

### **Endophytic fungi and Phytochemical profile of *Withania somnifera***

#### **Abstract**

*Withania somnifera* (Ashwagandha) is used in medicine from the time of Ayurveda. The roots of the plant are used traditionally in the treatment of nervous and some other disorders. Several effects like immunomodulation, hypolipidemic, antibacterial, cardiovascular protection, tolerance and dependence have also been studied. Many Studies show that ashwagandha comprises antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinsonian, antivenom, anti-inflammatory, antitumor properties. This article is presented to gather all the updated information on its phytochemical profile and its endophytic fungal communities. The review result indicates the herb of *Withania somnifera* (Ashwagandha) should be studied more extensively to confirm the earlier studied results and reveal other capable therapeutic effects.

**Keywords:** *Withania somnifera*, Endophytic fungi, Phytochemical profile.

#### **Introduction:**

*Withania somnifera* (WS) named as ashwagandha/Indian ginseng/winter cherry, is a herb in the Ayurvedic and home-bred medical systems for over 3000 years. This plant's roots are classified as rasayanas, which are claimed to increase health and longevity and retard the process of ageing. Historically, the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, antiinflammatory agent, astringent and more recently to treat ulcers, bacterial infection, venom toxins and senile dementia by traditional healers for years together. Clinical trials and animal research support the use of WS for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease<sup>[1]</sup>. WS chemo preventive properties make it a potentially useful candidate for patients undergoing radiation and chemotherapy<sup>[1]</sup>. Recently WS is also used to reduce the development of tolerance and dependence on chronic use of various psychotropic drugs<sup>[1]</sup>.

*Withania somnifera* (Solanaceae) which produces withanine and withanolides used to cure various diseases was selected for exploring the endophytes associated with it. Various endophytic fungi were isolated from different parts of plant like root, stem and leaf.

Endophytic fungi are defined as microbes that intercepts plant tissues in their life cycle i.e. resides on plant parts like leaf, bark, root etc without causing any external harm to their host. Endophytic fungi are found in every plant on earth. Novelty of bioactive compounds such as antifungal, antibacterial, anticancer, anti-inflammatory, antiviral, antioxidant, nematicidal/insecticidal, immunosuppressant etc have been isolated from fungal endophytes.

#### **Procedure of isolation of species:**

As per standard procedure, leaves, roots and flowers samples were washed in running water to remove particles and air dried. At first, samples were dipped in 70% ethanol (v/v) for 1 min then second plunge in sodium hypochlorite (3.5 % v/v) for 3 min. The samples were rinsed thrice in sterile distilled water and dried on sterile blotters under laminar airflow to ensure complete drying. Leaf, root and flower samples were excised with the help of a sterile scalpel and the inner

tissues were carefully placed on water agar plates<sup>[2]</sup>. After 21 days of incubation, morphological study, the fungi are removed and transferred to on potato dextrose agar (PDA) and kept for incubation for one week and noted the observation of growth appearance, front and back view of the plate. Unknown endophytic fungi are identified by studying their cultural characteristics, spore formation and mycelium. Slides were prepared by tease mount method using lactophenol cotton blue reagent and observed<sup>[3]</sup>. Endophytic Fungi were grown on synthetic media under standardized culture condition. Identification of the isolates recovered was done on the basis of their morphological and cultural characteristics<sup>[4]</sup>.

### Identified endophytic fungi

15 species of endophytic fungi were isolated from leaves, roots and stem of *W. somnifera* (Table 1). Various bioactivities were found to present in different taxa. The largest endophytic fungi was *Cladosporium sp*<sup>[5]</sup>.

**Table 1:** Endophytic fungi isolated from Different parts of *W. somnifera*<sup>[5]</sup>:

Root	Stem	Leaves
<i>Pseudallescheria boydii</i>	<i>Mycelia sterilia</i>	<i>Acremonium sp.</i>
<i>Cladosporium sp</i>	<i>Cladosporium sp.</i>	<i>Paecilomyces sp.</i>
<i>Cladosporium sp.</i>	<i>Cladosporium sp</i>	<i>Mycelia sterilia</i>
<i>Ochroconis sp.</i>		<i>Cladosporium sp.</i>
<i>Acremonium sp.</i>		
<i>Aspergillus sp.</i>		
<i>Curvularia sp.</i>		
<i>Penicillium sp.</i>		

20 species belongs to 12 genera of fungi were isolated during another study [6]. It included 9 fungi from leaves, 20 from stems and 4 from roots (Table 2). Most prevalent endophyte was *Alternaria alternata* which is not organ-specific. It has been isolated from leaf and stem tissues. It was isolated five times from four different plants at different times.

**Table 2:** Class Ascomycetes and Deuteromycetes isolated from fungi [6].

Sl. No.	Fungi
	<b>Ascomycota</b>
1	<i>Chaetomium bostrycodes</i>
2	<i>Eurotium rubrum</i>
3	<i>Melanospora fusispora</i>
4	Unidentified
<b>Sl. No.</b>	<b>Deuteromycota</b>
1	<i>Aspergillus awamori</i>

2	<i>Aspergillus auricomus</i>
3	<i>Aspergillus flavus</i>
4	<i>Aspergillus niger</i>
5	<i>Aspergillus pulvinus</i>
6	<i>Aspergillus terreus</i>
7	<i>Aspergillus terreus</i> var. <i>aureus</i>
8	<i>Aspergillus terricola</i>
9	<i>Aspergillus thomii</i>
10	<i>Alternaria alternata</i>
11	<i>Cladosporium cladosporioides</i>
12	<i>Curvularia oryzae</i>
13	<i>Drechslera australiensis</i>
14	<i>Fusarium moniliforme</i>
15	<i>Fusarium semitectum</i>
16	<i>Myrothecium roridum</i>
17	<i>Penicillium corylophilum</i>
18	<i>Penicillium</i> sp.
19	<i>Phoma</i> sp.
20	Unidentified

### Chemical Composition:

Laboratory analysis has published over 35 chemical ingredient comprised in the roots of *Withania somnifera* <sup>[7]</sup>. The active chemical components are alkaloids (isopelletierine, anferine), steroidal lactones (withanolides, withaferins), saponins comprising an additional acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitonidoside XI and X). *Withania somnifera* is also rich in iron. Mainly based on the compounds of the plant's roots, known as withanolides, are considered for its remarkable medicinal properties. Withanolides are steroidal and stand a similarity, both in their action and exterior, to the active constituents of Asian ginseng (*Panax ginseng*) known as ginsenosides. Ashwagandha's withanolides have been successively studied in a difference of animal studies search their effect on frequent situation, as well as immune function and even cancer <sup>[8]</sup>. Chemical analysis of Ashwagandha interpret its main constituents to be alkaloids and steroidal lactones. The main constituent is various alkaloids, withanine and the other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropene, choline, cuscohygrine, isopelletierine, anaferine and anahydrine. Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root. The leaves have steroidal lactones, which are called withanolides. The withanolides have C28 steroidal nucleus with C9 side chain, with a six membered lactone ring. Twelve alkaloids, 35 withanolides, and numerous sitoindosides from *Withania somnifera* have been isolated and studied. Asitoindoside is a withanolide containing a glucose molecule at carbon 27. Much of Ashwaganda's pharmacological activity has been credited to two main withanolides, withaferin A and

withanolide D. Additional chemical study has shown the existence of the following: Anaferine (Alkaloid), Anahygrine (Alkaloid), Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine (Alkaloid), Iron, Pseudotropine (Alkaloid), Scopoletin, Somniferinine (Alkaloid), Somniferiene (Alkaloid), Tropanol (Alkaloid), Withanine (Alkaloid), Withananine (Alkaloid) and Withanolides A-Y (Steroidallactones) [9].

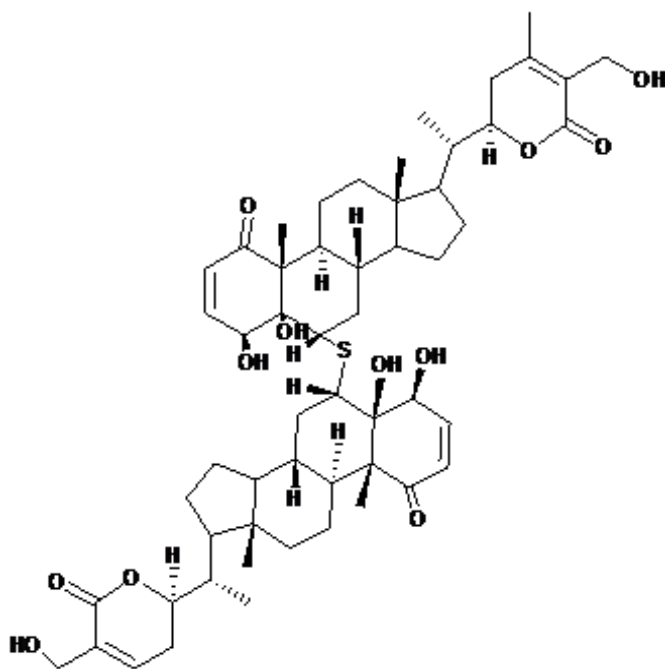
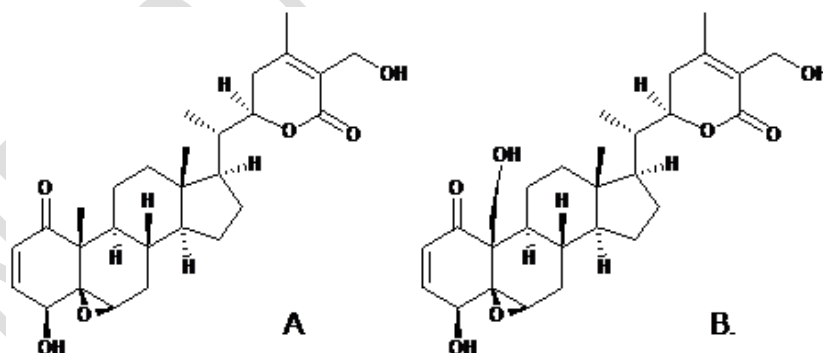


Figure 1: Ashwagandhanolide, a new compound isolated from *W. Somnifera*



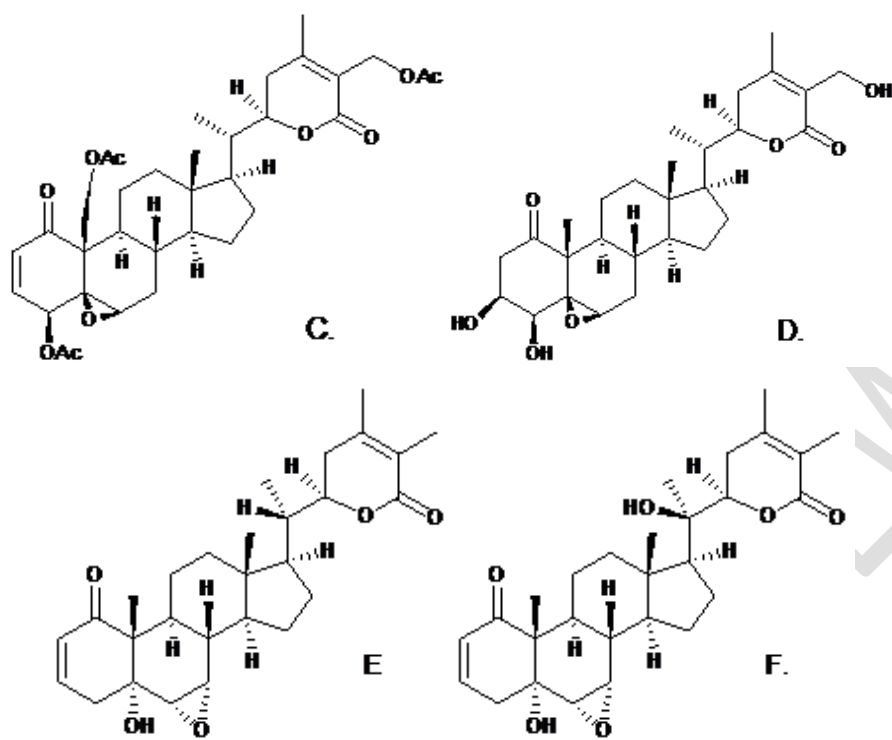


Figure 2: Structure of the Ashwagandha extract compounds from leaves, roots, stem and fruits (A) Withaferin A, (B) Withalongolide A, (C) Withaferin triacetate, (D) 2,3-Dihydro-3 $\beta$ -methoxy Withaferin A, (E) Withanone, (F) Withanolide A <sup>[10]</sup>.

#### Biological activity of Withaferin A (WFA):

WFA acts as an inhibitor of the chaperon p97 by its analogues can be a proteostasis modulator by retaining p97 activity and cytostatic activity in vitro <sup>[11]</sup>. Recently, Motiwala and co-authors have reported the synthesis and cytotoxicity of semisynthetic Withalongolide analogues where 24 compounds were tested on five cell lines (JMAR, MDA-MB-231, SKMEL-28, DRO81-1, and MRC-5) <sup>[12]</sup>. The other constituents of WFA have hepatoprotective, cardio-protective, immunosuppressive, anti-inflammatory, neuroprotective, anti-oxidative and anti-microbial activities. WFA treatment leads to apoptosis, evasion of anti-growth signaling and immune system by with sustained proliferative signaling and interactions with the tumor microenvironment <sup>[13]</sup>. The recent updates on anti-carcinogenic effects of WFA on different cancers such as breast, colon, prostate, lung, ovarian along with renal, head and neck, pancreatitis, liver and skin cancers are summarized with their mechanisms of action and plausible pathways (Table 3)

**Table 3:** Withaferin A (WFA), its role in cancer with the mechanism of actions <sup>[10]</sup>.

Types of cancer	Mechanism of action	References
Prostate cancer	Par-4-Dependent Apoptosis	[14]
Colorectal cancer cells ROS-dependent mitochondrial dysfunction-mediated apoptosis	ROS-dependent mitochondrial dysfunction-mediated apoptosis.	[15]
Leukemic cells of lymphoid and myeloid origin	Mitochondrial apoptosis by activating p38 MAPK cascade.	[16]
Myeloid leukemia HL-60 cells	Early ROS generation and mitochondrial dysfunction.	[17]
Glioblastoma multiforme (GBM)	Orthotopic mouse model showed GBM neurosphere collapsed at nM concentrations.	[18]
Breast cancer	FOXO3a (Forkhead box O3)- and Bim-dependent apoptosis.	[19]
Pancreatic cancer cells	Induction of proteasome inhibition and promotion the accumulation of ubiquitinated proteins, resulting in ER stress-mediated apoptosis.	[20]

**Conclusion:**

*Withania somnifera* (Ashwagandha) used in medicine from the time of Ayurveda. The plant also studied for their different pharmacological activities like antioxidant, anxiolytic, adaptogen, memory enhancing, antiparkinson, antiinflammatory, antitumor properties and other effects like immunomodulation, hypolipidemic, antibacterial, cardiovascular protection. The review indicates *Withania somnifera*'s use as a versatile medicinal agent. We are motivated to investigate the bioactive compounds from *Withania somnifera* and its associated endophytic fungi with an aim to find the fungal strains able to produce structurally novel and biologically active secondary metabolites that will contribute to the aim of establishing a new drug.

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