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Original Article

GC-MS Analysis of the Ethanol Extract of *Andrographis serpyllifolia* (Rottl. ex Vahl.) Wt. (Acanthaceae)

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ABSTRACT

In the present study, ethanol extracts from *Andrographis serpyllifolia* were subjected to GC-MS analysis to study the important phytochemical constituents responsible for the various pharmacological activities. The crude extracts of ethanol were obtained by soxlet method. The GC-MS analysis of ethanol extract from *A. serpyllifolia* revealed the presence of twenty-nine phytocompounds in the underground part and twenty six compounds in the aerial parts. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. E-2-Tetradecen-1-01, Pentadecanal and 1, 15-Pentadecanediol are prominent compounds in underground part. Three major phytochemical constituent's mass spectra in aerial part are Thiocyanic acid, 2-propynyl ester, 3(2H)-Pyridazinone and 3-Furanmethanol.

Key words: *Andrographis serpyllifolia,* bioactive compound, Gas Chromatography (GC), Mass Spectroscopy (MS).

1. INTRODUCTION

Knowledge of the chemical constituents of plants is developing in the scientific research not just for the invention of therapeutic agents, however conjointly getting such data could also be of important in revealing new sources of economic phytocompounds for the synthesis of complex chemical substances and for locating the particular significance of folkloric remedies (Milne, 1993) [7]. GC and GC-MS are accepted strategies for the analysis of volatile constituents of herbal medicines, attributable to their sensitivity, stability and high potency (Guo *et al*., 2006; Teo

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et al., 2008) [4, 12]. In "Flora of British India" by J. D. Hooker (C.B Clarke) reportable concerning forty nine genera, 504 species and 127 varieties of acanthus family from India. In "Flowering Plants of India" (Karthikeyan et al., 2009) [6] reported 47 genera, 475 species and 118 varieties of Acanthaceae in India. Many effective medicinal plants from Acanthaceae family, predominantly from the rural areas, are still unexplored which deserve attention and research. *Andrographis serpyllifolia* (Rottl. ex Vahl.) Wt. is an important plant, which did not have much research work so far. *Andrographis serpyllifolia* is a medicinal plant found in wild in Tirunelveli district of Tamilnadu. *Andrographis serpyllifolia* extracts are able to ameliorate biochemical damages induced by alloxan in diabetic rats (Sanjeevaiah and Jithan, 2013) [9]. It is used as a medicinal plant in traditional practices in India, where in cancer patients are treated using the leaves of Andrographis serpyllifolia (Smitha Jeyaram et al., 2011) [11]. Review of literature record that information on the GC-MS analysis of *Andrographis serpyllifolia* is totally lacking. Hence, the objective of the present study is to identify the phytochemical constituents with the aid of GC-MS technique. This work will help to identify the compounds of therapeutic value.

Description of selected plant

A trailing and rooting procumbent herb arise from a stout root stocks, leaves orbicular or subreniform, sessile, villous, up to 1 inch diam. the flowers pale with purple blotches on the lower lip. flowers solitary axillary or in few flowered raceme in upper axils; calyx - lobes very slender, 25 - 4 inch long; anthers much bearded; Capsule, acute at both ends, glabrous, 4 in long, 2 in broad, 8 - seeded; seeds small, pale, deeply rugose retinacula spoon-shaped.

2. MATERIALS AND METHODS

Plant material

The medicinal plant *Andrographis serpyllifolia* was collected from Tirunelveli District, Tamil Nadu, India. The identified plant species was confirmed with Voucher specimen available in the Survey of Medicinal Plant Unit (SMP), Govt. Siddha Medical College, Palayamkottai, Tirunelveli, Tamil Nadu. The taxonomic features of the plant confirmed with the Flora of Presidency of Madras (Gamble, 1915 – 1921) and The Flora Tamil Nadu Carnatic (Mathew, 1983 – 1988).

Soxhlet extraction

60 gram of shade dried *A. serpyllifolia* powder was refluxed with 250 ml of the ethyl alcohol for five hours on a steam bath. The collected extract was concentrated.

Procedure

Shimadzu GC – MS - QP 2010 was used for The GC - MS analysis. Analysis was performed with a DB1fused methylphenylsiloxane, (30 m × 0.25 mm i.d.) capillary column in Gas chromatograph. The column is directly coupled with mass spectrophotometer. Noble gas was used as carrier gas, with a flow of one ml/min. The column temperature was 70^{o}C. The column was programmed for 5 minutes in 180°C, 180-260°C at 3°C/min, 5 minutes in 260°C, 260-280°C at 0.2°C/min, and finally 5 minutes in 280°C. The sample was injected at temperature 280°C without splitting sample. The detector temperature was 290°C. 2 µL of sample was injected. The split magnitude relation was 10:1. The Mass Spectra operative parameters includes ionization potential seventy eV. The ion supply temperature was 200°C and quadrupole 100°C. The solvent delay was 6.0 minutes. Scan speed was 2000 amu/s. Total MS time period for scanning was 36 minutes. The reaction time of scan varies from 30 to 600 amu. The electron volt voltage was 3000 volts.

The targeted *A. serpyllifolia* extract is injected into the GC/MS instrument (Hewlett Packard 5890 GC/MS with Mass Selective Detector with Turbo mass gold-perkin Elmer). The ethanolic extract is vaporised at the injection port. By increasing temperature, the extract was eluted through a capillary column. Since the extract run all the way through the column, based on the affinity with the stationary phase of the column varied elements separated. It was known by retention time. Retention time means the time sample takes for a compound to move through the column and gas chromatograph system. Each chemical component in the sample has a unique retention time and it was measured in minutes. The result was revealed in a peak on a graph. It measures abundance on the ordinate against retention time on the abscissa. The integrated peak is correlated to the concentration of the chemical. A mass spectroscopic selective detector divides each chromatographic constituent into fragment ions. It was revealed by their abundance, with each particle depicted as a vertical line in the order of increasing relative molecular mass. The peak of each line has a close similarity to the abundance of that particle. The resulting spectrum is selective to particular chemical. In ''Scan'' mode all chemical constituents are present in the sample was listed first.

Compound Identification

Identification of components of the methnolic extracts was depend on the comparison of their mass spectra data and retention indices with those reported in the literature and by matching the data in the NIST 2005 MS computer library (Wiley).

3. RESULTS AND DISCUSSION

The results of the GC-MS analysis are useful to the identification of a variety of compounds in selected species. GC-MS chromatogram of the ethanolic extract of *A. serpyllifolia* aerial parts (Fig.1) recorded 26 peaks indicating the presence of the many phytochemical constituents (Table: 1). Three major phytochemical constituent's mass spectra are Thiocyanic acid, 2-propynyl ester, 3(2H)-Pyridazinone and 3-Furanmethanol (Fig. 2). Thiocyanic acid (Molecular

formula: CHNS, Molecular weight: 59.09034 g/mol) derivatives are particularly powerful fungicides. 3(2H)-Pyridazinone ($C_4H_4N_2O$) exhibits antimicrobial activity (Deniz *et al.*, 2008) [2]. 3-Furanmethanol ($C_5H_6O_2$) is one of the bioactive compounds in methanolic extract of *Holigarna grahamii* (Varsha Jadhav *et al.*, 2014) [13]. Alpha,-Chloroacrylic acid ($C_3H_3ClO_2$) (Fig 4.7) is synthesized as phytoalexin in plants. 1-Hepten-4-01 ($C_7H_{14}O$) have shown antifungal activity (Chen *et al.*, 1989) [1] Pentanal, 2-methyl-($C_6H_{12}O$) is an aldehyde compound used as antimicrobial agent (Jananie *et al.*, 2011) [5]. It is also reported in *Senna italica* and exhibits anti-inflammatory drug activity (Shamuga Jothi *et al.*, 2015) [10].

Table: 1 GC-MS studies on ethanol extract of Aerial part of *A. serpyllifolia*

S. No	RT value	Area	Chemical compound
1	16.43	3.31	Cyclopropene 1-Propyne, 3-Chloro- Thiocyanic acid , 2-Propynyl ester
2	16.87	0.76	Thiocyanic acid , 2-Propynyl ester N- Methyl-7-azabicyclo(2,2,1)hept-2 1-propen-1-one, 2-methyl-
3	18.39	0.86	Cyclopropane 4-Methyl-1, -oxazine- 2,6(3H)-dione 4-pentenenitrile, 3-hydroxy-
4	19.26	0.44	Propenal dimethylhydrazone 1-Butanol Taurine
5	19.47	0.55	2-propyn-1-01 Acetoacetic acid, 1-thio-, S-ally1 3,4-Furandicarboxylic acid
6	19.92	2.32	3(2H)-Pyridazinone, 6-methyl-1, 3- Butadiene-1-carboxylic acid 2-Phenyl 1- 3-butyn-2-01
7	20.94	3.68	Galacto-heptulose 1,2-Propanediol, 3-chloro-Ethanol, 2-(2- aminoethoxy)-
8	21.37	16.36	Alpha ,-Chloroacrylic acid 1-Hepten-4-01 Pentanal, 2-methyl-
9	21.73	2.11	dl-Malic disodium salt Ethylene maleic anhydride N-Hydroxymethylacetamide
10	22.01	3.86	(3-Methyl-oxiran-2-yl)-methanol Oxirane , 2,3-dimethyl- 1,2,4-Trioxalane, 3,5-dipropyl-
11	22.29	1.00	Propanal, 2,3-dihydroxy-, (s)- Ethanol, 2-(2-aminoethoxy)- Ethanol,2-methoxy-, acetate
12	22.49	1.66	Octanal 1,3-Cyclohexanediol Piperazine, 2-methyl-
13	22.76	7.57	4H-Pyran-4-one,2,3-dihydro-3,5-di
14	23.37	0.96	Phenol, 4-ethenyl-, acetate Benzenemethamine, N-methyl- Acetic acid, oxo-
15	23.62	0.54	Galacto-heptulose d-Glycero-d-ido-heptose Hexanohydroxamic acid
16	23.85	43.45	Thiocyanic acid, 2-propynyl ester 3(2H)-Pyridazinone 3-Furanmethanol
17	24.50	0.28	3(2H)-Pyridazinone, 4,5-dihydro-6- 2-Butyne-1,4-diol, diformate 3-Hydroxypyridine monoacetate
18	25.49	0.68	3(2H)-Pyridazinone , 4,5-dihydro-6- Acetoacetic acid, 1-thio-, S-ally Ethanol, 2-(hexyloxy)-
19	25.70	0.53	2H-Pyran-2-one, tetrahydro-6, 6-dim 6-Oxabicyclo(3.1.0)hexane
20	26.11	4.31	Benzene, (1-chloroethenyl)- Benzaldehyde, 2-nitroso- Benzenecarbothioic acid
21	26.41	0.99	Cyclopropanecarboxylic acid 1-Methoxy -1-buton-3-yne 2,2-Bipyridine-3,3-diol
22	26.82	0.23	Butanal, 3-hydroxy- 1,2-Hydrazinedicarboxylic acid, di 2-Pentanone, 5-methoxy-
23	28.41	0.42	1-Cyclohexane-1-methanol1 3-Cyclopropenoic acid,-1-butyl, me Benzene, (methylenecyclopropyl) su
24	28.96	0.63	5-Tridecene, (E)- 1-Hexadecene 2-Tridecene, (E)-
25	35.63	1.72	Decanedioic acid d-Glycero-d-ido-heptose Pentadecanoic acid
26	36.00	0.78	Acetic acid, ethoxy-, ethyl ester Butanoic acid, ethyl ester Hexanoic acid, ethyl ester

Table: 2 GC-MS studies on ethanol extract of underground part of *A. serpyllifolia*

S. No	RT value	Area	Chemical compound
1	17.51	1.67	Pyrimidine , 5-chloro-2-dimethylami Quinoline, 2,4-dimethyl- Quinoline, 2,4-dimethyl-
2	19.94	0.94	1,3-Decadiene-7, 9-dione Thero-4-Hydroxy-1-homoarginine 1ac 1H-4-Azacycloprop(cd) indene, octah
3	20.62	0.54	Silane, (bromomethyl)- 4-Camphenylbutan-2-one Spiro (2,5) octane, 3,3-dimethyl1-2-(
4	21.96	2.15	Dodecane, 1-chloro- Spiro (4,5)decane, 6-methylene- 3-Methyl-5-hydroxy-isoxazole
5	22.90	1.10	Benzene, 1,3,5-trimethoxy
6	23.84	1.10	5H-1,2,4-Thiazolo(4,3-b) (1,2,4)thi Dodecanal Trans-5-Methyl-2-isopropyl-2-hexen
7	24.36	0.91	3-buten-2-one, 4-(5,5-dimethyl-1-0 2R-Acetoxymethyl-1,3,5-trimethyl-4 1,4-Methanoazulene, decahydro-4,8,
8	25.22	3..07	13-Tetradece-11-yn-1-o1 3-Cyclohexen-1-carboxaldehyde, 3,4 1,2-15, 16-Diepoxyhexadecane
9	25.77	1.82	1,2-Cyclohexanediol, 3-methyl-6-(1 2-Butanone, 4-(2,2-dimethyl-6-meth 5,9-Dimethyl-9-decen-3-ol
10	26.60	8.57	Diethyl Phthalate
11	27.33	3.29	Tetradecane, 1-chloro- Undecane
12	27.82	3.83	Naphthalene, 1,4,6-trimethyl-
13	28.22	7.97	Hydroxylamine, 0-decyl- Silane, trichlorodocosyl- Dodecane, 1-fluoro-
14	28.70	2.48	Cyclotridecane 4-Tetradecene, (Z)-
15	29.07	1.67	Heptafluorobutyric acid, n-tetrade 2(3H)-Benzofuranone, hexahydro-4,4
16	29.24	2.02	9-Undecenal, 2,10-dimethyl- 1,3,6,10-Dodecatetraene, 3,7,11-tr 12-Methyl –E,E-2,13-octadecadien-1-
17	29.50	1.96	3-(2-Methyl-propenyl)-1H-indene Naphthalene, 1,4,6-trimethyl- 2,5-Cyclohexadien-1-one, 4,4-dimet
18	29.97	2.94	4,11,11-Trimethyltricyclo(5,3,1,0) Bicycle (3.1.1) heptanes, 2,6,6-trime 3-buten-2-one, 4-(5,5-dimethyl-1-0
19	30.66	3.97	Cyclotetradecane 2,5—Furandione, 3-dodecyl-

			2-Dodecen-1-yl(-)succinic anhydrid
20	31.13	11.08	2-Tetradecanone
21	31.65	4.01	9-Oxabicyclo(6.1.0)nonane 13-Octadecenal, (Z)- 13-Tetradecenal
22	31.92	2.37	7,11-Hexadecadienal 1,4-Methanoazulen-7-ol, decahydro- 12-Methyl-E, E-2, 13-octadecadien-1-
23	32.61	20.64	E-2-Tetradecen-1-01 Pentadecanal- 1, 15-Pentadecanediol
24	33.17	1.40	Tetradecane, 1-chloro- Dodecane
25	33.55	4.19	Tetradecanal 1-Tetradecene Cyclotetradecane
26	34.06	1.19	2,6-Dimethyl-8-(tetrahydropyran-2- trans-2-Hexenyl valerate 2-(4-Chloro-1-methylbutoxy)tetrahy
27	34.86	1.62	Bicyclo (3.1.1)heptanes, 2,6,6-trime 4(1H)- Isobezofuranone, hexahydro- p-Menth-8(10)-en-9-01, cis-
28	35.35	0.45	Bicyclo (3.1.1) heptanes, 2,6,6-trime Bicyclo (3.1.0) hexane-6-methanol, 2 Cyclohexanol, 5-methyl-2-(1-methyl
29	35.75	1.05	2-Pentadecanone, 6,10,14-trimethyl 2-Heptanone, 4-methyl-

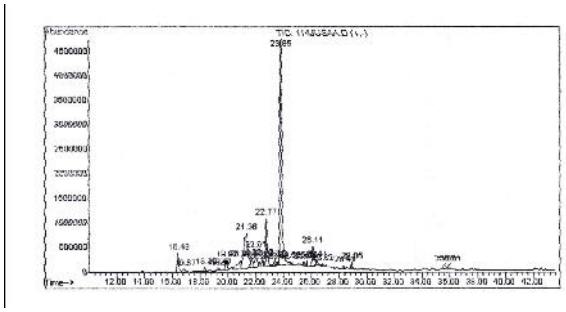

Fig 1: Chromatogram for ethanolic extract of *A. serpyllifolia* aerial part

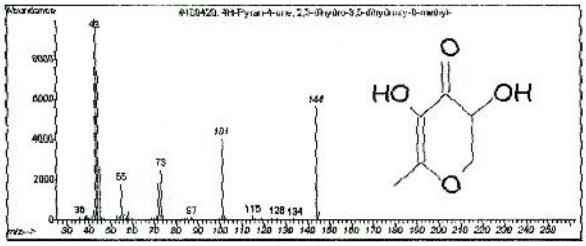

Fig 2: Mass spectrum for the ethanolic extract of *A. serpyllifolia* aerial part

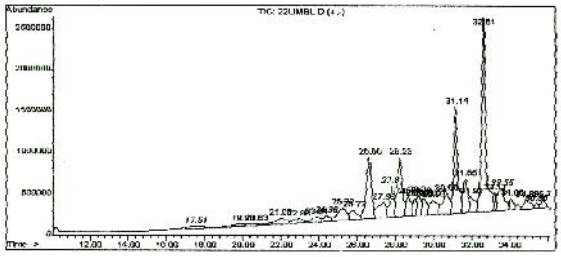

Fig 3: Chromatogram for ethanolic extract of *A. serpyllifolia* underground part

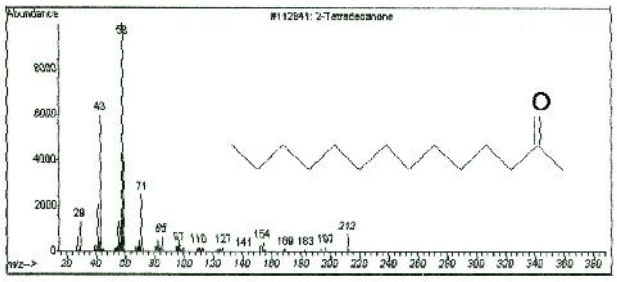

Fig 4: Mass spectrum for ethanolic extract of *A. serpyllifolia* underground part

By GC-MS analysis 29 compounds were known in ethanolic extract of *A. serphyllifolia* underground parts dried powder sample. The spectrum profile of GC-MS confirmed the presence of 29 bioactive components with the retention time. GS/MS chromatogram of *A. serpyllifolia* underground parts powder ethanolic extract give two prominent peak, one with 20.54 area and the retention time of 32.61 minutes indicating the presence of three compounds E-2-Tetradecen-1-01, Pentadecanal and 1, 15-Pentadecanediol. Another prominent peak with area of 11.08 and the retention time is 31.13 indicating the presence of 2-Tetradecanone (Table: 2; Figure: 3). 2 – Tetradecanone ($C_{14}H_{28}O$) (Fig. 4) and Pentadecanal ($C_{15}H_{32}O$) shows antimicrobial activity. -Pentadecanol, which is the active ingredient of the topical composition for treating acne Vulgaris (Yingngam and Brantner, 2015). Sebacic acid (Decanedioic acid) is a naturally occurring dicarboxylic acid with the structure (HOOC) $(CH_2)_8$(COOH). Sebacic acid is also used as an intermediate for aromatics, antiseptics, and painting materials (Patrick *et al.,* 2009) [8].

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