Research Article

GC-MS Analysis of Bioactive Compounds in the Whole Plant of Ethanolic Extract of *Ludwigia perennis* L.

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ABSTRACT

GC-MS analysis of bioactive compounds in whole plant of ethanolic extract of *Ludwigia perennis* L. revealed the presence of 29 bioactive compounds. The major bioactive compounds are 13-Docosenamide (19.87%);(4, 3', 4'-Tris (methoxy carbonyl methoxy)-6, 6'-dimethy lbiphenyl-3-yloxy) acetic acid methyl ester (16.51%); Ergost-7-en-3-ol, (3á) (11.72%). Hence, these bioactive compounds are responsible for the various pharmacological actions.

Keywords: *Ludwigia perennis*, GC-MS, ethanolic, bioactive compounds.

INTRODUCTION

Medicinal plants are widely used by the traditional medicinal practitioners to cure different diseases due to their world-wide availability and fewer side effects. Medicinal plants are valuable therapeutic agents; both in modern and traditional medicine[1]. Plants play a role in the prevention and treatment of diseases and reduce the adverse effects of treatments[2]. Throughout the world, they have been widely employed in all cultures, for the prevention and treatment of diseases[3]. The herbal medicines occupy distinct position right from the primitive period to present day. Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. The medicinal value of this plant lies in the bioactive phytochemical constituents that produce definite physiological effect on human body. These natural compounds signify the base of modern drugs as we use today. Natural products play a leading role in the development of innovative drug leads for the treatment and prevention of diseases[4-6]. Phytocomponents are the natural bioactive compounds found in the plants[7]. The side effects of the modern medicine, traditional medicines are gaining importance and are now being studied to find the scientific basis of their therapeutic actions [8]. Traditionally medicinal plants are used recently attracted the attention of the biological scientific communities. This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations [9]. Information of the chemical constituents of plant is helpful in the discovery of therapeutic agent as well as new cradles of economic materials like oil and gums. The most vital bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenolic compounds. Medicinal plants are at curiosity to the field of biotechnology as well as most of the drug industries depend on plant parts for the future production of pharmaceutical compounds[10].

GC-MS is used for analysis of compounds directly in traditional medicines and medicinal plants. In recent years this study have been applied for the analysis of medicinal plants to be a valuable method for the non polar compound analysis and volatile essential oil, fatty acid, lipids[11] and alkaloids[12]. Its feature is to identify different substances within a test sample. It is the best technique to identify the bioactive compounds of long chain hydrocarbons, alcohols, acids, esters, etc.,[13].

Hence the present study aimed to explore the bioactive compounds present in the ethanolic extract of *Ludwigia perennis* L. whole parts by GC-MS technique.

MATERIALS AND METHODS

Collection of Plant materials

The whole plant of *Ludwigia perennis* L. (Fig. 1) were collected from Erode district, Tamil Nadu, India and were authenticated by Botanical Survey of India, Coimbatore, Tamil Nadu, India. Fresh plants were collected and air-dried at room temperature and then homogenized to obtain coarse powder. The powdered test plants was extracted with the solvent ethanol by hot extraction using soxhlet apparatus, collected and stored in a vial for further analysis[14].

Figure 1: *Ludwigia perennis* L. - Habit

Solvent extraction

Crude plant extracts was prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of ethanol solvent separately. The process of extraction continues for 24 hours or 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 all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for their future use in phytochemical analysis.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Ethanolic extracts of whole plant of *Ludwigia perennis* was analyzed for the presence of different volatile compounds by Gas chromatography-Mass spectroscopy (GC-MS) technique. GC-MS analysis of some of the potent volatile constituents present in the extract was performed at The South India Textile Research Association (SITRA), Coimbatore (Tamil Nadu), India. GC analysis of the extracts was performed using a GC-MS equipped with a DB-35MS fused silica capillary column (30m length X outside diameter 0.25 mm X internal diameter 0.25 µm) and gas chromatograph interfaced to a Mass Selective Detector (MS-DSQ-II) with XCALIBUR software. For GC-MS detection, an electron ionization system with ionization energy of -70eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1ml/min and the sample injected was 1µl; Injector temperature 250°C; Ion source temperature 200°C. The oven temperature was programmed from 70° to 200°C at the rate of 10°C/min, held isothermal for 1minutes and finally raised to 250°C at 10°C/min. Interface temperature was kept at 250°C. Total GC run time was 37.50 min. The relative percentage of each extract constituent was expressed as percentage with peak area normalization.

Identification of components

The identification of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST[15], [16]WILEY library sources were also used for matching the identified components from the plant material.

RESULTS AND DISCUSSION

GC-MS chromatogram of the ethanolic extract of whole plant of *Ludwigia perennis* shows the presence of twenty nine compounds. The GC-MS chromatogram of the test plant is presented in Figures 1. The active principles with their retention time (RT), molecular formula, molecular weight (MW), and peak area are presented in Table 1. The most prevailing major compounds were 13-Docosenamide, (Z)–(19.87%),(4,3',4'-Tris(methoxy carbonylmethoxy)-6,6'-dimethylbiphenyl-3-yloxy)acetic acid methyl ester–(16.51%), Ergost-7-en-3-ol, (3á)-(11.72%), Hexadecanoic acid, methyl ester –(7.62%), 9-Octadecenoic acid (Z)-, methyl ester – (5.42%), 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester – (4.93%),3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyphenyl)ethyl]pyrrole 2,5dicarboxylic acid-(5.40%). Some minor compounds like 7,8-Epoxy-trans-syn-cis-tricyclo[7.3.0.0(2,6)]dodecane, (+-) (7S,8R) – (3.17%), 2-(2'-Furyl)-4-(trimethylsilyl)furan – (3.09%), 1,2-Benzenedicarboxylic acid, dibutyl ester – (2.95%), 1-Nonadecene – (2.89%), 1-Tetradecanol – (2.68%), 2,4-Dimethoxy-4',4"- bis (5,5-dimethyl-1,3-dioxan-2-yl) triphenylamine–(1.35%),2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)cyclohexanol–(1.25%),7,9-Di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione–(1.22%), 1,2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester – (1.15%), 1-Hexadecene – (1.12%), 3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyphenyl)ethyl]pyrrole-2,5-dicarboxylic acid – (1.10%),9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester, trans – (1.09%) were also obtained.

Among the identified bioactive compounds,13-Docosenamide, (Z)- has anti-microbial activity; (4,3',4'-Tris(methoxycarbonylmethoxy)-6,6'-dimethy lbiphenyl-3-yloxy)acetic acid methyl ester has anti-oxidant and anti-microbial activity;Ergost-7-en-3-ol, (3á) has anti-tumor, immunomodulatory activity, inhibitory hemolytic activity, anti-inflammatory and anti-viral activity; Hexadecanoic acid, methyl ester has anti-oxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, hemolytic,5-alpha reductase inhibitor, nematicide, pesticide and lubricant; 9-Octadecenoic acid (Z); methyl ester has hypocholesterolemic, nematicide, anti-arthritic, hepatoprotective, anti-androgenic, hypocholesterolemic, 5-alpha reductase inhibitor, anti-histaminic, anti-coronary, insectifuge, anti-eczemic, anti-acne, anti-

inflammatory, cancer preventive; 3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyph enyl)ethyl]pyrrole-2,5-dicarboxylic acid has antibacterial, antiviral and antitumor activity.

In this present study related to[17] Thanga Krishna Kumari *et al*., (2012) reported the presence of fourteen compounds in ethanol extract of whole plant of *Sarcostemma secamone*. Dihydrotachysterol (22.78%) was found to be major component followed by Methane, nitro (17.72%), Butanoic acid, 3,7-Dimethyl-6-octeny ester (11.39%), Phytol (10.76%), Squalene (10.13%), 9,12-Octadecadienoic acid (Z,Z)-, phenylmethyl ester (6.96%), 9-Octadecenoic acid (Z)-, phenylmethyl ester (6.96%) and Didodecyl phthalate (3.16%). Similarly [18]Varsha Jadhav *etal*., (2014) reported that the GC-MS analysis of the methanolic extract revealed the presence of forty four bioactive compounds with valuable biological activities, including the allergic Melamine. The major chemical constituents are Quinic acid (5.72%), 1,2,3-Benzenetriol (42.25%), Melamine (3.07%), Pentanoic acid, 4-oxo- (1.40%), Myristic acid (3.36%) and Oleic Acid (0.49%).

In the bark of *Solanum verbascifolium*21 phytocompounds were identified using three different extracts from the bark extract of the plant. Among these 13 constituents in ethyl acetate, 6 in methanolic and 2 in acetone extract were identified during the GC-MS analysis. Phytol and Linolenic acid which were identified in the plant is considered to have anti-cancerous properties[19].

[20]Balaji and Kilimozhi (2014) identified seven different compounds in the leaf extract of the plant *Clerodendrum phlomidis*, namely Isopropyl Linoneate, Hexadecanoic Acid, 2- Hydroxyl-1-[Hydroxymethyl] Ethyl Ester, 9-Octadecenoic Acid [Z]-, 2-Hydroxy-1- [Hydroxymethyl] Ethyl Ester, 1,11- Tridecadiene, Hexadecane, Benzene 1-methyl-4-nitroso, 1[2Acetoxyethyl] 3,6diazahomo adamantan-9-one oxime. [21]Suprava *et al.,* (2016) revealed that the presence of eight different components for 92.1% and 82.86% of the leaf and rhizome extract of *Kaempferia parishii*. In leaf extract phytol (72.55±0.5%), hexadecanoic acid methyl ester (4.94±0.2%), hexahydro farnesyl acetone (3.78±0.2%), dibutyl phthalate (3.31±0.2%) were found to be the major constituents and those of rhizome extract were totarol (74.96±0.86%), cembrene (2.83±0.2%), borneol (1.23±0.15).

[22]Mohamed and Benedict (2016) assured the presence of 30 compounds in the leaf *Leucaena leucocephala*. Among these the major compounds are Squalene (41.02%), Phytol (33.80%), 3, 7,11,15-Tetramethyl-2-hexadecen-1-ol (30.86%) and 3,7,11-Tridecatrienenitrile, 4,8,12-trimethyl (25.64%).[23] Varadharajan, 2016 reported in the *Cucumis callosus* extract 32 compounds were identified by GC–MS/MS. Doxorubicin is used to bring significant changes in biochemical parameters and antioxidants in the heart. The pretreatment with *Cucumis callosus* at two doses (250 mg/kg and 500 mg/kg) to DOX treated rats significantly prevented the altered enzymes SGPT, SGOT, CPK and LDH, lipid profile LDL, VLDL, TGs, HDL, TC and antioxidant SOD, GSH, CAT, GSH-Px and MDA to near normal level. Serum urea, uric acid, and ALP which are increased on DOX administration registered near normal values on pretreatment with *Cucumis callosus*. Similarly [24]Igwe (2016) reported the presence of 12 compounds in the ethanolic extract of the leaves of *Acalypha wilkesiana*. Among the 12 compounds, the most abundant were 2-Ethyl-1-hexene with 39.21 peak area %, RT 22.698 and molecular formula C_8H_{16} ; n-Haxadecanoic acid or plamitic acid with 20.92 peak area %, RT:20.92 and molecular formula $C_{16}H_{32}O_2$ and Butane 1,4-diol with 11.58%peak area RT:8.358 and molecular formula of $C_4H_{10}O_2$ which demonstrated various medicinal potentials.

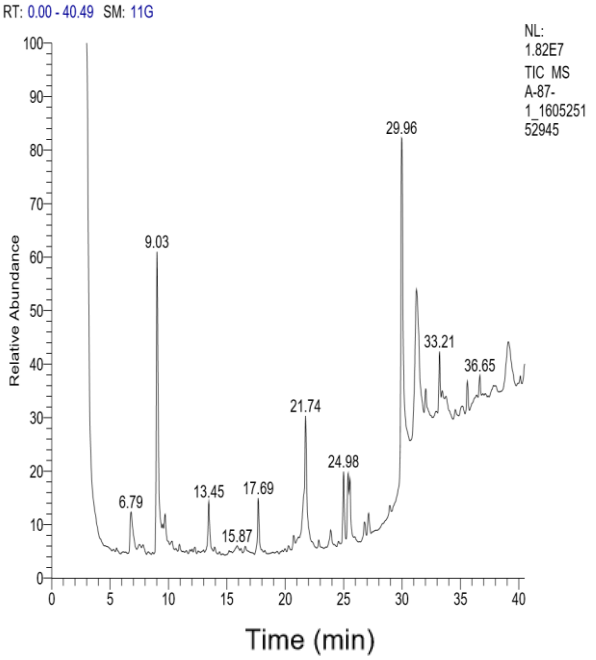

Figure 2: GC-MS Chromatogram of the ethanolic extract of whole plant of *Ludwigia perennis* L.

CONCLUSION

The GC-MS method is a direct and fast analytical approach for identification of phytochemicals and only few grams of plant material is required. The importance of the study is due to the biological activity of some of these compounds. In addition to this, the results of the GC-MS profile can be used as pharmacognostical.

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Table 1: Bioactive compounds identified in the ethanolic extract of whole plant of *Ludwigia perinnis* L. by GC-MS

S. No	R.T	Name of the compound	Molecular Formula	Molecular Weight	Peak Area%
1	3.07	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, trans	$C_{28}H_{44}O_4$	444	1.09%
2	6.79	2-(2'-Furyl)-4-(trimethylsilyl)furan	$C_{11}H_{14}O_2Si$	206	3.09%
3	7.53	4-Cyano-2-Methyl-N-phenylacetanilide	$C_{16}H_{14}N_{2O}$	250	0.44%
5	9.03	(4,3',4'-Tris(methoxycarbonylmethoxy)-6,6'-dimethy lbiphenyl-3-yloxy)acetic acid methyl ester	$C_{26}H_{30}O_{12}$	534	16.51%
6	9.71	1-Hexadecene	$C_{16}H_{32}$	224	1.12%
7	10.31	Cyclohexane, 1,4-dimethyl-2-octadecyl	$C_{26}H_{52}$	364	0.29%
8	12.25	2-Deuterio-4,5,5-trimethyl-1-pyrroline 1-oxide	$C_7H_{12}DN$	127	0.31%
9	13.45	1-Tetradecanol	$C_{14}H_{30}O$	214	2.68%
10	15.87	(cis)-2-(t-Butyl)-2-cyano-3-(1'-naphthyl) cyclobutanone	$C_{19}H_{19}NO$	277	0.62%
11	17.69	1-Nonadecene	$C_{19}H_{38}$	266	2.89%
12	20.70	8-Pentadecanone	$C_{15}H_{30}O$	226	0.47%
13	21.74	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	7.62%
14	22.86	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278	0.29%
15	23.89	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8 –dione	$C_{17}H_{24}O_3$	276	1.22%
16	24.98	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16}H_{22}O_4$	278	2.95%
17	25.38	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	5.42%
18	26.78	N-(2-Methoxy-4-phenylbutyl)hydroxylamine	$C_{11}H_{17}NO_2$	195	0.92%
19	27.14	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	1.15%
20	28.94	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	0.38%
21	29.96	13-Docosenamide, (Z)-	$C_{22}H_{43}NO$	337	19.87%
22	31.24	Ergost-7-en-3-ol, (3á)	$C_{28}H_{48}O$	400	11.72%
23	32.01	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadec a-3,7,11,15-tetraenyl)-cyclohexanol	$C_{30}H_{50}O$	428	1.25%
24	33.21	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	4.93%
25	35.14	4,4-dichloro-1,3-bis(trimethylsilyl)-2trimethylsilylamino-1,3-diaza-2-phos pha-4-silacyclobutane	$C_9H_{28}C_{l2}N_3PSi_4$	391	0.77%
26	35.59	2,4-Dimethoxy-4',4"-bis(5,5-dimethyl-1,3-dioxan-2 -yl)triphenylamine	$C_{32}H_{39}NO_6$	533	1.35%
27	36.65	7,8-Epoxy-trans-syn-cis-tricyclo[7.3.0.0(2,6)]dodec ane, (+-) (7S,8R)	$C_{12}H_{18}O$	178	3.17%
28	37.83	3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyph enyl)ethyl]pyrrole-2,5-dicarboxylic acid	$C_{33}H_{35}NO_{11}$	621	1.10%
29	39.11	3,4-Bis(3,4,5-trimethoxyphenyl)-1-[2-(4-methoxyph enyl)ethyl]pyrrole-2,5-dicarboxylic acid	$C_{33}H_{35}NO_{11}$	621	5.40%

REFERENCE

1. Krentz AJ, Bailey C J, Oral antidiabetic agents: current role in type 2 diabetes mellitus, Drugs, 65, 2005, 385-411.
2. Bachrach ZY, Contribution of selected medicinal plants for cancer prevention and theraphy, Acta Fac Medicae Naissensis, 29 (3), 2012, 117-123.
3. Huie C,A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants, Anal Bioanal Chem, 2002; 373, 23-30.
4. Tagboto S, Townson S. Adv Parasitol, Antiparasitic properties of medicinal plants and other naturally occurring products, 50, 2001, 199-295.
5. Evans WC, Trease and Evans Pharmacognosy WB, Saunders Company Ltd., London, (14th Edition) 2000, 19-20.
6. Cragg GM, Snadde KM, Natural products as sources of new drugs over the Newman period, 1981– 2002,J Nat Prod, 66 (7), 2003, 1022 -1037.
7. Krishnaiah GM and Prashanth GK, Phytochemical Studies and GC-MS Analysis of the Leaf Extracts of *Melia Azedarach* Linn., International Journal of Advancement in Engineering, Technology, Management & Applied Science, 1, 2014, 48-54.
8. Gupta Y K and Briyal S, Animal models of cerebral ischemia for evaluation of drugs, Indian Journal of Physiology and Pharmacology, 48, 2004, 379-394.

9. Mary Helen PA, Aswathy MR, Deepthi K, Rathi RM, Joseph JJ, Sree SJ, Phytochemical analysis and anticancer activity of leaf extract of *Mangifera indica* (Kotttukonam Varika), Int J. Pharma. Sci. & Res., 4 (2), 2013, 819-824.

10. Velmurugan P, Kamaraj M, Prema D, Phytochemical constituents of Cadaba Trifoliata Roxb. root extract International Journal of Phytomedicine, 2, 2010, 379-384.

11. Jie MSF and Choi, CYC. J. Int. Fed. Clin. Chem., 3, 1991, 122.

12. Betz JM, Gay ML, Mossoba MM, Adams S and Portz BS, Chiral gas chromatographic determination of ephedrine-type alkaloids in dietary supplements containing Ma Huang. J AOAC Int, 80, 1997,303-315.

13. Karuppasamy B, Antony N, Veerabahu RM, GC-MS analysis of *Polycarpaea corymbosa* (L.) Lam whole plant, Asian Pacific J. Trop. Bio. 2012, 1289-1292.

14. Mukherjee PK. Quality Control of Herbal Drugs. An approaches to evaluation of botanicals, edition 1st published by Business Horizons, New Delhi; 2002; 390 - 403.

15. Mc Lafferly FW. Registry of mass spectral data, 5ed, Wiley New York 1989.

16. Stein SE. National Institute of Standards and Technology (NIST) Mass Spectral Database and Software, Version 3.02, USA; 1990.

17. (17) Thanga Krishna Kumari S, Muthukumarasamy S and Mohan VR, GC-MS Analysis of Ethanol Extract of *Sarcostemma Secamone* (L) Bennet. (Asclepiadaceae), Science Research Reporter,2012, 187-191.

18. Varsha J, Vaibhav K and Poonam P, GC-MS analysis of bioactive compounds in methanolic extract of *Holigarna grahamii* (wight) Kurz, International journal of Herbal Medicine, 2 (4), 2014, 35-39.

19. Deepak P and Gopal GV, GC-MS analysis of various solvent extracts of bark in *Solanum verbascifolium* Linn. American Journal of Advanced Drug Delivery, 2014, 484-492.

20. Balaji K, Kilimozhi D, GC-MS analysis of various extracts of *Clerodendrum phlomidis* leaf, International Journal of Pharmacy and Pharmaceutical Sciences, 6 (1), 2014, 226-232.

21. Suprava S, Sudipta J, Ambika S, Asit R, Noohi N, Basudeba K, Sanghamitra N, GCMS analysis and evaluation of Bioactivities of *Kaempferia parishii*-A natural source of Totarol, International Journal of Pharmacy and Pharmaceutical Sciences, 8(5), 2016, 182-186.

22. Mohamed ZZ and Benedict S, Phytochemical constituents of the leaves of *Leucaena leucocephala* from Malaysia, International Journal of Pharmacy and Pharmaceutical Sciences, 8 (12), 2016, 174-179.

23. Varadharajan R, Rajalingam D, and Palani S, GCMS/MS analysis and Cardioprotective Potential of *Cucumis callosus* on Doxorubicin Induced Cardiotoxicity in Rats, International Journal of Pharmacy and Pharmaceutical Sciences, 8 (9), 2016, 239-245.

24. Igwe KK, Madubuike AJ, Chika Ikenga, Amaku FJ, Studies on the Medicinal Plant *Acalypha wilkesiana* ethanol Extract Phytocomponents by GCMS Analysis, International Journal of Advanced Research in Science, Engineering and Technology, 3, 2016, 1779-1785.

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