

GC-MS Analysed Phyto-Chemicals and Antibacterial Activity of *Withania Somnifera* (L.) Dunal Extract in the Context of Treatment to Liver Cirrhosis

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Withania somnifera (L.) Dunal (*Ashwagandha* in Sanskrit) is one of the important medicinal plants in *Ayurveda* with a wide spectrum of actions and applications against diseases including gastric, hepatic, cardiovascular and immunological disorders. The objective of this paper was to review and investigate the phytochemical constituents with GC-MS analysis and to establish the antibacterial property of the plant extract against the bacterial pathogens, *Escherichia coli* and *Klebsiella pneumonia* (which are proven causal organisms for all forms of Urinary Tract Infections (UTI) and also liver infections) for which *Withania somnifera* plant has been significantly used as a multidrug constituent. The qualitative phytochemical study of different parts of the plant confirms presence of 10 bioactive compounds like Alkaloids, Phenols, Steroids, Terpenoids, etc. The Gas Chromatography-Mass Spectrometry (GC-MS) analysis of whole plant crude extract further validates the qualitative phytochemical data. 11 compounds, importantly Oleic Acid, Phytol and n-hexadecanoic acid at molecular level were identified from the extract in the GC-MS analysis. These compounds are known for different therapeutic and antimicrobial effects. The extracts of the plant are found effective (showing larger zones of inhibitions) against these two bacteria. The findings and results of this paper could help to evaluate and assess the therapeutic multipurpose use of *Withania somnifera* more rationally and can create an awareness of the need of in situ conservation of this most wanted medicinal plant.

Keywords: Antibacterial; Ayurvedic; Gas Chromatography-Mass Spectrometry Analysis; Phytochemical; Therapeutic.

Withania somnifera (L.) Dunal is one of the most important medicinal plants in *Ayurveda* based medicines¹ with a wide spectrum of actions and applications. The whole plant as well as specific parts (roots, stems, leaves) of plant extract and its active constituents have been used for the treatments of a larger number of human ailments. In India, *Ashwagandha* producing major states are Maharashtra, Haryana, Gujarat, Rajasthan, Punjab,

Madhya Pradesh, and Uttar Pradesh. India produces more than 1500 tonnes of *Withania* roots which is much less than the requirement. Thus, a need arises for the enhancement in its cultivation and larger production.²

Studies indicate that *Withania* has immune-modulatory, stem cell and more vital properties besides positively influencing the other metabolic systems, and also acts on anti-

serotogenic and arthritis.³ As a popular Ayurvedic rejuvenative plant, *Ashwagandha* is used in many medicines and formulations by Ayurvedic pharma companies that helps to maintain the vitality and functioning of the body systems.⁴ *Charaka Samhita* describes *Ashwagandha* in the treatment of liver diseases.⁵ *Withania somnifera* being a multidrug constituent, it needs to be extensively studied from antibiotic property and phytochemical correlation point of view. The plant extract contains many bioactive compounds. Alkaloids, withanolides and several sitoindosides have been reported to be present in the roots of the plant. It also contains withanolides (withaferin A and withanolide D).⁴ The root extract plays some role in decreasing serum AST, ALT towards normal levels in gentamicin intoxicated rats; due to its free radical scavenging activity showing its hepatoprotective effect.⁶ Also Withanolides have anti-inflammatory property.⁶ Phytochemicals have several biological properties such as antimicrobial effect, antioxidant activity, and anti cancer property, etc. It is therefore necessary to find the type of bioactive compounds at molecular level through phytochemical GC-MS analysis in the plant parts of *Withania somnifera* for understanding its potential in multidrug actions.

Many individuals today are affected with UTI and liver disorders for which general medical practitioners treat the patients with antibiotics, whereas Ayurvedic practitioners deal individuals with several herbal formulations. Pharmacological industries developing medicines against most dreadful disease pathogens are becoming unsuccessful due to multi-resistance of the pathogens to many drugs.⁷ The herbal drugs sold in the market for various diseases do not explain the content of medicines properly like, correct plant names, plant parts, quantity of bio-compounds or active parts, etc. So, there is a need to understand the proper working principle of the herbal drugs for their efficient outcome in light of their biological/therapeutic activities. The present paper has focused on these two aspects scientifically.

MATERIALS AND METHODS

Collection and Identification of Plant material

The plant, *Aswagandha* (*Withania somnifera* (L.) Dunal) was collected from Sunrise

Agro Services, Pune, Maharashtra, India during the month of August, 2016. The Plant's identification and authentication was confirmed by Botanical Survey of India, Pune with reference to Voucher No. BSI/WRC/IDEN.CER./2016/455/SP-2.

Extraction and Phytochemical Analysis

The powdered plant material of whole part was taken separately and successively in each of different solvents (Methanol, Ethanol, Water, Petroleum Ether, Benzene and Chloroform) and subjected to Soxhlet extraction procedure.⁸ Preliminary phytochemical analysis was carried out for each solvent extract according to the standard procedure.^{9,10}

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

GC-MS analysis of whole plant extract (methanolic) was done at the Sophisticated Analytical Instrument Facility (SAIF) labs, IIT Bombay using standard GCMS model as explained below. The procedure followed was of Dandekar *et al.*¹¹

Instrument details

Agilent 7890 instrument was used for GC, detector used was Flame Ionization Detector (FID) and the total run time of GC was 35 min.

Joel Accu Time of Flight Analyzer (TOF) GCV instrument for MS was used, Specification: Mass range of 10-2000 amu and resolution is of 6000.

GC-MS analysis was performed by split less injection (split 20:80-8-200-5M-8-260-10M-10-280-HP5-ETOH) of 1.0 μ l of the sample in methanol on a Hewlett Packard 6890 (USA) gas chromatograph built-in with a cross-linked 5% phenyl methyl Siloxane HP-5 MS capillary column (length 30 mm x internal diameter 0.32 mm x film 0.25 μ m), joined with a mass detector.

GC-MS operating conditions

The initial column temperature was 35 °C with a hold time of 3 minutes. The temperature was programmed to rise by 8°C/min with a final temperature of 280°C. In the process, 1 μ l of the sample was injected into the port and immediately vaporized and moved down the column with helium as the carrier gas with flow rate of 1 ml/min. The MS Spectrum was taken at 70 eV. After the separation in the column, the components were identified and further analyzed by FID. The identification of the compounds was done by

comparing the spectrum of unknown compounds with the spectrum of known compounds in NIST MS 2.0 structural library to find out the names, molecular weight and structure.

Collection of Micro-Organisms and Maintenance

The bacterial pathogens, *Escherichia coli* Migula (1895) Castellani and Chalmers (1919) (AL 1980) and *Klebsiella pneumoniae* (Schroeter 1886) Trevisan 1887 (Approved Lists 1980) were obtained from stock culture kept in the Department of Biotechnology, College of Computer science and Information Technology (COCSIT), Latur, Maharashtra, India. These identified samples were already collected from Department of Microbiology, Maharashtra Institute of Medical Sciences and Research (MIMSR) Latur, Maharashtra, India. The organisms were cultured on nutrient agar (bacteria) and stored at 4°C until use.

Inhibitory Activity of Withania Whole Plant Extract against Causal Bacteria

Antibacterial activity of whole plant methanolic extract of *Withania somnifera* against *Escherichia coli* and *Klebsiella pneumoniae* (the causal organisms of liver disease) was determined by agar disk diffusion method. Agar plates (Mueller Hinton Agar) are inoculated with a standardized inoculum of the test microorganism (E. coli and K. pneumoniae). Dried methanolic extract of *Withania* whole plant was dissolved in 20 % Dimethylsulfoxide (DMSO) to make saturated solution of plant extract. Then, Whatman filter paper No. 1 discs (about 6 mm in diameter) soaked

in the saturated extract-DMSO solution were placed on the agar surface. The Petridishes were incubated under suitable conditions. Antibacterial agent diffuses into the agar and inhibits the growth of the microorganism.¹² Accordingly, in this experiment, inhibitory growth zones were also measured. Standard antibiotic (Erythromycin) disc was used as positive control for comparison of the results with that of inhibition shown by the plant extracts. Only DMSO solution soaked disc without plant extract or antibiotic was used as negative control.

RESULTS

Preliminary Phytochemical Analysis

The outcomes of extracted contents of whole plant (in different solvents) tested for presence or absence of various phytochemicals (in qualitative form) are noted in Table 1. The results show that *Withania somnifera* plant contains a maximum ten types of phytochemical groups, such as – alkaloids, flavonoids, proteins, carbohydrates, steroids, glycosides, Phenols, saponins and terpenoids.

GC-MS Analysis

The Chromatogram of GC-MS spectra analysis showing peaks of the number of compounds from the GC fractions of the methanol extract of whole plant of *Withania somnifera* is represented in Fig 1. In this observation, presence of 12 different bioactive compounds namely, Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl),

Table 1. Phytochemical tests results, where (+) sign indicates presence and (-) sign indicates absence of corresponding phytocomponents.

Sr no.	Phytochemical	Whole Plant extract					
		M	E	W	PE	B	C
1	Alkaloids	+	+	+	+	+	+
2	Tannins	-	-	-	-	-	-
3	Flavonoids	+	+	+	+	+	-
4	Proteins	+	+	+	+	-	-
5	Carbohydrates	+	+	+	+	+	+
6	Steroids	+	+	+	-	+	+
7	Glycosides	+	+	+	+	+	+
8	Phenols	+	+	+	+	+	+
9	Saponins	+	+	+	-	-	-
10	Terpenoids	+	+	-	+	+	+

(M= Methanol, E= Ethanol, W= Water, PE= Petroleum Ether, B= Benzene, C= Chloroform)

O-Bromoatropine, 2-Methoxy-4-vinylphenol, Sucrose, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Hexadecanoic acid, methyl ester, 17-Octadecynoic Acid, n-Hexadecanoic acid, 9-Octadecenoic acid (Z)-, methyl ester, Phytol, 9-Methyl-Z-10-

tetradecen-1-ol acetate and Oleic Acid were identified as major compounds. Table 2 shows the reported therapeutic /biological activities of these identified bio-compounds.

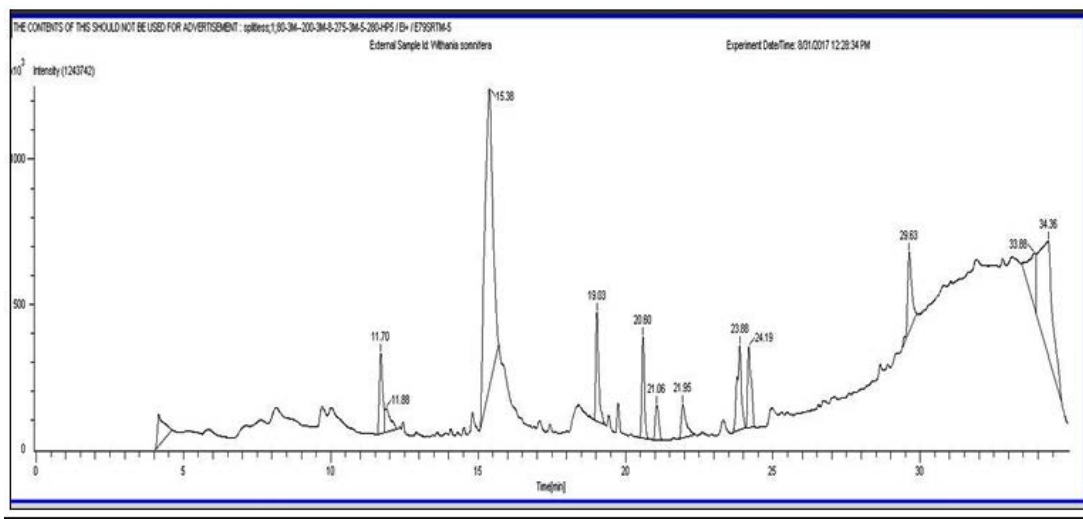


Fig. 1. Chromatogram of GC-MS Analysis

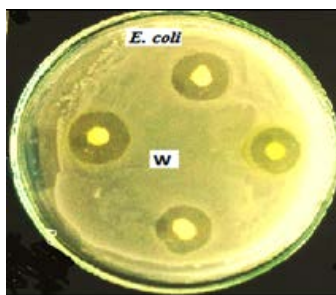


Fig 2 (a). Plant extract against *E. coli*

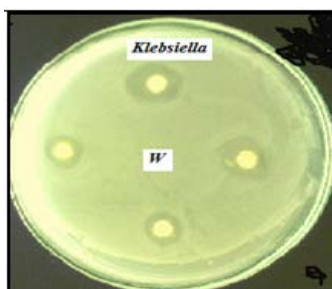


Fig 2 (b). Plant extract against *Klebsiella*

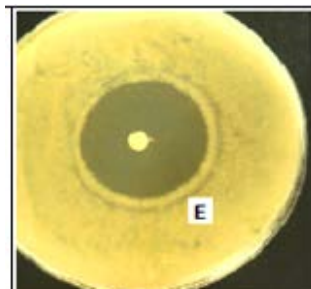


Fig 2 (c). Antibiotic (E) against *E. coli*



Fig 2(d). Antibiotic (E) against *Klebsiella*

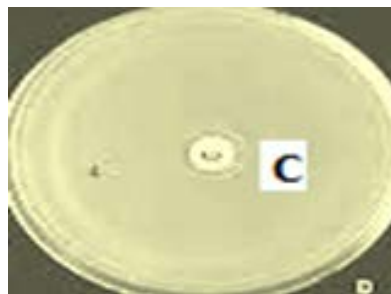


Fig 2 (e). Negative Control

*Note -E is Erythromycin and Negative Control C is with DMSO (Dimethyl sulfoxide)

Table 2. Compounds identified from *Withania somnifera* extract

Sr. No.	Peak Name	Retention Time (min)	Peak Area	Peak Width	Therapeutic/Biological Activity
1.	Name: Azetidin-2-one 3, 3-dimethyl-4-(1-aminoethyl) Formula: C ₇ H ₁₄ N ₂ O Molecular Weight: 142	4.16	16162524.44	0.4947	Anti-inflammatory ²⁰ Ulcerogenic ²⁰ Analgesic ²⁰
2.	Name: O-Bromoatropine Formula: C ₁₇ H ₂₂ BrNO ₃ Molecular Weight: 367	11.70	2197810.19	0.1268	No Activity Reported ²¹⁷
3.	Name: 2-Methoxy-4-vinylphenol Formula: C ₉ H ₁₀ O ₂ Molecular Weight: 150	11.88	1084132.93	0.2106	Antioxidant ²² Antimicrobial ²² Anti-inflammatory ²²
4.	Name: Sucrose Formula: C ₁₂ H ₂₂ O ₁₁ Molecular Weight: 342	15.38	19272455.50	0.3069	No Activity Reported ²³
5.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Synonym: Phytol Formula: C ₂₀ H ₄₀ O Molecular Weight: 296	19.03	2592272.21	0.0981	Antibacterial ^{21,24} Antifungal ^{21,24} Anticonvulsant ²¹ Antiarthritis ²¹ Insulin Sensitizing ²¹ Antidiabetic Effect ²¹ Anticancer ²⁴
6.	Name: Hexadecanoic acid, methyl ester Formula: C ₁₇ H ₃₄ O ₂ Molecular Weight: 270	20.60	2120062.11	0.0926	Anti-inflammatory ²¹ Vasodilator ²¹ Release of Insulin–Stimulation ²¹ Antidiabetic ²¹ Antibacterial ²¹ Antifungal ^{21,25}
7.	Name: 17-Octadecynoic Acid Formula: C ₁₈ H ₃₂ O ₂ Molecular Weight: 280	21.06	991489.30	0.1403	Alpha-glucosidase inhibitors ²¹
8.	Name: n-Hexadecanoic acid Formula: C ₁₆ H ₃₄ O ₂ Molecular Weight: 256	21.95	1352933.94	0.1629	Anti-inflammatory ^{25,26} Antioxidant ²⁵ Hypocholesterolemic ^{25,26} Nematicide ²⁵ , Pesticide ²⁵ , Anti-androgenic flavor ²⁵ , Hemolytic ²⁵ , 5-Alpha reductase inhibitor ²⁵ Potent mosquito larvicide ²⁵ Anticancer ²⁶ Antipesticide ²⁶ Antimicrobial ²⁶
9.	Name: 9-Octadecenoic acid(Z)-, methyl ester Formula: C ₁₉ H ₃₆ O ₂ Molecular Weight: 296	23.88	2949323.74	0.1750	Alpha-glucosidase inhibitors ²¹

10.	Name: 9-Methyl-Z-10-tetradecen-1-ol acetate Formula: C ₁₇ H ₃₂ O ₂ Molecular Weight: 268	29.63	2729768.65	0.1545	Anti-inflammatory ²⁶ Anti-cancer ²⁶
11.	Name: Oleic Acid Formula: C ₁₈ H ₃₄ O ₂ Molecular Weight: 282	33.88	2996434.50	0.4929	Hepatoprotective ²⁶ Antibacterial ²⁵ Apoptotic Activity ²⁷

Table 3. Inhibitory Activity of whole plant extract of *Withania somnifera*

Sr. No	Plant Part extract/ Antibiotic/Control	Zone Of Inhibition (mm) + SD	
		<i>E. coli</i>	<i>K. pneumoniae</i>
1.	<i>Withania somnifera</i> Whole Part	20.5 ± 2.40	10.0 ± 1.90
2.	Positive Control (Erythromycin)	26.25 ± 0.43	20.25 ± 0.43
3.	Negative Control (only DMSO disc)	0.00	

(Values expressed as Mean ± Standard Deviation of 4 observations of each)

Antibacterial Activity

The results of the present study indicated (Table 3) that whole plant extract of *Withania somnifera* is active against growth of bacteria *Escherichia coli* and *Klebsiella pneumoniae* as per the size of the zone of inhibitions.

Note –E is Erythromycin and Negative Control C is with DMSO (Dimethyl sulfoxide)

DISCUSSION

Results of phytochemical analysis observed in this study are in confirmation with Ayurvedic Pharmacopoeia of India (API)13 for alkaloids. The study reveals and confirms that *Withania somnifera* has the phytochemicals like alkaloids, steroids, flavonoids, carbohydrates, saponins, etc. are present in its whole plant extract (Table 1). These results are similar to the findings of other researchers' work.^{14,15} Result of Uddin *et al.*¹⁴ shows presence of all these phytochemicals in all the plant part extracts of *Withania somnifera*. According to result of Visveswari *et al.*¹⁵, Flavonoids are absent in aqueous extract of the root of *Withania somnifera*. Analysis carried out by GC-MS of the methanolic extracts of whole plant (present study) revealed presence of 11 compounds at molecular level viz. Oleic Acid, Phytol, n-hexadecanoic Acid, 9-Octadecenoic acid(Z)-, methyl ester, Hexadecanoic acid, methyl

ester, 2-Methoxy-4-vinylphenol, Azetidin-2-one 3,3-dimethyl-4-(1-aminoethyl), 17-Octadecyenoic acid, O-Bromoatropine, and Sucrose (as in Fig 1 and Table 2). All these bioactive compounds are responsible for the antibacterial property and other therapeutic uses of the plant, e.g. alkaloids have pharmacological effects and are used as medications. Also some phytochemicals have antioxidant and anticancer activity.¹⁶

The potentiality of *Withania somnifera* (L.) Dunal for multidrug action was rationally understood from the present study. The experiments indicated that *Ashwagandha* is active against bacterial strains of *Escherichia coli* and *Klebsiella pneumoniae* showing zones of inhibitions (Fig.2). The inhibition zones displayed by the whole plant extracts were found to be greater against *E. coli* than *Klebsiella*. The results (Table 3) were in agreement with other researchers.^{3,7,17} According to these authors, the plant extracts show inhibition against *E. coli* and the isolates of *K. pneumoniae*, but no action against *Pseudomonas aeruginosa*. In the present study, *Ashwagandha* plant extract against *E. coli* shows inhibition zone of 20.5 ± 2.40 mm, whereas according to a report¹⁸, the zone of inhibition was 0.63 ± 0.03 mm only. But results of Kaur *et al.*,¹⁹ show zone of inhibition as similar to present finding against *Klebsiella pneumoniae*, whereby zone of inhibition is 8.84 ± 0.16 mm, and is equally high.

CONCLUSION

The present study reveals that the plant extract of *Withania somnifera* (L.) Dunal is effective against growth of both the bacterial pathogens, *Escherichia coli* and *Klebsiella pneumoniae*, which are causal organisms for liver infection too. The chemical compounds responsible for making *Withania* plant as antibiotic and hepatoprotectant in nature are confirmed through GC-MS findings. The GC-MS identified phytochemicals are found reasonably responsible for the multi-therapeutic uses and effects of *Withania somnifera* in various health disorders including liver cirrhosis. The findings and results of this paper could help to evaluate and assess the therapeutic multipurpose use of *Withania somnifera* (L.) Dunal more rationally and further opened the scope for development of novel phytochemo-therapeutic drugs from the plant, which may serve as improved therapeutic agents and can create an awareness of the need of in situ conservation of this most wanted medicinal plant.

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