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META-ANALYSIS OF DISEASES CAUSING FACTORS IN ASHWAGANDHA

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Abstract— Ashwagandha scientifically known as *Withania somnifera* described in Ayurveda as a powerful rejuvenating herbal medicine. Their roots are thick and whitish brown, the leaves are somewhat oval in shape, hairless, and small. *Withania somnifera* is pharmacology as an adaptogen, antibiotic, abortion, aphrodisiac, astringent, anti-inflammatory, obtrusive, diuretic, narcotic, sedative, tonic, etc. Ashwagandha has been found to provide strong antioxidant protection. It stimulates the activation of immune system cells such as lymphocytes and phagocytic cells. *Withania somnifera* (L.) Dunal has been used to treat all kinds of illnesses and human illnesses. *Withania somnifera* is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3000 years.

Keywords— Ashwagandha, *Withania somnifera*, Withaferin A, Withanine somniferine, adaptogen, antibiotic, micro propagation.

I. INTRODUCTION

A member of the Solanaceae family *Withania somnifera* (WS) commonly known as Ashwagandha or Indian Jinsen or Winter Cherry ⁽¹⁾ is a two-foot-tall woody shrub. It grows throughout Africa, the Mediterranean region, and in Asia continent. Ashwagandha scientifically known as *Withania somnifera* described in Ayurveda as a powerful rejuvenating herbal medicine. It has been used for over 2,000 years in India, the Middle East, and parts of North Africa. *Withania somnifera* is cultivated in many of India's arid regions, such as Mandasaur, Madhya Pradesh, Punjab, Sindh, Gujarat, Kerala, Rajasthan. It also occurs in Nepal, China and Yemen. *Withania somnifera* is abundantly cultivated in Congo, South Africa, Egypt, Morocco, Jordan and Pakistan ⁽²⁾. This is an upright evergreen shrub, found in barren land and mountains in the arid regions. Plants have roots that are thick and whitish brown, the leaves are somewhat oval in shape, hairless, and small. The branches are widespread with leaves simple, alternating or opposite overall, the base is pointed. The flowers are discreet, greenish, or slimy yellow in the form of axillary umbels. The berries are small, spherical, orange-red when ripe and surrounded by permanent scabs. Yellow kidney-shaped seeds. The flowers are greenish-yellow and can be found in some axillary inflorescences. The maximum length of the flower stem is 4 mm. The sword is 5 mm long and has a star-shaped

hairy appearance. The length of the crown is 8 mm and it is divided in more than half. The filament is 3 mm long, thin and smooth, the anthers are wide and oval (almost circular), and the length is 1.25 mm. The ovaries and style are bald. The fruits are reddish-yellow berries, smooth, 6 mm in diameter, surrounded by bulging calix. It reaches over 5-8 mm in diameter, is spherical, slightly pentagonal, has a unified calix and is hairy on the outside. The seeds are 2.5 mm in diameter, yellow and slightly scabbed ⁽³⁾.

Various varieties cultivated around the Indian Sub-Continent like Posita and Lucknow are high-yielding varieties published by CSIR-CIMAP, Lucknow, Javahal 20 is cultivated in Madhya Pradesh. WSR is another variety published by CSIR-Area Studies, Jammu. Semi-arid tropical regions with rainfall of 500-750 mm are suitable for growing this crop. A dry season is required during the growing season. Rain at the end of winter promotes proper root development. For growth, well-drained sandy loam or light red soil with a pH of 7.5-8.0 is suitable. It grows better at altitudes of 600-1200 meters. The optimum temperature for cultivation is 20°C to 35°C.

Ashwagandha is harvest at the end of the rainy season. It requires a relatively dry season and the roots are fully developed by the late winter of a year or two. Areas with rainfall of 650 to 750 mm are ideal for cultivation.

II. PLANT IMPORTANCE

Centuries of Ayurvedic drug experience at *Withania somnifera* is pharmacology as an adaptogen, antibiotic, abortion, aphrodisiac, astringent, anti-inflammatory, obtrusive, diuretic, narcotic, sedative, tonic, etc. Ashwagandha has been found to provide strong antioxidant protection ⁽⁴⁻⁵⁾. It stimulates the activation of immune system cells such as lymphocytes and phagocytic cells ⁽⁶⁻⁷⁾. It counteracts the effects of stress and promotes general well-being ⁽⁸⁾.

a. Anti-inflammatory effect

Withaferin A has a fairly strong anti-arthritis and anti-inflammatory effect. The anti-inflammatory effect is due to the biologically active steroid, which is based on withaferin A. This is an effective dose as hydrocortisone sodium succinate ⁽⁹⁾. It has been shown to be effective in controlling arthritis syndrome without the effects of toxicity. In contrast to animals treated with hydrocortisone that lost weight, animals treated with withaferin A showed weight gain with arthritis syndrome. Asgand (*Withania somnifera*) exhibits anti-inflammatory



properties in many animal models of inflammation such as carrageenan-induced inflammation, cotton pellet granulomas, and adjuvant-induced arthritis. Detailed studies are being conducted to investigate two models of inflammation, the release of serum β -1 globulin during inflammation.

b. Anti-stress

Ashwagandha has been traditionally used to stabilize the mood of patients with behavioural disorders. Studies have shown that this herb provides anti-depressant and anxiolytic effects on rodents comparable to the antidepressant imipramine and the anxiolytic lorazepam (Ativan)⁽¹⁰⁾. In fact, Ashwagandha is one of the most widely used tranquilizers in India and has the same importance as Chinese ginseng. It acts primarily on the reproductive and nervous system, providing a rejuvenating effect on the body, improving vitality and helping recovery from chronic illness⁽¹¹⁾. Chronic stress can cause conditions such as cognitive impairment, immunosuppression, sexual dysfunction, gastric ulcers, irregular glucose homeostasis, and altered plasma corticosterone levels. In a rat model of chronic stress syndrome, extracts of *Withania somnifera* and *Panax ginseng* are compared and contrasted for their ability to improve some of the adverse effects of chronic stress⁽¹²⁾. Studies show that both Ashwagandha and *Panax ginseng* reduced the frequency and severity of stress-induced ulcers, reversed the suppression of stress-induced male sexual behavior, and suppressed the effects of chronic stress on the retention of learned tasks.

c. Antibiotic Activity

Antibiotic activity in both roots and leaves has recently been experimentally demonstrated. Withaferin A at a concentration of 10 μ g / ml suppressed the growth of various Gram-positive, acid-fast and aerobic bacilli and pathogens. It was active against *Micrococcus Pyogenes* var *aureus* and partially inhibited the activity of *Bacillus subtilis* glucose-6-phosphate dehydrogenase. Shrub extracts are effective against vaccinia virus and *Entamoeba histolytica*⁽¹³⁾. *Asgand* has shown a protective effect against systemic *Aspergillus* infection. This protective activity is likely to be associated with activation of macrophage function, as evidenced by the observed increase in phagocytosis induced by Ashwagandha treatment in mice and intracellular killing of peritoneal macrophages⁽¹⁴⁾. The antibiotic activity of withaferin A is due to the presence of unsaturated lactone rings. Lactones had a potent therapeutic effect on experimentally induced abscesses in rabbits and were slightly more potent than penicillin. It underpins the reputation of leaves as a treatment for ulcers and carbuncles in indigenous medicine⁽¹⁵⁾.

d. Antioxidant effect

The brain and nervous system are richer in lipids and iron, which are known to be important in the production of reactive oxygen species, making them more vulnerable to free radical damage than other tissues. Free radical damage to nerve tissue

may be involved in normal aging and neuro degenerative diseases such as Epilepsy, Schizophrenia, Parkinson's disease, Alzheimer's disease and other illnesses. The active ingredients of *Withania somnifera*, withaferin A and withanolide (glycowitanolide), use the major free radical trapping enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) and tested for antioxidant activity. Decreased activity of these enzymes leads to the accumulation of toxic oxidative free radicals and the resultant denaturing effect. Elevated levels of these enzymes represent increased antioxidant activity and protective effects on nerve tissue. The increase is comparable to that observed with the administration of deprenyl (a known antioxidant). This means that WS has antioxidant properties in the brain, which may be responsible for its multiple pharmacological properties⁽¹⁶⁾. In addition to hepatic lipid peroxide (LPO), the serum enzymes alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase were evaluated as indicators of hepatotoxicity. Iron overload induced a significant increase in hepatic LPO and enzyme serum levels, which was dose-dependently attenuated by glycowitanolide (*Withania somnifera* glycoprotein) and silymarin⁽¹⁷⁾.

e. Anti-parkinsonian properties

Parkinson's disease is a neurodegenerative disease characterized by the selective loss of dopamine neurons in the substantia nigra. However, the events that trigger or mediate the loss of substantia nigra dopamine neurons remain unknown. Neurorelaxant-induced catalepsy has long been used as an animal model for screening drugs for parkinsonism. *Withania somnifera* significantly suppresses haloperidol or reserpine-induced catalepsy and provides hope for the treatment of Parkinson's disease⁽¹⁸⁾. In another study, 6-hydroxydopamine (6-OHDA) is one of the most commonly used rat models of Parkinson's disease. There is typical evidence in the literature that 6-OHDA mediates its toxic symptoms through oxidative stress. The anti-Parkinson's disease effect of WS extract has been reported for its potent antioxidant and free radical inhibitory effects in a variety of diseases. The *Withania somnifera* extract significantly reversed all parameters in a dose-dependent manner⁽¹⁹⁾. Oxidative stress may play an important role in the pathophysiology of reserpine-induced abnormal oral movements⁽²⁰⁾. WS may significantly reverse the toxic symptoms induced by catalepsy, tardive dyskinesia, and 6-hydroxydopamine, providing a new therapeutic approach to the treatment of Parkinson's disease.

f. Cardiovascular protection

Withania somnifera may be useful as a general tonic because of its beneficial effects on the cardiopulmonary system, as reported. The effects of *Withania somnifera* on the cardiovascular and respiratory systems of dogs and frogs have been studied⁽²¹⁾. Alkaloids have shown long-term hypotensive, bradycardia and respiratory stimulation effects in



dogs. In this study, it was found that the antihypertensive effect is mainly due to autonomic ganglion blockade, and the inhibitory effect on higher brain centers also contributes to hypotension. Alkaloids stimulated the vasomotor and respiratory centers of the dog's brain stem. The direct effect of cardiac depressant in dogs was thought to be due to ganglion obstruction. Alkaloids provided an immediate predominant but short-lived cardiostimulant effect and a weak but sustained cardiostimulant effect in isolated normal and hypodynamic frog hearts. *Withania somnifera* showed a strong cardioprotective effect in an experimental model of isoprenaline-induced myonecrosis in rats. Increased endogenous antioxidants, maintenance of myocardial antioxidant status, and most significant recovery of altered hemodynamic parameters may contribute to its cardioprotective effect⁽²²⁾.

g. Immunomodulatory Activity

Ashwagandha showed a significant regulation of immune responsiveness in animal models. Administration of Ashwagandha was found to prevent myelosuppression in mice treated with three immunosuppressive drugs such as cyclophosphamide, azathioprine, prednisolone. Ashwagandha treatment was found to significantly increase Hemoglobin concentration, red blood cell count, platelet count, and body weight in mice⁽²³⁾. Administration of Ashwagandha extract was found to significantly reduce leukopenia induced by cyclophosphamide (CTX) treatment. Administration of Ashwagandha extract increased the number of β -esterase-positive cells in the bone marrow of CTX-treated animals compared to the CTX monotherapy group⁽²⁰⁾, also was found to significantly reduce sub lethal doses of gamma-induced leukopenia⁽²⁴⁾. Withaferin A and withanolide E showed specific immunosuppressive effects on human B and T lymphocytes and mouse thymocytes. Withanolide E had a specific effect on T lymphocytes, whereas withaferin A affected both B and T lymphocytes⁽²⁵⁾.

h. Anti-hyperglycaemic Effect

Since the hyperglycemic activity of Streptozotocin (STZ) is the result of decreased superoxide dismutase (SOD) activity in islet cells, which leads to the accumulation of denatured oxidants, this antihyperglycemic effect is associated with the activity of islet cells to remove free radicals and may be caused. Free radicals in beta islet cells lead⁽²⁶⁾.

i. Anti-carcinogenic activity

Ashwagandha is said to have an anti-carcinogenic effect. Studies in animal cell culture have shown that herbs reduce levels of nuclear factor Kappa B, suppress intracellular tumor necrosis factor, and enhance apoptotic signaling in cancer cell lines⁽²⁷⁾. One of Ashwagandha's most exciting potential uses its ability to fight cancer by reducing the size of tumors⁽²⁸⁻²⁹⁾. To study its use in the treatment of various types of cancer, researchers have studied the anti-tumor effect of *Withania somnifera*.

j. Antivenom

The toxic hyaluronidase aids in the rapid spread of the toxin by destroying the integrity of the extracellular matrix of the tissue of the victim. Hyaluronidase Inhibitor is purified from *Withania somnifera*. Glycoprotein inhibited the hyaluronidase activity of cobra (*Naja naja*) and viper (*Daboia russelii*) venom, as determined by zymogram assay and staining of different activity of skin tissue. *Withania somnifera* glycoprotein completely inhibited the activity of enzymes at a concentration of 1:1 w/w of venom against *Withania somnifera* glycoprotein. The topical use of plant extracts as antidotes in rural India for snakebite victims appears to be scientifically based⁽³⁰⁾.

k. Anxiety and depression

We evaluated the anxiolytic and anti-depressant effects of bioactive *Withania somnifera* glycoprotein isolated from *Withania somnifera* roots. *Withania somnifera* glycoprotein was orally administered once daily for 5 days and the results were compared to the anxiolytic effects induced by the benzodiazepine lorazepam and the tricyclic anti-depressant imipramine. *Withania somnifera* glycoprotein elicited anxiolytic effects comparable to trolazepam in tests of increased mazes, social interactions, and feeding latency in unfamiliar environments. *Withania somnifera* glycoprotein also showed anti-depressant effects comparable to those induced by imipramine in forced swimming, induced behavioral distress and learned helplessness tests. This study supported the use of *Withania somnifera* as a mood stabilizer in the clinical status of Ayurvedic anxiety and depression⁽³¹⁾.

III. DRUG FORMULATION

Traditional medicine has a long history of serving people around the world. It is the sum of accumulated knowledge⁽³²⁾. The World Health Organization (WHO) recognizes the importance of medicinal plants for public health in developing countries⁽³³⁾. In the current situation, herbal remedies play an important role in the healthcare system because these medicines are easily and cheaply available, safe and trusted by people⁽³⁴⁾. Traditional medicine-inspired approaches remain important for treating chronic diseases and facilitating the discovery of natural products⁽³⁵⁾. *Withania somnifera* (L.) Dunal has been used to treat all kinds of illnesses and human illnesses for over 2500 years⁽³⁷⁾. It is an adaptogen, a non-toxic herb that acts non-specifically to normalize physiological function by acting on the HPA axis and the neuroendocrine system⁽³⁸⁾. *Withania somnifera* is an important medicinal plant that has been used in Ayurvedic and indigenous medicine for over 3000 years⁽³⁹⁻⁴⁰⁾. The main components of *Withania somnifera* are alkaloids and steroid lactones such as Withanine, Somniferin, Somnin, Somniferinin, Withananin, Pseudowithanin Tropine, Pseudotropin, Choline, Anaferin, Anahydrin, Isopertierin⁽³⁶⁾. Ayurvedic classics state that Rasayana is described as a health tonic for children, a drug for middle-aged people, and a rejuvenating herbal or metal formulation for the elderly. Among the Ayurvedic Rasayana

herbs, Ashwagandha has the highest rank ⁽⁴¹⁻⁴⁴⁾ and just as nourishing the roots helps the tree to rejuvenate, activating the reproductive organs helps to rejuvenate the body ⁽⁴⁵⁾. Withania somnifera is generally said to have powerful aphrodisiac, sedative, rejuvenating, and life-prolonging effects ⁽⁴⁶⁻⁴⁷⁾. It is also used as a common energy-enhancing tonic known as Medhya Rasayana (a nootropic herb) ⁽⁴⁸⁾.

It has general irritation and regenerative properties and is used to treat nerve fatigue, memory loss, insomnia, malaise, potency problems, skin problems and cough, among others. It improves learning ability and memory. Ashwagandha favors the use of adjuvants in the treatment of various psychosomatic disorders, improving tissue vitality, physical and mental endurance, and neuromuscular strength ⁽⁴⁹⁾. Ashwagandha has long been used as Rasayana, especially for children, in the treatment of malnutrition ⁽⁵⁰⁻⁵¹⁾. It has also been reported to have immunomodulatory ⁽⁵²⁻⁵³⁾, anti-aging ⁽⁵⁴⁾, anti-stress ⁽⁵⁵⁾, and cardiovascular protection properties ⁽⁵⁶⁾. It has also been shown to be effective against hypothyroidism ⁽⁵⁷⁾, anxiety, and depression ⁽⁵⁸⁾.

Ayurvedic pharmacodynamic properties of Withania somnifera ⁽⁵⁹⁻⁶⁰⁾

Rasa: Tikta (Bitter), Katu (Pungent), Madhura (Sweet)

Guna: Laghu (Light), Snigdha (Unctuous)

Virya: Ushna (Hot)

Vipaka: Madhura (Sweet)

Doshakarma: Kapha-Vatashamaka (Alleviates Kapha and VataDosh)

The root part of this plant is Lasayanas improves immunity to disease, suppresses the aging process, rejuvenates the body in a debilitated state, enhances the individual's ability to resist harmful environmental factors, and enhances mental well-being. (Famous for promoting health and longevity by producing) ⁽⁶¹⁾. Anxiety is a common emotional phenomenon that represents human central nervous system disorders ⁽⁶²⁾. It is unpleasant in nature and is an emotional state with anxiety, and fear or anxiety about defined or undefined future threats ⁽⁶³⁾. Traditional uses of this plant include anti-arthritis, nerve and sexual disorders, nerves, anti-stress, sedation, anti-inflammatory, anti-hypertensive, nerve sedation, free radical scavengers, antioxidants, immunomodulators, anti-cancer and includes anti-stress treatment ⁽⁶⁴⁻⁶⁵⁾. Prolonged exposure to this disorder can upset an individual mental and physiological state and lead to other illnesses such as high blood pressure, metabolic disorders, depression and heart disease. These

diseases are becoming major global diseases and their prevalence is increasing rapidly. There are many plants used to treat anxiety disorders. Withania somnifera is one of these plants.

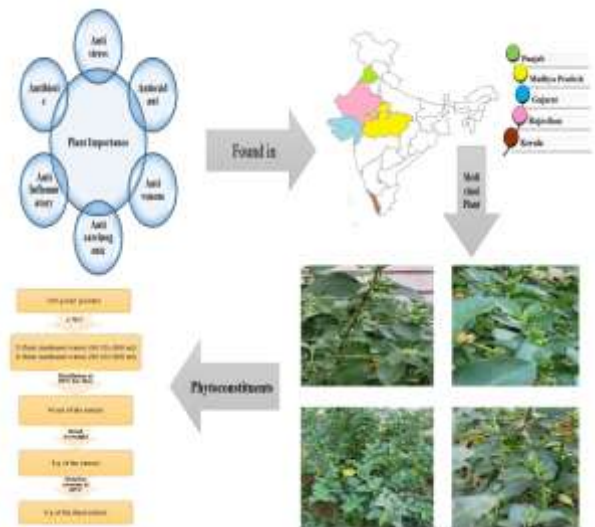


Fig. 1. Flow diagram of extraction drug from ashwagandha plant



Fig 2. Image of Ashwagandha Plant.



Sl. No.	Behavioural parameters	Dose (mg/kg)	Usages	Compound	Reference
1.	Effect of <i>Withania somnifera</i> on locomotor activity	100-200	Hypoactivity	3-NP-induced	Kumar P, Kumar A (2009), Possible neuro protective effect of <i>Withania somnifera</i> root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. <i>J Medicinal Food</i> ; 12: 591-600. Kumar A, Kalonia H (2007), Protective effect of <i>Withania somnifera</i> Dunal on behavioral and biochemical alteration in sleep-disturbed mice(Grid water over suspended method). <i>Indian J Exp Biol</i> ; 45: 524-528.
2.	Effect of <i>Withania somnifera</i> on motor coordination	100-200	Locomotory activity	3-NP-induced	Kumar P, Kumar A (2009), Possible neuro protective effect of <i>Withania somnifera</i> root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. <i>J Medicinal Food</i> ; 12: 591-600. Parihar MS et al (2004), Susceptibility of hippo campus and cerebral cortex to oxidative damage in streptozotocintreated mice: prevention by extracts of <i>Withania somnifera</i> and Aloe vera. <i>J Clinical Neurosci</i> ; 11: 397-402.
3.	Effect of <i>Withania somnifera</i> on anxiety	100-200	Anxiolytic activity	3-NP-induced	Khan ZA, Ghosh AR (2010), Possible nitricoxide modulation in protective effects of withaferin A against stress induced neurobehavioral changes. <i>J Medicinal Plants Res</i> ; 4: 490-495. Khan ZA, Ghosh AR (2011), Age related differences in stress-induced neuro-behavioral patterns in rats modulate by withaferin-A and other antioxidants. <i>J Pharmacy Res</i> ; 4:1281-1284
4.	Effect of <i>Withania somnifera</i> on muscle grip strength	100	Muscle grip strength	3-NP-induced, MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced	Kumar P, Kumar A (2009), Possible neuroprotective effect of <i>Withania somnifera</i> root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. <i>J Medicinal Food</i> ; 12: 591-600. Sankar SR et al (2007), The neuro protective effect of <i>Withania somnifera</i> root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables. <i>Cell Mol Biol Lett</i> ; 12:473-481. Chaudhary G et al (2003), Evaluation of <i>Withania somnifera</i> in middle cerebral artery occlusion model of stroke. <i>ClinExp Pharmacol Physiol</i> ; 30: 399-404.
5.	Effect of <i>Withania somnifera</i> on spatial reference memory & cognitive behavior	100-200	Anti-memory loss	3-NP-induced	Naidu PS et al (2006), Effect of <i>Withania somnifera</i> root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. <i>Phytother Res</i> ; 20: 140-146. Baitharu I et al (2013), <i>Withania somnifera</i> root extract ameliorates hypobarichypoxia induced memory impairment in rats. <i>J Ethnopharmacol</i> ; 145:431-441. Kumar P, Kumar A (2008), Effects of root extract of <i>Withania somnifera</i> in 3-Nitropropionic acid-induced cognitive dysfunction and oxidative damage in rats. <i>Int J Health Res</i> ; 1: 139-149. Trigunayat A et al (2007), Neuro protective effect of <i>Withania somnifera</i> (WS) in cerebral ischemia-reperfusion and long-term hypoperfusion induced alterations in rats. <i>J Natural Remedies</i> ; 7: 234-246.
6.	Effect of <i>Withania somnifera</i> on gait abnormalities	100	Anti-gait abnormalities	MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induced	Sankar SR et al (2007), The neuroprotective effect of <i>Withania somnifera</i> root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables. <i>Cell Mol Biol Lett</i> ; 12:473-481. Rajasankar S et al (2009), <i>Withania somnifera</i> root extract improves catecholamines and physiological abnormalities seen in a Parkinson's disease model mouse. <i>J Ethnopharmacol</i> ; 125: 369-373.

Table: Effect of Ashwagandha (*Withania somnifera*) on different dose and their usages.



Chemical Constituents of *Withania somnifera*

The biologically active chemical components of herb are proteins, amino acids, carbohydrates, steroids, alkaloids, oxalic acids, flavonoids, phenolic compounds, tannins, withanoloids, inorganic compounds and saponins⁽⁶⁶⁻⁶⁹⁾.

A plant from genus *Withania*

Among the plants known to have medicinal properties, the plants of the genus *Withania* belonging to the Solanaceae family are very important for their therapeutic potential. *Withaniagraduateens*, *Withaniasimonii*, *Withaniaadunensis*, *Withaniariebeckii* are examples of known important species of the genus *Withania* that grow in different parts of the world and are known for their medicinal properties⁽⁷⁰⁻⁷⁷⁾. Therapeutic uses of *Withania somnifera*. *Withania somnifera* Dunal and *Withania somnifera* Kaul are two subspecies of this plant.

Therapeutic uses of *Withania somnifera*

Withania somnifera is one of the major herbal ingredients of geriatric tonics mentioned in the Indian medical system. In traditional Ayurvedic medicine, plants are said to have powerful aphrodisiac, rejuvenating and life-prolonging effects. It is commonly irritating and regenerative and is used to treat nerve fatigue, memory loss, insomnia, malaise, erectile dysfunction, skin problems, coughing and more. It improves learning ability and memory. The traditional use of ashwagandha is to increase energy, youthful vitality, stamina, strength, health, maintenance of body time elements, vitality, muscle fat, blood, lymph, semen and cell production. Relieves chronic fatigue, weakness, dehydration, bone weakness, loose teeth, thirst, impotence, premature aging, weakness, recovery and muscle tension. It helps strengthen the body by rejuvenating the reproductive organs, just as the tree is strengthened by providing immune regulation and hematopoiesis to the roots⁽⁷⁸⁻⁸⁰⁾.

IV. PLANT TISSUE CULTURE

Plant tissue culture is a method for cultivating plant cells, tissues, and organs on artificial media in an aseptic environment with managed photoperiod, temperature, and humidity levels. The discovery and characterisation of plant hormones were intimately related to the development of plant tissue culture as a fundamental science, which has helped us better understand how plants grow and develop. Additionally, the capability of cultivating plant cells and tissues in culture and controlling their development serves as the foundation for numerous practical applications in horticulture, industrial chemistry, agriculture, and plant genetic engineering⁽⁸¹⁾.

A significant medicinal plant from the Solanaceae family is ashwagandha. It also goes by the name Winter Cherry⁽⁸²⁾.

A 99.75 percent success rate was found for the threatened medicinal herb *Withania somnifera*⁽⁸³⁾. In the Vedas, it is referred to as herbal tonic and health food, and in the

conventional Indian medical system, it is known as "Indian Ginseng"⁽⁸⁴⁾.

Numerous pharmacological investigations also point to the plant's potential for multiple medical uses, including adaptogenic, antioxidant, anticancer, and cardiovascular effects⁽⁸⁵⁾.

Because it may play a therapeutic role in disorders of the central nervous system, including Parkinson's disease, Alzheimer's disease, and epilepsy, these plants are propagated⁽⁸⁶⁾. The active ingredients in this plant's medicinal characteristics, which are found in various plant sections, include withanolides, withaferin, withanosides, withanine, somniferine and sitoindosides⁽⁸⁵⁾.

Numerous uses of this plant, which has therapeutic potential, have required its extensive accumulation as a source of raw materials for the pharmaceutical sector⁽⁸⁷⁾. Due to the limited viability of its stored seeds⁽⁸³⁾ and low seed germination rates, *Withania somnifera* is typically propagated primarily through seeds⁽⁸⁸⁾. This method is insufficient to meet commercial demand⁽⁸⁹⁾. One issue with commercial production is the slow productivity of seed germination strains.

Techniques used in tissue culture can be crucial in the growth and quality development of this crucial plant for medicine. *Withania somnifera* has been successfully micropropagated using a variety of explants, including shoot tips⁽⁹⁰⁻⁹²⁾, cotyledons⁽⁹³⁾, embryos, hypocotyl⁽⁹⁵⁾, leaf disc, roots⁽⁹⁴⁾, apical buds⁽⁹⁶⁾, nodal segments⁽⁹⁷⁾, and axillary buds⁽⁹⁸⁾. Thus, the quick multiplication and preservation of the herb's germplasm depend heavily on an effective in vitro propagation technique. From leaf discs grown on MS media supplemented with varied amounts of indole-3-acetic acid (IAA), 6-benzyladenine (BA), and kinetin (KN), they demonstrated direct shoot regeneration⁽⁹⁹⁾.

Material and methods:

Material required:

Explant of *Withania somnifera*, MS media, Sucrose, Agar, HgCl₂, Distilled water, Ethanol, Detergent, BAP, NAA.

Instrument required:

Laminar Air Flow, Autoclave, Tissue culture room with AC, weighing machine, pH meter.

Glassware required:

1 flask (1L), 10 flasks (100mL), 12 boiling tubes, 2 petri plates, 1 spatulla, and 1 forcep.

Steps:

1. Collection of plants

The plant *Withania somnifera* was employed as a test subject. Nodal segments and shoot tips were employed as explants for in vitro cultivation. From a plant nursery in Jaipur, Rajasthan, explants were obtained.

2. Surface sterilization

Explants were cut out from the plants grown in the field, washed thoroughly for 30 minutes while running tap water,

soaked in laborene (detergent) for 10 minutes, and washed several times with distilled water. The explants soaked in 0.1% HgCl₂ solution for 1 minute were washed again with distilled water 3-4 times to remove trace amounts of HgCl₂. Place the transferred explants in filter paper to remove excess water and cut off both ends of all explants.

3. Laminar Air Flow sterilization

Wipe the laminar air flow with cotton and ethanol, then turn on the UV light for 30 minutes before use.

4. Culture media and micro propagation

MS medium consists of salts and vitamins⁽¹⁰⁰⁾, 8 g / l agar and 30g/l sucrose, various combinations and concentrations of plant growth regulators (auxin and cytokinin) for callus induction and shoot regeneration. When autoclaved at 121 ± 2°C for 20 minutes using 0.1N NaOH or 0.1N HCl, the optimum pH was 5.8. The medium was then poured into test tubes and flasks (100 ml) and then waited for solidification. All explants were sequentially inoculated into solid medium under laminar air flow, then the inoculated solid medium was transferred to a tissue culture room to provide an alternating environment of light (16 hours) and dark (8 hours) period for growth. The regenerated callus and microshoot were transferred to callus regeneration medium to form adventitious shoot and shoot elongation media individually containing different concentrations of BAP and NAA and a combination of BAP, NAA. Single shoots grown to about 3-4 cm in length were transferred to semi-intensity MS medium containing BAP or NAA for rooting. 2.0 mg of L⁻¹ IBA and glucose, maltose, fructose, lactose, or sucrose were added to MS medium at 3% (w/v) to investigate the effect of carbon sources on in vitro rooting. The rooted seedlings were transferred to a plastic cup containing soil, sand and vermiculite (1: 1: 1). The saplings were maintained under the same controlled environmental conditions for 3 weeks, watered with half the strength of MS basal salt every 2 days, then transferred to a polyethylene cover and maintained in the greenhouse, after 4 weeks the saplings were maintained. It was moved to the field.



Fig 3. Culture of stem nodal segment of *Withania somnifera*.



Fig 4. Growth of nodal segment of *Withania somnifera* in MS media with different concentration of BAP, IBA and NAA after 2 weeks.



Fig 5. Shoot induction from nodal segment of *Withania somnifera* in MS media after 2 weeks.

V. CONCLUSION

Due to its richness of diverse therapeutic secondary metabolites, *Withania somnifera* has significant therapeutic value in many medical disorders (withanolides). The importance of the plant helps to determine the best strategy to increase plant output in response to rising demand. There are a number of experimental investigations on Ashwagandha and its components that provide the scientific underpinnings for the Ayurvedic activities. Since it has been shown to have strong antibacterial properties with amikacin and immunopotentiality with the DPT (Diphtheria, Pertussis, Tetanus) vaccine, enhancing their therapeutic benefits, the drug has been proven to be safe for long-term use and in higher amounts.

VI. REFERENCE

- [1]. Weiner M.A., Weiner J (1994), Ashwagandha (India ginseng). In Herbs that Heal. Quantum Books, Mill Valley, CA; 70-72.



- [2]. N Gaurav, Kumar A, Tyagi M, Kumar D, Chauhan U.K, Singh A.P (2015), Morphology of *Withania somnifera* (Distribution, Morphology, Phytosociology of *Withania somnifera* L. Dunal), International Journal of Current Science Research, 2454-5422.
- [3]. Mirjalili M H; Mayano E; Mercedes B and Cusido R M (2009), Palazón. Steroidal lactones from *Withania somnifera*, an ancient plant for novel medicine. Mol 2009; 14: 2373-2393.
- [4]. Abou-Douh AM (2002), New withanolides and other constituents from the fruit of *Withania somnifera*. Arch Pharm; 335:267-76.
- [5]. Panda S, Kar A (1997), Evidence for free radical scavenging activity of Ashwagandha root powder in mice Indian J PhysiolPharmacol.;424-426.
- [6]. Wagner H, Norr H, Winterhoff H (1994). Plant adaptogens, Phytomed;63-76.
- [7]. Singh B, Saxena AK, Chandan BK et al (2001), Adaptogenic activity of a novel, withanolide-free aqueous fraction from the roots of *Withania somnifera* Dunal Phytother Res.; 15:311-318.
- [8]. Singh B, Chandan BK, Gupta DK (2003), Adaptogenic activity of a novel withanolide -free aqueous fraction from the roots of *Withania somnifera* Dunal. (Part II). Phytother Res.;531-536.
- [9]. Khare CP (2007), Indian Medicinal Plants—An Illustrated Dictionary. First Indian Reprint, Springer (India) Pvt. Ltd., New Delhi. Kirtikar KR, Basu BD. Indian Medicinal Plants 2:717- 718.
- [10]. Archana R, Namasivayam A (1999), Antistressor effect of *Withania somnifera*. J Ethnopharmacol; 64:91-93.
- [11]. Bhattacharya S, Goel R, Kaur R, Ghosal S (1987), Antistress activity of sitoindosides VII and VIII, new acylsterylglucosides from *Withania somnifera* Phytotherapy Res.;1:32-39.
- [12]. Bhattacharya SK, Muruganandam AV (2003), Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. PharmacolBiochemBehav; 75:547-555.
- [13]. Anonymous (1982), The Wealth of India. Publications and Information Directorate, Council of Scientific and Industrial Research (CSIR), New Delhi; 580-85.
- [14]. Dhuley JN (1998), Effect of *Asgand* on lipid peroxidation in stress induced animals. J Ethnopharmacol, 7:173- 178.
- [15]. Anonymous (1982), The Wealth of India. Publications and Information Directorate, Council of Scientific and Industrial Research (CSIR), New Delhi; 580-85.
- [16]. SK Bhattacharya, KS Satyan, A Chakrabarti (1997), Effect of *Trasina*, an Ayurvedic herbal formulation, on pancreatic islet superoxide dismutase activity in hyperglycaemic rats. Indian J Exp Biol.;35(3):297-299.
- [17]. Visavadiya NP, Narasimhacharya AV (2006), Hypocholesteremic and antioxidant Effects of *Withania somnifera* (Dunal) in hypercholesteremic rats. Phytomed. (In Press).
- [18]. Kumar A, Kulkarni SK (2006), Effect of BR-16A (Mentat), a polyherbal formulation on drug-induced catalepsy in mice. Indian J Exp Biol.;44(1):45-48.
- [19]. Ahmad M, Saleem S, Ahmad AS, Ansari MA, Yousuf S, Hoda MN, et al (2005), Neuroprotective effects of *Withania somnifera* on 6-hydroxydopamine Induced Parkinsonism in rats. Hum ExpToxicol;24(3):137-147.
- [20]. Naidu PS, Singh A, Kulkarni SK (2003), Effect of *Withania somnifera* root extract on haloperidol-induced orofacial dyskinesia: possible mechanisms of action. J Med Food;6(2):107-114.
- [21]. Bhattacharya A, Bhattacharya D, Sairam K, Ghosal S (2002), Effect of *Withania somnifera* glycowithanolides on a rat model of tardive dyskinesia. Phytomed;9(2):167-170.
- [22]. Malhotra CL, Das PK, Dhalla NS, Prasad K (1981) Studies on *Withania ashwagandha*, Kaul. III. The effect of total alkaloids on the cardiovascular system and respiration. Indian J Med Sci.; 49:448-460.
- [23]. Mohanty I, Arya DS, Dinda A, Talwar KK, Joshi S, Gupta SK (2004) Mechanisms of cardioprotective effect of *Withania somnifera* in experimentally induced myocardial infarction. Basic ClinPharmacol Toxicol;94(4):184-190.
- [24]. Ziauddin M, Phansalkar N, Patki P, Diwanay S, Patwardhan B (1996), Studies on the immunomodulatory effect of *Asgandh*. J Ethnopharmacol;50(2):69-76.
- [25]. Aggarwal R, Diwanay S, Patki P, Patwardhan B (2012), Studies on immunomodulatory activity of *Withania somnifera* (*Ashwagandha*). J App Pharm Sci.;2(1):170-175.
- [26]. Bhattacharya SK, Satyan KS, Chakrabarti A (1997), Effect of *Trasina* (TR), an Ayurvedic herbal formulation, on pancreatic islet superoxide dismutase (SOD) activity in hyperglycaemic rats. Indian J Exp Biol.;35(3): 297-299.
- [27]. Ilayperuma I, Ratnasooriya RD, Weerasooriya TR (2002), Effect of *Withania somnifera* root extract on the sexual behaviour of male rats. Asian J Androl;4(4):295-98.
- [28]. Ichikawa H, Takada Y, Shishodia S, Jayaprakasam B, Nair MG, Aggarwal BB (2006), Withanolides potentiate apoptosis, inhibit invasion, and abolish osteoclastogenesis through suppression of nuclear factor-kappaB (NFkappaB) activation and NF-kappa B-regulated gene expression. Mol Cancer Therap.;1434-45.



- [29]. Prakash J, Gupta SK, Dinda AK (2002), *Withania somnifera* root extract prevents DMBA-induced squamous cell carcinoma of skin in Swiss albino mice. *Nutr Cancer*; 42:91-97.
- [30]. D.K. Machiah, K.S. Girish, T. V. Gowda (2006), A glycoprotein from a folk medicinal plant, *Withania somnifera*, inhibits hyaluronidase activity of snake venoms. *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.* 143(2): 158-161.
- [31]. S.K. Bhattacharya, A. Bhattacharya, K. Sairam, S. Ghosal (2000), Anxiolytic-antidepressant activity of *Withania somnifera* glycowithanolides: an experimental study. *Phytomedicine* 7(6): 463-469.
- [32]. Arun Raj GR, Shailaja U, Rao PN, Sharanesh T, Gokul J (2013), Review on the contribution of Dashapushpa, a traditional medicine in the management of cancer, *Global J Res. Med. Plants & Indigen. Med*; 2(9):656-663.
- [33]. Waddar S, Gopi KBJ, Rao PN, Raj AGR, Waddar S (2014), Standardization of Mulaka (*Raphenussativus* Linn.) Kshara: an herbal alkaline preparation. *Journal of Pharmacognosy and Phytochemistry*; 3(1):108-110.
- [34]. Sujata Waddar, Prasanna N Rao, BJ Gopi Krishna, Arun Raj GR, Shridhar Waddar (2014), Standardization of Mulaka (*Raphenussativus* Linn.) Kshara: a herbal alkaline preparation. *Journal of Pharmacognosy and Phytochemistry*; 3(1):108-110.
- [35]. Arun Raj GR, Shailaja U, Prasanna N Rao, Ajayan S, Nivya P Thomas (2014), Review on the contribution of Ura-Marunnu, a traditional baby care practice in southern India. *The Pharma Innovation*; 2(11):42-70.
- [36]. Arun Raj GR, Shailaja U, Rao Prasanna N, Mallanavar V (2013), Review on the therapeutic efficacy of an Ayurvedic compound drug in chronic tonsillitis in children. *Unique Journal of Pharmaceutical and Biological Sciences*; 01(02):2-11.
- [37]. Bhattacharya A, Ghosal S and Bhattacharya S (2001), Antioxidant effect of WS glycowithanolides in chronic foot shock induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *Journal Ethnopharmacol*; 74:1-6.
- [38]. Sharma V, Sharma S, Pracheta, Paliwal R (2011), *Withania somnifera*: A Rejuvenating Ayurvedic Medicinal Herb for the Treatment of various Human ailments. *International Journal of Pharm Tech Research*; 3(1):187-192.
- [39]. Sivamani S, Joseph B, Kar B (2014), Anti-inflammatory activity of *Withania somnifera* leaf extract in stainless steel implant induced inflammation in adult zebrafish. *Journal of Genetic Engineering and Biotechnology*; 12(1):1-6.
- [40]. Khyati S, Thakar AB, Shukla VJ, Harisha CR (2011), A preliminary physico-chemical assay of *Ashwagandha* granules a pilot study. *IJRAP*; 2(4):1026-1032.
- [41]. Shastri B. Guduchyadivarg (1999). In: Bhavprakash-Vidyotini HV. 9th ed. Varanasi: howkhamba Sanskrit Sansthan, 393-4.
- [42]. Tripathi I. Vaatvyadhiprakaran, Sootra 90 (1994), In: Chakrpanidatt VC, Vaidyprabha HV. 2nd ed. Varanasi: Chowkhamba Sanskrit Sansthan; 132-158.
- [43]. Sharma N, Sharma MD, Dhiman M, Koshy (2014), Micropropagation strategies for conservation of endangered medicinal plant *Withania somnifera* (L.) Dunal. *Journal of Cell and Tissue Research*; 14(2):4333-4338.
- [44]. Narendra Singh, Mohit Bhalla, Prashanti de Jager, Marilena Gilca (2011), An overview on *Ashwagandha*: A rasayana (rejuvenator) of Ayurveda. *Afr J Tradit Complement Altern Med*; 8(S):208-213.
- [45]. Kharela P, Manandharb MD, Kalaunia SK, Awalec S, Barala S (2011), Isolation, Identification and Antimicrobial Activity of a Withanolide [WS- 1] from the Roots of *Withania somnifera*. *Nepal Journal of Science and Technology*; 12:179-186.
- [46]. Savai J, Varghese A, Pandita N (2013), Lack of the cytochrome P450 3A interaction of methanolic extract of *Withania somnifera*, Withaferin A, Withanolide A and Withanoside IV. *J Pharm Negative Results*; 4:26-32.
- [47]. Verma SK. Kumar A (2011), Therapeutic uses of *Withania somnifera* (*Ashwagandha*) with a note on Withanolides and its pharmacological actions. *Asian Journal of Pharmaceutical and Clinical Research*; 4(1):1-4.
- [48]. Mirjalili MH, Moyano E, Bonfill M, Cusido RM, Palazon J (2009). Steroidal lactones from *Withania somnifera*, an Ancient plant for Novel medicine. *Molecules*; 14:2373-93.
- [49]. Shailaja U, Rao Prasanna N, Arun Raj GR, Mallanavar V (2013). Effect of Kumarabharana rasa on chronic tonsillitis in children: A pilot clinical study. *Int. J. Res. Ayurveda Pharm*; 4(2):153-157.
- [50]. Shailaja U, Rao PN, Girish KJ, Arun Raj GR (2014), Clinical study on the efficacy of Rajayapana Basti and Baladi Yoga in motor disabilities of cerebral palsy in children. *Ayu.*; 35:294-9.
- [51]. Raj GRA, Shailaja U, Rao PN, Ajayan S (2014), Review on the concept of Immunomodulation in Ayurveda with special emphasis on Prakara yoga. *Int J Pharm Sci Res.*; 5(4):1116-23.
- [52]. Davis L, Kuttan G (2000) Immunomodulatory activity of *Withania somnifera*. *J Ethnopharmacol*; 71:193-200.
- [53]. Arun Raj GR, Shailaja U, Rao Prasanna N (2013), Preventive Medicine in Children: An Ayurvedic



- Approach Highlighting Native Vaccinations. *IJIRD*; 2(6): 886-893.
- [54]. Jayaprakasam B, Zhang Y, Seeram N, Nair M (2003) Growth inhibition of tumor cell lines by withanolides from *Withania somnifera* leaves. *Life Sci.*; 74:125-132.
- [55]. Bone K (1996), *Clinical Applications of Ayurvedic and Chinese Herbs. Monographs for the Western Herbal Practitioner.* Australia: Phytotherapy Press, 137-141.
- [56]. Bhattarcharya SK, Muruganandam AV (2003) Adaptogenic activity of *Withania somnifera*: an experimental study using a rat model of chronic stress. *Pharmacol Biochem Behav*; 75: 547-555.
- [57]. Andallu B, Radhika B (2000) Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (*Withania somnifera*) root. *Indian J Exp Biol*; 38:607-609.
- [58]. Bhattacharya A, Ghosal S, Bhattacharya SK (2001), Antioxidant effect of *Withania somnifera* glycowithanolides in chronic foot shock stress induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. *J Ethnopharmacol*; 74:1-6.
- [59]. Weiner MA, Weiner J (1994), *Ashwagandha (India ginseng).* In: *Herbs that Heal.* Mill Valley, CA: Quantum Books, 70–72.
- [60]. S Sharma, S Dahanukar, SM Karandikar (1985), Effects of long-term administration of the roots of ashwagandha and shatavari in rats. *Indian Drugs.* 133–139.
- [61]. A Vijayan, V B Liju, J V John, Reena, B Parthipan et al. (2007), *Indian Journal of Traditional Knowledge*, 6: 589-594.
- [62]. Gavande Kalpana, Jain Kirti, Jain Bharti, Mehta Rakesh, (2013-2018), *Comprehensive Report on Phytochemistry and Pharmacological Prominence of Withania somnifera*, *Pharmaceutical and Biosciences Journal*, ISSN: 2347-9442.
- [63]. Nema Rajeev, Khare Sarita, Jain Paruland, Pradhan Alka, (2013), *Anticancer Activity of Withania Somnifera (Leaves) Flavonoids Compound*, *Int. J. Pharm. Sci. Rev. Res.*, 19: 103-106.
- [64]. Kaur Narinderpal, Niazi Junaid, Bains Raman (2013), *A review on pharmacological profile of Withania somnifera (Ashwagandha)*, ISSN: 2320-0189.
- [65]. Singh G, Sharma P K, Dudhe R, Singh S (2010), *Biological activities of Withania somnifera*, *Scholars Research Library, Annals of Biological Research*, 1:56-63.
- [66]. Gavande Kalpana, Jain Kirti, Jain Bharti, Mehta Rakesh, (2013-2018), *Comprehensive Report on Phytochemistry and Pharmacological Prominence of Withania somnifera*, *Pharmaceutical and Biosciences Journal*, ISSN: 2347-9442.
- [67]. Nema Rajeev, Khare Sarita, Jain Paruland, Pradhan Alka (2013), *Anticancer Activity of Withania Somnifera (Leaves) Flavonoids Compound*, *Int. J. Pharm. Sci. Rev. Res.*, 21: 103-106.
- [68]. Atal CK, Kapoor BM (1989), *Cultivation and utilization of medicinal plants (Eds. PID CSIR).*
- [69]. WHO survey (1993) *In medicinal plants (Eds. Haq. I.) Hamdard Foundation Press, Karachi, 13.*
- [70]. Sanyal, PK (1989), *Homeopathic Pharmacy in India. In: Cultivation and utilization of medicinal plants.* Editor: Atal CK and Kapoor BM (Published by PID CSIR).
- [71]. Chopra RN, Chopra IC, Handa KL, Kapoor LD (1993) *Indigenous drugs of India (Published by UN Dhar, Pvt. Ltd., Calcutta).*
- [72]. Chopra RN, Nayar SI, Chopra IC (1956), *Glossary of Indian Medicinal Plants (Published by CSIR, New Delhi).*
- [73]. Satyavati GV, Raina MK, Sharma M (1976), *Medicinal Plants of India (Published by ICMR, New Delhi).*
- [74]. Nadkarni AK, Nadkarni KM (1976), *Indian Materia Medica (Published by Popular Prakashan Pvt. Ltd., Bombay).*
- [75]. Sirkar NN (1989), *Pharmacological basis of Ayurvedic therapeutics. In: Cultivation and utilization of medicinal plants. Editors: Atal CK and Kapoor BM (Published by PID CSIR).*
- [76]. *Charaka Samhita, Chikitsa Sthana (1997), Second Chapter, Chowkambha Publishers, 38.*
- [77]. Sharma PV, Dravyaguna Vigyan, Chowkambha Sanskrit Sansthan, (1997).
- [78]. Vaidyaratnam P.S (1994) Varier's, "Indian Medicinal Plants, a compendium of 500 species", (Warrier.P.K. Nambiar V.P.K, Ramankutty Eds.), Part II; 52-55, by Orient Longman Publications, Hyderabad.
- [79]. Nadakarni (1993), *Indian Materia Medica*, 1; 1292.
- [80]. Shastri V.D, Bhavaprakasha Nighantu, Motilal Banarasidas Publications, Chowkambha Devi PU, Sharada AC, Solomon FE and Kamath MS (year.?) *In vivo growth inhibitory effect of Withania somnifera (Ashwagandha) on a transplantable mouse tumor, Sarcoma 180. Ind. J. Exp. Biol. 30: 169-172.*
- [81]. Abhyankar G, Chinchankar G. (1996), *Response of Withania somnifera Dunal leaf explants in vitro. Phytomorphology 46(3), 249-252.*
- [82]. Andallu B, Radhika B. (2000.) *Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera, Dunal) root; 38(6):607-609.*
- [83]. Siddique N, Bari M, Shahnewaz S, Rahman M, Hasan M, Khan M, Islam M. (2004), *Plant Regeneration of Withania somnifera (L.) Diurni*



- (Ashwagandha) from Nodal Segments Derived Callus an Endangered Medicinal Plant in Bangladesh. *Journal of Biological Sciences* 4(2), 219-223.
- [84]. Singh B, Saxena A, Chandan B, Gupta D, Bhutani K, Anand K. (2001), Adaptogenic activity of a novel, withanolide free aqueous fraction from the roots of *Withania somnifera* Dunal. *Phytotherapy Research* 15(4), 311-318.
- [85]. Kulkarni S, Dhir A. (2008), *Withania somnifera*: An Indian Ginseng Progress in neuro-psychopharmacology and biological psychiatry 32(5), 1093-1105.
- [86]. Tohda C, Kuboyama T, Komatsu K. (2005), Search for natural products related to regeneration of the neuronal network *Neurosignals* 14(1-2), 34-45.
- [87]. Antonisamy R, Manickam V. (1999), Conservation through micropropagation and restoration of selected rare and endangered medicinal plants of South India, XVI International Botanical Congress, 1-7.
- [88]. Kattimani K, Reddy Y, Rao BR (1999), Effect of pre-sowing seed treatment on germination, seedling emergence, seedling vigour and root yield of Ashwagandha (*Withania somnifera* Dunal). *Seed Science and technology* 27(2), 483-488.
- [89]. Vakeswaran V, Krishnasamy V. (2003), Improvement in storability of Ashwagandha (*Withania somnifera* Dunal) seeds through pre-storage treatments by triggering their physiological and biochemical properties. *Seed Technology* 203.
- [90]. Sen J, Sharma A. (1991), Micropropagation of *Withania somnifera* from germinating seeds and shoot tips. *Plant cell, tissue and organ culture* 26 71-73.
- [91]. Furmanowa M, Gajdzis-Kuls D, Ruzkowska J, Czarnocki Z, Obidoska G, Sadowska, A. Upadhyay SN. (2001). In vitro propagation of *Withania somnifera* and isolation of withanolides with immunosuppressive activity. *Planta Medica* 67(02), 146-149.
- [92]. Ray S, Jha S. (2001), Production of withaferin A in shoot cultures of *Withania somnifera*. *Planta Medica* 67(05), 432-436.
- [93]. Rani G, Virk G, Nagpal A. (2003), Callus induction and plantlet regeneration in *Withania somnifera* (L.) Dunal. *In Vitro Cellular & Developmental Biology-Plant* 39(5), 468-474.
- [94]. Kulkarni AA, Thengane S, Krishnamurthy K. (1996), Direct in vitro regeneration of leaf explants of *Withania somnifera* (L.) Dunal. *Plant Science* 119(1-2), 163-168.
- [95]. Rani G, Grover I. (1999), In vitro callus induction and regeneration studies in *Withania somnifera*. *Plant cell, tissue and organ culture* 57(1), 23-27.
- [96]. Sivanesan I. (2007), Direct regeneration from apical bud explants of *Withania somnifera* Dunal.
- [97]. Sivanesan I, Murugesan K. (2008), An Efficient Regeneration from Nodal Explants of *Withania somnifera* Dunal. *Asian Journal of Plant Sciences* 7(6), 551-556.
- [98]. Saritha K, Naidu C. (2007), In vitro flowering of *Withania somnifera* Dunal. - an important antitumor medicinal plant. *Plant Science* 172(4), 847-851.
- [99]. D E Evans, JOD Coleman and A Kearns (2003), *Plant Cell Culture*, Bios Scientific Publishers, Taylor & Francis Group, London, p.1.
- [100]. Murashige T, Skoog F. (1962), A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiological Plantarum* 15(3), 473-497.

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