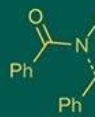


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Medicinal plant *Withania somnifera* (L.) Dunal and pharmacological active compounds: A systematic review

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Abstract

India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants including more than 2000 species. Among the plants known for medicinal value, genus *Withania* belonging to family Solanaceae is very important for its therapeutic potentials. The major biochemical constituents of Ashwagandha roots and leaves are steroidal alkaloids and steroidal lactones which belong to class of constituents called withanolides which include withanolide A, withanolide D, withaferin A and the sitoindosides. Mycorrhizal associations confer benefits like better nutrition acquisition, enhanced growth and secondary metabolite production, defense enhancement and improved abiotic and biotic stress tolerance in plants. To meet the growing demand of herbal medicine the utmost need is to explore the means by which these bioactive metabolites can be enhanced. The symbiotic association of Arbuscular mycorrhizal fungi with medicinal plants like Ashwagandha can be useful for enhancing the quantity and quality of phytochemicals or secondary metabolites of pharmacological, therapeutic, and cosmetic interest.

Keywords: Phytochemical, Indian herbs, antioxidant, arbuscular mycorrhizal fungi

Introduction

In India, the Ayurveda concept appeared and developed between 2500 and 500 BC, which means “science of life”. This traditional knowledge forms the basis of modified system of medicine and exists in the form of Ayurveda, Unani, Siddha and Swa-riga (Tibetan) system of medicine. However, very few plants have been explored chemically and pharmacologically for their potential medicinal value (Thenmozhi *et al.*, 2013) [43]. According to World Health Organization (WHO), there are 21,000 plant species around the world used for medicinal purpose while in India around 2,500 medicinal plant species belonging to more than 1,000 genera are being used for treating different ailments (Yadav *et al.*, 2006) [50]. WHO reported that more than 80% of the world’s population depends on traditional medicine for their primary health care needs (Ammara *et al.*, 2009) [12]. Now days, there has been a resurgence in the consumption of herbal medicines due to lack of curative modern remedies for several chronic diseases, side effects of chemical drugs and microbial resistance as well as the abandoned investment in pharmaceutical research and development (R&D). Medicinal plants are getting extensive recognition due to their less toxic effect, better safety, economically more affordability, less chances of relapse of disease and easy availability in rural areas. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Thenmozhi *et al.*, 2010) [42].

Recent bans and restrictions on the use of animal antibiotic products have been diverted attention towards plant bioactive secondary metabolites (Greathead, 2003) [12]. The plant chemicals are classified as primary and secondary metabolites. Medicinal plants generally produce many secondary metabolites which are biosynthetically derived from primary metabolites and constitute an important source of microbiocides, pesticides and many pharmaceutical drugs. In contrast to primary metabolites, they are synthesized in specialized cell types and at distinct developmental stages, making their extraction and purification difficult. As a result, secondary metabolites that are used commercially as biologically active compounds, are generally high value-low volume products

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(e.g. steroids, quinones, alkaloids, terpenoids and flavonoids), which are used in drug manufacturing by the pharmaceutical industries. These are generally obtained from plant materials by steam distillation or by extraction with organic or aqueous solvents and have molecular weight generally less than 2000 Dalton. From a long period of time medicinal plants or their secondary metabolites have been directly or indirectly playing an important role in the human society to combat diseases. Medicinal plants produce a diverse type of chemical compounds with various biological functions and defend against attack from predators such as insects, fungi and herbivorous mammals (Divekar *et al.*, 2022) ^[9]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenols. Plant constituents may be isolated and used directly as therapeutic agents or as starting materials for drug synthesis or they may serve as models for pharmacologically active compounds in drug synthesis. The general research methods include proper selection of medicinal plants, preparation of crude extracts, biological screening, detailed chemical and pharmacological investigations, toxicological and clinical studies, standardization and finally use of active moiety as the lead molecule for drug design. Approximately, 12,000 such compounds have been isolated so far, which is less than 10% of the total compounds present in the nature (Elumalai and Eswariah, 2012) ^[10].

Plants produce incomprehensible varieties of phytochemicals but most are derivatives of a few biochemical motifs. Alkaloids are a class of chemical compounds containing a nitrogen ring. These are produced by a large variety of organisms, including bacteria, fungi, plants and animals. Alkaloids are a part of the group of natural products (also called secondary metabolites). They can be purified from crude extracts by acid-base extraction. Several alkaloids are toxic to other organisms. They often have pharmacological effects and are used as medications, recreational drugs or in entheogenic rituals. Examples are the local anesthetic and stimulant cocaine; the psychedelic psilocin; the stimulant caffeine; nicotine; the analgesic morphine; the antibacterial berberine; the anticancer compound vincristine; the antihypertension agent reserpine; the cholinomimetic galatamine; the spasmolysis agent atropine; the vasodilator vincamine; the anti-arrhythmia compound quinidine; the anti-asthma therapeutic ephedrine and the antimalarial drug quinine. Although alkaloids act on diverse metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste (Behl *et al.*, 2023) ^[4]. Polyphenols (also known as phenolics) are compounds containing phenol rings. Examples are the anthocyanins which are present in the purple grapes (Xia *et al.*, 2010) ^[49], the isoflavones and the phytoestrogens from soy and the tannins present in the tea giving astringency are phenolics (Pandey and Rizvi, 2009) ^[29]. Glycosides are the molecules in which a sugar is bound to a non-carbohydrate moiety, usually a small organic molecule. Many plants store chemicals in the form of inactive glycosides, which can be activated by enzyme hydrolysis by breaking their sugar moiety. Many such plant glycosides are used as medications (Rahman *et al.*, 2022) ^[35]. In animals and humans, poisons are often bound to sugar molecules as part of their elimination from the body. An example is the cyanoglycosides in cherry pits that release toxins only when bitten by an herbivore. Terpenes are large and diverse classes of organic compounds, particularly produced by

conifers also have a protective function. They are the major components of resin and turpentine produced from resin. The name "terpene" is derived from the word "turpentine". Terpenes are major biosynthetic building blocks within nearly every living creature. Steroids, for example, are derivatives of the triterpene squalene. When terpenes are modified chemically by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids. Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and flowers. Essential oils are used widely as natural flavor additives for food, as fragrances in perfumery and in traditional and alternative medicines such as aromatherapy (Masyita, 2022) ^[23]. Synthetic variations and derivatives of natural terpenes and terpenoids also greatly expand the variety of aromas used in perfumery and flavors used in food additives. Vitamin A is an example of a terpene. The fragrance of rose and lavender is due to monoterpenes. The carotenoids impart red, yellow and orange colour to pumpkin, corn and tomatoes.

The Indian flora offers great possibilities for the discovery of new compounds with important medicinal applications in combating infection and strengthening the immune system. The antimicrobial compounds found in plants may prevent bacterial infections and therefore, these may have clinical value in treating resistant microorganism strains. The indiscriminate use of antibiotics has resulted in many bacterial pathogens rapidly becoming resistant to a number of originally discovered antimicrobial drugs. Thus, there is a continuous search for new antibiotics and medicinal plants which may offer a new source of antibacterial agents. This is indeed very important because *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* are some of the important human pathogens that have developed resistance to antimicrobial antibiotics (Stanley *et al.*, 2014) ^[40].

Indian herbal market is nearly 5000 crores with an annual growth of 14%. India's share in the export of herbal is 2.5% of the total global herbal market. While with the same biodiversity and ancient culture, the China has 13% share of the global herbal market. This shows that there is a massive scope for Indian manufacturers in the worldwide growing opportunities in herbal pharmaceutical business. According to research by Global Industry Analysts (GIA), the global herbal supplement and remedy industry is forecast to exceed \$105 billion by 2017. WHO predicts that overall pharmaceutical market will reach US \$ 5 trillion by 2050 (Bhattacharya *et al.*, 2014) ^[6].

Ashwagandha- a medicinal plant: an overview

Scientific classification

Kingdom	: Plantae
Division	: Angiosperma
Class	: Dicotyledoneae
Order	: Tubiflorae
Family	: Solanaceae
Genus	: <i>Withania</i>
Species	: <i>somnifera</i>
Sanskrit name:	Ashwaganda
Hindi name	: Asgandh
English name	: Winter cherry

Withania somnifera (L.) Dunal, commonly known as Ashwagandha, Indian ginseng, Poison Gooseberry or Winter

Cherry, belongs to Solanaceae. The symbiosis of AM with the favorable characteristics of medicinal plants leads to improvement in the production and accumulation of important active ingredients of medicinal plants such as terpenes, phenols and alkaloids which ultimately improve the quality of herbal materials (Zeng *et al.*, 2013)^[51]. Zubek *et al.* (2015)^[52] testified that there is an increase in production of secondary metabolites in *Viola tricolor* shoots which may be due to improved mineral nutrition in presence of AMF and/or a result of general plant defense reaction to fungal colonization.

Withania somnifera (L.) Dunal, commonly known as Ashwagandha, Indian ginseng, Poison Gooseberry or Winter Cherry, belongs **Error! Bookmark not defined.** probably refers to its extensive use as a remedy against stress. Ashwagandha possesses several health benefits and therefore also known as 'Rasayana' as "Sattvic Kapha Rasayana" in Indian Ayurveda. It is used since centuries for its miraculous advantages (Sharma 1938)^[36]. Rasayana is defined as an herbal or metallic preparation that promotes a youthful state of physical and mental health and expands happiness. Most of the Rasayana herbs are adaptogen / anti-stress agents. Rishi Punarvasu Atriya was the first who recognized and taught the use of Ashwagandha long back over 3000 to 4000 years ago, (Tiwari *et al.*, 2014)^[44]. Chinese, Unani, Ayurveda and Siddha literatures appreciated the therapeutic merits of Ashwagandha derived medicines against almost all ailments. Thus 'Indian ginseng' of Ayurvedic medicine, considered as 'adaptogen' which improves physical energy and athletic ability, increase immunity to colds and infections and increase sexual capacity and fertility.

Morphological features of Ashwagandha: *W. somnifera* (L.) Dunal is an erect, branched, greenish, stellate-tomentose under-shrub, around 30-150 cm high with long tuberous roots. The roots are stout, fleshy, whitish and aromatic. The fleshy roots when dry are cylindrical, gradually tapering down with a brownish white surface and pure white inside when broken. Leaves are simple, petiolate with the leaf blade varying in shape from elliptic-ovate to broadly ovate, entire along margins, acute to obtuse at apex 4-10 cm long and 2-7 cm broad (Mir *et al.*, 2012)^[26]. Flowers inconspicuous, greenish or lurid-yellow, in axillary, umbellate cymes, hermaphrodite (have both male and female organs); berries small, globose, orange-red when mature, enclosed in the persistent calyx; seeds yellow and reniform. Beneficial parts in the plant may be whole plant, roots, leaves, stem, green berries, fruits, seeds and bark which are used. The roots are the main portion of the plant used therapeutically (Gupta *et al.*, 2007)^[13]. High pollen load on the stigma and stiff pollen competition within a flower strongly favours self-pollination (Mir *et al.*, 2012)^[26].

In the traditional Ayurvedic system of medicine, this plant is claimed to have potent, aphrodisiac, rejuvenative and life prolonging properties. It has general stimulating and regenerative qualities and is used for the treatment of nervous exhaustion, memory related conditions, insomnia, tiredness potency issues, skin problems and coughing. It improves learning ability and memory capacity. The traditional use of 'Ashwagandha' was to increase energy, youthful vigor, endurance, strength, health, nurture the time elements of the body, increase vital fluids, muscle fat,

blood, lymph and semen and cell production. It helps counteract chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotency, premature aging emaciation, debility, convalescence and muscle tension (Williamson 2002; Devi *et al.*, 2012; Mishra *et al.*, 2000)^[48, 8, 27].

The roots are used as a nutrient and health restorative in pregnant women and old people. The decoction of the root boiled with milk and ghee is recommended for curing sterility in women. The roots are also used in constipation, senile debility, inflammation, immunomodulatory, hypertension, anxiety, cognitive, rheumatism, general debility, nervous exhaustion, neurological disorder, Parkinson's disease, hepatoprotection and spermatorrhoea. The roots of Ashwagandha consist primarily of compounds known as withanolides, which are believed to account for its extraordinary medicinal properties. The leaves of the plant are bitter in taste and used as an antihelmantic. The infusion is given in fever. Bruised leaves and fruits are locally applied to tumors and tubercular glands, carbuncles and ulcers. The fresh berries have been used as an emetic (Kushwaha *et al.*, 2012; Nadkarni, 1976; Harikrishnan *et al.*, 2008; Verma and Kumar, 2011)^[18, 28, 14, 47]. Fruits and seeds of *W. somnifera* (L.) Dunal are diuretic, hypnotic, masticatory and employed in curdling plant milk to prepare vegetarian cheese. The seeds are also used as a substitute of soap being rich in saponins (Vashistha *et al.*, 2010)^[46].

W. somnifera (L.) Dunal grows well in sandy loam or light red soil, having pH 7.5-8.0 with good drainage. It can be cultivated between 600-1200 m altitudes. The semi-tropical areas receiving 500-750 mm rainfall are suitable for cultivation of this crop. The crop requires dry season during its growing period. Temperature between 20 °C to 35 °C is most suitable for cultivation. It is grown as late rainy season (kharif) crop. Late winter rains are conducive for the proper development of the plant root. The plant start flowering and bearing fruits from December onwards. The crop is ready for harvest in January-March at 150 to 180 days after sowing. The maturity of crop is judged by drying out of leaves and yellow or red berries. Root is harvested in the autumn and dried for some time for later use. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring. The seed is diuretic and hypnotic.

Origin and cultivation of Ashwagandha

Ashwagandha grows mainly in dry region of Africa, Central Asia and South Asia, mainly in India, Pakistan, Bangladesh, Sri Lanka, Afghanistan, South Africa, Egypt, Congo and Jordan. The genus *Withania* has been estimated to consist of 23 species distributed widely in South Asia and Eastern mediterranean area. Out of these only two species *W. somnifera* (L.) Dunal and *W. coagulans* (L.) Dunal have been reported in India. In India, *W. somnifera* genotypes have been reported from the mountain regions of Jammu and Kashmir, submountain areas of Punjab and Uttaranchal and in the plains of Rajasthan (Nagour), Punjab, Haryana, Uttar Pradesh, Gujarat, Maharashtra and Madhya Pradesh covering Manasa, Neemuch, and Jawad tehsils of Mandsaur and Ratlam districts and adjoining villages of Rajasthan. Neemuch in Mandsaur district is a wholesale market for this crop. Commercial cultivation of Ashwagandha in India is carried out on 5,000 ha of land in Manasa, Madhya Pradesh, and in some parts of Rajasthan, Andhra Pradesh and Uttar

Pradesh. The estimated production of Ashwagandha roots in India is about 1500 tonnes and the annual requirement is about 7000 tonnes, thereby demanding an increase in its cultivation and higher production (Thakur *et al.*, 2014) [41].

Phytochemistry (Bioactive compounds present in Ashwagandha)

Chemical characterization started with Power and Salway (1911) [32] who isolated an amorphous alkaloid ($C_{12}H_{16}N_2$) from a South African strain of *W. somnifera*. Later, Majumdar and Guha (1933) [21] investigated a plant from Bengal (India) and confirmed the presence of the alkaloid. Later on, they reported presence of nicotine and seven other alkaloids from the roots which they named (without structural information) as somniferine, somniferine, somnine, withamine, pseudowithamine, withanmine and withanamine (Majumdar, 1955) [22]. Kirson and Glotter (1980) [17] reported the presence of other alkaloids such as anaferine [bis (2-piperidylmethyl) ketone], isopelletierine, tropine, pseudotropine, 3a-tigloyloxtrapine, 3-tropyltigloate, cuscohygrine, dlisopelletierine, anahygrine, hygrine, mesoanaferine, choline, somniferine, withanine, withanamine, hentriacontane, visamine, withasomnine, pseudowithanine and ashwagandhine in *W. somnifera* (L.) Dunal.

The presence of catechin was reported in *W. somnifera* by high performance liquid chromatography analysis (Alam *et al.*, 2011) [1]. At present, more than 12 alkaloids, 40 withanolides, and several sitoindosides (a withanolide containing a glucose molecule at carbon 27) have been isolated and reported from aerial parts, roots and berries of *Withania somnifera* species (Table 1a). The concentration of withanolides usually ranges from 0.001 to 0.5% dry weight (DW) (Hmood and Salim 2017) [15].

Table 1 a: Phytoconstituents of Ashwagandha

Plant part	Phytochemicals
Leaves	The leaves of plant are reported to contain 12 withanolides, 5 unidentified alkaloids, glycosides, chlorogenic acid, many free amino acid, glucose, condensed tannins and flavonoids. Withaferin A, a steroidal lactone is the most significant withanolide present in the leaves (Khare, 2007) [16]
Fruits	The green berries contain proteolytic enzyme, amino acids, condensed tannins and flavonoids. The occurrence of a proteolytic enzyme, chamase in the berries may be responsible for the high content of the amino acids (Qamar <i>et al.</i> , 2012) [33]
Root	The roots are reported to contain amino acids, alkaloids, steroids, starch, reducing sugars, hentriacontane, glycoside, cuscohygrine, tropine, anaferine, withanamine, pseudo withanine, somnine, somniferine (Khare, 2007) [16]
Stem	The stem of the plant contain condensed tannins and flavonoids (Qamar <i>et al.</i> , 2012) [33]
Shoot	The tender shoot is rich in crude protein, calcium and phosphorus. They are reported to contain scopoletin (Qamar <i>et al.</i> , 2012) [33]

The biologically active chemical constituents in the *Withania somnifera* (L.) Dunal are alkaloids (isopelletierine, anaferine); steroidal lactones (withanolides, withaferins); saponins containing an additional acyl group (sitoindoside VII and VIII) and withanolides with glucose at carbon 27 (sitonidoside XI and X). It is also rich in iron,

copper, nickel, manganese, and zinc which are known for their significant role in therapeutic properties (Mishra *et al.*, 2000; Shirin *et al.*, 2009) [27]. Apart from these contents, plant also contain chemical constituents like withanol, acylsterylglucosides, starch, reducing sugar, hantreacotane, ducitol, a variety of amino acids including aspartic acid, proline, tyrosine, alanine, glycine, glutamic acid, cysteine and tryptophan (Gupta *et al.*, 2007) [13]. The roots of *Withania somnifera* (L.) Dunal consists primarily of compounds known as withanolides, which are believed to account for its extraordinary medicinal properties. Withanolides are steroidal and bear a resemblance, both in their action and appearance, to the active constituents of Asian ginseng (*Panax ginseng*). A large number of withanolides have been identified and different therapeutic activities are associated with different constituents viz., withaferine A associated with anti-inflammatory activity and is a safe radiosensitizer and chemotherapeutic agent, withanolide A and withanoside IV known for treatment of neurodegenerative diseases and withanone for anti-cancer and anti-aging activities etc. Lal *et al.* (2006) [19] recognized that the chloroform extract of the fresh berries of *Withania somnifera* (L.) Dunal has been investigated to afford stigmaterol, its glucoside, withanone, 27-hydroxywithanolide A along with two new withanolides, namely, iso-withanone (1) and 6 α ,7 α -epoxy-1 α ,3 β ,5 α -trihydroxy-witha-24-enolide (2). Compound 1 possesses an uncommon 17 α -oriented side chain whereas compound 2 has 1 α ,3 β -dihydroxy group which is a molecule of genetic significance for withasteroids. Chatterjee *et al.* (2010) [7] identified 62 major and minor primary and secondary metabolites from leaves and 48 from roots. Twenty-nine of these were common to the two tissues. Few of these included fatty acids, organic acids, amino acids, sugars and sterol based compounds. Eleven bioactive sterol-lactone molecules were also identified. Twenty-seven of the identified metabolites were quantified.

Withanolides are generally defined as C-28 steroidal lactones. The presence of a lactone ring with C-22 and C-26 oxygen functions to form a six or five membered lactone ring on an ergostane skeleton. Withanolides may be divided into the following groups like withanolide glycoside, withaphysalins, physalins, nicandrenones or ring D aromatic withanolides, jaborols, acnistins, perculactones and withajardines (Mathur and Agarwal, 2011) [24]. Withaferin A (4 β , 27-dihydroxy-1-oxo-5 β , 6 β -epoxywitha-2-24-dienolide) was the first member of this group of compounds to be isolated from *W. somnifera* (L.) Dunal (Tursunova *et al.*, 1977) [45]. Seven new withanolide glycosides called withanosides I, II, III, IV, V, VI and VII had been identified and isolated (Matsuda *et al.*, 2001) [25], which come under saponins containing an additional acyl group (sitoindoside VII and VIII).

All terpenoids, including both primary metabolites and more than 25,000 secondary compounds, are derived from the five-carbon precursor isopentenyl diphosphate (IPP). A total of about 12,000 known alkaloids, which contain one or more nitrogen atoms, are biosynthesized principally from amino acids and 8000 phenolic compounds are formed by shikimic acid pathway or the malonate/acetate pathway.

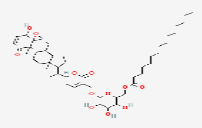
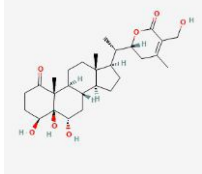
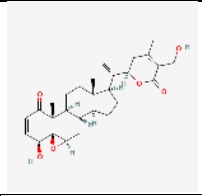
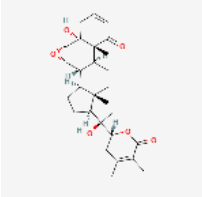
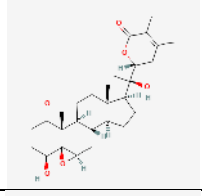
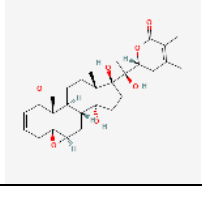
Pharmacological Activity of Ashwagandha

Two major classes of compounds are identified in the Ashwagandha viz., steroidal alkaloids and steroidal lactones which are responsible for its wide range of beneficial effects

of Ashwagandha. the root of Ashwagandha is mainly used in aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic and stimulant. It is also found useful in neurodegenerative diseases such as Parkinson's, Huntington's and Alzheimer's diseases (Table 1b). Ashwagandha has been also used as liver tonic, anti-inflammatory agent, astringent and to treat bronchitis,

asthma, ulcers, emaciation, insomnia and senile dementia. Withaferin- A is therapeutically active withanolide reported to be present in leaves. In animal studies, Withaferin-A has shown significant anticancer activity. Clinical trials and animal research support the use of Ashwagandha for anxiety, cognitive and neurological disorders, inflammation and Parkinson's disease (Singh *et al.*, 2010) [37].

Table 1b: Pharmacological activities of different withanolides, glycowithanolides and alkaloids (Mir *et al.*, 2012) [26]

Sr. No.	Chemical constituent	Chemical Structure	Molecular Formula	Biological activity
1	Glycowithanolides (Sitoindosides X)		C ₅₀ H ₇₈ O ₁₂	Immunomodulatory, Central Nervous System (CNS) effect, antioxidant, antistress
2	Total alkaloids (somniferin)		C ₂₈ H ₄₂ O ₇	CNS system, respiration, cardiovascular system, hyptonic activity, hypothermic and nictinolytic
3	Withaferin A		C ₂₈ H ₃₈ O ₆	Antibacterial, antifungal, antibiotic, antimitotic, antitumor, radio sensitizer, cytotoxic, anti-inflammatory, antiarthritic, Alzheimer immunosuppressant
4	Withanolide A		C ₂₈ H ₃₈ O ₆	Sedative and hypnotic
5	Withanolide D		C ₂₈ H ₃₈ O ₆	Antitumour
6	Withanolide E		C ₂₈ H ₃₈ O ₇	Antibacterial, Insect antifeeder, Immuno suppressive

Sukanya *et al.* (2011) assessed one hundred and eight selected accessions of *W. somnifera* (L.) Dunal for chemical diversity in relation to withanolide content. The accessions differed both quantitatively and qualitatively for withanolides estimated by High Performance Liquid Chromatography (HPLC). The total withanolide content ranged from 0.066 to 0.588% on dry root weight basis in the accessions and as many as 13 accessions recorded more than 0.30%, which are considered as commercially viable. The analysis of withanolide profile in these accessions paved the way for identification of superior chemotypes, which recorded higher content of specific withanolides of therapeutic importance, thus indicating considerable diversity in terms of phytochemicals.

Enhancement of secondary metabolites via Arbuscular mycorrhizal (AM) fungi

Mycorrhiza (Greek: *mykós*, "fungus" and *riza*, "roots") is a symbiotic association composed of a fungus and roots of a vascular plant. Frank (1885) [11], German scientist, around 100 years ago, was probably the first to recognize the widespread nature of associations between plant roots and mycorrhizal fungi (Frank & Trappe, 2005) [11]. Arbuscular mycorrhizal (AM) fungi have been widely used now a day in agriculture to improve the cultivation of medicinally important metabolites in different medicinal plants. AM symbiosis was found beneficial for the cultivation of medicinal plants by improving content of secondary metabolites, the rate of photosynthesis, antioxidants and nutrition uptake of plants (Raei *et al.*, 2013) [34].

In a mycorrhizal association, the fungus colonizes the host plant's roots, either intracellular as in Arbuscular Mycorrhizal Fungi (AMF or AM), or extracellular as in ecto mycorrhizal fungi. They are an important component of soil life and soil chemistry. Mycorrhizal associations are the most important and prevalent mutualistic symbiosis which involves three-way interactions between host plants, mutualistic fungi and soil factors. Arbuscular Mycorrhizal (AM) fungi [or Vesicular-Arbuscular Mycorrhiza, (VAM fungi)] belongs to the Phylum Glomeromycota and are symbionts with terrestrial plant roots. The association is generally mutualistic, but occasionally weak pathogenic. This mutualistic association provides the fungus with relatively constant and direct access to carbohydrates, such as glucose and sucrose. The carbohydrates are translocated from their source (usually leaves) to root tissue and finally to the plant's fungal partners (Bago *et al.*, 2003) [3]. In return, the plant gains the benefits through mycelium's higher absorptive capacity for water and mineral nutrients due to the comparatively large surface area of mycelium with root, thus improving the plant's mineral absorption capabilities (Smith and Smith, 1997; Smith and Read, 2008) [38, 39]. This two-way exchange of nutrients in AM symbiosis takes place between the plant root cortical cell and the fungal arbuscule that has penetrated it. This is the symbiotic interface and on the one side it is bordered by the plant plasma membrane and its extracellular matrix, and on the other side by the fungal plasma membrane and its extracellular matrix. Plant roots alone may be incapable of taking up phosphate ions that are demineralized in soils with a basic pH. The mycelium of the mycorrhizal fungus can, however, access these phosphorus sources and make them available in the soluble form to the plants they colonize (Li *et al.* 2006). Therefore, plants with mycorrhizal association will have higher efficiency for nutrient absorption such as nitrogen, phosphorus, potassium, calcium, magnesium, zinc and copper. It also increase plant resistance to diseases, parasites, water drought, salinity and heavy metal toxicity. The symbiosis of AM with the favorable characteristics of medicinal plants leads to improvement in the production and accumulation of important active ingredients of medicinal plants such as terpenes, phenols and alkaloids which ultimately improve the quality of herbal materials (Zeng *et al.*, 2013) [51]. Zubek *et al.* (2015) [52] testified that there is an increase in production of secondary metabolites in *Viola tricolor* shoots which may be due to improved mineral nutrition in presence of AMF and/or a result of general plant defense reaction to fungal colonization.

Arbuscular mycorrhizal fungi (AMF) increase the growth and concentration of primary and secondary metabolites in a medicinal plant Ebil (*Anadenanthera colubrine*) by increasing the phosphorus supply to the plant (Pedone *et al.*, 2013) [31].

Significance of plant fungus symbiosis

Mycorrhizal infection enhances plant growth by

- Increasing the surface area of absorption within the soil;
- Mobilizing sparingly-available nutrient sources from unavailable compounds;
- Excretion of chelating compounds or ectoenzymes;
- Alleviation of physical stresses;
- Changes in antagonist or deleterious microbe populations in the mycorrhizosphere;

- Change in biochemical compounds and hormones related with plant response.

Conclusion

Ashwagandha is an important anti-cancerous, immunomodulatory, adaptogen, anti-aging and antioxidant plant. Although a large amount of literature has been cited on the physiological effects of the active constituents present in the plant, but few reports are available methods of enhancement of secondary metabolites in the medicinal plant. Arbuscular mycorrhizal fungi (AMF) increase the growth and concentration of primary and secondary metabolites in a medicinal plant Ebil (*Anadenanthera colubrine*) by increasing the phosphorus supply to the plant (Pedone *et al.*, 2013) [31]. Therefore, Arbuscular mycorrhizal fungi (AMF) can also be apply on medicinal plant *W. somnifera* (L.) Dunal to enhance the production of important phytochemicals associated with treatment of many diseases.

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