

COMPARATIVE GC-MS ANALYSIS OF LEAF AND ROOT EXTRACT OF MEDICINAL PLANT *WITHANIA SOMNIFERA*

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ABSTRACT

Medicinal plants are enormous significance in the present research, drug industries depend directly and indirectly in medicinal plants for the production of pharmaceutical compounds. *Withania somnifera* is important medicinal plant having sources for many chemicals used as pharmaceutical chemicals. Present study is based on identification of bioactive compounds from leaf and root of *Withania somnifera* in ethanol and methanol solvent system by using Gas chromatography and Mass spectroscopy (GC-MS). GC-MS analysis of methanolic and ethanolic leaf extract was done and found 35 and 49 phytochemical compounds respectively. For the root both solvent system was used and found 68 and 29 phytochemical compounds. The GC-MS

analysis revealed the presence of various compounds such as withanolide B, rosifoliol, dodecanoic acids, hexadecanoic acid Guanosine, squalene, phytol, retinol and sitosterol etc in the leaf and root of *W. somnifera*. Hence, the *Withania somnifera* may have anticancer, anti-microbial activity, antioxidant and anti-diabetic activity due to the presence of secondary metabolites.

KEYWORD: *Withania somnifera*, phytochemical, anti-diabetic.

INTRODUCTION

Ashwagandha is also known as Indian Ginseng, is the medicinal plant of Solanaceae family. It is one of the major medicinal plant botanical known as *Withania somnifera*.^[1] It is found in Madhya Pradesh, Rajasthan, parts of Punjab, Himachal Pradesh and Uttar Pradesh. Ashwagandha is found to be cultivated in India and North America. It is well informed in Madhya Pradesh including Manasa, Neemuch, Jawad tehsils of Mandsaur and Ratlam districts and also the adjoining villages of Rajasthan.^[2] Medicinal properties are found in leaves and roots of Ashwagandha. Withanolides are present in its roots which possess anti-tumour activity.^[3,4] It is basically a shrub whose roots have been used for thousands of years. Some of its species bear edible fruits while some fruits are used for medicinal purposes.^[5] For the treatment of various diseases like asthma, arthritis, hypertension, rheumatism, syphilis, dyspepsia, the roots of this plant are used.^[6]

Withanolides are the major active constituents of *W. somnifera*, which are isolated from its root and leaves. Withaferin A and Withanolide D are the two main Withanolides that contribute to most of the biological activity of *W. somnifera*.^[7] The total alkaloid content of Indian root varies from 0.13% to 0.31%. *W. somnifera* has been used as an anti-oxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, astringent and antibacterial agent.^[8] *Withania somnifera* Dunal (Solanaceae) is used as rejuvenator in Ayurvedic System of Medicine. It is widely utilized for therapeutic management of various clinical ailments including arthritis, rheumatism as well as to prevent disease in athletes, the elderly and during pregnancy.^[9]

Withania somnifera also known as Indian ginseng or ashwagandha is a well known medicinal plant utilized for treatment of several diseases and ailments since ages.^[1] Ashwagandha is found to be cultivated in India and North America. It is basically a shrub 60-200 cm high whose roots have been used for thousands of years some of its species bear edible fruits while some fruits are used for medicinal purposes.^[10] The plant is widely utilized in modern as well as traditional system of medicine owing to its several biological activities and pharmaceutical properties. Some of the crucial biological properties of *Withania somnifera* include anti cancer, anti-inflammatory, anti aging, anti oxidant. The immense medicinal potential of plant is attributed to various bioactive metabolites synthesized in the plant, among which withanolides are most important phytochemical compound produced by the plant. Roots

remain to be most commonly utilized part of plant for medicinal purposes however, other parts of plants such as leaves and seeds also having medicinal potential.

The objective of the present study were to analysis of plant extract in different solvent system to identified chemical compound and its biological activity by using Gas chromatography and mass spectrometry.

MATERIALS AND METHODS

Plant Material

Mature plants of *Withania somnifera* were procured from Botanical garden, Dehradun in the month of February and maintained in Department of Biotechnology Uttaranchal College of Applied and Life Sciences, Uttaranchal University Dehradun.

Extract Preparation

Leaf and root part was used for preparation of plant extract of *Withania somnifera*, were used for GC-MS analysis. 10 g of dry plant was macerated in ethanol and methanol: water (90:10), for 24 hour. The extract was filtered and then concentrated in a rotary evaporator for 15 min and dried in lyophilizer. Powder was weighed to calculate the yield and kept at – 20°C for further utilization. For GC-MS analysis powder was dissolved in ethanol and methanol solvent system.^[11]

GC-MS analysis of the sample

GC-MS analysis of plant extracts was performed using a regular Perkin Elmer Auto System XL GC-MS analyzer. For GC-MS detection, an electron ionization energy system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1.51 ml/min and an injection volume of 2µl was employed. Total GC running time was 22 min. Software adopted to handle mass spectra and chromatograms were Turbo Mass.

Identification of compounds was based on the molecular structure, molecular mass. Interpretation on mass spectrum GC-MS was conducted using the database of NIST (National Institute Standard and Technology) having more than 62,000 patterns and Wiley library. The name, molecular weight and structure of the components of the test material were ascertained by correlating with the library. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas.

RESULT AND DISCUSSION

GC-MS analysis of ethanolic and methanolic plant leaf extract

GC-MS analysis of ethanolic and methanolic plant extract obtained from plant *Withania somnifera*, revealed the presence of many phytochemical compounds in plant (**Figure 1 and Figure 2**). In ethanolic extract of leaf 49 phytochemical compounds were identified, Some of these are 2,6,10- Trimethyl, 14-Ethylene-14 pentadecne (10.37%), hexadecanoic acid (14.11%) cis, cis, cis,-7,10,13-Hexadecatrienal (10.69%) with retention time 21.803, 23.771, 26.177 respectively (**Table 1**). Among these hexadecanoic acid was most abundant. Compound such as 3-cyclopentylpropionic acid (0.53%), docosanoic acid, ethyl ester (0.36%), gamma tocopherol (0.26%) and phosphoric acid, diethyl octyl ester (0.21%) with retention time 27.857, 26.731, 37.576, 16.937 respectively were found to be present in comparatively lesser amount in ethanolic extract of plant.

Irrespective of the amount or concentration (high or low) in which these compounds were found to be present, almost all these compound have been reported to possess some pharmacological or biological activity. In the ethanolic leaf extract having 29 compounds that show many biological and pharmacological activities whereas in methanolic leaf extract having 17 compounds for the same activities (**Table 2 and Table 4**).

On the other hand methanolic extract of the plant was found 35 phytochemical compounds including 1,3,4,5-Tetrahydroxy-cyclohexanecarboxy (13.80%), 1- Tetradecamine (20.28%), hexadecanoic acid (9.35%) and guanosine (6.26%) by way of retention time 18.867, 19.803, 23.708 and 15.732 respectively as the major compounds. Compounds such as Farnese epoxide (0.48%), 9-Octadecanoic Acid (0.40%), Nootkaton-11, 12 epoxide (0.37%) and Isophthalic acid (0.45%) with retention time 18.042, 20.755, 32.333, 32.283 respectively were present in small amounts (**Table 3**). The compounds identified were found to belong to different classes such as steroids, acid, phytosterols, alkaloids, ketones, ester, etc.

Almost all the compound identified have been reported to exhibit antibacterial, antifungal, Antioxidant and Antiviral activities against several pathogenic bacteria, fungal and viral specie.^[12,12,14] Beside antioxidant activity hexadecanoic is also reported to possess hypocholesterolemic and antiandrogenic activity.^[13] Antioxidant property is one of the crucial properties possessed by plant, in the present study compounds such tetradecanoic acid, 2-hexadecen-1-ol,3,7,11,15-tetramethyl, 9,12,15-octadecatrien-1-ol and hexadecanoic acid identified to be present in both plant extract of *Withania somnifera* have been reported to

possess potential antioxidant activity.^[12,13,15,16] Identified compounds tetradecanoic acid, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, gamma tocopherol, stigmast -5-en-3-ol have been reported as Anticancer and are utilized in treatment of breast cancer and antitumor agent.^[12,17,18] Compound like 9-12-octadecadienoic acid have been reported to possess biosurfactant activity.^[18] Beside these, Isopropyl linoleate is utilized as skin care and n hexadecanoic acid is antiinflammatory in function.^[19,20] Hexadecanoic acid have possessed antiandrogenic and hypercholesterolemic activity.^[13]

Table 1: Identified compounds, Area and Retention time of Ethanolic leaf extract of *Withania somnifera*.

Peak	R.Time	Area	Area%	Name
1	15.689	2403235	1.88	Guanosine
2	16.937	272418	0.21	Phosphoric acid, diethyl octyl ester
3	17.545	1134599	0.89	Dodecanoic acid
4	19.874	526717	0.41	1-TetradecanamineN,N-dimethyl
5	20.759	1346610	1.05	Tetradecanoic acid
6	21.595	220516	0.17	Isopropyl myristate
7	21.696	581509	0.45	2-Hexadecene,3,7,11,15-tetramethyl-
8	21.803	13275226	10.37	2,6,10 Trimethyl, 14-Ethylene-14-pentadecne
9	21.883	901880	0.70	1-Decanol,2-octyl
10	22.163	2445616	1.91	2-Hexadecen-1-ol
11	22.438	4521616	3.53	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	23.392	1222181	0.95	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-
13	23.771	18062172	14.11	Hexadecanoic Acid
14	24.057	898952	0.70	Ethyl Ester
15	25.509	300073	0.23	2-methyltetracosane
16	25.665	9273272	7.24	Phytol isomer
17	26.177	13682608	10.69	cis,cis,cis-7,10,13-Hexadecatrienal
18	26.380	1897301	1.48	9,12,15-Octadecatrien-1-ol
19	26.731	466443	0.36	Docosanoic acid, ethyl ester
20	27.857	680494	0.53	3-Cyclopentylpropionic acid
21	28.310	1351093	1.06	2H-Pyran,2-(2-heptadecyloxy) tetrahydro-
22	30.085	1494380	1.17	2- dimethyl amino ethyl ester
23	30.595	1332471	1.04	1-Octadecene
24	30.937	356600	0.28	Methyl 2-O-acetyl-3,4-di-o-methyl-alpha-D-xylopyranosid
25	31.150	491817	0.38	1,2-Benzenedicarboxylic acid
26	31.630	627323	0.49	2,5-Cyclohexadiene-1,4-Dione
27	32.483	174541	0.14	Cyclododecanone-2-methylene
28	32.783	3484607	2.72	Isopropyl linoleate
29	33.021	2155678	1.68	2-methylhexacosane
30	33.473	583766	0.46	2-[12-(2-oxiranyl) dodecyl] oxirane
31	34.213	1196686	0.93	Squalene
32	34.417	1188174	0.93	Phytol
33	34.561	3486811	2.72	Neophytadiene

34	35.276	864010	0.67	Pentatriacontane
35	36.197	372201	0.29	3alpha,5alpha-cyclo-ergosta-7,9(11),22triene-6beta-ol
36	36.533	365456	0.29	Retinol
37	37.169	1145042	0.89	Trihydroxyholanic acid
38	37.576	333930	0.26	Tocopherol
39	37.710	447306	0.35	Celidoniol
40	38.297	1709251	1.34	Stigmast-5-en-3-ol
41	39.002	5885998	4.60	Vitamin E
42	39.657	317545	0.25	Ergosta-7,22-Dien-3-ol
43	39.891	320503	0.25	Pivalate
44	40.910	324812	0.25	Propylidenecholesterol
45	41.119	4928961	3.85	Ergost-5-en-3-ol, (3.beta.)-
46	41.719	2012321	1.57	Stigmasta-5, 22-Dien-3-ol
47	42.408	3814139	2.98	24-Propylidene-(3 beta)
48	43.320	5393968	4.21	Sitosterol
49	43.770	7738059	6.04	Fucosterol

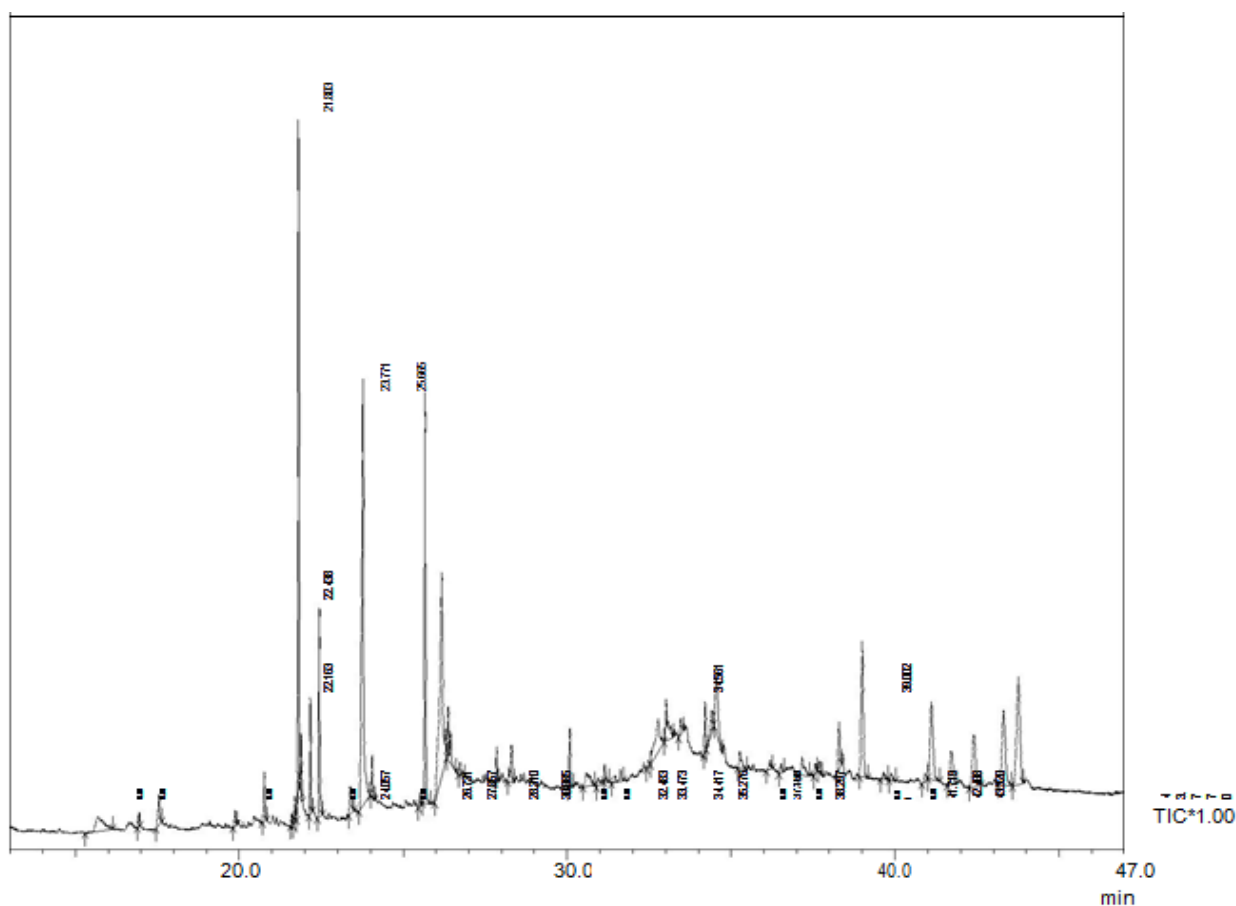


Figure 1: GC-MS Chromatogram of *Withania somnifera* plant leaf of Ethanol extract.

Table 2: Biological Activity of Identified Compounds of leaf ethanolic extract of *Withania somnifera*.

S.No	Compound	Biological Activity	References
1	Dodecanoic acid	Anti-fungal and anti-infective agent	[11]
2	Tetradecanoic acid	Antioxidant, Lubricant, Hypercholesterolemic, Cancer-preventive, Cosmetic	[12]
3	2,6,10Trimethyl,14-Ethylene-14-pentadecne	Antiproliferative	[21]
4	2-Hexadecen-1-ol	Antituberculosis, Insecticidal, Anti-Inflammatory, Antioxidant, Antimicrobial	[15]
5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic	[12]
7	9,12,15-Octadecatrien-1-ol	Analgesic, Ansthetic, Allergenic, Antibacterial, Anticonvulsant, Antiinflammatory, Antioxidant, Antipyretic, Anti-salmonella, Antiseptic, Antistaphylocolli	[16]
8	Hexadecanoic Acid	Antioxidant; Hypocholesterolemic Nematicide; Pesticide, Lubricant; Antiandrogenic Flavor; Hemolytic	[13]
9	Ethyl Ester	Antioxidant; Hypocholesterolemic Nematicide; Pesticide, Lubricant; Antiandrogenic Flavor; Hemolytic	[13]
10	2-methyltetracosane	Free radical scavenging activity	[22]
11	Phytoliseromer	Antituberculosis, Insecticidal, Anti-Inflammatory, Antioxidant, Antimicrobial	[15]
12	cis,cis,cis-7,10,13-Hexadecatrienal	Antioxidant activity	[23]
13	9,12,15-Octadecatrien-1-ol	Analgesic, Ansthetic, Allergenic, Antibacterial, Anticonvulsant, Antiinflammatory, Antioxidant, Antipyretic, Anti-salmonella, Antiseptic, Antistaphylocolli	[16]
14	2H-Pyran,2-(2-heptadecynyloxy) tetra hydroxy	Antimicrobial Anti-inflammatory Antioxidant	[24]
15	1-Octadecene	Antibacterial, antioxidant	[25]
16	1,2-Benzene dicarboxylic acid	No activity reported	[26]
17	Isopropyl linoleate	formulation of face and skin care products, hair care products, eye 4. Isopropyl linoleate and facial makeup	[20]
18	Squalene	Antimicrobial, Antioxidant, Anticancer, Neutralize different xenobiotics, Anti-Inflammatory, Anti-Atherosclerotic and Anti-Neoplastic, Role In Skin Aging And Pathology, and Adjuvant Activities	[15]
19	Phytol	Antimicrobial; Anti-inflammatory, Anticancer; Diuretic	[13]
20	Neophytadiene	Antimicrobial; Anti-inflammatory, Anticancer; Diuretic	[13]

21	Pentatriacontane	Antibacterial, Antiviral	[14]
22	Tocopherol	Anticancer, antioxidant, antitumor, anti-inflammatory, hypocholesterolemic, cardioprotective	[17]
23	Stigmast-5-en-3-ol	Antimicrobial activity	[27]
24	Vitamin E	Antiageing, Analgesic, Antidiabetic, Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary	[13]
25	Ergosta-7,22-Dien-3-ol	Antioxidant Antidiabetic Anticancer Cholesterol-lowering	[28]
26	Ergost-5-en-3-ol, (3.beta.)-	Antioxidant, hypocholesterolemic	[17]
27	Stigmasta-5, 22-Dien-3-ol	Antiviral, antioxidant, cancer preventive, hypocholesterolemic, antihepatotoxic	[29]
28	24-Propylidene-(3 beta)	Antibacterial, antioxidant	[30]
29	Sitosterol	No activity reported	[31]

Table 3: Identified compounds, Area and Retention time of Methanolic leaf extract of *Withania somnifera*.

Peak	R.Time	Area	Area%	Name
1	12.929	321829	1.41	2-Methoxy-4-vinylphenol
2	15.732	1430935	6.26	Guanosine
3	18.042	110255	0.48	Farnesene epoxide, E-
4	18.867	3153283	13.80	1,3,4,5- Tetra hydroxyl cyclo hexane carboxy
5	19.803	4632621	20.28	1-Tetra decan amine,N,N-Dimethyl
6	20.443	444808	1.95	Hexadecadienoic acid,
7	20.755	90869	0.40	9-Octadecenoic acid(Z)-
8	21.687	104372	0.46	(2E)-3,7,11,15-Tetra methyl-2- hexa decene
9	21.797	735183	3.22	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
10	21.883	198801	0.87	(2E)-3,7,11,15-Tetramethyl-2-hexadecene
11	22.167	150008	0.66	Cyclopropanenonanoic acid
12	22.436	283000	1.24	2-Hexadecane-1-ol
13	22.984	104235	0.46	3,Sec-Butyl-4-(2.2.3.3 Tetra Methyl cyclo propane
14	23.097	251750	1.10	Methyl Ester
15	23.186	263525	1.15	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydrox
16	23.708	2136564	9.35	Hexadecanoic acid
17	25.503	499168	2.18	8,11,14-Eicosatrienoic acid, methyl ester
18	25.657	977320	4.28	Phytol
19	26.104	406553	1.78	9,12-Octadecadienoic Acid(Z,Z)-
20	27.841	107697	0.47	2,6-Bis[2-(dimethylamino) ethoxy] pyridine
21	28.281	326051	1.43	Alloaromadendrene oxide-(1)
22	30.703	118134	0.52	Androsta-1,4,6-trien-3-one, 17-hydroxy-, (17.beta.)-
23	31.617	203989	0.89	2,5-Cyclohexadiene-1,4-Dione, 2-[(Decahydro)]}
24	32.333	84835	0.37	Nootkaton-11,12-epoxide
25	33.283	102327	0.45	Isophthalic acid, di(-)-menthyl ester

26	34.769	207755	0.91	3,5-Cyclo-33-Norgorgost-24(28)-Ene, 6-Methox
27	36.182	174048	0.76	17.alpha.-Hydroxypregnenolone
28	37.160	410695	1.80	Neoisolongifolene, 1,8-dibromo-
29	37.674	409893	1.79	Stigmast-5-en-3-ol
30	38.291	1941187	8.50	Fucos
31	38.636	438009	1.92	Cyclo pentane carboxamide, 3-ethenyl-2-(3-pentenylidene)-N
32	41.066	227475	1.00	3,7,7-Trimethyl-8-(2-Methyl-1-propenyl) bicyc
33	42.370	454890	1.99	3-Cyclo hexane-1- carboxy aldehyde,1,3,4-Trim
34	43.297	864118	3.78	Sitosterol
35	44.080	481459	2.11	Humulane-1,6-dien-3-ol

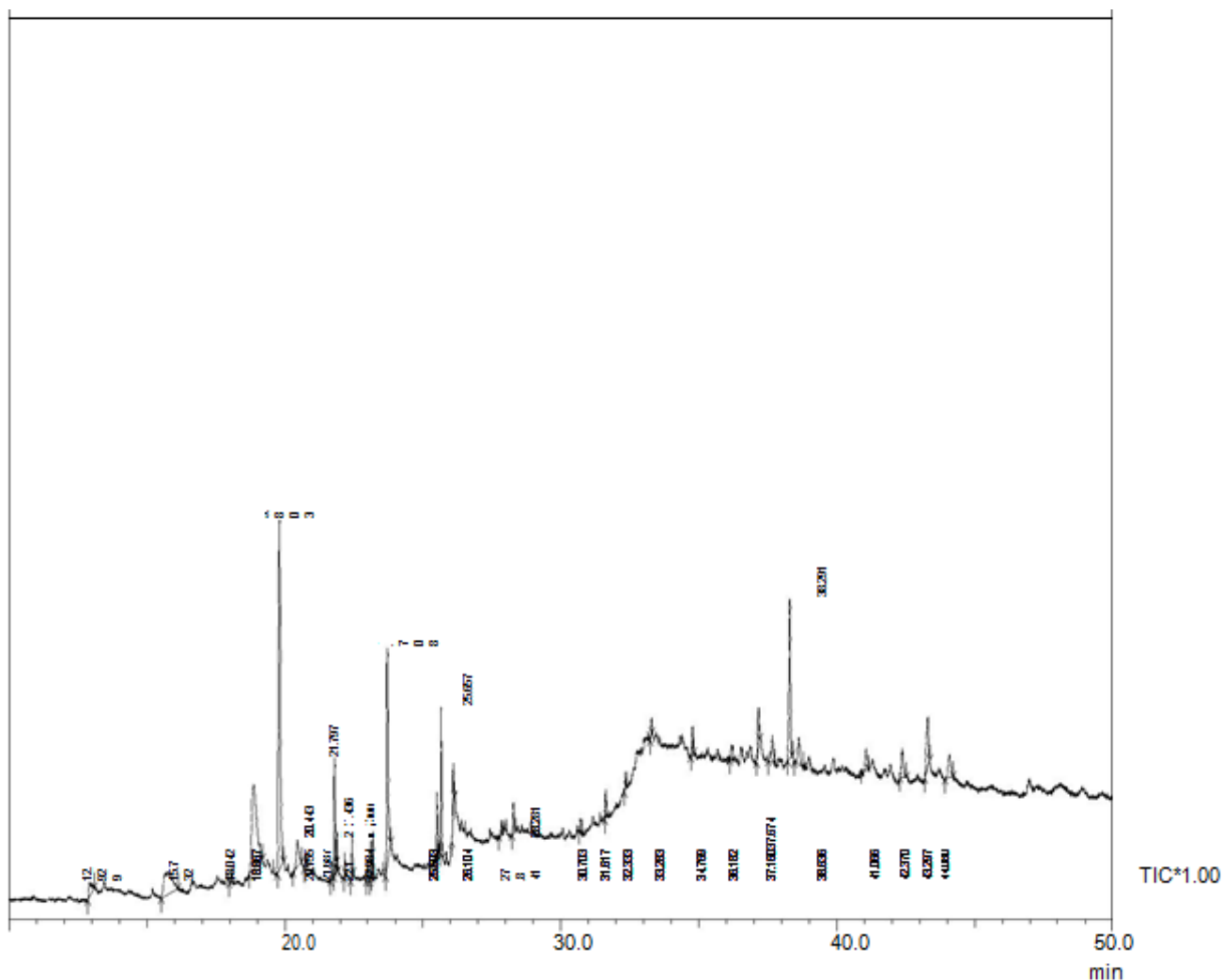


Figure 2: GC-MS Chromatogram of *Withania somnifera* plant leaf of Methanolic extract.

Table 4: Biological Activity of Identified Compounds of leaf Methanolic extract of *Withania somnifera*.

S.No.	Compound	Biological Activity	References
1	2-Methoxy-4-vinylphenol	Flavor and Perfumery	[32]
2	1,3,4,5-Tetrahydroxy-Cyclo hexane carboxy	Antimicrobial activity, anti-inflammatory	[33]
3	Hexadecadienoic acid	Antioxidant, hypocholesterolemic, Anti androgenic, hemolytic, Alpha reductase inhibitor	[12]
4	9-Octadecenoic acid	Anti inflammatory, Antiandrogenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic, Insectifuge	[32]
5	(2E)-3,7,11,15-Tetramethyl-2-hexadecene	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic	[12]
6	2,6,10-Trimethyl,14-Ethylene-14-pentadecne	Antiproliferative	[34]
7	(2E)-3,7,11,15-Tetramethyl-2-hexadecene	Cancer-Preventive Antimicrobial anti-inflammatory anti-diuretic	[12]
10	Hexadecanoic acid	Antioxidant, hypocholesterolemic, Anti androgenic	[12]
11	Benzene propanoic acid	flavoring, food additives, spices, fragrance, and medicines as it acts as a fixative agent,	[35]
13	8,11,14-Eicosatrienoic acid, methyl ester	anti-inflammatory, Insectifuge, Hypatoprotective, Antihistaminic, Antieczemic, Antiacen, Antiarthritic, Anticoronary	[23]
14	Phytol	Antimicrobial, Anticancer, Anti-Inflammatory, Anti-Diuretic, Immunostimulatory and Anti-Diabetic	[15]
15	9,12-Octadecadienoic acid	5- α reductase inhibitor, hypocholesterolemic, suppository, cosmetic, lubricant, surfactant & softening agent, perfumery, propepic, flavour.	[18]
16	Stigmast-5-en-3-ol	Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive, Hypocholesterolemic	[18]
17	Ethylcholest-5-en-3beta-ol	Antihepatotoxic, Antiviral, Antioxidant, Cancer preventive, Hypocholesterolemic	[18]

GC-MS analysis of plant root of ethanolic and methanolic extract

GC-MS chromatogram of methanolic extract of roots of *W. somnifera* revealed presence of 68 phytochemical compounds (**Figure 3**) as compared to 29 compounds found to be present

in ethanolic extract of roots of *W. somnifera* (Figure 4). Tropine and its derivatives; cedrane 8,13diol; cedrol; stigmasterol; N, Ndimethyl pentadecanamine and propoxy cedrane are some of the main compounds detected in methanolic extract of roots of *W. somnifera* (Table 5). However, major phytochemical compounds found to be present in methanolic extract of of *W. somnifera* were Cedrane; Benzidine; Hexadecanoic acid; octadecanoic acid and 3,4 dichloroatropine (Table 6). 3,4 Dichloroatropine; α - Selinine; Cedrane; Doconexent; Soalvetivone; 2,7 Ocatne dione 4,5 di iso propyl; Cadala 1(10),3,8 triene; Tetradecanoic acid; Vidirifloral; Ocatadecanoic acid and diethylene glycol dibenzoate were found to be present in both the methanolic extract and ethanolic extract of roots. Compounds namely Benzidiene; 4-chlorobutyric acid; Tridecanoic acid and Cycloartanol were found to be present in the ethanolic extracts of roots *W. somnifera*. 3-Tropanol; Naphthaline; Hydrazine; Iso vanillin; Decahydro quinoline; Cis Linaloxide; Methyl Steviol; Glycerol myristate; Benztropine; Grindelana; Fucosteral and Stigmasterol, were found to be present in only the methanolic extracts of *W. somnifera* only.

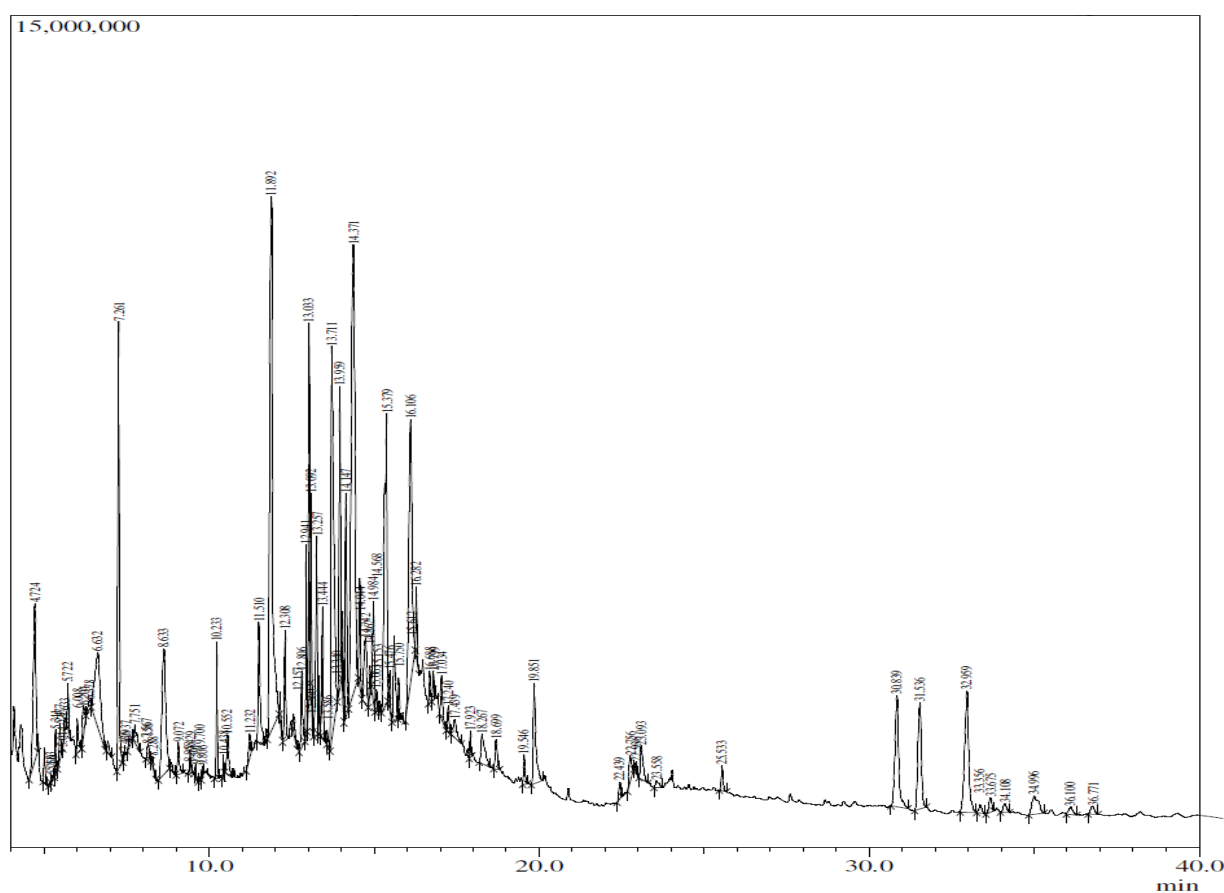


Figure 3: GC-MS chromatogram of Methanolic roots extract of *Withania somnifera*.

Table 5: Phytochemical compounds identified in methanolic extract of roots of *Withania somnifera*.

Peak	R. Time	Area %	Name	Molecular formula	Molecular weight
1	4.724	2.91	Withanolide B	C ₆ H ₈ O ₄	144
2	5.014	0.27	1,3-Cyclohexanedione, 4-Propyl-	C ₉ H ₁₄ O ₂	154
3	5.183	0.02	3,4-Anhydro-d-galactosan	C ₆ H ₈ O ₄	144
4	5.250	0.04	Naphthalene	C ₁₀ H ₈	128
5	5.341	0.34	Tropanone	C ₈ H ₁₃ NO	139
6	5.477	0.35	Tropine	C ₈ H ₁₅ NO	141
7	5.722	0.34	Pseudoecgoninol	C ₉ H ₁₇ NO ₂	171
8	6.008	0.92	3,Tropanol	C ₈ H ₁₅ NO	141
10	6.937	0.16	2-Methoxy-4-vinylphenol	C ₉ H ₁₀ O ₂	150
11	7.261	4.38	3,4-dichloroatropine	C ₁₇ H ₂₁ Cl ₂ NO ₃	357
12	7.426	0.05	decahydro quinoline	C ₉ H ₁₇ N	139
13	7.567	0.15	decanoic acid	C ₁₀ H ₂₀ O ₂	172
14	7.751	0.32	2,pentyl peridine	C ₁₀ H ₂₁ N	155
15	8.156	0.22	isovanillin	C ₈ H ₈ O ₃	152
16	8.288	0.11	benzene, (2-methyloctyl)-	C ₁₅ H ₂₄	204
17	8.633	3.37	dithio diglycol	C ₄ H ₁₀ O ₂ S ₂	154
18	8.858	0.09	3-hydroxybenzyl hydrazine	C ₇ H ₁₀ NO	138
19	9.072	0.40	9,12 octadecanoic acid	C ₁₈ H ₃₂ O ₂	280
20	9.429	0.15	phenol, 2,4-bis(1,1-dimethylethyl)-	C ₁₄ H ₂₂ O	206
21	9.591	0.10	benzoic acid, 4-ethoxy-, ethyl ester	C ₁₁ H ₁₄ O ₃	194
22	9.700	0.04	1-(2-aminophenyl)pyrrole	C ₁₀ H ₁₀ N ₂	158
23	9.806	0.08	.alpha.-selinene	C ₁₅ H ₂₄	204
24	10.233	1.15	3,4 dichloro tropine	C ₁₆ H ₂₉ NO ₂	267
25	10.552	0.56	2,cis- linaloloxide	C ₁₀ H ₁₈ O ₂	170
26	11.232	0.28	rosifoliol	C ₁₅ H ₂₄ O	220
27	11.892	10.97	cedrane 8,13diol	C ₁₅ H ₂₆ O ₂	238
28	12.157	0.24	ethanone,1-(4-hydroxy-3,5 dimethoxyphenyl	C ₁₁ H ₁₆ O ₃	196
29	12.308	1.17	tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228
30	12.806	0.80	Doconexent	C ₂₂ H ₃₂ O ₂	328
31	12.941	1.18	Solavetivone	C ₁₅ H ₂₂ O	218
32	13.033	2.78	2,7-Octanedione, 4,5-diisopropyl-	C ₁₄ H ₂₆ O ₂	226
33	13.092	1.11	Ledol	C ₁₅ H ₂₆ O	222
34	13.257	1.64	Cadala-1(10),3,8-triene	C ₁₅ H ₂₂	202
35	13.340	0.35	9 methoxy calamenene	C ₁₆ H ₂₄ O	232
36	13.444	1.08	Veridiflorol	C ₁₅ H ₂₆ O	222
37	13.711	6.95	1-Pentadecanamine, N,N-dimethyl-	C ₁₇ H ₃₇ N	255
38	13.959	2.47	5,6 dihydro stigmasterol acetate	C ₃₁ H ₅₂ O ₂	456
39	14.044	0.40	2,7-Octanedione, 4,5-diisopropyl-	C ₁₄ H ₂₆ O ₂	226
40	14.147	2.15	Murolan-3,9(11)-diene-10-peroxy	C ₁₅ H ₂₄ O ₂	236
41	14.371	11.05	8, Propoxycedrane	C ₁₈ H ₃₂ O	264
42	14.568	1.98	Methyl steviol	C ₂₁ H ₃₂ O ₃	332
43	14.862	0.24	Nerolidol isobutyrate	C ₁₉ H ₃₂ O ₂	292
44	14.984	1.06	Beta- sitosterol acetate	C ₃₁ H ₅₂ O ₂	456
45	15.379	5.37	Cedrol	C ₁₅ H ₃₆ O	222
46	15.612	1.06	Lonovar	C ₁₉ H ₃₀ O ₃	306
47	16.106	5.62	8 methyl-9 tetradecen-1 ol acetate	C ₁₇ H ₃₂ O ₂	268
48	16.282	0.55	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284
49	16.688	0.29	9,19-Cyclolanostan-3-ol, acetate	C ₃₂ H ₅₄ O ₂	470

50	17.034	0.50	Terpinyl formate	$C_{11}H_{18}O_2$	182
51	17.439	0.45	4-methyl androst-4-en 17-ol, 3-acetate	$C_{22}H_{32}O_3$	344
52	18.267	0.75	Malonic acid, 2-butyl undecyl ester	$C_{18}H_{34}O_4$	314
53	18.699	0.40	8,propoxycedrane	$C_{18}H_{32}O$	264
54	19.546	0.31	Diethylene glycol dibenzoate	$C_{18}H_{18}O_5$	314
55	19.851	1.80	Glycerol myristate	$C_{17}H_{34}O_4$	302
56	22.439	0.19	Benztropine	$C_{21}H_{25}NO$	307
57	22.756	0.45	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester	$C_{21}H_{38}O_4$	354
58	23.093	0.66	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$	358
59	23.558	0.15	Grindelane	$C_{20}H_{36}O$	292
60	25.533	0.31	terpinyl formate	$C_{11}H_{18}O_2$	182
61	30.839	2.79	Witheferin A	$C_{28}H_{48}O$	400
62	31.536	2.44	Stigmasterol	$C_{29}H_{48}O$	412
63	32.959	3.31	stigmast-5-en-3-ol, (3.beta.)-	$C_{29}H_{50}O$	414
64	33.356	0.16	Fucosterol	$C_{29}H_{48}O$	412
65	33.675	0.29	cedrenol	$C_{15}H_{24}O$	220
66	34.108	0.21	cholest-4-en-3-ol	$C_{27}H_{46}O$	386
67	34.996	0.78	pregna-4,17(20)-dien-3-one, 20,21-[(methylborylene)bis(oxy)]	$C_{22}H_{31}BO_3$	354
68	36.771	0.40	stigmast-4-en-3-one	$C_{29}H_{48}O$	412

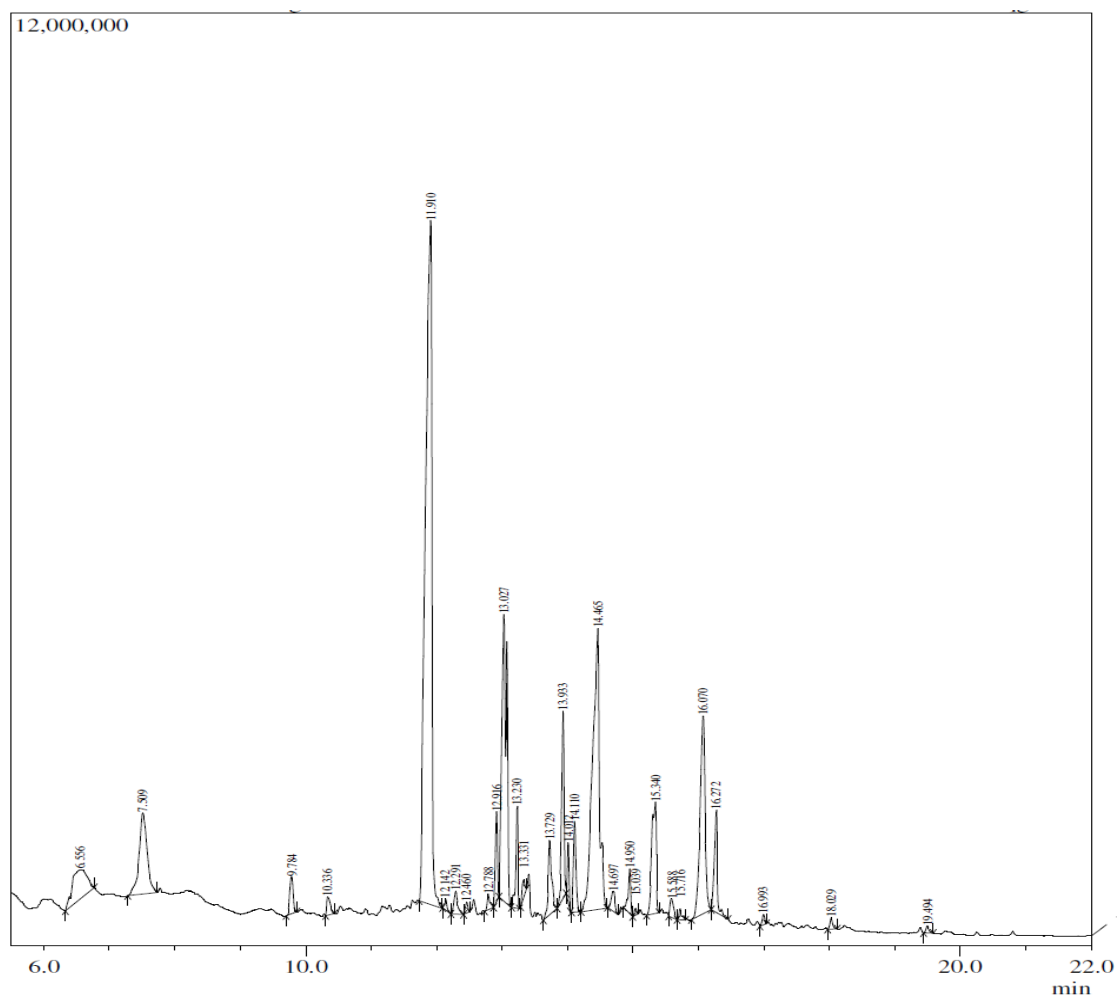


Figure 4: GC-MS chromatogram of ethanolic root extract of *Withania somnifera*.

Table 6: Phytochemical compounds identified in ethanolic extract of roots of *Withania somnifera*.

Peak	R. Time	Area %	Name	Molecular formula	Molecular weight
1	6.556	3.41	8-Methyl-8-Azabicyclo[3.2.1]OCTAN-3-OL	C ₁₅ H ₁₈ NO	141
2	7.509	5.15	3,4-Dichloroatropine	C ₁₇ H ₂₁ C ₁₂ NO ₃	357
3	9.784	0.95	Alpha .-selinene	C ₁₅ H ₂₄	204
4	10.336	0.49	3,4-Dichloroatropine	C ₁₇ H ₂₁ C ₁₂ NO ₃	357
5	11.910	33.82	Cedrane	C ₁₅ H ₂₅ I	332
6	12.142	0.21	Gibberellic acid	C ₁₀ H ₁₂ O ₄	196
7	12.291	0.64	Tridecanoic acid	C ₁₃ H ₂₆ O ₂	214
8	12.460	0.20	4-chlorobutyric acid	C ₂₂ H ₄₃ ClO ₂	374
9	12.788	0.36	Doconexent	C ₂₂ H ₃₂ O ₂	328
10	12.916	1.38	Solavetivone	C ₁₅ H ₂₂ O	218
11	13.027	11.01	2,7-Octanedione, 4,5-diisopropyl-	C ₁₄ H ₂₆ O ₂	226
12	13.230	1.76	Cadala-1(10),3,8-triene	C ₁₅ H ₂₂	202
13	13.331	0.45	Tetradecanoic acid	C ₁₄ H ₂₈ O ₂	228
14	13.729	2.02	Benzidine	C ₁₂ H ₁₂ N ₂	184
15	13.933	3.50	5,9-Dimethyl-2-(1-methylethylidene)-1-cyclodecanol	C ₁₅ H ₂₈ O	224
16	14.012	0.69	2,7-Octanedione, 4,5-diisopropyl-	C ₁₄ H ₂₆ O ₂	226
17	14.110	1.75	Viridifloral	C ₁₃ H ₂₀ O	192
18	14.465	15.27	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256
19	14.697	0.58	Eudesm 4(en) 11-ol	C ₁₅ H ₂₀ O	216
20	14.950	0.82	Cycloartanol	C ₃₀ H ₅₂ O	428
21	15.039	0.12	Ehyle isoalcolate	C ₂₆ H ₄₄ O ₅	328
22	15.340	3.89	8.Beta.H-cedran-8-ol	C ₁₅ H ₂₆ O	222
23	15.588	0.51	Lonavar	C ₂₁ H ₃₄ O ₂	318
24	15.716	0.24	Acetic acid 3(6,6-Dimethyl)	C ₁₆ H ₂₄ O ₂	248
25	16.070	7.76	9,12-octadecadienoic acid (Z,Z)-	C ₁₈ H ₃₂ O ₂	280
26	16.272	2.40	Octadecanoic acid	C ₁₈ H ₃₆ O ₂	284
27	16.993	0.19	Z-8-Methyl-9-tetradecenoic acid	C ₁₅ H ₂₈ O ₂	240
28	18.029	0.26	Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256
29	19.494	0.15	Diethylene glycol dibenzoate	C ₉ H ₁₁ NO	149

CONCLUSION

Results obtained provide an insight into the active components of plants belonging to Solanaceae family. Also identification of several phytochemical compounds found to be present in ethanolic and methanolic extract of leaf and root part of *withania somnifera* plant with several biological properties reveals immense medicinal potential of the plant.

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