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GCMS PROFILE OF ETHANOLIC CRUDE EXTRACT OF *WITHANIA SOMNIFERA* (ASHWAGANDHA) DUNAL- WHOLE PLANT (LEAVES, ROOTS, AND STEM)

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ABSTRACT

Withania somnifera also known as Ashwagandha or Amukkara kilangu (in tamil) is an important medicinal herb used for more than 3000 years. Ashwagandha possesses some important medicinal properties such as– anti microbial, anti-stress, anti-cancer, anti-tumor, anti-oxidant, and anti-inflammatory properties, such properties are due to the presence of secondary metabolites. In the present investigation- whole plant parts (including leaves, roots, and stems) of *Withania somnifera* were used to analyze phytochemicals. The prepared extract was used to identify and verify with the help of GCMS. The present finding showed a total of 104 peaks, out of 104 peaks 45 peaks were found to indicate some important phytochemicals / phyto compounds are- 3,7-Dimethylocta-1,3,6-triene. 1,3,6-Heptatriene, 2,5,5-trimethyl-, Bicyclo[3.1.1] heptane, 6,6-dimethyl-2-, beta.-Myrcene, D-Limonene, t rans-2-Dodecen-1-ol, Decanal, Benzaldehyde, 2,4,5-trimethyl-, Benzene, 1-(1,5-dimethyl-4-hexenyl)-, Tridecanoic acid 4-, .beta.-Bisabolene, Carotol, Dodecanoic acid etc. The present investigation indicates that the crude ethanolic extract of *Withania somnifera* possesses some important phytochemicals that can be used to develop some novel plant-based anti-cancer drugs.

Keywords: *Withania somnifera*, GCMS, 3,7-Dimethylocta-1,3,6-triene, beta-Myrcene, Carotol

INTRODUCTION

Developing countries especially South Asian countries are dependent on medicinal plants for food, and folk medication. Plants synthesize secondary metabolites like- alkaloids, flavonoids, terpenoids, tannins, and other secondary metabolites. These secondary metabolites possess many health benefits includes- anti-cancer, anti-inflammatory, anti-oxidants, anti-stress, anti-viral, and anti-microbial activities. *Withania somnifera* also known as Ashwagandha has been an important medicinal plant of Ayurveda- an Indian traditional system for more than 3000 years. Ashwagandha belongs to the Solanaceae family **[1]** and grows up to 1.75 meters **[2]**. Ashwagandha is also an important medicinal plant in Siddha and Unani systems.

Roots and leaves have been used in various formulations but in the present investigation stem part is also used as it contains many bioactive compounds. *Withania somnifera* roots are used for the manufacturing of Ayurvedic tonic, astringent and rejuvenating drugs **[3-5]**. in Siddha medicinal system Ashwagandha roots are used to treat fever **[6]** and inflammation. According to Unani literature Ashwagandha root is effective against Arthritis, Asthama, Leucoderma and bronchitis, and emmenagogue **[7-8]** and according to Folklare medicinal system roots are effective against cold, fever, Asthama and Tuberculosis **[9]**. Ashwagandha Dry root powder contains some important properties such as – Anticancer, Radiosensitizer, Antiulcerogenic, Psyco-physiological, Antistress. Dry root of Ashwagandha can treat Pulmonary tuberculosis, Epilepsy Glandular swellings in bubonic plague, and can be used as a Nervine tonic, and General tonic in seminal disease. Hypoglycemic diuretic properties. Ayurveda leaves are effective against Ulcers, and painful swelling **[10]**. Siddha system leaves are effective against Fever, chest pain, sores, and swelling **[4]**.

MATERIAL AND METHODS-

Collection of plant samples- 20 varieties (including both wild and developed) of *Withania somnifera* were collected from different states – Gujarat, Madhya Pradesh, Karnataka, Andhra Pradesh, Uttar Pradesh and Tamil Nadu. Plant samples were identified and verified by Dr. Prof. P Jayaraman PhD. Director- Plant anatomy Research center, Retd. Professor Presidency College, Chennai-5, Reg. No. of certificate- PARC/2021/4565. Collected plant seeds were washed with distilled water and cultivated in 2 different agro-climatic conditions i.e. Madhya Pradesh (Jabalpur) and Tamil Nadu (Chennai). Crop cultivated in Chennai was used for the ethanolic extract preparation to check the phyto-molecule identification.

Extract Preparation- Plant samples (leaves, roots, and stem) were washed with distilled water Extraction method plays a crucial role in plant extract preparation and identification. Ethanol (95% v/v) was used as a solvent for the extract preparation by the Soxhlet apparatus. Obtained *Withania somnifera* plant extract was further concentrated by rotatory vacuum evaporator.

GCMS analysis- The obtained extract was further diluted in ethanol and filtered with the help of Whatman filter paper number 41 for the particle-free extract solution of *Withania somnifera* . The active molecules /compounds were identified by Gas chromatography coupled with Mass spectroscopy. A Shimadzu GC-2010 gas chromatograph unit furnished with a 2 mm direct inject liner along with a 15 meter Altech EC – 5 column (250µ I.D., 0.25µ film thickness). A split injection was program to 10:1 ratio for the sample introduction. The oven temperature was programmed to begin the process at 35°C, (for 2 minutes), then increase the temperature 20°C per minute to 450°C (for 5 minutes). Helium gas was used as a carrier gas and programmed to a constant flow rate of 2 ml/minute flow rate.

Mass Spectrum: A Direct connection with capillary column metal quadrupole mass filter period mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver. 2.6 was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 1000 at 0.3 seconds per scan with a 0.2-second inter-scan delay. High-resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan.

Mass spectrometry library search

Identification of the components of the crude extract matched with their recorded spectra available in data bank mass spectra of NIST library V 11 furnished by the instrument's software. GC/MS metabolomics Database was used for the similarity search with the retention index.

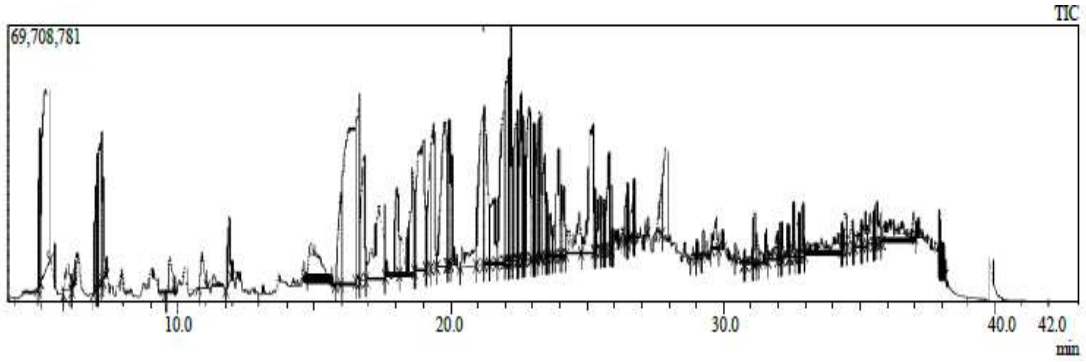

Figure 1: Chromatogram of Ethanolic extract of *Withania somnifera* whole plant (leaves, root, and leaves)

RESULTS AND DISCUSSION

In the present investigation 104 peaks were observed and identified with the help of NIST library V 11 provided by the instrument's software. Phytomolecules, retention time, area, area %, Height, and their properties were identified and compared with the known constituents present in the NIST library. Some important compounds identified with the NIST library are - 3, 7-Dimethylocta-1,3,6-triene. 1,3,6-Heptatriene, 2,5,5-trimethyl-, Bicyclo[3.1.1] heptane, 6,6-dimethyl-2-, beta.-Myrcene, D-Limonene, t rans-2-Dodecen-1-ol, Decanal, Benzaldehyde, 2,4,5-trimethyl-, Benzene, 1-(1,5-dimethyl-4-hexenyl)-, Tridecanoic acid 4-, .beta.-Bisabolene, Carotol, Dodecanoic acid etc. Rehman *et al* (2024) also reported 1,3, 6-heptatriene, 2,5,5-trimethyl- as an important compound found in *Origanum majorana* controls diarrhea and hyperactivity.

Table 1: Phytochemical composition of Ethanolic extract of *Withania somnifera* whole plant (includes- roots, leaves, and stem)

S. No.	Compound Name	R. Time	Area	Area %	Height%	Properties
1.	3,7-Dimethylocta-1,3,6-triene	4.952	153917907	1.03	1.95	Antioxidant property, Inhibits the growth of some bacteria including *Staphylococcus aureus*, enzyme modulator, affects metabolism of some compounds. [11]
2.	1,3,6-Heptatriene, 2,5,5-trimethyl-	5.150	631016024	4.22	2.30	Also found in microalgae *Scenedesmus falcatus* and *Chlorella sorokiniana* [12] Calcium channel inhibitory effect, controls diarrhea and Gut hyperactivity (Rehman *et al* 2024) [13]
3.	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-	5.961	80175943	0.54	0.36	Neuroprotective compound, anti-Parkinson's and anti-Alzheimer's activity [14]. Selective Prostaglandin D_2 Receptor [15]
4.	beta.-Myrcene	6.150	4320839	0.03	0.11	Antioxidant, anti-inflammatory, anti-aging, anti-aging, analgesic effects [16], Cardioprotective effect [17]
5.	o-Cymene	7.011	117005730	0.78	1.34	Found in Anise, basil, oregano, Sage, and thyme [18]
6.	Cyclobutene, bis(1-methyl ethylidene)-	7.130	293424175	1.96	1.73	
7.	1,3,8-p-Menthatriene	7.217	131417112	0.88	1.90	Found in the essential oil extracted from Aniseed *Pimpinella* anisum Linn. [19]
8.	D-Limonene	7.277	66183578	0.44	1.30	Effective against cancer,

						bronchitis, and obesity [20]
9.	.alpha.-Campholenal	9.720	86455616	0.58	0.48	[21]
10.	t rans-2-Dodecen-1-ol	10.890	68753142	0.46	0.44	Anti-bacterial, anthelminthic drug [22]
11.	Decanal	11.899	100890486	0.67	0.84	Antifungal [23]
12.	Benzaldehyde, 2,4,5-trimethyl-	14.911	228297895	1.53	0.42	Used as an intermediate compound for pharmaceutical drugs [23]
13.	Benzaldehyde, 2,4,5-trimethyl-	15.990	166513856	1.11	1.15	-
14.	Benzaldehyde, 2,4,5-trimethyl-	16.170	1167020097	7.80	1.76	-
15.	10-Chloro-1-decanol, pentafluoropropio	16.669	327334191	2.19	2.38	-
16.	1H-Cycloprop[e]azulene, 1a,2,3,5,6,7,7	16.843	299892566	2.00	1.57	-
17.	Dodecanal	17.411	406506619	2.72	0.92	Occurs in citrus oil, used in soap and fragrances [25]
18.	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-	18.041	200207064	1.34	1.09	[26]
19.	(E)-.beta.-Famesene	18.618	353192383	2.36	1.32	alarm pheromone and a metabolite [27]
20.	2-Dodecenal	19.013	686176421	4.58	1.65	Antibacterial agent [28]
21.	Benzene, 1-methyl-4-(1,2,2-trimethylcy	19.275	215398326	1.44	1.42	
22.	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-	19.423	307726554	2.06	1.82	Antimicrobial and anti-inflammatory effects [29]
23.	9-Bromo-10-[2-pyrimidylamino]-deoxo	19.823	502929546	3.36	1.83	-
24.	Tridecanoic acid	19.925	101822872	0.68	1.61	Energy source, Energy storage, membrane stabilizer [29]
25.	.beta.-Bisabolene	19.981	155350635	1.04	1.84	Anti-cancer can be used to treat breast cancer [30]
26.	.alpha.-Ionone	20.089	106651228	0.71	1.39	-
27.	Naphthalene, 1,2,3,4-tetrahydro-1,6-dim	20.420	115259633	0.77	0.25	
28.	Isoshyobunone	21.130	476137672	3.18	1.78	-
29.	Isoshyobunone	21.263	336100425	2.25	2.00	-
30.	Benzoic acid, 2,4,5-trimethyl-	21.665	216467880	1.45	0.81	
31.	Carotol	21.935	351002614	2.34	1.86	Antifungal, herbicide, and insecticide [31]
32.	2(1H)-Naphthalenone, octahydro-4a,7,7	22.025	165065056	1.10	2.21	-
33.	2-Isopropenyl-5-methylhex-4-enal	22.050	290842989	1.94	2.28	-
34.	Acetic acid, 4-hydroxy-4-methyl-2-phen	22.160	151625791	1.01	2.54	
35.	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,	22.229	285006309	1.90	2.99	
36.	Dodecanoic acid	22.420	306319071	2.05	1.77	Plant and algal metabolite, anti-bacterial agent [32]
37.	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trim	22.481	75139063	0.50	1.90	

38.	**Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trim**	**22.625**	**273404179**	**1.83**	**2.14**	
39.	**Ionone**	**22.687**	**118784739**	**0.79**	**1.83**	**Useful in pharmaceutical and fragrance industries [33]**
40.	**(+)-3-Carene, 4-isopropenyl-**	**22.724**	**58732924**	**0.39**	**1.29**	
41.	**Daucol**	**22.928**	**370100137**	**2.47**	**1.92**	**The highest concentration is found in wild carrots and can be used as a biomarker [34]**
42.	**Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trim**	**23.056**	**264066353**	**1.76**	**1.73**	
43.	**3-(4-Cyanomethyl-1H-pyrrol-3-yl)-prop**	**23.175**	**119533788**	**0.80**	**1.37**	
44.	**trans-2-Dodecenoic acid**	**23.293**	**194228774**	**1.30**	**1.87**	**Intermediate metabolite synthesized in fatty acid synthesis. [35]**
45.	**2-Dodecenal**	**23.335**	**108413747**	**0.72**	**1.76**	**Plant metabolite, anti-anthelminthic drug, anti-bacterial molecule [36]**
46.	**1,5-Heptadien-4-one, 3,3,6-trimethyl-**	**23.489**	**178860694**	**1.19**	**1.31**	**-**
47.	**Cadala-1(10),3,8-triene**	**23.565**	**62547796**	**0.42**	**0.80**	**-**
48.	**Tridecanoic acid**	**23.714**	**86832371**	**0.58**	**0.55**	**Also reported in Leea guineensis, Inula grandis [37], Used as a standard for lipid analysis [38]**
49.	**2,6,10-Dodecatrien-1-ol, 3,7,11-trimeth**	**23.835**	**41467058**	**0.28**	**0.45**	**Biochemical pesticide, insect attractant [39]**
50.	**(E)-2-Isopropyl-5-methylphenyl 2-meth**	**24.003**	**197447747**	**1.32**	**1.36**	**-**
51.	**1,5-Heptadien-4-one, 3,3,6-trimethyl-**	**24.100**	**57915974**	**0.39**	**0.90**	**-**
52.	**Diepicedrene-1-oxide**	**24.168**	**91025316**	**0.61**	**0.86**	**Reported in *Cyperus rotundus* L [40]**
53.	**Isoaromadendrene epoxide**	**24.746**	**168166603**	**1.12**	**0.52**	**Reported in essential oil of Carduncellus Helenioides (Desf.) [41]**
54.	**10,11-Dimethyl-tricyclo[4.3.1.1(2,5)]un**	**25.246**	**428655834**	**2.86**	**1.62**	**-**
55.	**cubedol**	**25.328**	**49466920**	**0.33**	**0.79**	**-**
56.	**8-Isopropenyl-1,3,3,7-tetramethyl-bicyc**	**25.472**	**75072702**	**0.50**	**0.71**	**-**
57.	**Oxirane, 2,2-dimethyl-3-[3,7-dimethyl-**	**25.580**	**86594375**	**0.58**	**0.66**	**Anti-diarrhoeal activity [42]**
58.	**1,3-Dioxolan-2-one, 3-methyl-3-(4,8-di**	**25.683**	**39715121**	**0.27**	**0.65**	**-**
59.	**Shyobunone**	**25.834**	**153554386**	**1.03**	**1.25**	**Metabolite, Reported as an important compound in *Alpinia calcarata*, *Acorus calamus*, and *Daucus carota*. [43]**
60.	**2-Pentadecanone, 6,10,14-trimethyl-**	**25.906**	**44284147**	**0.30**	**0.81**	**antibacterial, anti-nociceptive, and anti-inflammation activity.**

						[44]
61.	9-Isopropyl-1-methyl-2-methylene-5-ox	26.160	47597266	0.32	0.15	-
62.	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	26.310	15747085	0.11	0.13	One of the main products of geraniol isomerization process [45]
63.	1a,2,5,5-Tetramethyl-cis-1a,4a,5,6,7,8-h	26.465	46021115	0.31	0.53	-
64.	(-)-Globulol	26.515	28674053	0.19	0.76	Anti-microbial activity is also found in fruits of *Eucalyptus globulus* Labill (Myrtaceae) fruits [46]
65.	2,15-Hexadecanedione	26.655	47933130	0.32	0.32	-
66.	2,5,5,6,8a-Pentamethyl-trans-4a,5,6,7,8,	26.770	33048451	0.22	0.75	-
67.	Perhydrocyclopropa[e]azulene-4,5,6-tri	27.182	10663254	0.07	0.19	-
68.	4,8,12-Tetradecatrienal, 5,9,13-trimethy	27.670	74281090	0.50	0.27	Inhibit sterol biosynthesis, and anti-fungal activity [47]
69.	Pentadecanoic acid	27.913	163034960	1.09	1.15	Supports metabolic heart health and also lowers the risk of cardiometabolic diseases. [48]
70.	Succinic acid, di(cyclohex-2-enylmethy	28.905	9550999	0.06	0.13	
71.	5,5-Diethylpentadecane	29.076	11343496	0.08	0.24	
72.	Phytol	29.303	23839588	0.16	0.33	Precursor molecule for the preparation of synthetic forms of vitamin E and vitamin K1. [49]
73.	cis-9-Hexadecenal	29.803	75946439	0.51	0.39	Growth factor, cell wall organization and Virulence of Aspergillus fumigatus [50]
74.	Octadecanoic acid	30.010	22855268	0.15	0.22	Useful in Cosmetics and candle making. [51]
75.	Z-2-Octadecen-1-ol	30.469	8530475	0.06	0.22	
76.	5-Phenoxymethyl-3-(p-tolyl)-2-oxazolid	30.818	10116605	0.07	0.20	
77.	Naphthalene, 1-(1-decylundecyl)decahy	31.100	32280850	0.22	0.20	
78.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	31.180	38084270	0.25	0.65	
79.	Formamide, N,N'-[1,4-phenylenebis(me	31.278	30435611	0.20	0.38	
80.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	31.614	47718341	0.32	0.42	
81.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	31.995	55161541	0.37	0.33	
82.	Ethanone, 1-(1,3-dimethyl-3-cyclohexe	32.106	35690080	0.24	0.46	
83.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	32.176	22801026	0.15	0.46	
84.	1,37-Octatriacontadiene	32.414	35972216	0.24	0.43	
85.	3-[1-(4-Methoxy-phenyl)-1H-tetrazol-5-	32.465	11490259	0.08	0.25	Selective antitubercular agents. [52]
86.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	32.632	51631951	0.34	0.69	

87.	Acetic acid, [4-(1-hydroxy-1-methylethy	32.770	36050152	0.24	0.40	
88.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	32.821	23303835	0.16	0.56	
89.	1-Ethyl-trans-2-butenyl 2,4,6-trimethylb	32.971	42079160	0.28	0.68	
90.	4-Acetyloxyimino-2-carene	34.220	156968331	1.05	0.21	
91.	(E, E, E)-3,7,11,15-Tetramethylhexadeca	34.390	18188066	0.12	0.34	Flavor and fragrance agent [53]
92.	Benzenamine, 2-(1-methylcyclopropyl)-	34.502	51126381	0.34	0.46	Helps in plant growth [54]
93.	N,2,4,6-Tetramethylbenzenamine	34.765	50913626	0.34	0.37	
94.	trans-Geranylgeraniol	34.815	15798801	0.11	0.30	Nutritional supplement in food. Raw material to synthesize vitamins A and E [55]
95.	Cyclopentane-3'-spiropentacyclo[9.1.0.	35.104	57946070	0.39	0.33	
96.	1,3,2-Benzoxazaborole, 2-ethyl-2,3-dih	35.290	59369074	0.40	0.42	Antifungal activity [56]
97.	1,3,2-Benzoxazaborole, 2-ethyl-2,3-dih	35.425	66555113	0.44	0.38	
98.	1,6,10,14,18,22-Tetracosahexaen-3-ol, 2	35.574	21260302	0.14	0.43	
99.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	35.646	37803316	0.25	0.52	
100.	9,19-Cyclolanost-24-en-3-ol, acetate, (3	35.748	40002697	0.27	0.39	larvicidal and anti-mosquito activity. [57]
101.	Longiverbenone	35.832	26942826	0.18	0.44	Anti-bacterial and cytotoxic activity. [58]
102.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	37.040	209683356	1.40	0.31	
103.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	37.185	17370567	0.12	0.22	
104.	Benzoic acid, 2,4,6-trimethyl-, 2,4,6-tri	37.990	50111731	0.33	0.55	

DISCUSSION

Previous investigations also support that *Withania somnifera* contains many active phytochemicals responsible for curing many diseases and boosting immunity. Naher *et al* (2012) also reported 1,3,8-p-Menthatriene as an active compound found in the essential oil extracted from the 2 varieties of Aniseed (Pimpinella anisum Linn. Ghavam (2022) *also reported*. alpha.-Campholenal as an important compound in the GCMS study of essential oil extracted from 2 major plant species- *Hymenocrater incanus* Bunge and *Dracocephalum kotschyi.* 1,3-Cyclohexadiene is an important compound in ginger. The highest concentration of Daucol found in wild carrots and also found in root vegetables can be used as a biomarker [(24)]. Tridecanoic acid is also found and reported in Leea guineensis, and Inula grandis. The present investigation suggests that these compounds are pharmaceutically important and can be used

for the formulation of some plant-based novel drugs.

Conflict of Interest- No

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