IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 9, May 2026
Impact Factor: 8.2

Development of Herbal Silver Nanoparticals by using Ashwagandha (Withania somnifera)

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Abstract: *Herbal formulation is a dosage form consisting of one or more herbs or processed herbs(s) in specified quantities to provide specific nutritional, cosmetic benefits, and/or other benefits meant for use to diagnose treat, mitigate disease of human beings or animals and / or to alter the structure or physiology of human beings or animals In recent years, there has been a significant surge in reports on the health-promoting benefits of winter cherry (Withania somnifera), also known as Ashwagandha. Its current research covers many aspects of human health, including neuroprotective, sedative and adaptogenic effects and effects on sleep. There are also reports of anti-inflammatory, antimicrobial, cardioprotective and anti-diabetic properties. Furthermore, there are reports of reproductive outcomes and tarcicidal hormone action.*

This growing body of research on Ashwagandha highlights its potential as a valuable natural remedy for many health concerns. This narrative review delves into the most recent findings and provides a comprehensive overview of the current understanding of Ashwagandha's potential uses and any known safety concerns and contraindications.

Keywords: Development of Herbal Silver Nanoparticals by using Ashwagandha (Withania samnifera)

I. INTRODUCTION

Indian ginseng is also known as Indian winter cherry, Ashwagandha, or the herb Vitania sluggard (Withania somnifera). The raw material used in medicine is the root, and name "Ashwagandha" is derived from the word "ashwa", meaning horse. It is believed that after consuming the root, one gains powers similar to that of a horse.

Fig: Ashwagandha roots

The second part of the name "gandha," means fragrance and refers to the characteristic smell of the fresh root of the plant . (1) Since ancient times, it has been traditionally used in Ayurvedic medicine as a substance that strengthens the nervous system. This is evidenced by its adaptogenic effects and medicinal uses—the so-called "rasayana". Figure 1 below shows the comprehensive health benefit of Ashwagandha.The history of its use in traditional Indian medicine dates back nearly 3000 years. Its root has been used as an aphrodisiac, narcotic, tonic, diuretic, anthelmintic and

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stimulant. It is naturally native to India, but it is also cultivated in other areas such as the Mediter ranean countries, the Himalayan areas, Africa, Canary Islands, Cape of Good Hope and Australia.

In recent years, there has been a growing interest in the potential health benefits of Ashwagandha, particularly in the areas of stress management, cognitive function, and physical performance. Several studies have suggested that Ashwagandha supplementation may exhibit neuroprotective activity, be helpful in obsessive-compulsive disorder, and exhibit anti-inflammatory, immunomodulatory and antibacterial properties. Additionally, there is evidence to suggest that Ashwagandha supplementation may be helpful in infertility, anticancer and antidiabetic treatment. Studies have suggested that Ashwagandha may exhibit cardioprotective properties, be helpful in the treatment of sleep-disorders, improve stress resilience, reduce anxiety, be helpful in hypothyroidism, andmanagement, cognitive function, and physical performance.enhance muscle strength and recovery (2- 4)

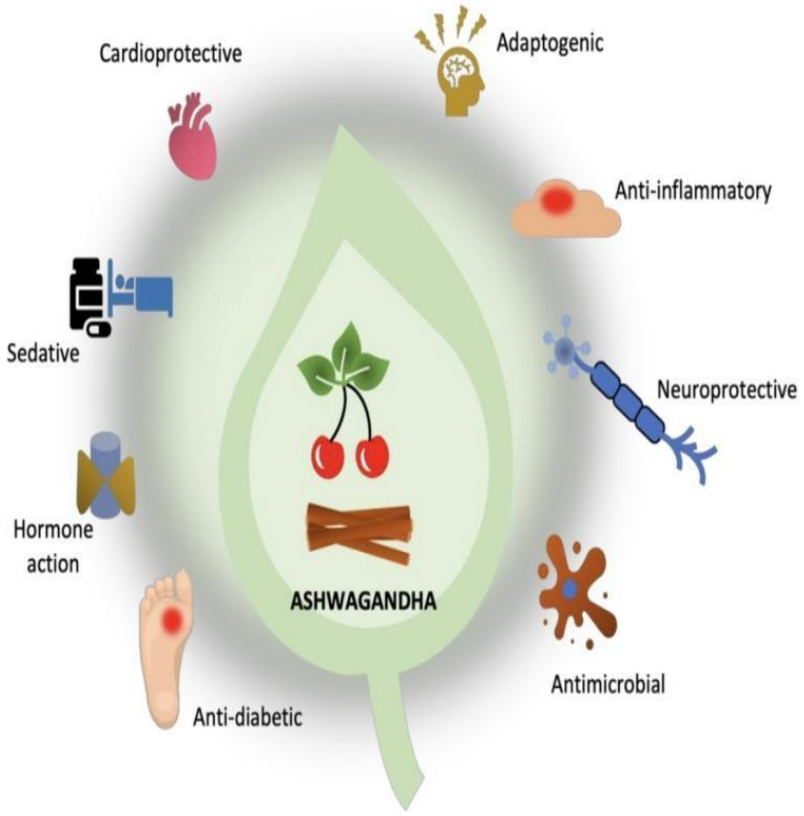

Figure: . The comprehensive health benefit of Ashwagandha.

Nanoparticle

Nanotechnology was considered one of the most emerging fields of science and it deals with the synthesis of nanoparticles and nanomaterials, which have dimensions of 1 to 100 nanometers (5). The most important and distinct property of nanoparticles is that they have a larger surface to volume ratio (6). The properties of nanoparticles, such as high diffusion, durability and versatile chemical and biological activities have gained importance in technological applications (7). A metal nanoparticle synthesis study has increased in number due to possible applications in nanotechnology (8). The nanoparticles can be synthesized by physical-chemical and biological method, but the green synthesis of silver nanoparticles has several advantages over physical and chemical method as it is cheaper, can be achieved with a single process and eco-friendly (9 - 14). Silver Nanoparticles (AgNPs) have attracted growing interest

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due to their unusual physical, chemical and biological properties, including high electrical and thermal conductivity, Surface-Enhanced Raman Scattering, chemical stability, catalytic activity, and nonlinear optical behavior. AgNPs have wide applications in pharmaceuticals, cosmetics, medical devices, footwear, and textile industries. It has been also AgNPs were helpful in the purification of drinking water/effluent water by efficient removal of the water-born pathogen (15). The synthesis of AgNPs is speedy, cost-saving, eco-friendly and single step synthesis procedure (16) 626 Medico-legal Update, April-June 2021, Vol. 21, No. 2 The roots of Withania Somnifera (L.) Dunal (Family-Solanaceae)-a vital Rasayana herb is traditionally known as 'Ashwagandha' or winter cherry. In Ayurveda, it is called 'Indian ginseng'. Ashwagandha is widely used in most Indian herbal drugs and nutraceuticals for the treatment of various diseases including nervous, infectious diseases, diabetes, cancer, ulcer, immunological disorders, stress, arthritis, etc(17) . Withania Somnifera (Ashwagandha) as Medicinal plants has therapeutic potential due to the presence of natural antioxidants functioning as reducing agents, free radical scavengers and quenchers of singlet oxygen. Majority of different medicinal plant and their antioxidant activity are due to bioactive compounds viz. flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins and isocatechins . The aim of this work, Ashwagandha root extract was used for the production of AgNPs by green mothed. The different properties of biosynthesized AgNPs have
been investigated by XRD, SEM, UV-visible, and FTIR to evaluate their shape, distribution of particle size distribution, and functional groups. The biosynthesized formulation of AgNPs was obtained to investigate the antibacterial against Gram-positive and Gram-negative bacterial strains in the inhibition zone compared to the standard antibacterial drug Meropenem.

Active compound

Ashwagandha is characterized by a rich phytochemical composition. Depending on the location of the raw material, it exhibits a diverse composition of chemical compounds.

Its active substances that play a crucial role in pharmacological action are witanolides and alkaloids. Witanolides are compounds whose essential structure is that of ergostane, which has a six-membered lactone ring at the C-8 or C-9 position. The group of witanolides includes witanopherin A, witanolides A-Y, witanone, witadomniferin A, and witasomniferols A-C. Figure 2 shows the main active compounds present. Alkaloids include witanin, somniferin, somnin, tropin, somniferinin, pseudowitanin, pseudotropin, choline, kuskohigrin, isopeletierin, and anaferin . Also present in the raw material are flavonoids which include 3-O-rutinoside, 6,8-dihydroxycemferol, quercetin and its glycosidic derivative, 3-0rutinoside-7-O-glucoside. Additionally, witanolid glycosides, which have a structure that contains a glucose moiety at position C-27, are also present in the raw material. This group of compounds includes sitoindoside IX and sitoindoside X. Ashwagandha also contains steroidal saponins that contain an acyl group-sitoindoside VII and VIII. Saponins, coumarins (scopoletin), sterols, chlorogenic acid, resins, lipids, carbohydrates and fatty acids have also been identified in the raw material (18)

Biological Activity

1 Neuroprotective and Anti-Neurodegenerative Effects

Ashwagandha Use in Alzheimer's Disease

For many years, the phenomenon of aging populations has been observed, which also implies a significant increase in the percentage of people suffering from dementia syndromes. Dementia is a syndrome with a multifactorial aetiology characterized by a range of symptoms caused by a brain disease, typically with a chronic and progressive course. This condition affects higher cortical functions, including memory, thinking abilities, orientation, comprehension, learning abilities, and emotional control. Neurodegenerative diseases cause the destruction of the central nervous system, resulting in irreversible damage. Over the course of Alzheimer's disease, an abnormal deposition of β-amyloid protein in the brain is observed. In its fibrillar form, it has a neuro-toxic effect because it induces the formation of free radicals and impairs glucose transport in neurons, which leads to cell damage and death. Hyperphosphorylated τ proteins in

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Alzheimer's disease form clusters surrounding the core of the senile plaque, consisting of β-amyloid. Physiologically, τ proteins stabilize microtubules, along with other proteins. Accumulating senile plaques are accompanied by microglia (inflammatory response cells), attempting to break down and remove damaged and dead neurons as well as senile plaques. Microglia cells produce toxins, destroying both diseased and healthy cells and enhancing the brain's inflammatory response (19)

Ashwagandha Use in Parkinson's Disease

In Parkinson's disease, the degeneration of the dopaminergic neurons of the nigros-triatal system is observed. This leads to an imbalance between dopamine's inhibitory action and acetylcholine and glutamic acid's excitatory action. Factors that induce the degeneration of nigrostriatal cells include:

- Genetic conditions;
- Endo- and exogenous toxic factors;
- Neuroinfections; o Oxidative stress;
- Reduced growth tactors;
- The sum of the action of several of the above factors.

The disease is slightly more common in men than in women, and although the caus is not known, it is thought that this may be due to the protective role of estrogen

A study was conducted on rats with 6-hydroxydopamine-induced Parkinson's disease. Prior to an injection of 6-hydroxydopamine into the striatum, the rats were orally administered a Withania somnifera extract at doses of 100, 200, and 300 mg/kg body weight for 3 weeks. It was observed that administration of Ashwagandha significantly reduced lipoperoxidation, increased glutathione concentration, increased glutathione S-transferase, glutathione reductase, glutathione peroxidase, superoxide dismutase and catalase activities, catecholamines, and dopamine D2 receptor binding and enhanced tyrosine hydroxylase expression (20)

Use of Ashwagandha in the Treatment of Huntington's Disease

Huntington's Disease is an incurable disease. Current medications only work on the symptoms and slow down the progression of the disease. The disease is inherited in an autosomal dominant manner, which means that statistically, half of the offspring will receive the disease-causing allele. A mutation in the IT15 gene encoding the huntingtin (htt) protein on chromosome 4 leads to a conformational change in huntingtin, into its insoluble form. The N-terminal part of the mutant huntingtin protein, containing expanded polyglutamine repeats, accumulates leading to accelerated neuronal apoptosis. This leads to an imbalance of dopamine, GABA, serotonin, and acetylcholine (21)

Treatment of Obsessive-Compulsive Disorder, Alcohol Withdrawal Syndrome

Obsessive-compulsive disorder (OCD) is a chronic psychiatric disorder that involves patients experiencing symptoms in the form of intrusive thoughts and imagery. Patients perceive them as undesirable, unwanted, compulsive, and irrational. Although the severity of the cognitive disturbance can vary considerably from patient to patient, OCD makes daily life significantly more difficult-especially in its severe form, where it can significantly impair psychosocial functioning . Genetic and psychological factors play a significant role in the aetiology of OCD, but structural and functional abnormalities within the central nervous system are equally important . Obsessive-compulsive disorder is thought to be associated with the dysregulation of the serotonergic system

Anti-Inflammatory/Immunomodulatory Effects

Due to its properties, Withania somnifera is being studied for the treatment of many diseases associated with inflammation in the body, such as cardiovascular, pulmonary, and autoimmune diseases and diabetes, cancers, and neurodegenerative diseases. Preclinical studies have demonstrated the ability of this plant to regulate mitochondrial function and apoptosis and reduce inflammation by inhibiting inflammatory markers such as cytokines (including IL-6

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and TNF-a), nitric oxide, and reactive oxygen species. Meanwhile, in a mouse model with lupus, a potential inhibitory effect of Ashwagandha root powder was demonstrated in conditions such as proteinuria and nephritis .Ashwagandha is also being investigated for its efficacy in rheumatoid diseases. In a study conducted in an animal model, Withania somnifera root powder was administered orally to rats for three days, one hour before inflammation was induced by an injection of CFA (complete Freund's adjuvant). In the control group (positive control), rats were administered phenylbutazone. Changes in the concentrations of a number of serum proteins, such as a2 glycoprotein, acute phase protein a1 and prealbumin, were demonstrated, along with a significant reduction in inflammation . In a study using the HaCaT human keratinocyte cell line, an aqueous solution from Ashwagandha root was found to inhibit the NF-B and MAPK (mitogen-activated protein kinase) pathways by decreasing the expression of pro-inflammatory cytokines, including interleukin (IL)-8, IL-6, tumour necrosis factor (TNF-x), IL-1ß, and IL-12, and increasing the expression of anti-inflammatory cytokines. Based on these results, it can be concluded that the anti-inflammatory effects of Ashwagandha could potentially be used in the prevention of skin inflammation . In a preclinical study of the anti-neuroinflammatory effects of Ashwagandha water extract (ASH-WEX) against lipopolysaccharide-induced systemic neuroinflammation, animals treated with ASH-WEX showed an inhibition of reactive gliosis; production of inflammatory cytokines such as TNF-a, IL-1ß, and IL-6; and expression of nitro-oxidative stress enzymes. The underlying molecular mechanisms for the antiinflammatory potential of ASH-WEX appear to involve inhibition of lipopolysaccharide (LPS)-activated NFkB, P38 and JNK/SAPK MAPK pathways. The results of this study suggest the potential use of Withania somnifera in suppressing nervous system inflammation associated with various neurological disorders . Evidence presented in a study by Kanjilal et al. showed that Ashwagandha extract applied for a period of 8 to 12 weeks can be useful in managing arthritis symptoms in patients. The immunomodulatory effect was confirmed in a study on the effect of Withania somnifera root powder on the stimulation of immune activity in immunodeficient mice. Administration of Withania somnifera was found to increase the total number of whiteblood cells and bone marrow cells, as well as to increase the titre of circulating antibodies and antibody-producing cells and to stimulate the production of immune cells and the phagocytosis of macrophages . A randomized, double-blind, placebo-controlled trial with an open-label extension was conducted to evaluate the effect of Ashwagandha extract on the immune system of healthy participants. The results of the study showed that Ashwagandha extract significantly increased natural killer cell activity and cytokine levels, compared to placebo.

Antibacterial Properties

Drug resistance in micro-organisms is a major and growing threat, although now widely recognized. In recent years, a significant increase in infections caused by drugresistant strains has become a major problem. It is known that the reckless and often unwarranted use of antibiotics has resulted in the development of drug-resistant strains, and in some situations, these drugs have become completely ineffective. Ashwagandha, therefore, appears to be a valuable addition to pharmacotherapy in the treatment of bacterial infections. Many of the drugs currently used to treat bacterial infections, despite their efficacy, have many dangerous side effects related to their toxicity. Ashwagandha is a safe, non-toxic plant with almost no side effects. In the studies that have been conducted, it has been proven to effectively inhibit the growth of methicillin-resistant Staphylococcus aureus and Enterococcus spp.]. Withania somnifera root extract has also been shown to effectively inhibit the growth of the Gram-negative bacteria Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Citrobacter freundi, and Klebsiella pneumoniae (22)

Antitumor Properties

To investigate its use in treating various forms of cancer, the antitumor and radiosensitizing effects of WS have been studied. In one study, WS was evaluated for its anti-tumor effect in urethane-induced lung adenomas in adult male albino mice. Simultaneous administration of WS (ethanol extract of whole plant, 200 mg/kg daily orally for seven months) and urethane (125 mg/kg without food biweekly for seven months) reduced tumor incidence significantly (tumor incidence: untreated control, 0/25; urethane treated, 19/19; WS treated, 0/26, and WS plus urethane treated,

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6/24, p<0.05). The histological appearance of the lungs of animals protected by WS was similar to those observed in the lungs of control animals. No pathological evidence of any neoplastic change was observed in the brain, stomach, kidneys, heart, spleen, or testes of any treated or control animals. In addition to providing protection from carcinogenic effects, WS treatment also reversed the adverse effects of urethane on total leukocyte count, lymphocyte count, body weight, and mortality.The growth inhibitory effect of WS was also observed in Sarcoma 180 (S-180), a transplantable mouse tumor.12 Ethanol extract of WS root (400 mg/kg and up, daily for 15 days) after intra-dermal inoculation of 5x105 cells of S-180 in BALB/c mice produced complete regression of tumor after the initial growth. A 55-percent complete regression was obtained at 1000 mg/kg; however, it was a dose in some cases. WS was also found to act as a radio- and heat sensitizer in mouse S180 and in Ehrlich ascites carcinoma.Anti-tumor and radiosensitizing effects of withaferin (a steroidal lactone of WS) were also seen in mouse Ehrlich ascites carcinomain vivo. 15 Withaferin A from WS gave a radiosensitizer ratio of 1:5 for in vitro cell killing of V79 Chinese hamster cell at a non-toxic concentration of about 2 mM/L.1214 These studies are suggestive of antitumor activity as well as enhancement of the effects of radiation by WS.

Antistress Effect

To evaluate the antistress effect of WS,an alcohol extract from defatted seeds of WS dissolved in normal saline was given (100 mg/ kg intraperitoneally as a single dose) to 20-25g mice in a swimming performance test in water at 28º-30ºC.10 Controls were given saline. The extracts approximately doubled the swimming time when compared to controls. In another study, WS prevented both a weight increase of the adrenals and a reduction in ascorbic acid content of the adrenals normally caused by this swimming test. The authors suggested that WS induced a state of nonspecific increased resistance during stress. Glycosides of WS (sitoindosides VII and VIII, 50 to 100 mg/kg) exhibited significant antistress activity in forced swimming-induced immobility in mice, restraint stress-induced gastric ulcers in rats, restraint-induced auto-analgesia in rats, restraint stress effect on thermic response of morphine in rats, and morphine-induced toxicity in aggregated mice.The alcohol extract of WS (100 mg/ kg, twice daily orally on day 1, 4 or 7) reduced stress-induced increases in blood urea nitrogen levels, blood lactic acid, and adrenal hypertrophy, but did not affect changes in thymus weight and hyperglycemia in rats.7 Ws reversed the cold swimming-induced increases in plasma corticosterone, phagocytic index, and avidity index to control levels. WS root powder (100 mg/kg orally as an aqueous suspension daily for seven days) given before the swimming test in water at 10ºC also increased total swimming time, indicating better stress tolerance in rats.8 These results indicated a significant increase in plasma corticosterone level, phagocytic index, and avidity index in control rats, whereas these levels were near normal in WS rats subjected to the same test. In a comparative study for antistress activity, finely powdered roots of WS and Panax ginseng (PG), suspended in 2-percent acacia (100 mg/kg in 1.00 mL orally) were given to 18-20 g mice daily for seven days; the swimming test was given on day 8.25 Significant antistress activity, as measured by the swimming endurance test, was found for both compounds. The swimming time was 536.6 minutes for PG, 474.1 minutes for WS, and 163.3 minutes for controls; all differences between groups were significant (p<0.05). Anabolic activity, measured as an increase in body weight, was significant for both herbal extracts but was better in the WS group than in the PG group. If these results could be reproduced in humans, it would support the use of WS in nervous exhaustion due to stress and in cachexia to increase body weight.

Antioxidant Effect

The brain and nervous system are relatively more susceptible to free radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species. The brain also uses nearly 20 percent of the total oxygen supply.Free radical damage of nervous tissue may contribute to neuronal loss in cere-bral ischemia and may be involved in normal aging and neurodegenerative diseases, e.g.,epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases. Since traditional Ayurvedic use of WS has includedmany diseases associated with free radical oxidative damage, it has been considered likely the effects may be due to a certain degree

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of antioxidant activity. The active principles of WS, sitoindosides VII-X and withaferin A (glycowithanolides), have been tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain fronta lcortex and striatum.

Decreased activity of these enzymes leads to accumulation of toxic oxidative free radicals and resulting degenerative effects. An increase in these enzymes would represent increased antioxidant activity and aprotective effect on neuronal tissue. Active glycowithanolides of WS (10 or 20 mg/kg intraperitoneally) were given once daily for 21 days to groups of six rats. Dose-related increases in all enzymes were observed; the increases comparable to those seen with deprenyl (a known antioxidant) administration (2 g/kg/day intraperitoneally). This implies that WS does have an antioxidant effect in the brain which may be responsible for its diverse pharmacological properties.30 Further studies on other parts of the brain (e.g., cerebellum, medulla, and hypothalamus) may provide information with respect to the effects of WS oncognitive behavior and other functions of the brain, in both healthy and diseased individuals.In another study, an aqueous suspension of WS root extract was evaluated for its effect on stress-induced lipid peroxidation (LPO) in mice and rabbits.9 LPO blood levels were increased by IV administration of 0.2 mg/kg of lipopolysaccharides (LPS) from Klebsiella pneumoniae and 100 mg/kg of peptidoglycans (PGN) from Staphylococcus aureus. Simultaneous oral administration of WS extract (100 mg/kg) prevented an increase in LPO. The authors indicated that the almost innocuous doses of LPS and PGN used in this study that induced elevated levels of LPO were comparable to a mild bacteremia which may follow tooth extraction, streptococcal angina, etc.

Immunomodulatory Properties

The use of WS as a general tonic to increase energy and prevent disease may be partially related to its effect on the immune system. Glycowithanolides and a mixture of sitoindosides IX and X (Figure 2) isolated from WS were evaluated for their immune modulatory and central nervous system effects (antistress, memory, and learning) in Swiss mice (15-25 g, 5-6 months old) and Wistarstrain albino rats (120-150 g and 250300 g).31 Both materials produced statistically significant mobilization and activation of peritoneal macrophages, phagocytosis, and increased activity of the lysosomal enzymes. Both compounds (50-200 mg/kg orally) also produced significant antistress activity in albino mice and rats, and augmented learning acquisition and memory retention in both young and old rats.

Hemopoetic Effect

Administration of WS extract was found to significantly reduce leukopenia induced by cyclophosphamide (CTX) treatment in Swiss albino mice. Total white blood cell count on the 12th day of the CTX-treated groupwas 3720/mm3; that of the CTX-plus-WS group was 6120/mm3. In the CTX-plus-WS mice, the cellularity of the bone marrow was significantly increased (13.1 x 106 /femur) ($p<0.001$) compared to the CTX-alone treatedgroup (8 x 106/femur). Similarly, the number of alpha-esterase positive cells (1130/4000 cells) in the bone marrow of the CTX-plus-WS mice increased compared to the CTX- alone mice (687/4000 cells). The major activity of WS may be the stimulation of stem cell proliferation. These studies indicated WS reduced CTX-induced toxicity and may prove useful in cancer chemotherapy. Further studies need to be conducted to confirm the hemopoetic effect with other cytotoxic agents and to determine its usefulness as an adjuvant in cancer chemo therapy.

Nervous System Effects

Total alkaloid extract (ashwagandholine, AG) of WS roots has been studied for its effects on the central nervous system. AG exhibited a taming effect and a mild depressant (tranquilizer) effect on the central nervous system in monkeys, cats, dogs, albino rats, and mice. AG had no analgesic activity in rats but increased Metrazol toxicity in rats and mice, amphetamine toxicity in mice, and produced hypothermia in mice. It also potentiated barbiturate-, ethanol-, and urethane-induced hypnosis in mice. Effects of sitoindosides VII-X and withaferin isolated from aqueous methanol extract of roots of cultivated varieties of WS were studied on brain cholinergic, glutamatergic and GABAergic receptors in male Wistar rats.38 The compounds slightly enhanced acetylcholinesterase (AChE) activity in the lateral

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septum and globus pallidus, and decreased AChE activity in the vertical diagonal band. These changes were accompa nied by enhanced M1-muscarinic-cholinergic receptor-binding in lateral and medial septum as well as in frontal cortices, whereas the M2 muscarinic receptor-binding sites were in- creased in a number of cortical regions includeing cingulate, frontal, piriform, parietal, anretrospinal cortex. The data suggest the compounds preferentially affect events in the cor-tical and basal forebrain cholinergic-signal cascade. The drug-induced increase in cortical muscarinic acetylcholine receptor capacity might partly explain the cognitionenhancing and memory-improving effects of WS extracts in animals and in humans.Ashwagandholine, total alkaloids extracted from extract of WS roots, caused relaxant and antispasmodic effects against various agents that produce smooth muscle contractions in intestinal, uterine, tracheal, and vascular muscles.The pattern of smooth muscle activity was similar to that of papaverine, but several-fold weaker, which indicated a directmusculotropic action. These results are consistent with the use of WS to produce relaxation.

Effects on the Endocrine System

Based on the observations that WS provides protection from free radical damage in the mouse liver, studies were conducted to determine the efficacy of WS in regulating thyroid function. Mice were given WS root extract (1.4 g/kg by gavage, daily for 20 days). The treatment significantly increased the serum levels of 3,3',5triiodothyronine (T3) and tetraiodothyronine (T4), while the hepatic con centrations of glucose 6-phosphatase activity and hepatic iodothyronine 5'-monodeiodinase activity did not change significantly. WS significantly reduced hepatic lipid peroxidation and increased the activity of superoxide dismutase and catalase. The results suggest WS stimulates thyroidal activity and also promotes hepatic antioxidant activity.

A combination formula of WS Tinospora cordifolia, Eclipta alba, Ocimium sanctum, Picorrhiza kurroa, and shiled was found to cause a dose-related decrease in streptozotocin-induced hyperglycemia.None of the herbs given individually, however, produced any effect on the hyperglycemia, indicative perhaps of why Ayurvedic medicine generally prefers combinations of herbs rather than single herbs.

Effects on the Cardiopulmonary System

WS may be useful as a general tonic, due in part to its beneficial effects on the cardiopulmonary system, as reported in the following studies. The effect of AG was studied on the cardiovascular and respiratory systems in dogs and frogs.The alkaloids had a prolonged hypotensive, bradycardiac, and respiratory-stimulant action in dogs.

The study found that the hypotensive effect was mainly due to autonomic ganglion blocking action and that a depressant action on the higher cerebral centers also contributed to the hypotension.The alkaloids stimulated the vasomotor and respiratory centers in the brain stem of dogs.The cardio-inhibitory action in dogs appeared to be due to ganglion blocking and direct cardio-depressant actions. The alkaloids produced immediate predominant but short-lived cardio-depressant effects and a weak but prolonged cardiotonic effect in isolated normal and hypodynamic frog hearts. The pharmacological actions of the total extract of WS roots on the cardiovascular and respiratory systems appeared to be due to its alkaloid content. The total alkaloids were more than twice as active as the 70-percent alcohol extract of WS root. These studies were found to be consistent with the use of WS as a tranquilizing agent. (23)

Anticancer Effects

Cancer is a group of diseases in which cell division proceeds in an uncontrolled manner. This is due to mutations in gene-encoding proteins that are involved in the cell cycle, such as proto-oncogenes and anti-oncogenes. Statistics show that cancer is a serious and growing health and social problem. Despite worldwide research efforts in the fight against cancer, it remains a major cause of death. Studies have shown that various compounds isolated from parts of Ashwagandha, such as the root, stem, and leaves, exhibit anti-cancer properties. Therefore, they can be used to treat cancer alone or in combination with other chemotherapeutic agents . Wi-tanolides are alkaloids present in the plant that show great anti-cancer potential. They are also the most promising compounds showing this action, as they play a

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major role in the induction of apoptosis. Ashwagandha is effective against cancers such as breast, colon, lung, prostate, and blood cancers .It acts as a chemotherapeutic agent against many different types of breast cancer, especially ER/PR-positive breast cancer and triple-negative breast cancer . In addition to its treatment, it also shows properties that prevent it. Research also suggests the potential of Ashwagandha in improving the quality of life of breast cancer patients . According to research, withaferin A derived from Ashwagandha is also effective in the treatment of melanoma. This compound induces apoptosis and also reduces cell proliferation and inhibits melanoma cell migration . The antitumor mechanisms of withaferin A in glioblastoma multiforme (GBM) were investigated.

RNA-seq analysis, Western blot, immunofluorescence staining, qRT-PCR, and siRNA gene silencing were performed to determine the signaling pathways affected by withaferin A. It significantly inhibited GBM growth in vitro and in vivo and triggered intrinsic apoptosis of GBM cells It arrested GBM cells in the G2/M phase of the cell cycle by defosphorylating Thr161 CDK1. This finding is important for the optimization of withaferin A-based regimens for the prevention and/or treatment of glioblastoma multiforme . Jawarneh et al. demonstrated that a combination of Ashwagandha extract and intermittent fasting has potential as an effective breast cancer treatment that may be used in conjunction with cisplatin. The combination was found to decrease cancer cell proliferation through apoptosis induction, while also reducing cisplatin-induced toxicity in the liver and kidney. Azab et al. found that the extract had a protective effect against the harmful effects of radiation exposure, reducing oxidative stress and inflammation in the liver and spleen tissues. These findings suggest that Withania somnifera root extract may have potential therapeutic applications in protecting against radiotherapy-induced damage to vital organs such as the liver and spleen.

Treatment of Sleep Disorders

Insomnia is defined as a situation in which the patient sleeps too little in relation to his or her needs, and this further leads to a deterioration in daytime functioning. Contrary to popular belief, insomnia is not just a condition in which nocturnal sleep is shortened, as the problem is much more complex and involves numerous complications. It is believed that insomnia can be related to difficulty initiating sleep, difficulty maintaining sleep or waking up too early. All of these symptoms can occur even when maintaining good sleep hygiene. The occurrence of these disorders leads to a poor sense of well-being, problems concentrating, emotional disturbances, cognitive disorders, and lack of motivation, which affects professional as well as social life. Researchers around the world agree that insomnia affects women more often than men. Old age is also a factor in insomnia. Unfortunately, there has been a significant increase in the proportion of people taking sleep medication, which indicates the scale of the insomnia problem. Nevertheless, it is believed that the condition is underdiagnosed. Epidemiological data differ by countries, which may be the reason for different methods of diagnosing this disorder. It is also worth noting that sleep constitutes approximately 30% of human life, hence the conclusion that the occurrence of any disorders in this area significantly disrupts the homeostasis of the organism .

Increase Muscle Strength

Ashwagandha supplementation has been shown to significantly increase muscle strength and to stimulate muscle renewal processes. In one study conducted, young healthy men were orally administered 300 mg of Withania somnifera root extract twice daily for eight weeks. These men also performed physical exercise-subjects participated in a structured resistance training program based on the publications of the National Strength and Conditioning Association (NSCA). A significant increase in muscle strength was observed in the treated patients, as well as an increase in muscle mass in the arms and chest. It was noted that in the Ashwagandha-supplemented patients, the level of exercise-induced muscle myocyte damage was significantly lower than in the placebo group, as indicated by the stabilisation of plasma creatine kinase levels. In addition, a significant increase in testosterone levels and a significant decrease in body fat were noted in the treated group in their study, confirmed that the group receiving Ashwagandha supplementation had significant improvements in several measures of cardiorespiratory endurance compared to the placebo group. Specifically, the Ashwagandha group showed a significant increase in maximal aerobic capacity, time to exhaustion,

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International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 9, May 2026 Impact Factor: 8.2

and ventilatory threshold. Additionally, the Ashwagandha group had lower levels of serum cortisol, a hormone associated with stress. A study was also conducted on a group of adult athletes who were given a strictly defined dose of Ashwagandha, while the other group received a placebo. At the end of the study, a significant increase in VO2 max (maximum aerobic capacity) was observed in the treated group of athletes, compared to the placebo group. The athletes treated with Ashwagandha had significantly higher Total Quality Recovery Scores (TQR). An improvement in quality of life was observed in Ashwagandha-treated athletes (this was investigated based on results obtained from the DALDA-Daily Analysis of Life Demands for Athletes-questionnaire). It was estimated from the Recovery Stress Questionnaire (RESTQ) scores that treated athletes recovered more easily from exercise-they were less tired and had more energy—compared to the placebo group. A significant increase in antioxidant levels was also noted in the treated group. No adverse effects were observed throughout the study, indicating that this plant can be used safely .In addition, an aqueous extract of Withania somnifera effectively increased muscle strength and induced fat growth. The study found that after 8 weeks of supplementation with Ashwagandha, the participants had significantly greater increases in muscle strength and power, compared to those who received a placebo. Additionally, the participants who took Ashwagandha had faster muscle strength recovery following a muscle-damaging exercise compared to the placebo group.

Other Effects of Ashwagandha

Some medical studies suggest that Ashwagandha may also have applications in the treatment of COVID-19 as it exhibits many valuable properties such as the maintenance of immune homeostasis, regulation of inflammation, suppression of pro-inflammatory cytokines, protective effects on many organs, and anti-stress, anti-hypertensive, and antidiabetic effects It may therefore be a valuable adjunct therapy for COVID-19 sufferers and may also have a beneficial effect on co-morbidities. However, more research is still needed. Studies indicate that witanoside V and somniferin, isolated from Ashwagandha, may be potential inhibitors of the major SARS-CoV-2M protease .In addition, wi-tanolides isolated from Ashwagandha appear to be valuable phytochemicals with antiviral activity in the context of COVID-19 treatment . Withanone from Ashwagandha demonstrated antiviral activity in vitro by targeting the host's major viral protease (MPro) and transmembrane TMPRSS2.(24)

II. MATERIALS AND METHOD

Extraction procedure –

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Extraction by Maceration and Hot Continuous Percolation Process 100 g of W. somnifera dried roots were exhaustively extracted with various solvents (alcohol, water, hydro alcohol (50:50)), using different drugs - solvent ratios (1:6, 1:8, 1:10) by hot continuous percolation (10 hours) and maceration methods (10 hours). The extracts were dried and the percentage yields of extracts were determined. Preliminary phytochemical test was carried out to identify the nature of phytoconstituents present in the extracts.(25)

Preparation of nano particle

Synthesis of silver nano particle: 5 mg of ashwagandha aqueous extract was taken in a beaker and paced on a magnetic stirrer with hot plate. Then this 50 ml of 1 mM silver nitrate solution was added drop wise with constant stirring at 120 rpm. The colour change of the solution was checked periodically. Separation of silver nano particle: The nano particle were separated by centrifugation using REMI centrifuge at 500 rpm for 15 minute. Then pellets were collected and store.

Phytochemical Investigation:

Test for Starch: Dissolved 0.015 gm of iodine and 0.075 gm of potassium iodide in 5 ml of distilled water and add 2 - 3 ml of an aqueous extract of the drug, the blue color is produced.

Test for Steroids:

A) Salkowski Test: 2 - 3 drops of concentrated sulphuric acid was added to chloroform solution, shaken and allowed to stand, the appearance of red color in the lower layer indicates the presence of sterols.

Test for Flavonoids:

A) Shinoda Test: To the extract, a few fragments of magnesium ribbon and concentrated hydrochloric acid was added. The appearance of red to pink color after a few minutes indicates the presence of flavonoids. Lead acetate test: To the extract added few drops of aqueous basic lead acetate solution. Formation of a yellow precipitate indicates the presence of flavonoids.

Test for Alkaloids: The extract was basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid, shaken well and filtered. The filtrate was used for testing the alkaloids. A) Hager's Test: The filtrate was treated with a few drops of Hager's reagent. Formation of a yellow precipitate indicates the presence of alkaloids.

Test for Tannins:

A) Gelatin Test: To the extracts of the drug added 1% solution of gelatin containing 10% sodium chloride. Formation of a white precipitate indicates the presence of tannins. Test for Carbohydrates: Small amount of extracts of the drug were dissolved in little quantity of distilled water and filtered separately. The filtrates were used to test the presence of carbohydrates. A) Molisch's Test: The filtrate of the drug was treated with Molisch reagent and concentrated sulphuric acid was added from the sides of the test tube to form a layer. A reddish violet ring shows the presence of carbohydrates.

Test for Phenols:

Phenolic Compounds: Extract was dissolved in alcohol, and 1 drop of neutral ferric chloride was added to this. The intense color indicates the presence of the phenolic compound.

Glycosides: 0.5 ml of extract was taken in a test tube and added with 1 ml glacial acetic acid containing traces of ferric chloride. To this solution, 1 ml of concentrated sulphuric acid was added and observed for the formation of reddish

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brown color at the junction of two layers and the upper layer turned bluish green in the presence of glycosides.

Terpenoid: 2 ml of chloroform and 1 ml of conc. H2SO4 was added to 1mg of extract and observed for reddish brown color that indicates the presence of terpenoids.

Resins: 1 ml of extract was diluted with water. Formation on bulk black precipitate indicates the presence of resins.

Cardiac Glycoside: 100 mg of extract was dissolve in 1ml of glacial acid containing 1 drop of ferric chloride solution. This was then under layer with 1 ml of conc — sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardenolide. Coumarins: 1 ml of extract was treated with alcoholic 10% NaOH. Dark yellow color shows the presence of coumarins. (26)

III. DISCUSSION

In the present study, herbal silver nanoparticles were successfully developed using aqueous extract of Ashwagandha by green synthesis method. The change in color from light yellow to brown confirmed the formation of silver nanoparticles. The phytochemicals present in Ashwagandha such as flavonoids, alkaloids, and phenolic compounds acted as reducing and stabilizing agents during nanoparticle synthesis.

The study indicates that Ashwagandha-mediated silver nanoparticles can be prepared by a simple, eco-friendly, and cost-effective method. These nanoparticles may have potential applications in pharmaceutical and biomedical fields because of their enhanced biological activity and reduced toxicity.

IV. RESULT

Preliminary Phytochemical Screening:

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF ASHWAGANDHA CHURNA

S. no.	Phyto-constituents	Phytochemical test	Result
1	Starch	Iodine test	-
2	Steroids	Salkowski test	+
3	Flavonoids	Ferric chloride test	+
		Alkaline reagent text	-
4	Alkaloids	Hager's test	++
		Wagner's test	+
		Mayer's test	+
		Dragendorff's test	++
5	Tannins	Ferric chloride test	+
6	Saponins	Froth test	+
		Form test	-
7	Carbohydrates	Molisch's test	+
8	Glycoside	10% NaOH test	++
9	Terpenoids	Salkowski's test	+
10	Resins	Aqueous solution test	++
11	Cardiac glycosides	Keller killiani's test	++
12	Coumarins	10% NaOH solution	+
13	Phenol	Ferric chloride test	-

(+) Positive, (++) Moderated Positive, (-) Negative

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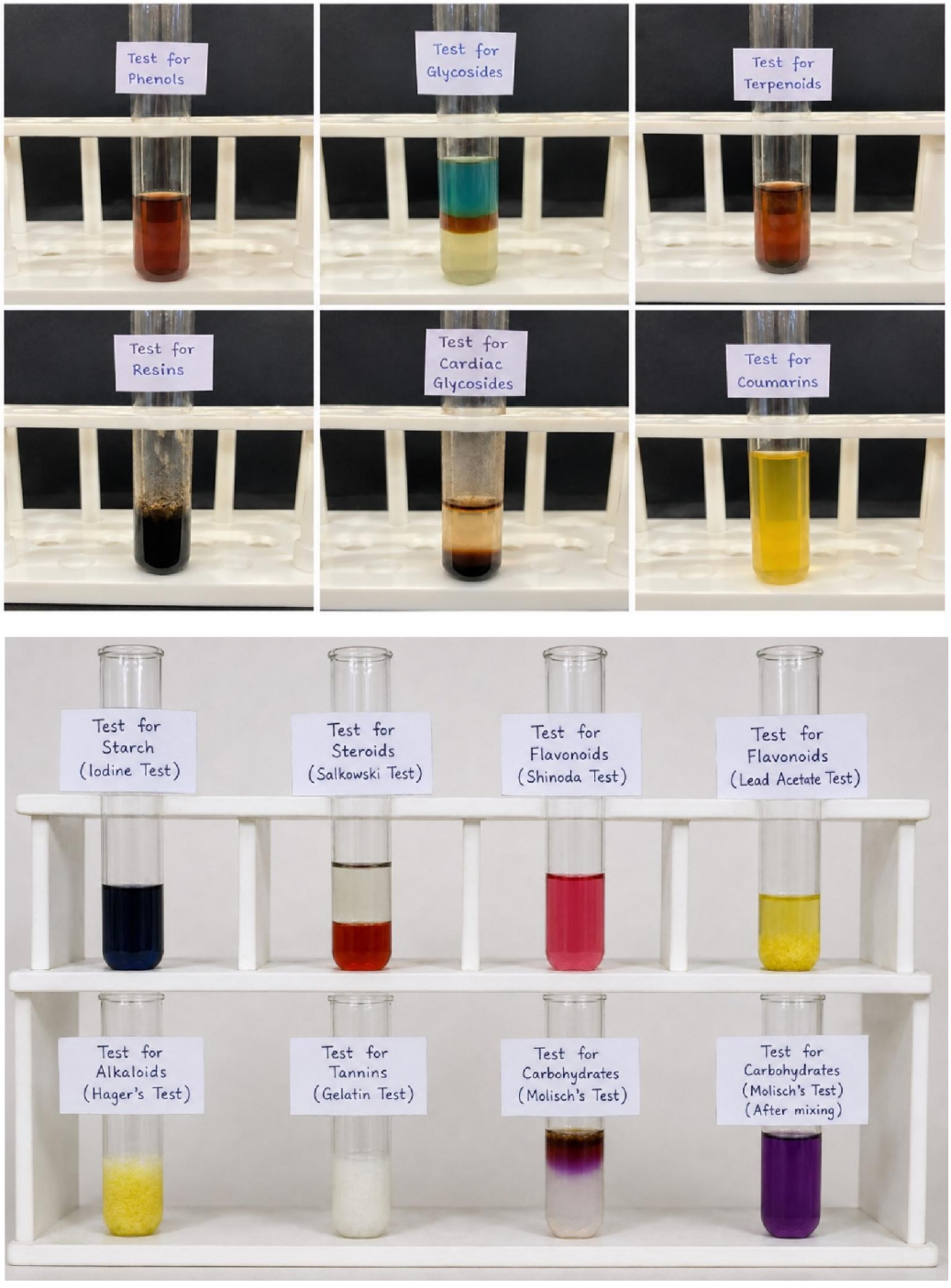

Fig- Pytochemical tests of ashwagandha

DOI: 10.48175/IJARSCT-35648

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
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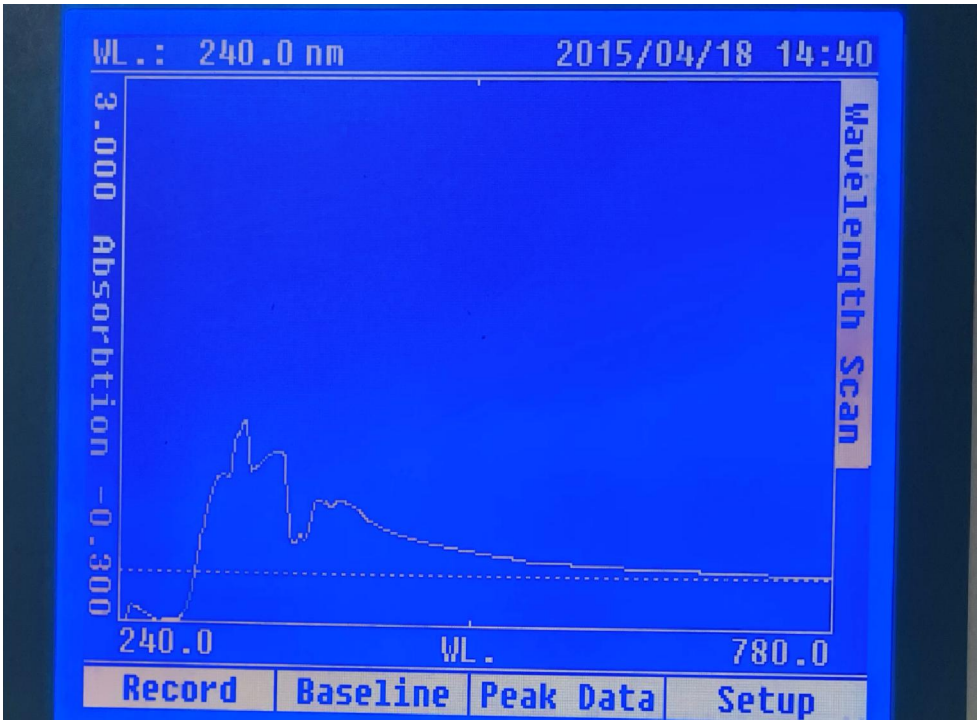

Figure: XRD pattern of synthesized AgNPs from Ahwagandha roots

UV-Visible Spectrophotometer Analysis:

The formation of silver nanoparticles was easily monitored with color change and it is due to excitation of surface plasmon vibration of metal nanoparticles. When colorless AgNO3 solution mixed with Ashwagandha roots at 0 hour time watery pale yellow color was shown. The mixed solution color at time 4 hours, 24 hours,28 hours, 48 hours, and 72 hours, was gradually from light yellow to dark reddish-brown. The color change indicates the synthesis of AgNPs by UV–Visible spectrum analysis as shown in (Figure 3). In our result, maximum absorption was observed at 430 nm (Figure 3), the optical properties of silver nanoparticles change which depends upon the collective oscillation of free electron when particles aggregate and the conduction electrons near each particle surface become delocalized and are shared amongst neighboring particles. The resulted observe the spectrum shifts the surface plasmon resonance (SPR) to lower energies. i.e. The absorption peaks move to red shift of plasmon resonance (increases the wavelength at which plasmon resonance occurs) with wide and lower intensity spectrum towards blue shift because of the accumulation effect. The increase of color intensity and (SPR) band sharpness clearly indicates the reduction of Ag+ into Ag0

V. CONCLUSION

The present research work conclusively establishes that green synthesis of silver nanoparticles using Ashwagandha (Withania somnifera) is a simple, cost-effective, and environmentally friendly approach. The use of plant extract eliminates the need for hazardous chemicals, making the process safer and more sustainable compared to conventional physical and chemical methods. The bioactive phytoconstituents present in Ashwagandha, such as alkaloids, flavonoids, tannins, and phenolic compounds, played a dual role as reducing as well as stabilizing agents in the formation of nanoparticles.

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Volume 6, Issue 9, May 2026
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