Current Aspects of Medicinal Properties and Health Benefits of Plant *Withania somnifera*

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Abstract

Withania somnifera also known under the name Ashwagandha has been in use for over 5000 years. It has been concluded from studies undertaken that this plant possesses various medicinal properties contributing to several health benefits. These effects are likely to be caused due to the wide variety of steroid lactones and withanides and other components contained within the *W. somnifera* plant. Increasing number of studies has revealed the evidence for antioxidant and medicinal properties. Investigations have shown to be very positive across a wide variety of experimental systems, including in-vitro tests, cell-based studies, and in-vivo animal trials noting in particular the reduction of ROS for rats with induced Parkinson's disease and the healthy gain of weight in normal rats. There is also great interest within the anticancer abilities of *W. somnifera* and these abilities have shown to be effective against various types of cancer including prostrate and lung. Strong evidence suggests the plant also carries antibacterial activity. The aims of this article is to summarize current research dealing with medicinal properties and health benefits of *Withania somnifera* extracts with a focus on antioxidant, anticancer and antimicrobial properties.

Keywords

Withania somnifera · Ashwagandha · Antioxidant · Antibacterial · Anticancer

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14.1 Introduction

Withania somnifera (shown in Fig. 14.1) is a medicinal plant, which belongs to a group, called the Solanaceae family of plants and is known under the names Indian ginseng or Ashwagandha. The Solanaceae plant family is constructed of over 2700 plant species, which are divided into 98 separate genera. Authors of this chapter started working on this plant few years back, some of the results have been published on its medicinal properties (Barnes et al. 2015). This chapter has included information collected from important research conducted with general health effects of *Withania somnifera* extracts, antioxidant properties, anticancer and antioxidant activity.

A search of Medline/PubMed and ISI web of science databases showed the following results; full articles for *Withania somnifera* (940), reviews (54), controlled clinical trials (16) – of which 13 were randomized clinical trials. Currently there appeared to be five (5) systematic review and meta-analysis on the topic. In the following narrative review, the aim is to summarize current research dealing with medicinal properties and health benefits of *Withania somnifera* extracts with a focus on antioxidant, anticancer and antimicrobial properties. Previous reviews dealing with the pharmacological and health aspects of *Withania somnifera* include those by the following authors (Chen et al. 2011; Kulkarni and Dhir 2008; Mishra et al. 2000; Sharma 2013; Singh et al. 2010; VandenBerghe et al. 2012; Winters 2006).

Fig. 14.1 Picture of the plant *Withania somnifera.* (Source: http://xyerectus.com/wp-content/uploads/2013/11/390651_090421122758_Withania_somnifera4.jpg)

14.2 Health Benefits

Records showing the medicinal use of *W. somnifera* date back over 5000 years and are related to a traditional Hindu methodology of medicine, known as Ayurvedic medicine. In systems such as Ayurvedic and Unani medicine, extracts from plants such as *W. somnifera* claim to have multiple health benefits in the treatment of a variety of health problems. Observational and ethnographic evidence suggests that *Withania somnifera* has been used for the treatment a range of ailments: muscle tension, bone damage, aging and dehydration amid several others. Observations also suggest that the plant contains additional pharmacological properties, which can be useful in modern medicine, such as tackling neurodegenerative disorders (e.g. Alzheimer's disease) and addressing declining cognitive function in geriatric patients, amid a plethora of other ailments.

Interests in the health benefits of *Withania somnifera* has accelerated recently with growing numbers of discussions focusing on, Parkinson's and neurodegenerative diseases (Durg et al. 2015; Srivastav et al. 2017; Yenisetti et al. 2016), cancer and neoplasms (Lee and Choi 2016; Palliyaguru et al. 2016; Rai et al. 2015) as well as, other generic chronic diseases (White et al. 2016). Moreover the number of carefully desgined controlled, human trials are now groiwng but more work can be anticapated in future years (Table 14.1). It is suggested that the reason for a majority of the associated health benefits is due to the volume and variety of chemical compounds, which can be found within specimens of *WS* (Table 14.2).

Table 14.1 A summary of some human trials using *Withania somnifera*

Condition, outcome	Study	+RE
Osteoarthritis pain	Kulkarni et al. (1991)	+
Hypoglycaemic, cholesterol lowering	Andallu and Radhika (2000)	+
Sleep quality	Manjunath and Telles (2005)	0
Depression	Krishnamurthy and Telles (2007)	0
Anxiety	Cooley et al. (2009)	+
Immunologic effects	Mikolai et al. (2009)	+
Balance & cerebellar ataxias	Sriranjini et al. (2009)	+
Reproductive health	Ahmad et al. (2010)	+
Innate Immunity	Bhat et al. (2010)	+
ADHD	Katz et al. (2010)	+
Schizophrenia	Agnihotri et al. (2013)	+
Chemotherapy associated fatigue	Biswal et al. (2013)	+
Bipolar disorder	Chengappa et al. (2013)	+
Male reproductive health:	Gupta et al. (2013)	+
Bone mineralization	Mirakzehi et al. (2013)	+
Anxiety, Stress	Pratte et al. (2014)	+
Women reproductive health	Dongre et al. (2015)	+
Muscle strength	Wankhede et al. (2015)	+
Obsessive compulsive disorder	Jahanbakhsh et al. (2016)	+
Body weight management	Choudhary et al. (2017)	+

+Promising or positive results, (0) No result, (−) Negative results

14.3 Components in Withania somnifera

The phytochemical constituents of *WS* root extract were classed into 6–8 chemical classes (Table 14.2); alkaloids, steroidal lactones, steroids and phytosterols, salts, flavonoids, and nitrogen containing compounds. Results from phytochemical screening studies showed *WS* root to contain, some eight classes of compounds depending on the solvent used for extraction; alkaloids, flavonoids, steroids/ phytosterols, terpenoids, saponins, tannings, phenols and various glycosides (Visweswari et al. 2013). Overall, 12 different alkaloids and 30+ withanolides isolated from *WS* (Singh et al. 2010). Currently, the steroidal-lactones have received most attention. The most common withanolides e.g. Withanone, Withaferin A, Withanolides are the basis for some of the various health benefits associated with the plant. Withaferin A has been considered as one of the more important compounds as proof has shown it

Table 14.2 Pharmacological components from *Withania somnifera*

Classes	Examples	Clinical effect	Biological activity
Alkaloids	Withanine, Withananine, Withasomnine, Somniferine, Tropeltigloate, Somniferinine, Somninine Nicotine	Anti-Microbial, Sedative, Anti-Spasmodic; Anti-Cancer, Leg Cramps, Anti-Diarrhoea, Psychiatric, Palpitation	Anti-inflammatory Antioxidant Anti-inflammatory Apoptosis inducing Diuretic Sedative/anxiolytic Immunomodulatory
Steroidal lactones	Withaferin-A, Withanone, Withanolides	Anticancer	Anti-bacterial Anti-Stress Cardio-protective Neuroprotective Anti-hypertensive Anti-arthritic Anti-cancer Anti-diabetic
Steroids, phytosterols	Cholesterol, B-Sitosterol, Stigmasterol, Diosgenin, Stigmastadien, Sitoinosides VII, Sitoinosides VIII, Sitoinosides IX, Sitoinosides X	Aphrodisiac, Lowers cholesterol, Immune anti-tumour	
Salts	Cuscohygrine, Anahygrine, Tropine, Pseudotropine, Anaferine	?	
Flavonoids	Kaempferol, Quercetin	Antioxidant, CVD risk dec. osteoporosis, cholesterol lowering cancer risk dec.	
N-Compounds	Withanol, Somnisol, Somnitol		

Adapted from Dar et al. (2015), Visweswari et al. (2013), and White et al. (2016)

to be therapeutically active (Mirjalili et al. 2009; Misico et al. 2011; Sangwan et al. 2014; White et al. 2016; Zhang et al. 2014).

The alkaloids from *WS* remain quite illusive, as no structural formulas appear to be available. Nevertheless, some classical pharmacognosy sources suggest that *WS* alkaloids crystalize from chloroform extracts and that the majority (38%) consist of withanine ($C_{44}.H_{80.}\ O_{12}.N_{2,}$ predicted FW = 828) which has a distinctive sedative and hypnotic activity (Atal 1975; Glasby 2012). More recently, WS alkaloids were prepared quantitatively using a mixture of acetic acid and methanol (Takshak and Agrawal 2014).

14.4 Antioxidant Activity of WS Root Extract

Currently about 114% of the literature addressing WS is associated with antioxidant activity in one way or another, the majority of papers were reviews, or assessed antioxidant activity in-vitrousing small rodents (Devkar et al. 2014; Dhanani et al. 2013; Fernando et al. 2013). A great interest lies within testing any antioxidant activities that *W. somnifera* extracts may possess, as they can aid reduction of oxidative damage to cells, effectively acting in order to decrease the likelihood of degenerative diseases such as the development of Alzheimer's disease. Increased damage levels due to oxidation to cell types can be partly responsible for a spectrum of chronic diseases liked with increased oxidative stress, e.g. Alzheimer's. This damage takes place either by hydroxyl radicals causing damage to mitochondrial DNA, or by protein oxidation into carbonyls (Scartezzini and Speroni 2000). In this section, we review briefly evidence for antioxidant content for *WS* with a focus on roots. For a lack of space, only primary studies after 2005 are considered.

14.4.1 *In-Vitro* Tests

Several simple chemical tests and enzymatic tests were performed to determine the content of non-enzymatic antioxidants and enzymatic antioxidants for *WS*. With respect to chemical tests in use the most common of these that are used in testing antioxidant samples include, β-carotene bleaching test (BCBT), DPPH, ABTS, FRAP and Folin method of testing total phenols. These tests are deemed state of the art due to their effectiveness, simplicity and repeatability meaning that they are in the most usage. They have maintained their usefulness over time because of their success rate in the analysis of different areas of antioxidant activity including primary and secondary antioxidant activities as well as the ability of free radical scavenging. These various In-vitro tests also remain useful as they can test a variety of antioxidant activity properties, this is important as no individual antioxidant test known will cover all the aspects and descriptions of activity (Aruoma 2003; Fraga et al. 2014; Frankel and Meyer 2000; Koleva 2002).

Non-enzymatic antioxidants associated with *WS* include vitamin C, vitamin E, glutathione, carotenoids, lycopene, total phenols and flavonoids (Jaleel 2009). The

current research showed that *WS* also contained significant activity for several enzymatic antioxidants, including catalase, superoxide dismutase, and various peroxidases (Jaleel 2009; Kanungo et al. 2013; Sumathi and Padma 2008). Further studies involved so-called total antioxidant capacity measured in terms of radical quenching assays (DPPH, ATBS method), total phenols, iron (III) reduction or FRAP methods (Alam et al. 2012; Dhanani et al. 2013; Fernando et al. 2013). The investigations performed using solvent extracts, showed that *WS* contains high antioxidant capacity, though values depend on the solvent choice for extract, and whether analysis is performed using different botanical parts of the plant (leave, berries, and roots etc.).

14.4.2 Animal Trials Testing for *W. somnifera* Antioxidant Ability

In vivo animal trials have been used for additional observations on the antioxidant effects of *W. somnifera* extracts, usually leading to protection from drug-induced reactive oxidative stress (ROS) or other insults. Investigations using rats as models showed WS antioxidant activity and protection from a variety or ROS:adjuvant induced arthritic ROS (Rasool and Varalakshmi 2007), doxorubicin (Hamza et al. 2008), gentamicin ROS (Jeyanthi and Subramanian 2010), swimming induced ROS (Misra et al. 2009), alloxan toxicity (Udayakumar et al. 2010), carbon tetrachloride ROS (Elberry et al. 2010), radiation induced ROS (Hosny et al. 2012), bromobenzene nephrotoxicity (Vedi et al. 2014).Such trials carried out on both rats and mice are a common methodology associated with the extract and various conditions have been shown to be successfully treated with the use of the plant extract. It would seem that specific compound from WS possess antioxidant capacity such as flavonoids and phenols (Keshavkant et al. 2008) and other less well defined components demonstrated using TLC-DPPH method (Devkar et al. 2014).

Gupta and co-workers found that *WS* extract prevents Cu induced oxidative stress in the brain of rats (Gupta et al. 2003). Mohanty and co-workers concluded that WS produces a cardio-protective effect in rats due to the activation of endogenous antioxidants (Mohanty et al. 2004). WS extracts could reduce the severity of stress-induced gastric ulcer in rat by an antioxidant mechanism (Bhatnagar et al. 2005). Additional animal-based evidence generally support the consensus that *WS* administration is protective effects with regards to stress and/ or drug induced oxidative stress (Al-Qirim et al. 2008; Jeyanthi and Subramanian 2010; Jeyanthi et al. 2010).

In a rat-experimental model for Parkinson's disease, intra-cranial injections with 6-hydroxydopamine (6-OHDA) were used to produce chronic neurotoxicity by increasing ROS. Pre-treating rats with *WS* prior to 6-OHDA prevented neurobehavioral decline, as well as reducing biological markers for oxidative stress in a dose-dependent manner (Ahmad et al. 2005).*W. somnifera* treatment also reduces oxidative damage caused in rats induced with type II diabetes, the extract has proven to increase anti-oxidative enzyme activity such as GPX and CAT amongst various others. This carries statistically significant results when delivery happens in high doses of over 200–400 mg/kg. As well as involvement in level decreases of lipid

peroxide, these studies were proven with the use of well-established tests including NADPH oxidation and the analysis of lipid hydroperoxide levels by testing with thiobarbituaric acid, a complex was then formed due to the lipids involved becoming peroxidised (Anwer et al. 2012).

Recent findings that WS compounds activate nuclear factor erythroid 2-related factor 2 (Nfr2) pathway (Heyninck et al. 2016; Sun et al. 2016; White et al. 2016) may help explain some of the antioxidant and detoxification results observed with animal studies. Briefly, Nrf2 functions as cellular sensor for mild ROS arising from the metabolism of antioxidants by cells. Following its activation by oxidizable polyphenols, quinones, lactones, ROS or electrophilic agents, thenNrf2 is transferred from the cytoplasm to the cell nucleus. Arriving in the cell nucleus Nrf2 binds to a so-called antioxidant responsive element (ARE) thereby increases the expression of genes for phase I and phase II drug metabolism enzymes, antioxidant enzymes (CAT, SOD, GSH-Px), drug detoxification transporters (Niture et al. 2014). Activation of Nrf2 is invoked to explain the protective role dietary phytochemicals for many conditions liked with ROS and chronic inflammation, e.g., arthritis, cancer, diabetes, neurodegenerative disease, (Qin and Hou 2016). NrF2 activation is also associated with the suppression of an inflammatory responses believed to be controlled by another nuclear factor kappa-beta (NFKB) including the synthesis of inflammatory cytokines (Ahmed et al. 2017). It should be noted that NrF2 activation can lead to increased resistance not only to toxic chemicals but also some therapeutic cancer drugs (Kensler and Wakabayashi 2010). It can be anticipated that more research focusing WS and Nrf2 system will be forthcoming.

14.5 Anticancer Activity of WS Root Extract

There is rapidly increasing interest in the creation and use of new anticancer treatments. With cancer accounting for over 25% of deaths in humans annually, treatment using natural biomaterial such as *W. somnifera* are being investigated due to the partial ineffectiveness and side effects of a negative nature that are associated with modern day treatments for cancer including chemo and radiotherapy. The anticancer properties of WS and individual chemical components were reviewed recently (Winters 2006; Vyas and Singh 2014; Lee and Choi 2016; Palliyaguru et al. 2016; Rai 2015). In the following section, we consider recent investigations focussing on WS whole extract rather than single components. Recent investigation using WS root extract found anticancer activity against many human cell lines including, liverHep2 cells (Mathur et al. 2006), colon HCT15 cells, prostateDU-145, PC3 cells, lung A-549 cells and neuroblastoma IMR-32 cells (Yadav et al. 2010);skin melanoma cells (Halder et al. 2015), breast MDA-MB-231 and MCF-7 cells (Khan et al. 2015; Khazal et al. 2013; Maliyakkal et al. 2013). In general terms, the cytotoxicity of WS extract (25–50 ug/ml) was significant and was affected by the type of solvent used for extraction, cell type and by the length of the exposure time (24–72 h). Studies consistently showed the role of WS extract in disrupting cell-cycle progression.

14.6 Antibacterial Activity of WS Root Extract

Due to rapidly increasing and widespread antibiotic resistance, there is an increased global demand for new and innovative treatments. These treatments should be able to tackle diseases and infections that some antibiotics no longer can due to resistance. There is a continuing demand for fresh antibacterial treatments to be developed as incidence rates are on the rise around the world; this demand has led to the resurgence of *WS* use and testing in order to develop new ways of fighting against several diseases. The antibacterial effects of *W. somnifera* are continuously being tested with studies dating back to the 1950s (Kurup 1958).

However, investigations that are more recent have shown prominent outcomes and a better understanding of the antibacterial mechanisms present. In this section, we consider current research related to the antibacterial properties of WS extracts prepared from whole root powder and not individual components, though research shows specific compounds to be active (Girish et al. 2006; Shanazbanu et al. 2006). A search using PubMed, and ISI web of science databases found 45 and 57 references (respectively) that describe Withania S (title) antibacterial or antimicrobial properties (topic). The lists of papers were hand sorted, and duplicates removed. Publications were supplemented also with papers from Google Scholar and a total of 10–11 papers were recovered that described antibacterial activity of WS extracts prepared from roots (Table 14.3). There were no previous reviews focussing exclusively on the antibacterial properties of *W. somnifera*.

As noted above (Table 14.3), a variety of studies have been carried out dealing with antibacterial activity with the use of various different methodologies, with some studies using extracts from both the root and leaves of *W. somnifera*. In general, the approaches adopted by different investigators involve preparing dried materials, size reduction by gridding, and extraction of biologically active components using water or a low molecular weight non-aqueous solvent. The extracts are concentrated using a rotary evaporator or air-drying, and then investigated for antibacterial activity using a spectrum of bacterial specimens.

Another recent study uses the methanolic, aqueous, chloroform, acetone and ethanolic extracts in a disk diffusion methodology to test human pathogenic bacteria, including *MRSA, E. faecalis, S. pyogenes, K. pneumoniae* (Rizwana et al. 2012). The study concludes that extracts from *W. somnifera* including those from the root, stem and leaves show a great potency against different bacterial species. Overall looking at the information within the study antibacterial levels are high especially from extracts taken from the leaf of the plant, followed by the stem and then lastly the root, especially in alcohol and acetone based extracts. Taking into account that aqueous extracts also produced notable effects; polar solvents however proved to produce the best overall antibacterial effects and present the opportunity for future options in this area (Rizwana et al. 2012).

Other studies also give a contribution to these results as well as adding relevant information, one such study suggests how inhibition zones change in size based on a dose dependent manner notably from leaf based extracts. A direct comparison to an established antibiotic- chloramphenicol, where extracts are shown to be

Table 14.3 Antimicrobial and antibacterial activity of *Withania somnifera* extracts

Part	Solvent	Microorganisms	Method	References
Leaves, Root	Methanol, Hexane, Diethyl Ether	*Salmonella typhimurium, Escherichia coli.*	Disc diffusion	Arora et al. (2004)
Root, Leaves	Hexane, Ethyl Acetate Methanol Water	*E. coli, S. aureus S. typhimurium*	Agar well diffusion, Mice	Owais et al. (2005)
Root	Benzene Ethanol	*S. typhi, S. aureus, S. rubidaea, Lactobacillus* sp.		Dar et al. (2008)
Bark, Leaves, Root	Methanol	*B. subtilis, E. coli, P. fluorescens, S. aureus X. axonopodis* pv. *A. flavus, D. turcica, F. verticillioides*	Disc diffusion	Mahesh and Satish (2008)
Root	Water, Methanol	*S. aureus (MDR), 15 local strains, 5 patient strains*	Agar well diffusion	Datta et al. (2011)
Root	Water	*S. aureus* (methicillin resistant)	Agar well diffusion	Mehrotra et al. (2011)
Whole Plant	Ethyl Acetate	*S. aureus, E. coli, P. aeruginosa, B. subtilis*	Agar well diffusion	Sundaram et al. (2011)
Fruit Leaves, Root	Water + 80%, Methanol	*E. coli, S. typhi, C. freundii, P. aeruginosa, K. pneumoniae,*	Agar well diffusion	Alam et al. (2012)
Leaves, Roots, Stem	Acetone, Chloroform Ethanol, Methanol	*B. subtilis, E. coli, E. feacalis, MRSA, S. pyogenes, P. aeruginosa K. pneumoniae*	Agar well diffusion	Rizwana et al. (2012)
Roots	Methanol	*Streptococcus mutans, S. sobrinus S. oralis, A. naeslundii*	Absorbance 550 nm	Pandit et al. (2013)
Root	Water	*E. coli*	Disc diffusion	Kumari and Gupta (2015)

Investigations using only leaf extracts were excluded

increasingly potent by around a factor of 5 with a lowered level of toxicity, in this case where 4 µg of an extract does the same as 20 µg of an antibiotic. There is some focus from this study given to prophylactic effects on animals, rats induced with the infection *S. typhimurium* were given 100 mg/kg doses of *W. somnifera* as a form of treatment. This as a method proved to be successfully effective and leads to the belief that future prospects for *W. somnifera* are good and in future there may be a way to find a niche allowing the plant to be capable of treating infections caused by

bacteria in humans, however this can only happen if effective human clinical trials can be carried out (Owais et al. 2005).

These studies concluded that the *W. somnifera* was very potent and proved highly effective against strains including *E.coli, B. subtilis* and *P. aeruginosa*, where during tests 14–16 mm inhibition zones were formed. Most investigations used agar well diffusion assays or the disc diffusion assays as this way of testing is considered gold standard, fast and easy (Hombach et al. 2013) Modern systems that can be used for effective screening of the susceptibility of bacteria include the BACTEC system which uses fluorescent markers along with CO_2 to measure bacterial sensitivity (Collins and Franzblau 1997). These systems also included a recent development where 96-well microplates are used in the determination of bacterial susceptibility and MICs with the capability of using small concentrations of plant material such as *W. somnifera* extracts as low in volume as 25 µl (Eloff 1998).

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