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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 Mandsaur, 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 20^0C to 35^0C.

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 µg / ml suppressed the growth of various Gram-positive, acid-fast and aerobic bacilli and pathogens. It was active against Micrococcus Pyogenesvar 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, citoindode VII-X and withaferin A (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 cardiotonic effect and a weak but sustained cardiotonic 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, suppressess 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 (Daboiarusselii) 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

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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 pharmaco dynamic 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 VataDosha)

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.

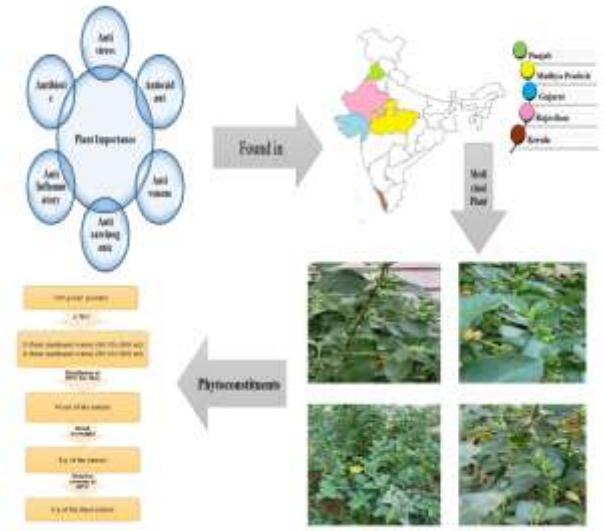

Fig. 1. Flow diagram of extraction drug from ashwagandha plant

Fig 2. Image of Ashwagandha Plant.

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Vol. 7, Issue 5, ISSN No. 2455-2143, Pages 241-251
Published Online September 2022 in IJEAST (http://www.ijeast.com)

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

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

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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, $HgCl_2$, 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 spactulla, 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,

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soaked in laborene (detergent) for 10 minutes, and washed several times with distilled water. The explants soaked in 0.1% $HgCl_2$ solution for 1 minute were washed again with distilled water 3-4 times to remove trace amounts of $HgCl_2$. 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 immunopotentiation 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.

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