT	Name of the compound	Molecular formula	MW	Peak area	Chemical structure
0.098	Formamidinium acetate	C3 H8 N2 O2	104	6.86	
0.300	(2S,3S)-2,3-Epoxy-1-hexanol Oxiranemethanol	C6 H12 O2	116	3.29	
1.140	Beta.-ionone epoxide	C13 H20 O2	208	5.75	
29.052	Propane, 2-methoxy-2-methyl- (CAS)	C5 H12 O	88	3.42	MeOBu-t
31.108	1H-Cyclopropa[b]naphthalene-2,7-dion	C15 H22 O3	250	3.32	
31.307	Tetraethylsuccinic acid dinitrile Butanedinitrile	C12 H20 N2	192	3.03	NCCEt 2CEt 2CN
31.518	Furfural	C5H4O2	96	4.53	
31.608	Furfural	C5H4O2	96	5.53	
31.850	1-(.alpha.-Carbethoxy)ethylazetidin-2-	C8 H13 N O3	171	3.08	
31.950	4-brom-5-(phenylthio)tricyclo[4.1.0.0(2	C13 H11 BR S	278	3.10	
32.325	Nonane, 4-methylene- 1-Heptene, 2	C10H20	140	11.33	
32.401	2(3H)-Furanone, dihydro-5-methyl-	C5H8O2	100	3.93	
32.567	Cyclohexanone, 2,2,6-trimethyl-	C9H16O	140	5.21	
32.759	Dodecanoic acid, 2-hexen-1-yl ester	C18H34O2	282	4.89	
33.001	cis 3 hexenyl lactate	C9 H16 O3	172	6.98	
33.125	4-dimethylamino-2,3,4,6-tetra	C8 H15 N O2	157	3.58	
33.423	n-Hexyl acrylate 2-Propenoic acid, hexyl	C9H16O2	156	6.91	
33.625	(2H)Pyrrole_2_carbonitrile, 5_amino_3,4_	C5H7N3	109	6.15	
33.983	Hexanenitrile (CAS) Capronitrile Tricapronile	C6 H11 N	97	5.04	
34.897	8-Pentadecanol	C15H32O	228	4.09	

Table 4. Length of shoots and of flax seeds grown in hydroponics for 5 weeks

Parameters	Week after sowing					
	1	2	3	4	5	Total
Length of shoots						
Mean	2.396	4.328	9.88	12.44	18.44	9.497
Standard deviation.	1.022	0.9689	3.6208	4.1037	4.9166	6.6568
Number of leaves						
Mean	3.16	5.64	9.88	12.44	18.44	9.912
Standard deviation	2.6721	2.8994	3.6208	2.8994	4.9166	6.5044

Fig. 1. Multiple shoots of *Linum usitatissimum* L. grown in hydroponic unit

complex of five secoisolariciresinol diglycoside residues held together by four HMGA (3-hydroxy-3-methylglutaric acid) residues in the outer layers of the seed (Muir 2006).

The studies on the active principles in the flax root of methanolic extract by GC-MS analysis clearly showed the presence of twenty compounds with their retention time (RT). molecular formula, molecular weight (MW), and concentration (peak area%)(Table-. The major component present in *Linum usitatissimum* L. Root methanolic extract are Formamidinium acetate (6.86%), Beta. -ionone epoxide (5.75%), Furfural (4.53%), Nonane, 4-methylene-1-Heptene, 2 (11.33%), Cis 3 Hexenyl Lactate (6.98%), Hexanenitrile (CAS) Capronitrile Tricapronile (5.04%) and 8-Pentadecanol (4.09%). Conventionally grown *Linumusitatissimum* revealed the presence of seventeen compounds of which squalene (45.27 %) and 9, 12, 15-octadecatrienoic acid (z,z,z) (24.67) are the phytocomponents with high peak areas (Dharshini et al 2013). These alkaloids have been found to possess significant bioactivities such as antiviral, antiprotozoal, antitumor and cholinesterase inhibitory activities. (Zou G et al 2009). Flavonoids are antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect the cell against all stages of carcinogenesis (Okwu2004). 1-Monolinoleoylglycerol trimethylsilyl ether has many biological activities such as Antiarthritic, Anticancer, Hepatoprotective, Antimicrobial, Antiasthma, Diuretic, antioxidant, anti-inflammatory and anti-diabetic (Senthil et al 2016). Several other compounds were also detected through GC/MS chromatogram having notable medicinal property. Among the identified phytochemicals, n-Hexadecanoic acid, Hexadecanoic acid, ethyl ester, Palmitic acid has the property of antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitor (Jegadeeswari et al 2012. Upgade and Anusha 2013). Almost the results output to the availability of nutrients is a vital factor influencing plant growth (Lazcano et al 2009).

CONCLUSION

The major constituents identified in the leaf methanolic extract were Silane, Trimethyl-2-Propyne- etc., whereas the stem extract shows Carbonochloridic acid, Furfuryl glycidyl ether, Goitrin, Nerinine etc., whereas in roots of Flax herbs seven compounds were shown and they are Ethane and many other compounds at low level. These investigations can prove that *Linum usitatissimum* would be used as alternative medicine for treating various diseases.

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