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GC-MS analysis and identification of daidzein by High Performance Thin Layer Chromatography (HPTLC) of *Pluchea lanceolata* - a bone healing plant of Semi-Arid Land

Kavi Gour *, Raghunandan Singh Nathawat, Deepika Arya, and Vidya Patni

Plant Pathology, Tissue Culture and Biotechnology Laboratory, Department of Botany, University of Rajasthan, Jaipur-302004,India

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ABSTRACT

Gas chromatography mass spectroscopic investigation of methanolic extract of *Pluchea lanceolata* – a semi-arid land plant is investigated using GCMS-QP 2010 Plus while the mass spectra of the compounds found in the extract are matched with the standard library of NIST. Maximum % area are found for hexadecanoic acid (19.93 %) with Rt = 24.598 min. and Ethanol, 2-(vinyloxy)- (16.46%) with Rt = 6.974 min. for stem and callus respectively. A simple HPTLC method is used for the qualitative determination of daidzein. The samples are dissolved in methanol and development is carried out in Camag twin trough chamber saturated with mobile phase consisting of Toluene: Ethyl acetate: Acetone: Formic acid (20:4:2:1v/v/v/v). Spectrodensitometric scanning is performed by TLC scanner III (CAMAG) in absorbance mode. The system are found to give compact spot for daidzein (Rf = 0.21 ± 0.03). The data of the results of stem and callus of *Pluchea lancolata* revealed that the samples also contain daidzein with maximum Rf = 0.20 & 0.21 respectively which is a potent bone healer compound. Data obtained could be useful in the identification and authentication of traditional drug for the international acceptance.

Key words: GCMS-QP 2010 Plus, NIST, Daidzein, Bone healer, HPTLC.

INTRODUCTION

The plant species *Pluchea lanceolata* known vernacularly as Rasayan, Rasna, Raasna, Yuktaa, Elaparnee, Sarmei or by its trade or popular names as Rasana, Tuktarasa, Surasa belongs to the family Asteraceae. Source taxon is a xerophytic common in sandy soil and often found on the slope in gregarious patches. There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease [1]. In the recent years, the interest for the study of the organic compounds from plants and their activity has increased. A lot of extraction methods and analytical methods as spectrophotometry, high performance thin layer chromatography (HPTLC), high performance liquid chromatography (HPLC), capillary electrophoresis, gas chromatography–flame ionization detection (GC-FID), gas chromatography–mass spectrometry (GC–MS) have been developed for the study of plant active compounds [2].The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for analysis for volatile and semi-volatile compounds and HPTLC is also more accurate for identification of compounds.

The plant contains numerous therapeutic uses. Hot decoction of herb is used in arthritis, rheumatism, bronchitis, dyspepsia, cough, psoriasis, piles and neurological diseases [3]. Leaves are aperient and used as a laxative, analgesic, antipyretic while roots are bitter, thermogenic, alexiteric, antipyretic and laxative in nature [4]. Plant also contains secondary metabolites viz. quercetin, quercitrin, beta sitoseterol, pluchine [5,6,7]. The petroleum extract of the flowers, roots, stems and leaves showed anti-inflammatory activity in test with rat and yielded the triterpenes moretenol acetate and moretenol [8,9,10,11,12].

There are no previous reports on the phytochemical information of *Pluchea lanceolata* by GC-MS and HPTLC. In this paper we report the chemical composition of the methanolic extracts of *Pluchea lanceolata* from semi-arid lands of Rajasthan by using GC-MS analysis and identification of daidzein (a phytoestrogen) through HPTLC. Data obtained in the present work could be useful in the proper identification and authentication of the traditional drug which is the primary pre requisite for the international acceptance and recognition of herbal medicines.

*** Corresponding author.**
Kavi Gour
Plant Pathology,
Tissue Culture and Biotechnology Laboratory,
Department of Botany,
University of Rajasthan,
Jaipur-302004,India

MATERIAL AND METHODS

GC-MS description

The GCMS-QP 2010 Plus consisted of radioisotope nuclide 63 Ni Quaintly 370 MBq (10mci) equipped with detector FTD, Injection Mode Split with Flow Control Mode :Linear Velocity. A Shimadzu column RTX-5 MS Cross Bond with 5% diphenyl/ 95% dimethyl polysiloxane 60 meter × 0.25 mmID × 0.25 um df was used.

The GC-MS Column was equilibrated at ambient temperature, and the detector stabilized with mobile phase (methanol 1 µl). The carrier gas was helium at a constant column flow rate 1.21 ml/min. held by electronic pressure control. Injector, temperature was 280°C, and splitless injection mode was used. The oven temperature programme was 100°C (held for 2 min.) to 250°C (hold time 10 min), to 300°C (hold time 30 min.). The MS operating condition were as follows: positive electron ionization mode using automatic gain control (AGC). The ion source temperature was 250°C and interface temperature 280°C, gain voltage was set to 0.00 kV. Solvent cut time was 6 min. One microliter of the extract was injected on the system. GCMS-QP 2010 Plus automatic software was used for instrument control and to record chromatograms.

Plant material and extraction

Aerial parts of *Pluchea lanceolata* were collected during the month of July from the forest regions of Jaipur and adjacent areas. The plant material was authenticated by Department of Botany, University of Rajasthan, Jaipur. A voucher specimen has been deposited in the Herbarium of the Department. The plant materials (stem and callus) were air dried at room temperature and under shade, and then powdered to a fine grade by using a laboratory scale mill. These shade dried parts of the plant were powdered which was kept in air tight plastic bag until use. The powder was defated with petroleum ether and the resulting marc was then soxhlet extracted with 80% methanol. After extraction the samples were filtered and the solution was evaporated to obtain the residue. This residue was dissolved in 5 ml of methanol. This was used as the test solution for GC-MS-QP 2010 Plus.

Identification of the compound

The identification of the compounds present in the methanolic extracts were based on the direct comparison of the peaks by retention times and mass spectral data with those for standard compounds, and by computer matching with the online standard library of NIST, Willey, Permury & Drugs.

HPTLC description

TLC Al sheets silica gel 60F254 precoated cut to 10 x 10 cm. were used as a stationary phase and Toluene: Ethyl acetate: Acetone: Formic acid (20:4:2:1) were used as a mobile phase for analysis. Sample/Standard were applied with the help of Linomat 5,10 µl of test solution and standard solution on precoated layer, 10mm from the bottom edge. Band length 8 mm., distance between

tracks 14 mm., distance from the side 15 mm. Application position 10mm with solvent position 80 mm. Measurement mode were UV absorbance / reflectance with scanning wavelength 254 nm., tank saturation 10 minutes with filter paper. Total no. of tracks was 13 with position of first track X 14.8 mm, distance between tracks 14.1 mm. , scan start position Y 5.0 mm. , scan end position Y 85.0 mm. , slit dimensions 6.00× 0.30 mm. Micro with scanning speed 20 mm/s and data resolution 100µm/step. Detector was used as an automatic mode with sensitivity 33%, peak threshold min. Slope 10, min. height 10 AU, min. Area 50, max. height 990 AU.

Plant material and extraction

Daidzein was purchased from Sigma Chemical Co. All the solvent and chemicals used were analytical grade, obtained from Merck India.

10 mg of daidzein was weighed in a 10 ml vol. flask and dissolved in methanol. That was used as standard solution for analysis. 5 gm. of sample (stem and callus) was weighed in a 50 ml standard flask. 1ml methanol was added and shaken well, and the final volume was made up to 50 ml with methanol filter and evaporated. The solution was filtered and evaporated residue dissolved in 1 ml of methanol. This was the test solution for analysis.

RESULTS AND DISCUSSION

In the first part of the experiment which is GC-MS analysis of methanolic extract of *Pluchea lanceolata*, a rapid and simple method outlined the active principles in the studied herbs which are responsible for some therapeutic effects. The extraction method presented is simple, rapid and inexpensive, with reduced solvent consumption. GC-MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principle in herbs used in drugs, cosmetic, pharmaceutical or food industry. The metahnolic fractions were defattigated with petroleum ether and subjected to GC-MS investigation. It is evident from the tables that all the fractions have a complex chemical composition. Some of the GC-MS peaks remained unidentified, because of lack of library data of corresponding compounds.

GC-MS investigation of stem and callus powder of *Pluchea lanceolata* is summarized in Table 1 & 2. The results showed that many constituents with significant commercial or medicinal importance were present in high concentrations. Seventeen compounds were identified in stem powder of the plant (Figure 1, Table 1) representing the various functional groups like acid (-COOH), alcoholic (-OH), ester (R-O-R), carbonyl (-CO-), alkanes (Compounds containing only single bonds) and alkenes (double bonds) and maximum number of compounds showed acid and carbonyl group. The major components of the extract are hydrocarbons. Ten compounds were identified as oxygen containing compounds in which the oxygen atom varies from 1-2. Maximum % area was found for hexadecanoic acid (19.23%) with retention time 24.598 min. and minimum was found for decanoic acid (1.14 %) with retention time 21.180 min..

Table 1: Compounds identified in GC-MS analysis of stem powder of *Pluchea lanceolata*.

Peak#	R.Time	Area	Area %	Name
1	13.601	243301	2.27	2,7-Octadiene-1,6-diol, 2,6-dimethyl-
2	14.071	348312	3.25	2-Furanmethanol, 5-ethenyltetrahydro-. alpha.,.alpha.,5-trimethyl-, trans-
3	15.108	126424	1.18	1-Butanol, 3-methyl-, benzoate
4	21.18	121665	1.14	DECANOIC ACID
5	24.322	677517	6.32	9-Octadecenoic acid
6	24.598	2135930	19.93	Hexadecanoic acid
7	27.412	1283965	11.98	Cyclopentadecanone, 2-hydroxy-
8	27.475	788903	7.36	9-Hexadecenoic acid
9	27.736	2061290	19.23	n-Octadecanoic acid, Stearic acid
10	28.133	522840	4.88	1-Decanol, 2-ethyl-
11	30.005	392426	3.66	Heptadecane
12	32.051	539317	5.03	Hexadecane, 2,6,10,14-tetramethyl-
13	34.55	460151	4.29	tetracosane
14	37.609	333168	3.11	n-Nonadecane
15	40.161	372196	3.47	Tetratetracontane
16	42.145	174417	1.63	Docosane
17	43.803	134995	1.26	Heptadecane, 2,6,10,15-tetramethyl-
		10716817	100	

Table 2: Compounds identified in GC-MS analysis of callus of *Pluchea lanceolata*.

Peak#	R.Time	Area	%Area	Name
1	6.108	1824140	3.9	2-Furancarboxaldehyde, 5-Methyl-
2	6.348	334930	0.72	Butanoic acid, 3-hydroxy-3-methyl-
3	6.542	538908	1.15	1,2,3-PROPANETRIOL
4	6.607	1258914	2.69	1H-Pyrrole-2,5-dione
5	6.713	4174780	8.92	1,2,3-Butanetriol
6	6.974	7698350	16.46	Ethanol, 2-(vinyloxy)-
7	7.284	1865216	3.99	Benzeneacetaldehyde
8	7.442	494716	1.06	2(3H)-Furanone,dihydro-3-hydroxy-4,4-dimethyl-,(+/)-
9	7.683	106276	0.23	OXIRANEMETHANOL, 2,3-DIMETHYL-, TRANS-
10	8.241	183773	0.39	2,5-Dimethyl-4-hydroxy-3(2H)-furanone
11	9.333	3911579	8.36	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
12	9.59	575765	1.23	BENZOIC ACID
13	10.084	911139	1.95	6-Methyl-1,5-diazabicyclo[3.1.0] hexane, ,4-Anhydro-d-galactosan
14	10.599	1501337	3.21	2-Ethylbutyl acrylate
15	12.667	185330	0.4	2-Methoxy-4-vinylphenol
16	13.418	244004	0.52	Phenol, 2,6-dimethoxy-
17	14.853	230172	0.49	2,5-Monomethylene-l-rhamnitol
18	15.49	1390402	2.97	Benzaldehyde, 2-hydroxy-6-methyl-
19	16.961	1375950	2.94	D-Allose
20	17.457	158899	0.34	Dodecanoic acid
21	17.867	194579	0.42	Propylphosphonic acid
22	18.283	222092	0.47	1,2-Benzenedicarboxylic acid, diethyl ester
23	19.497	4831192	10.33	1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)
24	21.401	764176	1.63	1,6-Anhydro-.beta.-D-glucopyranose (levoglucosan)
25	23.209	233880	0.5	1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester
26	24.192	239145	0.51	Ethyl (2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate
27	24.322	544620	1.16	9-Hexadecenoic acid
28	24.45	460366	0.98	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
29	24.612	3033968	6.49	n-Hexadecanoic acid
30	24.779	706231	1.51	1,2-Benzenedicarboxylic acid, dibutyl ester
31	27.423	1782898	3.81	Oleic Acid
32	27.485	1279181	2.73	9-OCTADECENOIC ACID (Z)-
33	27.754	1769637	3.78	n-Octadecanoic acid
34	32.071	170657	0.36	Tetratriacontane
35	34.56	192554	0.41	Heptadecane, 2,6,10,15-tetramethyl-
36	37.612	181969	0.39	Heptadecane, 2,6,10,15-tetramethyl-
37	40.156	182858	0.39	Heptadecane, 2,6,10,15-tetramethyl-
38	42.142	106997	0.23	Eicosane
39	44.675	675148	1.44	2,3-Bis(4-hydroxy-3-methoxybenzyl)-1,2,4-butanetriol
40	52.648	241350	0.52	Androstan-17-ol, 4-[1-naphthyl]-
		46778078	100	

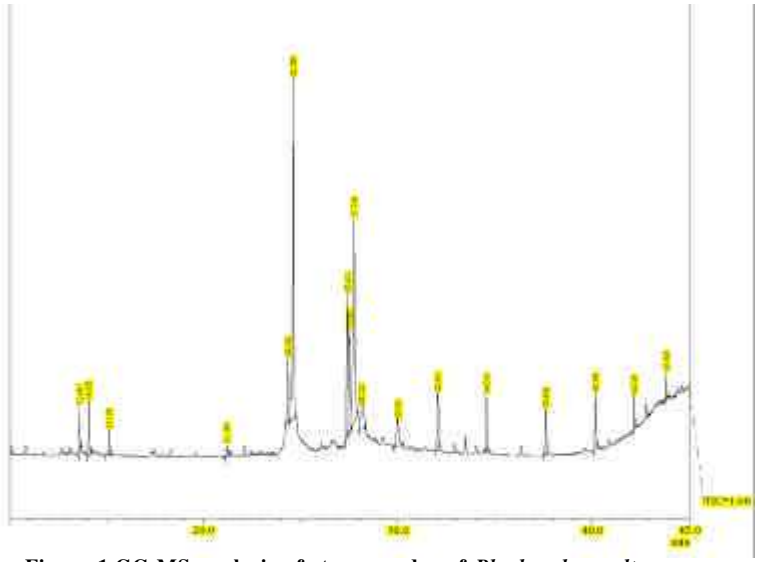

Figure 1.GC MS analysis of stem powder of *Pluchea lanceolta*

Forty compounds were also identified in callus (Figure 2, Table 2) in which thirty four compounds showed oxygen atom and oxygen varied from 1-7 atoms. Nitrogen was also found in 2 compounds (1-2 atom) and fluorine was found only in one (1 atom). Maximum % area was found for Ethanol, 2-(vinyloxy)- (16.46%) with retention time 6.974 min. and minimum was oxiranemethanol, 2,3-dimethyl-, trans- (0.23%) with retention time 7.683 min..

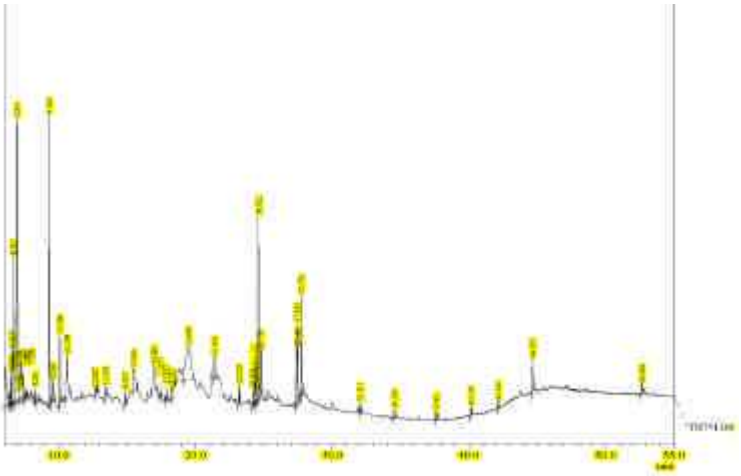

Figure 2.GC MS analysis of callus powder of *Pluchea lanceolta*

In the second part of the experiment HPTLC analysis of daidzein, selection of mobile phase was carried out on the bases of polarity. Different composition of the mobile phase for HPTLC were tested and the desired resolution of the compound, together with symmetrical and reproducible peak, was achieved toluene-ethyl acetate-acetone-formic acid (20:4:2:1) gave good separation of daidzein from its matrix. The chromatographic conditions produced a well-defined compact spot of standard daidzein with optimum migration at Rf = 0.24 ± 0.03. (Figure 3)

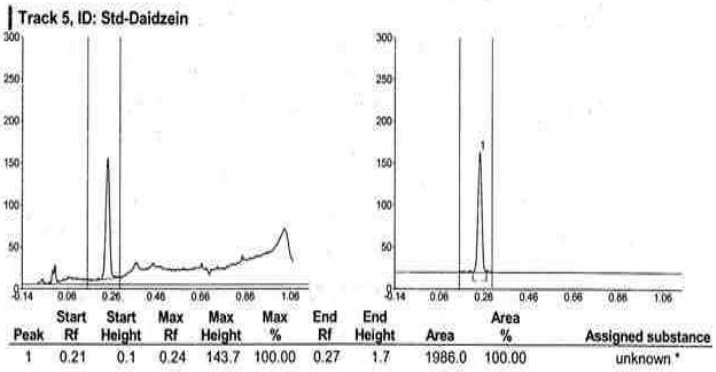

Figure 3.HPTLC analysis of standard compound daidzein.

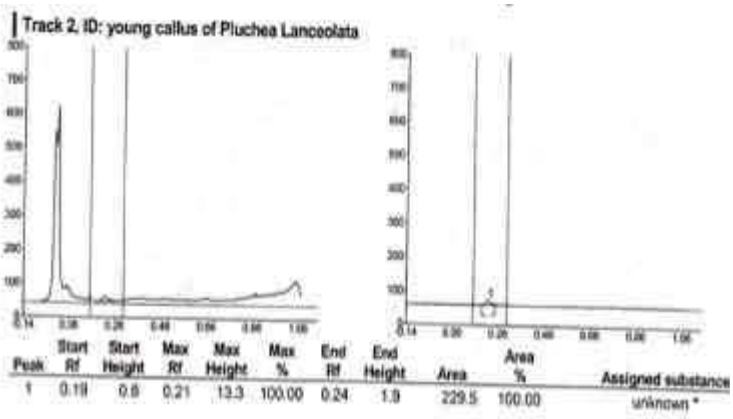

Figure 4.HPTLC analysis of callus powder of *Pluchea lancolata.*

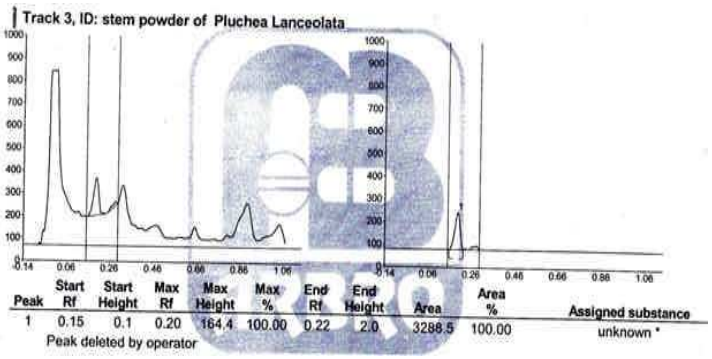

Figure 5.HPTLC analysis of stem powder of *Pluchea lancolata.*

Figure 4 shows chromatogram of callus of *Pluchea lanceolata* with the starting Rf value 0.19, max. Rf 0.21, end Rf 0.24 with starting height 0.6, maximum height 13.3 and area 229.5. Figure 5 shows chromatogram of stem with starting Rf 0.15, max. Rf 0.20, end Rf 0.22 with starting height 0.1, max. height 112.5 and area 3288.5. The identity of the band of daidzein in the sample extract was confirmed by overlaying the UV absorption spectra of sample with that of reference standard which is shown in figure 3.

Daidzein belongs to the group of isoflavones which is also called phytoestrogens because of some estrogen activity. They have recently been shown to be used as bone substitutes and supplemental bone graft materials [13,14,15]. In the present study we have demonstrated that methanolic extract of *Pluchea lanceolata* possess various phytochemicals specially daidzein which has a potent bone healing property. This study may be useful to explore the pharmacological activity of the extract and individual phytochemicals present in the extract.

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