Ambient Science, 2017: Vol. 04(2); 01-06
DOI:10.21276/ambi.2017.04.2.rv01

Published by: National Cave Research and Protection Organization, India

ambient SCIENCE
Vol. 4(2):01-06
Year 2017

REVIEW ARTICLE

An Update on Phytochemicals Analysis and Medicinal Prospects of Indian Herb *Withania somnifera*

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Key words: Ashwagandha, Withaferin-A, Anti-cancer, Anti-oxidant Potential.

Abstract

Universally the Ashwagandha (Indian Ginseng) could be referred as an extensively researched medicinal plant. It also holds an important place in the ***Charaka Samhita***. It contains highly potent phytochemicals which are meticulously used as a tonic in ***Ayurveda*** for various ailments. Various aspects of this useful plant with reference to the phytochemicals, various biochemical activities, the extraction methods, and its antioxidant and antimicrobial activity have been tried to discuss in this paper. Information generated in this study shall be useful for undertaking research on genomic, transcriptomic, proteomic, metabolomics, and *in vitro* studies on *Withania somnifera*.

Introduction:

Ashwagandha (*Withania somnifera*) or 'Indian Ginseng' (Sharma *et al.*, 1992, Chopra *et al.*, 2000), is an important medicinal plant that has been used in Indian Ayurvedic system of medicine since a long time (Chopra *et al.*, 2000). The name Ashwagandha a Sanskrit word is a combination of the word ashva, meaning horse, and gandha, meaning smell. Along with its varied therapeutic potential, it has also been the subject of considerable scientific interest. It is used for various kinds of ailments like cancer, arthritis, anti-inflammatory agent, anxiety, senile dementia, astringent, trouble sleeping (insomnia), tumors, tuberculosis, asthma, a skin condition marked by white patchiness (leukoderma), bronchitis, backache, fibromyalgia, menstrual problems, hiccups, and chronic liver disease (Tirtha *et al.*, 2005, Kaushik *et al.*, 2017). It is also known to enhance mucus secretion in airway glands (Nagashyana *et al.*, 2000).

It grows well in dry and sub-tropical regions. *Withania somnifera* is a hardy and drought tolerant plant. The state Madhya Pradesh, Gujarat, Haryana, Maharashtra, Punjab, Rajasthan and Uttar Pradesh are the main production region of this crop in the Indian subcontinent. As a result of its wide growing range, considerable morphological and phenotypical variations in terms of local species is quite obvious (Sharma *et al.*, 1992). The traditional Indian system of medicine has a long history of use of plants in various forms (Lodha & Bagga, 2000). Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties (Carkeet *et al.*, 2012, Kennedy & Wightman, 2011).

Chemical Composition:

The roots and leaves of *W.somnifera*, have a number of compounds including, two newly discovered monohydric alcohols, withaniol and somnirol (Chaudhuri *et al.*, 2012). The physicochemical properties, as well as qualitative phytochemical analysis of this plant, confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, saponins, terpenoids and Quinone (Kumar *et al.*, 2013). Phenolics content of it is very important constituents as they can act as reducing agents, hydrogen donors and metal chelator (Rice-Evans *et al.*, 1995). Flavonoids show their antioxidant action through scavenging or chelating process (Kessler *et al.*, 2003).

The most important of such natural bioactive constituents of this plant are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952), alkaloids (isopelletierine, anaferine, cuseohygrine, anahygrine, etc., steroidal lactones (withanolides, withaferins) and saponins (Mishra *et al.*, 2000). Sitoindosides and acylsterylglucosides in *W.somnifera* are anti-stress agents. Active principles of *Withania somnifera*, for instance the sitoindosides VII–X and Withaferin-A, have been shown to have significant anti-stress activity against acute models of experimental stress (Bhattacharya *et al.*, 1987). Many of its constituents support immuno-modulatory actions (Ghosal *et al.*, 1989). The aerial parts of *W.somnifera* is known to find 5-dehydroxy withanolide-R and withasomniferin-A (Atta-ur- Rahman *et al.*, 1993).

Classical uses of *Withania somnifera*:

Ayurveda, the traditional system of medicine practiced in India can be traced back to 6000 BC (***Charak Samhita***,

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ISSN- 2348 5191 (Print) & 2348 8980 (Electronic)

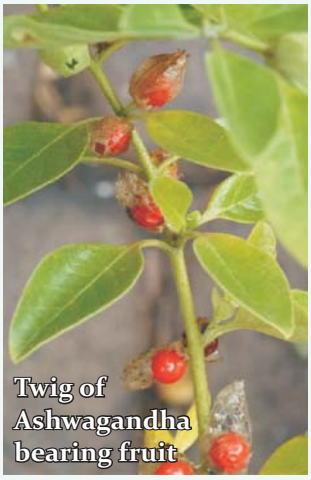
Ambient Science, 2017: Vol. 04(2); 01-06
DOI:10.21276/ambi.2017.04.2.rv01

1949). For most of these 6000 years, *Withania somnifera* has been used as the most potent ***Rasayana***. The root of *W.somnifera* is regarded as a tonic for an aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic and stimulant. As per a belief, on consuming its root, it gives the power of a horse, thus it refers as Ashwagandha. It is commonly used in emaciation of children (while given with milk, it works as the best tonic for children vigor), debility from old age, rheumatism, vitiated conditions of arthritis, leucoderma, constipation, insomnia, nervous breakdown, goiter etc. (Sharma, 1992). The paste formed when roots are crushed with water is applied to reduce the inflammation at the joints (Bhandari, 1970). It is also locally applied to cure carbuncles, ulcers and painful swellings (Kirtikar & Basu, 1918). The root in combination with other drugs is prescribed to overcome snake venom as well as in scorpion-sting (Misra, 2004). It also helps in leucorrhoea, boils, pimples, flatulent colic, worms and piles (Misra, 2004). The Nagori *W.somnifera* is the supreme among all other *W.somnifera* varieties. Maximum benefit appears when fresh *W.somnifera* powder is used (Singh, 2011).

The leaves are bitter and are recommended in fever, painful swellings. The flowers are astringent, depurative, diuretic and aphrodisiac. The seeds are anthelmintic and combined with astringent and rock salt remove white spots from the cornea. *Withania somnifera* is also used in hysteria, anxiety, memory loss, syncope, etc. and also as a stimulant and increases the sperm count (Sharma, 1938).

Scientific Studies on *Withania somnifera:*

Adaptogenic / Anti-stress effect of *W.somnifera* is compatative in activity to both Eleutherococcus senticosus (Siberian Ginseng) and Panax Ginseng (Chinese / Korean Ginseng) for its adaptogenic properties, and hence it is popularly known as Indian Ginseng (Kaushik *et al*. 2017; Singh *et al*., 2011). The extensive studies on the animals as biological model for the adaptogenic / anti-stress properties of *W.somnifera* (Abbas & Singh, 2006; Kalsi *et al*., 1987; Singh *et al.*, 2011) have shown it to be effective in increasing the stamina (physical endurance) and preventing stress-induced gastric ulcer, carbon tetrachloride (CCl_4) induced hepato-toxicity and mortality. *Withania somnifera* have similar anti-stress activity in rats (Archana & Namasivayam, 1999). It is used in the crude form and its aqueous extract is a potent anti-stress agent. But, the alcoholic extract was found to be ineffective (Kaushik *et al*., 2017). The above studies lend support to the hypothesis of tonics, vitalizers and rejuvenators of Ayurveda which indicate the clinical use of *W.somnifera* in the prevention and treatment of many stress-induced diseases like arteriosclerosis, premature aging, arthritis, diabetes, hypertension and malignancy (Singh, 1986, Singh & Misra, 1993).


Twig of Ashwagandha bearing fruit

Anti-tumor effect (Effect on Chinese Hamster Ovary (CHO) cells carcinoma): Roots of *Withania* caused the inhibitory effect of about 49% on colony forming efficiency of CHO cells. It inhibits the cell growth and prevents the cell attachment. It induced long-term growth inhibition of CHO cells which was dependent on the cell density and duration of *W.somnifera* exposure (Sumantran *et al*., 2007).

Anti-cancer Activity: *Withania somnifera* has anti-tumor effect on Chinese Hamster Ovary (CHO) cell carcinoma and urethane-induced lung-adenoma in mice (Yankee *et al.*, 2012). Antitumor and radiosensitizing effects of alcoholic root extract of *W.somnifera* and their modification by heat were studied *in vivo* on Sarcoma-180 grown on the dorsum of adult BALB/c mouse by Devi *et al.* (1992). Gamma radiations were used along with *W. somnifera* for the treatment. *Withania somnifera* was seen to increase the effect of radiation on tumour regression as well as growth delay, a better tumour cure, much higher than that produced by radiation alone. Devi (1996) further proved the efficacy of this plant extract to fight tumour cells. The studies so far indicate that *W. somnifera* could prove to be a good natural source of a potent and relatively safe radiosensitizer/ chemo-therapeutic agent (Devi *et al.*, 1995; Sharada *et al.*, 1996).

The antiproliferative effect of Witharin-A against the human breast cell line is also evident (Silvia *et al.*, 2008). The mechanism of action of Withaferin-A in combating cancer cells was evident by the study of (Challa *et al.*, 2012).

Lung-adenoma in mice and other studies: *Withania somnifera* was found to be very useful in experimental carcinogenesis in the crude form. It prevented urethane-induced lung-adenomas in mice along with leucopoenia. Besides this, it is found to reduce the side effects of chemotherapy, increases immunity and also as immunomodulator (Singh *et al.,* 2011) and immuno-modulator agent (Dixit *et al.*, 1995).

The research and studies of *W. somnifera*'s activities in the inhibition and reduction of tumour growth have shown encouraging evidence that this remarkable herb may prove to be extremely effective in the treatment of tumor type diseases including cancer (Singh & Gilca, 2010). It also improves the white cell count (WBC) and function, which are depleted in the chemotherapeutic treatment of cancer. *Withania somnifera* in the treatment of fibroid tumors of the uterus showed a reduction of

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DOI:10.21276/ambi.2017.04.2.rv01

uterine bleeding tendencies and disappearance of fibroids after long treatment (Abbas *et al.,* 2006).

Effect on Central Nervous System: Singh & Udupa (1993) have reported the effectiveness of *W. somnifera* in promoting memory and mental capacity. Dickson & Vickers (2001) reported that *W.somnifera* extracts help patients against cognitive neurodegenerative impairments and diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, and Creutzfeldt– Jakob disease, the atrophy of neuritis etc., regardless of the stage of the disease. According to Bhattacharya *et al.* (1995) Glycowithanolides withaferin- A and sitoindosides VII-X isolated from the roots of *W.somnifera* have significantly reversed ibotenic acid induced cognitive defects in Alzheimer's disease model. Singh *et al.* (1988) have referred *W.somnifera* as a nervine tonic (Singh *et al.*, 1988, 1993) and thus it is considered as a common ingredient of Ayurvedic tonic. Tonics, rejuvenators, and vitalizers of Ayurveda appear to allay disease and induce immunity (Singh *et al.,* 1986) and longevity in the users.

Cognitive dysfunction studies: in a recent study, *W.somnifera* dried leaf powder was found to alleviate cognitive dysfunction by enhancing hippocampal plasticity in high-fat diet induced obesity model (Manchanda & Kaur, 2017). The experimental animals during behavioural studies exhibited considerable improvement in their working memory and locomotors coordination.

Anxiolytic effect: *Withania somnifera* has been compared with the drug Lorazepam for anxiety tests by Abdel-Magied *et al.* (2001) where both were able to reduce the levels of tribulin in the brain which is a marker of clinical anxiety when the levels were increased following administration of the anxiogenic agent, pentylenetetrazole. The effect of *W.somnifera* on glycosamino-glycan synthesis in the granulation tissue of carrageenin-induced air pouch granuloma was studied by Begum & Sadique (1987). *Withania somnifera* is shown to exert significant inhibitory effect on incorporation of ribosome -35S into the granulation tissue. The uncoupling effect on oxidative was observed in the mitochondria of granulation tissue. Further, Mg2+ dependent ATPase and succinate dehydrogenase activity was found to be influenced by *W. somnifera*.

Anti-arthritic effect: *Withania somnifera* has been thoroughly researched for its property as an analgesic that soothes nervous system from pain response (Twajj *et al.*, 1989). The powerful anti-arthritic properties (Singh *et al.* 1986; 2011) of *W. somnifera* are now widely accepted and documented along with its antipyretic as well as analgesic properties too. The contribution of *W.somnifera* as one of the key ingredients and its toxicity to determine the safe dose in anti-arthritic herbal formulations is studied by Chaudhary *et al.* (2015).

Anti-oxidant Potential: the extract is known to exhibit total antioxidant activity and has potential efficient activities for scavenging ions like hydroxyl, superoxide, nitric oxide, singlet oxygen radicals, hypochlorous acid and inhibition of lipid peroxidation. The 70% methanolic extract of the same also has the possibility as an antioxidant and free radical scavenging agent. Various reactive oxygen species (ROS), which are generated during various biological redox reactions like superoxide radical (O_2-), hydroxyl radical (.OH), hydrogen peroxide (H_2O_2) and nitric oxide (NO.) (Aruoma *et al.,* 1989) which have the capacity of directly reacting with biological macromolecules such as proteins, lipids and DNA of healthy human cells and cause cell membrane disintegration, DNA mutation and protein damage. Deregulation of these reactive oxygen species (ROS) can lead to serious fatal conditions like cancer, atherosclerosis, cardiovascular disease, liver injury, aging and inflammatory disease (Braca *et al.*, 2002). Antioxidants are able to act as oxygen scavengers by interrupting the oxidation process by reacting with free radicals and chelating catalytic metals (Gulcin *et al.*, 2002). Some synthetic antioxidants have also been developed in the past few decades but they are suspected of having some adverse effects. Thus, in search of suitable alternative natural antioxidants has received much attention to identify and develop more potent antioxidants of natural origin to replace synthetic ones. Different kinds of plant material have already been reported as the natural antioxidant (Packer *et al.*, 1997).

Anti-ulcer activity: *Withania somnifera* extracts have shown activity similar to the synthetic drug Rantidine for the treatment of Gastric ulcers (Bhatnagar *et al.,* 2005). The *W.somnifera* extracts were given orally to experimental models along with another plant extract called *Asparagus rasamose* gave for 15 days. In experimental rats, it leads to significant reduction in the ulcer index, the volume of gastric secretion, free acidity, and total acidity. However, they found *A. racemosus* to be more effective in reducing gastric ulcer in indomethacin-treated gastric ulcerative rats, whereas *W.somnifera* was effective in the stress-induced gastric ulcer. Reproducible results were also generated by Raghuveer *et al.* (2013). *Withania somnifera* was found to be effectively protecting the stomach of experimental animals from peptic ulcers (Nair *et al.*, 2010). In a detailed study, *W.somnifera* along with a few number of herbal plants knowing to have anti-ulcerative properties were given to experimental models before inducing the ulcers. The other plants include *Oscimum sanctum, Triphala, shilajith* and *Camellia sinensis*. The study supported the cytoprotective, anti-secretory and antioxidant properties of the mentioned

herbal medicines. The formulation of the extracts was developed by a herbal medicine company in Banglore (Nair *et. al.*, 2010).

Neurogenetic activity: *Withania somnifera* is also having positive effect on children having memory deficit and in old age people having loss of memory. Clinical trials and animal research support the use of *W.somnifera* for anxiety, cognitive and neurological disorders, inflammation, and Parkinson's disease (Deocaris *et al.*, 2011). It has been developed as an immunosuppressive agent for the inflammatory diseases too (Davis & Kuttan 2000; Rasool & Varlakshmi, 2006).

Applications of Biotechnological tools on *W. somnifera:* genetic transformation and *in-vitro* culture of W. somnifera have been extensively studied since last few decades. Various parts of the plant like Seedlings, embryos, cotyledon, epicotyl, hypocotyl, petiole, leaves, nodes, internodes, stem, shoot tips and roots have been used in different experiments for callus induction, adventitious root induction, regeneration, differentiation, flower inductionetc (Singh *et al.,* 2017). Attempt on standardization of cell culture media for encapsulation of shoot tips of *W.somnifera* along with optimization of media composition has also been taken (Singh *et al.*, 2006). The effects of in vitro conditions on accumulation of withanolides, involving organ and callus culture, cell suspension culture and *Agrobacterium tumefaciens* as well as *A.rhizogene* mediated transformation gave an interesting insight on tissue culture of *W.somnifera* (Singh *et al.,* 2017). Modulated gene expression pattern was studied by modification in conditions of culture techniques which resulted in modulated accumulation of different withanolides. Most of the in vitro tissue culture conditions of *W.somnifera* have been briefly summarized recently by Singh *et al.* (2017).

Embryoids have also been successfully raised from anther induced callus cultures (Vishnoi *et al.*, 1979). Sen & Sharma (1991) pioneered in describing the in-vitro shoot multiplication followed by rhizogenesis from aseptically germinated seedlings using MS medium enriched with BAP. Morphogenetic studies were reported by Roja *et al.* (1991) from axillary bud derived callus of W. somnifera. Baburaj & Gunasekaran (1995) for the first time reported differentiation of shoots from leaf callus cultures. In another study Kulkarni *et al.* (2000) studied the *in-vitro* propagation through organogenesis using various explants.

Genes involved in Withanolide synthesis have been extensively studied by Gupta *et al.* (2015). Variation in gene expression pattern in relation to tissue culture and stress related conditions leading to withanolide accumulation were studied by Sabir *et al.* (2013). Ray & Jha (1999) have performed various experiments by infecting leaves of *W.somnifera* by *A.tumifaciens* (wild type nopaline and octopine) and have extracted two principal types of Withanolides, D and A I different parts of the plants. The ARNA silencing studies have been extensively done by Saema *et al.* (2015) on the gene WsSGRL1 gene in transgenic W. somnifera plant and various parameters of growth were studied. All the above types of attempts indicate that in future we could be also able to improve the variety of *W.semnifera* which would serve the medical science in much better ways.

Synthesis of Nanoparticles from *Withania somnifera* extract: Nanoparticles from *W.somnifera* extracts have been synthesised by Marshil *et al.,* 2015 and formulated in a cream base and studied its antimicrobial activity against human pathogens (*Staphylococcus aureus, Pseudomonas aeruginosa, Proteus vulgaris, Escherichia coli, and Candida albicans*) and a plant pathogen (*Agrobacterium tumefaciens*). The traditional use of *W.somnifera* (Ashwagandha) has a logical and scientific basis. Large-scale clinical studies are needed to prove the clinical efficacy of this herb, especially in stress-related diseases, neuronal disorders and cancers. Moreover, phytochemical compounds or the bioactive constituent in this plant might prove to be an extensive reservoir of bioactive compounds of substantial medicinal merit.

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