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.
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, Ashwagandha (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/IDENT.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 20:80-8-200-60-10-280-5E5-ETO) of 1.0 µl 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 µm), 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, 1µl 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 pneumonia* (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 pneumonia* (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),

| 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.

*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) | 4.16 | 16162524.44 | 0.4947 | Anti-inflammatory\(^{20}\) Ulcerogenic\(^{20}\) Analgesic\(^{20}\) |
|         | Formula: C\(_7\)H\(_{14}\)N\(_2\)O | Molecular Weight: 142 |          |            |                                  |
| 2.      | Name: O-Bromoatropine | 11.70 | 2197810.19 | 0.1268 | No Activity Reported\(^{217}\) |
|         | Formula: C\(_{17}\)H\(_{22}\)BrNO\(_3\) | Molecular Weight: 367 |          |            |                                  |
| 3.      | Name: 2-Methoxy-4-vinylphenol | 11.88 | 1084132.93 | 0.2106 | Antioxidant\(^{22}\) Antimicrobial\(^{22}\) Anti-inflammatory\(^{22}\) |
|         | Formula: C\(_9\)H\(_{10}\)O\(_2\) | Molecular Weight: 150 |          |            |                                  |
| 4.      | Name: Sucrose | 15.38 | 19272455.50 | 0.3069 | No Activity Reported\(^{23}\) |
|         | Formula: C\(_{12}\)H\(_{22}\)O\(_11\) | Molecular Weight: 342 |          |            |                                  |
| 5.      | Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol | 19.03 | 2592272.21 | 0.0981 | Antibacterial\(^{21,24}\) Antifungal\(^{21,24}\) Anticonvulsant\(^{21}\) Antiarthritis\(^{21}\) Insulin Sensitizing\(^{21}\) Antidiabetic Effect\(^{21}\) Anticancer\(^{24}\) Anti-inflammatory\(^{21}\) Vasodilator\(^{21}\) Release of Insulin–Stimulation\(^{21}\) Antidiabetic\(^{21}\) Antifungal\(^{21,25}\) |
|         | Synonym: Phytol | | | | |
|         | Formula: C\(_{30}\)H\(_{40}\)O | Molecular Weight: 296 | | | |
| 6.      | Name: Hexadecanoic acid, methyl ester | 20.60 | 2120062.11 | 0.0926 | Alpha-glucosidase inhibitors\(^{21}\) |
|         | Formula: C\(_{18}\)H\(_{32}\)O\(_2\) | Molecular Weight: 270 | | | |
| 7.      | Name: 17-Octadecyanoic Acid | 21.06 | 991489.30 | 0.1403 | Alpha-glucosidase inhibitors\(^{21}\) |
|         | Formula: C\(_{18}\)H\(_{32}\)O | Molecular Weight: 280 | | | |
| 8.      | Name: n-Hexadecanoic acid | 21.95 | 1352933.94 | 0.1629 | Anti-inflammatory\(^{25,26}\) Antioxidant\(^{25}\) Hypcholesterolemic\(^{25,26}\) Nematicide\(^{25}\) Pesticide\(^{26}\) Anti-androgenic flavor\(^{25}\) Hemolytic\(^{25}\) 5-Alpha reductase inhibitor\(^{25}\) Potent mosquito larvicide\(^{25}\) Anticancer\(^{26}\) Antipesticide\(^{26}\) Antimicrobial\(^{26}\) |
|         | Formula: C\(_{16}\)H\(_{34}\)O\(_2\) | Molecular Weight: 256 | | | |
| 9.      | Name: 9-Octadecenoic acid(Z)-methyl ester | 23.88 | 2949323.74 | 0.1750 | Alpha-glucosidase inhibitors\(^{21}\) |
|         | Formula: C\(_{18}\)H\(_{36}\)O\(_2\) | Molecular Weight: 296 | | | |
Table 3. Inhibitory Activity of whole plant extract of *Withania somnifera*

| Sr. No | Plant Part extract/ Antibiotic/Control | Zone Of Inhibition (mm) + SD | Zone Of Inhibition (mm) + SD |
|--------|---------------------------------------|-------------------------------|-------------------------------|
|        |                                       | *E. coli*                     | *K. pneumoniae*               |
| 1.     | *Withania somnifera* 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) 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. Result of Uddin *et al.* shows presence of all these phytochemicals in all the plant part extracts of *Withania somnifera*. According to result of Visveswari *et al.*, 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-Octadecynoic 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.

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. 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 report, the zone of inhibition was 0.63 ± 0.03 mm only. But results of Kaur *et al.*, 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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