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A review: Phytochemicals and bioactivities of Ashwagandha (*Withania somnifera)*

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Abstract: Ashwagandha is scientifically known as *Withania somnifera* (WS); a crucial medicinal plant reported in the Indian traditional medicinal system, Ayurveda. It possesses a diverse range of phytochemicals, making it abundant in bioactive compounds. Its bioactive substances are withanolides and alkaloids which play a crucial role in pharmacological actions. Owing to this, ashwagandha possesses a broad spectrum of pharmacological activities to be shown including anti-inflammatory, anti-cancer, anti-stress, immunomodulatory, reproductive stimulator, antioxidant, adaptogenic, neuroprotective, anti-aging, anti-diabetic, and endocrinological activities and among others. This review compiles the chemical composition and biological role of this wonderful medicinal plant in the treatment of various ailments. The combinatorial therapies of this plant extract with other bioactive compounds have been also addressed.

Keywords: Ayurveda, ashwagandha, phytochemicals, pharmacological, bioactive compounds.

History:

Over the last three thousand years, Ashwagandha has been utilized as a Rasayana in Ayurvedic and indigenous medicine (Madhuri, S., & Govind, P. (2009); Wiciński, M., et. al (2023)). It is a popular plant for promoting youthful energy, longevity, and overall well-being. Ashwagandha is scientifically known as *Withania somnifera* (WS) or "Winter Cherry" that belongs to the *Solanaceae,* or nightshade family.

It is a little evergreen shrub native to the drier region of India, the Middle East, and some areas of Africa. This shrub can reach heights of 2 meters and widths of 1 meter, and evergreen, xerophytic, woody, short, tender, and perineal. Its flowers are tiny and green, while the leaves are oval, dull, simple and hairless. Its fruit is an orange-red, hairless berry that measures 5–8 mm in diameter and is encased in a persistent, balloon-like calyx made of membranous tissue. The many kidney-shaped, light-brown seeds found inside its fruits are abundant. The Ashwagandha

roots have a pungent fragrance along with bitter and caustic taste, and it gives rise to fiber-like subsidiary branches. Roots of Ashwagandha are regarded as tonic, aphrodisiac, narcotic, diuretic, anthelmintic, astringent, thermogenic, and stimulant. (Madhuri, S., & Govind, P. (2009)).

The word "horse's smell" (ashwa means horse and gandha means smell) describes Ashwagandha in Sanskrit, and the herb is thought to provide strength comparable to that of a horse. Scabies, senile debility, lumbar aches, dyspepsia, miscarriage, inflammation, dropsy, ulcers, and hiccups are just some of the conditions that benefit from this commercially important medicinal crop's overall stimulant, sedative, and tonic properties (Bashir, A., et. al (2023).

It is a drought-tolerant annual crop and is largely grown in dryland areas. It is cultivated under rainfed conditions by small and marginal farmers of Rajasthan, Andhra Pradesh, Madhya Pradesh, and Karnataka states in India (Dar, N. J., et. al (2015)). The main constituents of Ashwagandha are withanolides, which are a group of chemicals encompassing steroidal alkaloids and lactones. Researchers have successfully extracted and identified more than twelve distinct alkaloids, forty different withanolides, and several sitoindosides from various components of the Withania genus, including leaves, stems, roots, and fruits (Mishra, L. C., et. al (2000)).

Numerous pharmacological benefits such as anti-inflammatory, anti-cancer, anti-stress, immunomodulatory, antioxidant, adaptogenic, neuroprotective, and endocrinological effects have been observed in preclinical studies of ashwagandha and its components (fig.1.) (Bhattacharya, et. al (2000); Sahni, Y. P., et. al (2014)). When compared to other forms of Ashwagandha, the Nagori Ashwagandha (also known as Indian Winter Cherry) is generally considered the best due to highly effective its raw as well as formulated materials. People who have recently used Ashwagandha powder have reported feeling healthier and more energetic (Abdelwahed, et. al (2023)).

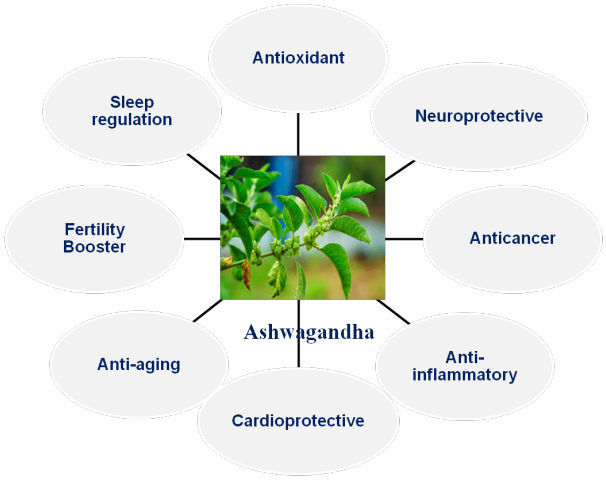

Fig.1: Various pharmacological activities of Ashwagandha

Chemical Composition:

Ashwagandha possesses a diverse range of phytochemicals, making it abundant in bioactive compounds. Depending on the location of the raw material i.e. Ashwagandha, it exhibits a diverse composition of chemical compounds. Withanolides and alkaloids are the primary components responsible for its pharmacological actions. Chemicals belonging to the withanolide family share the structural characteristics of ergostane,

which consists mostly of a "six-membered lactone" at either the "C-8 or C-9 position." (John, J. (2014)). Compounds like withanopherin A, withanolides A-Y, withanone, withadomniferin A, and withasomniferols A-C are all included in the class of substances known as withanolides (some depicted in fig.2.). Withanin, somniferin, somnin, tropin, pseudowithanin, pseudotropin, choline, kuskohigrin, isopeletierin, and anaferin are all examples of the class of chemicals known as alkaloids. Several flavonoids, including quercetin and its glycosidic derivative, 3-O-rutinoside-7-O-glucoside, are also found in Ashwagandha. In addition, withanolide glycosides found in Ashwagandha, have a glucose moiety at carbon position C-27. The chemical compounds such as sitoindoside IX and X are part of this combination (Abdelwahed, et. al (2023)).

The steroidal saponins found in this plant extract include the acyl group-containing compounds sitoindoside VII and VIII. Numerous bioactive substances, including saponins, coumarins (in particular scopoletin), sterols, chlorogenic acid, resins, lipids, carbohydrates, steroidal lactones, β-sitosterol, scopoletin, anahygrine, cysteine, and fatty acids, have been found in Ashwagandha (Dutta, R., (2019)).

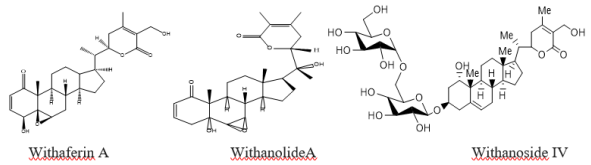

Fig.2. Chemical structures of the main active components present in Ashwagandha.

Pharmacological properties of Ashwagandha:

Indian medicinal system reported the ashwagandha as a medicinal plant that exhibits a broad range of pharmacological effects such as anti-inflammatory, antioxidant, anticancer, antimicrobial, neuroprotective, antistress, antiulcer, fertility, cardiovascular, and others (Singh, N., & Gilca, M. (2010); Abbas S. S., & Singh N. (2006)).

Besides it has limited clinical use due to lack of documentation in the modern scientific platforms. There exists a need to explore the scientific parameters for enhanced clinical performance in good health. The physiological consequences of Ashwagandha have been the subject of several investigations, many of which have been published by independent researchers (Gaurav, H., et. al (2023)). The authors want to compile the results of some pharmacological effects in the following manner:

Anti-inflammatory activity:

The therapeutic potential of WS is currently under investigation for several medical conditions characterized by bodily inflammation, including cardiovascular, pulmonary, and autoimmune illnesses. Additionally, WS is being explored as a potential treatment for diabetes, malignancies, and neurological disorders (Bhattacharya, S. K., et. al (1997)). Some of the Preclinical studies have shown that WS can inhibit inflammatory biomarkers as well as modulate mitochondrial processes and induce apoptosis. Powder of its roots was found to be a potent inhibitory effect on inflammatory markers such as cytokines including interleukin (IL)-6 and tumor necrosis factor (TNF)-a, nitric oxide (NO), ROS and proteinuria, nephritis

in a mouse model of lupus (Mikulska, P., et. al, (2023); Merecz-Sadowska, A., (2021)).

In one study, to examine the effect of Ashwagandha in the treatment of rheumatic diseases, the oral administrations of WS root powder was given to rats in an animal model experiment which are being induced inflammation with an injection of CFA (Complete Freund's adjuvant) before three days of starting treatment (Rasool, M., & Varalakshmi, P. (2006)).

Phenylbutazone served as a positive control and was given to the experimental group of rats. The results showed a significant reduction in inflammation and changes in the amounts of many serum proteins, such as a 2glycoprotein, acute phase protein1, and prealbumin.

In this study, we used the human keratinocyte cell line HaCat to test the effects of an Ashwagandha root aqueous solution. The results showed that the Ashwagandha root solution had inhibitory effects on the NF-B and MAPK pathways. This was achieved by simultaneously upregulating the expression of anti-inflammatory cytokines and downregulating the expression of proinflammatory cytokines such as interleukin (IL)-8, IL-6, tumor necrosis factor (TNF)-1, and IL-1 and IL-12. This study's findings that Ashwagandha has anti-inflammatory properties suggest it could be used to treat or prevent skin inflammation (Sikandan, A., et. al (2018); Gupta, M., & Kaur, G. (2018)).

A preclinical model was used to evaluate the potential anti-neuroinflammatory effects of ashwagandha water extract (ASH-WEX). The results showed that ASH-WEX administration significantly suppressed reactive gliosis, decreased the expression of nitro-oxidative stress enzymes, and reduced the production of inflammatory cytokines like (TNF)-1, interleukin-1 (IL-1), and IL-6. ASH-WEX showed anti-inflammatory effects by blocking the activation of NF-B, PB38, and JNK/SAPK MAPK pathways in response to lipopolysaccharide (LPS). The findings of this study suggest that WS can reduce the neuroinflammation associated with several neurological disorders. Kanjilal et. al (2021), studied the effects of Ashwagandha extract on arthritis symptoms and reported positive results from utilizing the supplement for 8 to 12 weeks.

Ashwagandha's immunomodulatory effects have been verified in several studies employing immunodeficient animals. For instance, administration of powdered WS root resulted in a significant rise in the number of white blood cells and bone marrow cells. It also caused an increase in the number of immunological cells, an increase in the phagocytic activity of macrophages, and a general increase in the concentration of antibodies in the blood (Davis, L., & Kuttan, G. (2000); Alanazi, H. H. and Elfaki, E. (2023)).In another study, the methodology of investigation was a randomized, double-blind, placebo-controlled trial with an open-label follow-up. Ashwagandha extract significantly improved natural killer cell activity and cytokine levels compared to the placebo group, according to the study's findings (Tharakan, A., et. al (2021)).

Anti-oxidant activity:

WS contains many powerful antioxidant phytochemicals such as polyphenols, sitoindosides VII–X, withaferin A, and glycowithanolides which are mainly responsible for its pharmacological effects (Gaurav, H., et. al (2023)).

The Egyptian Ashwagandha's leaves are full of antioxidants against an HCC cell line HepG2 that belongs to chemotype III, which is different from the Indian Ashwagandha regarding extent of antioxidant activity exhibited. According to recent studies, Ashwagandha water extract (ASH-WX) has been reported as a powerful antioxidant and can also inhibit the growth of cancer cells (Singh, G., et. al (2010)). ASH-WX has a strong cytotoxic effect on HepG2 cells.

It also showed a marked effect on the cells causing shrinkage and accumulation of dead HepG2 cells when compared with control untreated cells (Arora, S., et. al (2004)). A study also found an increase in the levels of superoxide dismutase, catalase, and glutathione peroxidase when the antioxidant compounds extracted from ASH-WX tested on rat brains (Christina, A. J. M., et. al (2004)).

Anti-stress/Influence on Adaptation:

Adaptogens refer to a category of herbal substances that possess the capacity to augment an individual's capacity to effectively manage and respond to stressors, as well as adapt to various environmental and physiological changes.

The latest characterization of adaptogens delineates them as hormones that control metabolism and help the body adapt to its surroundings by reducing the risk of negative effects. An optimal adaptogen should effectively alleviate the adverse consequences induced by stress exhibit a high level of safety, even when administered in higher dosages, and have minimal adverse side effects (Amir, M., et. al (2023)).

Based on these characteristics, Ashwagandha can be considered an adaptogen. Adaptogens are supposed to enhance the body's ability to withstand physiological and psychological stressors. Insomnia, aging, anxiety, and various other disorders are among the applications for which it is utilized (Dickson, T. C., & Vickers, J. (2001)).

Ashwagandha demonstrates favorable comparability to Eleutherococcussenticosus (Siberian Ginseng) and Panax Ginseng (Chinese and Korean Ginseng) in terms of its adaptogenic characteristics, therefore earning the colloquial designation of Indian Ginseng. Numerous scientific investigations have been performed to investigate Ashwagandha's adaptogenic and anti-stress properties using an animal model of biology. A study demonstrated the efficacy of Ashwagandha in enhancing stamina, as well as its ability to mitigate stress-induced gastric ulcer, carbon tetrachloride (CCl_4)-induced hepatic toxicity, and mortality. An extract of Ashwagandha roots in water were administered orally at a dosage of 100 mg. Its roots have demonstrated comparable anti-stress effects in rodent models (Gaurav, H., et. al (2023)). The findings exhibited a statistically significant elevation in the plasma corticosterone level, phagocytic index, and avidity index in rats exposed to cold swimming stress. The findings of

this study suggest that the use of WS in its unrefined state exhibits strong anti-stress properties. Furthermore, these studies provide evidence that aligns with the hypothesis of Ayurvedic tonics, vitalizers, and rejuvenators, which propose the clinical application of WS for the prevention and treatment of various stress-related ailments such as arteriosclerosis, premature aging, arthritis, diabetes, hypertension, and malignancy (Panossian, A., & Wikman, G. (2009)).

A research investigation was undertaken on a cohort of equine subjects who were administered Ashwagandha's root extract. The mice were exposed to a range of stressors including vigorous physical activity, isolation, and auditory disturbances. Throughout the experiment, parameters including hematological, biochemical, hormonal, and immunological factors were monitored. Following 21 days, the group that received treatment demonstrated notable and statistically significant decrease in levels of glucose, cortisol, epinephrine, creatinine, triglycerides, IL-6, alanine aminotransferase, and aspartate aminotransferase. The aforementioned results suggest that Ashwagandha possesses adaptogenic, antioxidant, and immunostimulating properties (Bhattacharya, S. K., & Muruganandam, A. V. (2003)).

The present study additionally examined the adaptogenic properties of standardized extracts derived from Panax ginseng and WS root in rats subjected to chronic stress using the Footshock technique. Research has linked chronic stress to a host of unfavorable health effects, such as the development of hyperglycemia, impaired glucose tolerance, increased levels of corticosterone, the formation of gastric ulcers, sexual dysfunction, cognitive impairments, compromised immune function, and the onset of mental depression. Nonetheless, the administration of extracts derived from WS and Panax ginseng before the stressor effectively alleviated the many illnesses stated earlier.

Additionally, an investigation was conducted on the adaptogenic properties of a water-based fraction obtained from the root of Ashwagandha, specifically focusing on its lack of withanolides.

The primary objective of this study was to investigate the effects of this aqueous fraction on rats. The findings of the study revealed notable anti-stress properties, as seen by enhanced swimming endurance and decreased weight of the adrenal glands, without any observed negative consequences (Singh, B., et. al (2001)).

Feel Young/ Skincare:

Herbal adaptogens are used to improve attention, improve physical stamina, strength, and energy levels, increase endurance in scenarios where fatigue is present, reduce stress, improve sexual dysfunction, restore cognitive performance that has been affected by stress, and maintain cortisol (the stress hormone) and other hormone levels under control (Singh, N., et. al (2011)).

These properties ultimately affect the skin cells and help to look radiant. Using Ashwagandha for skincare helps to get skin-calming properties. The latter refreshes the body and induces a peaceful sleep. It also controls the depletion of

collagen production and enhances it, which effectively helps flatten fine lines and wrinkles, maintain skin elasticity, and making the skin plump naturally (Jezierska, A., & Sykuła, A. (2023)).

The various causes of skin pigmentation include prolonged exposure to UV rays and harmful environmental elements, which can cause excess melanin production in the skin. Ashwagandha benefits in melanin production in the skin cells, reduces fine lines, and helps the skin retain its natural softness. Prolonged exposure to cosmetics infused with toxic chemicals can trigger keratosis in skin, leaving it dull and dehydrated. Ashwagandha for skin is an effective antidote for controlling the production of keratosis and relaxes the skin tissues. It also imparts deep moisturization and hydration by promoting hyaluronan production (an enzyme or linear polysaccharide that softens the skin tissues)

It also includes for cleansing impurities from the skin layers, thus preventing the formation of acne and other signs of aging (Maloh, J., et. al (2022); Kumar, S., et. al (2022)).

Anti-aging Effect:

• Ashwagandha has historically been employed as a substance with anti-aging properties (Singh, N., et. al (2011); Kumar, N., et. al (2016)). Ashwagandha can defend combat the deleterious effects of stress and boost general health and immunological function, increasing vitality, and elongating life expectancy. Ashwagandha has been studied for its purported ability to promote healthy aging by reducing the rate of cell death and enhancing tissue regeneration. KSM-66 Ashwagandha, a famous ayurvedic herb, is clinically proven to reduce stress, enhance memory and cognition, improve sexual function in both men and women, and increase strength, and the immune system of the body. Its root extract significantly was increased lifespan by about 20 % in a nematode model (Caenorhabditis elegans) (Sharma, R., & Amin, H. (2015)). The longevity of C. elegans was significantly lengthened by the bioactive chemical Withanolide, by 29.7 % on average. In addition, it showed no activity or inactivity in this organism while yet being able to influence the insulin/IGF-1 signaling (IIS) pathway. Experiments with varied doses of ashwagandha root extract on human HeLa cell lines found a dose-dependent increase in telomerase activity of around 45%. In addition, genotoxicity caused by H2O2-induced DNA damage in human peripheral blood cells was inhibited by the Ashwagandha extract (Kumar, R., et. al (2013); Akhoon, B. A., et. al (2016); Raguraman, V., & Subramaniam, J. R. (2016)).

Reproductive stimulation:

Sexual dysfunction and infertility have become a critical health concerns worldwide. The absence of gonadotropin, also known as Follicle Stimulating Hormone; a hormone in the functions of men and women's reproductive system affects the normal qualitative and quantitative spermatogenesis (Sharma et. al (2015)).

Several studies have been confirmed the spermatogenic, aphrodisiac, and fertility effect of the root, stem, leaf, and fruit extracts of *WS* both in humans and

in animals (Tandon, & Yadav, (2020); Majeed et. al (2023); Kaspate et. al (2015)).

In a study, the extracts of *WS* mildly stimulate the release of gonadotropin hormones in adult rats (Kataria et. al (2015)) and found to improve human menopausal syndrome (Mandlic, & Namdeo, (2020)).

In humans, clinical investigations on the efficacy of *WS* have been reported and studied the permatogenic activity of standardized capsule of *WS* root extract on 46 male patients with oligospermia (< 20 million/ mL) focusing on estimating their semen parameters and serum hormone levels. In this study, capsule of *WS* root extracts (225mg) was administered orally-3-times daily for 12 weeks to 21 patients and compared with 25 patients on placebo. The results demonstrated that the sperm count, sperm motility and semen volume increased significantly by 167%, 57% and 53% respectively (Dongre et. al (2015); Khalil et. al (2015)). Similarly, a very recent triple blind randomized clinical study compared the effects of *WS* with pentoxifylline on sperm parameters of 100 idiopathic infertile male patients for 90 days (Nasimi et. al (2018a)). The result demonstrated that *WS* root extract improved sperm parameters without any adverse effect. The mechanism of action of *WS* on male infertility patients is by suppressing oxidative stress (Tahvilzadeh et. al (2016)).

Neuroprotective Effect:

Ashwagandha, a member of the Rasayana subclass known as MedhyaRasayanas, enjoys widespread recognition for its beneficial effects. Commonly, "Medhya" refers to one's mental powers and capacities for thought. This is why Medhya Rasayana is used to improve brain function and memory, such as the use of Ashwagandha (Purohit, S, et. al (2024)).

People with memory impairments, such as children with memory deficiencies, people with limited memory owing to head injuries or lengthy illnesses, and elderly folks, are the most likely to benefit from the cognitive-enhancing effects of Medhya Rasayanas. Ashwagandha exhibits neuroprotective properties, which means it helps to protect brain cells from damage and degeneration. It has antioxidant and anti-inflammatory effects that combat oxidative stress and inflammation, both of which are involved in neuro diseases like "Alzheimer's and Parkinson's". Ashwagandha may also promote the growth of nerve cells and it also enhances neuronal connectivity (Vyazovskiy, V. V., & Delogu, A. (2014); Aguiar, S., & Borowski, T. (2013)).

In another study, the aqueous extract of WS's root has proved to be a neuroprotective agent by shielding PC-12 cells from the cytotoxicity brought on by Aβ (1–42) and H2O2 (Kumar et. al (2010)). Withanolide A protects the AD by increasing the expression of neuroprotective protein hemeoxygenase-1 and promoting neuritogenic activity and inhibiting secretase activity (Nitti et. al (2018); Bhat et. al (2022)). Ethanolic root extracts of WS provides nigrostriatal dopaminergic neuro-protection against MB–PQ induced Parkinsonism by suppressing the expression of iNOS (Prakash et. al (2014)).

Anti-cancer activity:

Cancer refers to illnesses caused by unchecked cell division, primarily caused by genetic mutations in proteins implicated in the regulation of the cell cycle, including proto-oncogenes and tumor suppressor genes. The available statistical data indicates that cancer is a substantial and increasing issue in terms of both health and societal impact, remaining a leading cause of death despite extensive global research efforts (Mehta, V., et. al (2021)).

Various substances derived from diverse components of Ashwagandha, including the root, stem, and leaves, have exhibited notable anti-cancer characteristics (Singh, N., et. al 2021)).

These compounds can be utilized alone or in combination with other chemotherapeutic agents for cancer treatment (Vashi, R., et. al (2021); Tang, Q., et. al (2020)).

Among them, witanolides, alkaloids found in the plant, show significant anti-cancer potential and play a crucial role in inducing apoptosis, making them the most promising compounds in this regard (Nagy, Z., et. al (2020)).

It has been shown that Ashwagandha is effective in treating various types of cancer, including breast, colon, lung, prostate, and hematological cancers (K Thakur, A., et. al (2015)).

The major constituents of WS, withaferin A and withanolide D, are mainly responsible for its anticancer effects by blocking the synthesis of RNA and proteins, respectively. Cancer cells may be killed off by inducing apoptosis through the inhibition of RNA and protein synthesis (Tekula, S., et.al (2018)).

When given to tumor-ridden rats, the drug increased liver and skin levels of glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) (Abdallah, E. M. (2011)).

Melanoma cells' ability to proliferate, migrate, and undergo apoptosis are all hampered by this chemical. Withaferin A's anticancer properties have been studied in the setting of glioblastoma multiforme GBM (Surya Ulhas, R., & Malaviya, A. (2022)).

To determine how withaferin A affects signaling pathways, multiple techniques were used, including RNA-seq analysis, Western blotting, immunofluorescence labeling, quantitative real-time polymerase chain reaction, and siRNA gene silencing. The Ashwagandha plant is the source of the chemical Withaferin A, which is highly effective in inhibiting the proliferation of GBM cells in both *in-vitro* and *in-vivo* conditions and activates a preexisting apoptotic pathway. Dephosphorylation of Thr161 CDK1 may also cause to stop the cell cycle during the G2/M transition. These results have major implications for the improvement of withaferin A treatment and prevention protocols for GBM (Andallu, B., & Radhika, B. (2000); Visavadiya, N. P., & Narasimhacharya, A. V. R. L. (2007)). A study conducted by Jawarneh et.al (2022), indicated that a therapy strategy combining Ashwagandha extract and intermittent fasting may be effective in the management of breast cancer. In combination with cisplatin and WS,

this method of treatment could be quite effective. Inducing apoptosis in cancer cells and reducing the toxic effects of cisplatin on the liver and kidneys were also hallmarks of this combined treatment approach. Additionally, Azab et al. (2022) have demonstrated that the administration of Ashwagandha extract exhibits a protective impact against the deleterious consequences of radiation exposure.

The study also revealed that the intervention of WS effectively mitigates oxidative stress and inflammation in both the liver and spleen. This study concludes that the therapeutic utilization of WS root extract may have potential benefits in preserving essential organs such as the liver and spleen against radiation-induced damage (Jain, S., et. al (2006)).

Anti-fungal activity:

Ashwagandha contains several bio-active compounds which include alkaloids, withanolides, and withaferins. All these compounds are believed to contribute to its anti-fungal properties through various mechanisms which are the disruption of fungal cell membranes, Inhibition of fungal growth, and Modulation of fungal biofilms (Singh, G., et. al (2010)).

Some research studies have investigated the anti-fungal activity of ashwagandha against various pathogens like Candida species, *Aspergillus species* and *Dermatophytes*, where Candida species is a common fungal pathogen associated with infections. Ashwagandha extracts and compounds such as withanolides and withaferin A can inhibit the growth of the *Candida* species including the drug-resistant strains. *Aspergillus* species are a common cause of invasive fungal infections and Withaferin A was used to inhibit the growth and biofilm formation of these species (Arora, S., et. al (2004)). The extracts of ashwagandha have demonstrated anti-fungal activity against *Dermatophytes* which inhibits their growth and reduces the severity of skin lesions caused by fungi (Christina, A. J. M., et. al (2004)).

Anti-bacterial activity:

The emergence of drug-resistant strains as a major health problem is a relatively recent development of great concern. Drug-resistant microorganisms have developed due to the widespread and often unnecessary use of antibiotics, and leaving some treatments completely ineffective. In the treatment of bacterial infections, Ashwagandha has recently emerged out as an effective complementary therapy (Bisht, P., & Rawat, V. (2014)).

Both Gram-positive and Gram-negative bacteria are vulnerable to the antibacterial effects of ashwagandha be efficient. These bacterial strains are *Agrobacterium tumefaciens, Acinetobacter baylyi, Bacillus cereus, Bacillus thuringiensis, Bacillus subtilis, Citrobacter freundii, Corynebacterium diphtheriae, Escherichia coli, Enterobacteraerogens, Klebsiella pneumonia, Lactic acid bacterial strains, Micrococcus luteus, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus* and few others (Nandeshwar, Rout, J., et. al (2023); Akroum, S., et. al (2010); Li, X. S., et. al (2007)).

Despite their efficacy, many of the pharmacological medicines now used to

treat bacterial infections are associated with serious adverse responses due to their intrinsic toxicity. Alternatively, Ashwagandha is a plant that has potential to show immunomodulatory effects that boost immunological response (immunopotentiation), cytotoxic effects, and gene silencing have all been credited with ashwagandha's antibacterial activity. Evidence from animal model research showed that WS is a successful therapeutic intervention for *salmonellosis*, reducing the severity of sickness after exposure to this pathogen (Jin, K., et. al (2015)).

Furthermore, there is evidence suggesting that it possesses potential as an agent for combating dental caries. This is achieved by its ability to impede the growth of oral bacteria, specifically *Streptococcus mutans* and *Streptococcus sobrinus*, as well as hinder their production of acid, acid tolerance, and proliferation of biofilms. Significantly, ashwagandha demonstrates remarkable effectiveness against *Salmonella typhi* (Kumari, M., & Gupta, R. Á. (2015); Singh, G., & Kumar, P. (2011); Alam, N., et. al (2012)). A separate study has demonstrated that ashwagandha exhibits significant antifungal effects by effectively reducing the growth of *Candida albicans* (Mwitari, P. G., et. al (2013)).

However, it appears that certain fungal species, such as *Aspergillus flavus* and *Aspergillus niger* may be resistant to its compounds. Nonetheless, WS glycoprotein isolated from its root tubers has shown both antifungal properties against *Aspergillus flavus, Fusarium oxysporum,* and *Fusarium verticillioides* and bacterial properties against *Clavibacter michiganensis subsp. Michiganensis* (Owais, M., et. al (2005)). The antibacterial action of ashwagandha extract against *Psedomonas aeruginosa* has been demonstrated through morphological study and membrane stabilization studies, which revealed that the extract damages the cell membrane. (Chandrasekaran, S., et. al (2013); Girish, K. S., et. al (2006)). Studies conducted on mice have also demonstrated the effectiveness of WS extracts, particularly at higher concentrations in treating malaria by significantly reducing parasitemia (Dikasso, D., et. al (2006); Mahdi, A. A., et. al (2011)).

Cardioprotective activities:

Ashwagandha's effects on a group of albino rats with isoprenaline-induced cardiac necrosis were examined in the present investigation. The experimental results showed that the levels of glutathione and the activities of superoxide dismutase, catalase, creatine phosphokinase, and lactate dehydrogenase dropped after WS administration (Mohanty, I. R., et. al (2008)).

Furthermore, a notable reduction in lipid peroxidation levels was observed. The results of this study suggest that WS exhibits cardioprotective effects in a rat model of isoprenaline-induced necrosis (Khalil, M. I., et. al (2015); Michalik, A., & Jarzyna, R. (2016)).

Further studies were performed on mouse Animals in this study had cardiac ischemia induced on purpose, which resulted in significant myocardial necrosis, a disruption in the equilibrium of oxidation-antioxidation systems, and an increase in lipoperoxidation. Histopathological examinations

confirmed that the administration of WS injection significantly mitigated ischemia-induced heart damage. Ashwagandha's ability to restore oxidative balance and its anti-apoptotic properties are responsible for its cardioprotective effects (Guo, R., et. al (2019)).

Researchers found that withaferin A has a cardio-protective effect at low doses when tested on rats. This was accomplished by increasing the ratio of Bcl-2 to Bax proteins and phosphorylating AMP-activated protein kinase (AMPK) to boost the activity of the mitochondrial anti-apoptotic pathway. The enzyme AMP-activated protein kinase (AMPK) is involved in a wide variety of processes, including the regulation of energy balance at the cellular and systemic levels. The endocrine system is essential for homeostasis because it controls the amounts of carbohydrates, proteins, and lipids in the brain and other tissues. It also shows sensitivity to hormonal stimuli, which in turn affects satiety and thermogenesis. Many processes associated with aging and age-related diseases are thought to be influenced by AMPK activation, which has been connected with caloric restriction. It has been hypothesized that AMPK's role in reestablishing energy balance contributes to increased human lifespan and well-being. The results of this study surprised researchers by showing that higher doses of withaferin A (5 mg/kg) did not produce the same positive effect as lower levels (1 mg/kg) (Kaushik, M. K., et. al (2017)).

The antioxidant and anti-apoptotic properties of the *WS* extracts are mainly responsible for a significant cardio-protective effect based on the myocardial and antioxidant histopathological evaluations and preventing the myocardial infarction and ischaemia–reperfusion injury to the heart. (Afewerky, H. K., et. al (2021)).

Further, an *in-vivo* study on the biochemical and histopathological parameters showed that the extracts of *WS* protect the myocardial cell membrane due to its anti-lipoperoxidation and antioxidants effects (Khalil et. al (2015)). The acute toxicity of the *WS* extract at 2000 mg/kg is determined practically safe, and its administration possesses low toxicity (Patel et. al (2016)), however significant to combat many patho physiological diseases.

Sleep Regulation:

Insomnia is characterized by a lack of sleep relative to an individual's rest needs, which in turn leads to impaired daytime functioning. The symptoms of insomnia range from trouble falling asleep to problems staying asleep to waking up too early. Even if a person practices good sleep hygiene, they may still have these problems. The symptoms of these diseases have far-reaching consequences, affecting not only one's well-being but also one's ability to concentrate, control one's emotions, think clearly, and be motivated in one's work and relationships (Siemiński, M., et. al (2018)).

There is variation in epidemiological data about this condition across different countries, which may be attributed to probable differences in diagnostic methodologies. It is imperative to acknowledge that sleep accounts for around 30% of the human lifespan,

emphasizing the fact that any disruptions in this realm profoundly alter the body's state of equilibrium (Kaushik, M. K., et. al (2017)).

Herbal remedies are being considered as a feasible therapeutic option for the treatment of insomnia that are associated with existing sleep medicines. In a study, a dosage of 300 mg of Ashwagandha root extract was given to patients twice a day for 10 weeks. According to the findings, sleep quality was significantly improved, and the process of falling asleep was aided (Kelgane, S. B., et. al (2020)). The acceptability, efficacy, and safety of Ashwagandha root extract in an aged population from 65 to 80 years have evaluated (Vernon, M. K., et. al (2010)).

Improvements in sleep quality, morning mental sharpness, and overall health were found. The study showed that the drug was very safe, quite effective, and very well tolerated by the people who used it. Owing to its anxiety-reducing and sleep-inducing effects, Ashwagandha has been used for centuries as a sleep aid. In another study, the aqueous extract of WS was found most effective at combating sleeplessness.

Nonetheless, extensive research on an aqueous extract predominantly made up of triethylene glycol uncovered its significant capacity to induce NREM sleep. Triethylene glycol sold commercially showed the same effects seen in this study; however, the extent of these effects was varied with the dose (Roth, T. (2010)).

According to Baker et. al (2022), the research indicated that Ashwagandha could benefit college students by helping them relax, sleep better, and have more energy with focused thought structures. The study used qualitative analysis to assess participants impressions of Ashwagandha's effect on the aforementioned traits. The participants who took Ashwagandha supplements performed better in these categories than those who took a placebo, according to the study's findings.

The results of this study show that Ashwagandha has a significant and positive effect on lowering stress and improving the quality of sleep. However, the results were inconclusive in terms of reducing occurrences of hunger (O'Connor et al. (2022); Roth, T., et. al (2010)).

Cognitive Enhancement:

Ashwagandha has been associated with improved cognitive function and memory. It may enhance cholinergic activity which supports the release and availability of acetylcholine which is an important neurotransmitter involved in learning and memory processes. Ashwagandha also attenuates oxidative stress and inflammation, which basically contribute to cognitive decline (Bostrom, N., & Sandberg, A. (2009); Carskadon, M. A., & Dement, W. C. (2005); Kreider, R. B. (2018); Sumanran, V. N., Boddul, S., & Madhuri, D. (2007)).

Anxiolytic effect:

Across the three standard measures of anxiety: the social interaction, elevated plus-maze, and feeding delay in a novel environment; WS treatment exhibited the calming anxiolytic effect that demonstrated similarities to

the pharmaceutical drug Lorazepam (Alzheimer's Association (2010)). Tribulin, an endocoid marker associated with anxiety was reduced in the brains of rats by both Ashwagandha and Lorazepam (Ross, S. M. (2023)). Tribulin levels dropped after the anxiogenic medication pentylenetetrazole was given to the subjects. Further, two widely used tests, the forced swim-induced "behavioral despair" test and the "learned helplessness" test, showed antidepressant effects comparable to imipramine (Wadhwa, R., & Kaul, S. C. (2023); Schliebs, R., et. al (1997); Khabiya, R., et. al (2023); Mikulska, P., et. al (2023)).

Therefore, Ashwagandha is a crucial Indian medicinal plant that plays an important role in the health benefits especially helps us to feel energetic, younger, and healthy. This review compiles the various pharmacological effects of it and its other components and may provide a compendium of enriched information on Ashwagandha. Table 1 also helps to explain the biological role of it with different parameters.

Table 1: The effect on biological properties of some combinatorial therapy of Ashwagandha

Sr. No.	**Combinations with Ashwagandha (*Withania somnifera*)**	**Disease**	**Outcomes**	**Reference**
1.	WS + *Bacopa monnieri* (Brahmi)	Alzheimer's	Strengthens the functional activity of nervous system, enhances memory & cognition and recalling capabilities.	Bredesen, D. E. (2009); Abascal, K., & Yarnell, E. (2004). 99-103
2.	WS+ *Asphaltumpunjabianum* (Shilajit)	Asthma, allergies, diabetes, and diabetic neuropathy; sexual dysfunction; weariness; stress; generalized weakness.	It strengthens the immune system, making the body more resistant to illness and more robust under pressure, and helps to build sperm quality & quantity as well.	Rao, T. S., et. al (2015); Husain, S. A., & David, J. (2018).
3.	WS+*Ocimumsanctum* (Tulsi)	Various skin illnesses, infections of the throat, nausea, loss of appetite, chest pain, and hiccups.	Balances Biologically, each individual responds to stress in a unique way to prevent vitiligo.	Edwards, S. E., et. al (2015); Khare, C. P. (2007).
4.	WS +*Zingiber officinale Rosc.* (Ginger)	Enhances stamina, fatigue, stress, treats sleep disorders and augments fertility, and urinary disorders.	Reduces stress-induced fatigue, helps in rejuvenation and growth of the whole body, promotes mitochondrial health, stimulates sexual desires, and increases urinary output.	Samy, R. P., et. al (2008); Byadgi, P. S., & Pandey, A. K. (2013).

5.	WS+*Chlorophytum borivilianum* (Safed musli)	Premature ejaculation and erectile dysfunction, stress.	Helps the body in stressful conditions, increases sperm content, increases the testosterone level, and increases muscle strength.	Rajani, K., et. al (2018).
6.	WS+*Phyllanthus emblica* (Amla)	Boosts immunity, improves memory, and enhances intelligence, and hair re-growth.	Delivers vital nutrients to body cells, increases the rate of hair growth, and enhances intelligence.	Kayne, S. B. (2009); Larson, R. A. (1988).
7.	WS+*Lavandula angustifolia* (Lavender)	Balance stress hormones promote a healthy sleep cycle.	Stabilizes the mood, for the treatment of epilepsy, and helps as an analgesic medicine.	Moon, T., et. al (2004).
8.	WS+*Aloe barbadensismiller* (Aloe vera)	Boosts immunity, promotes digestive health, stress relief, balances hormones,and detoxifies the body.	Stimulates cell growth, inhibits bacterial & fungal infections, and inhibits itching, and inflammation, used in many sunscreens, bath oils, and skin creams.	Van Wyk, B. E., & Wink, M. (2018).
9.	WS +*Angelica archangelica*(Garden angelica)	Asthma, bronchitis, cold, cough, flu.	It strengthens the immune system, making the body more resistant to illness and more robust under pressure, and inhibits bacterial & fungal infections.	Sathyaprabha, G., Kumaravel, S., et. al (2010).
10.	WS + *Chlorophytum borivilianum* (Safed musli) + *Glycyrrhiza glabra*(Licorice)	Menopausal symptoms, cough, digestive problems, viral and bacterial infections.	Acts as a tonic for women's reproductive system, cure joint pain & swelling and balances estrogen level in women.	Davies, J. (1994).
11.	WS + *Ocimumsanctum* (Tulsi) + *Foeniculum vulgare*(Fennel seeds)+ *Mentha piperita L.*(Peppermint)	Calming anxiety, digestion, cold&flu.	Helps in digestion, protects from microbial infections, and reduces stress.	Pawar, V. S., & Shivakumar, H. (2012).
12.	WS + *Chlorophytum borivilianum* (Safed musli) + *Asparagus racemosus*(Shatavari)	Promotes fertility, and hormone balance, strengthens the immune system, diabetes, and age healing.	Promotes cardiac functioning, manages diabetes, helps in promoting fertility hormones, protects from bacterial &viral infections.	Abascal, K., & Yarnell, E. (2003).

Future Perspective and conclusion:

Ashwagandha is a significant medicinal plant that exhibits various biological effects and used for the treatment of various ailments since antique age. It possesses diverse range of phytochemicals making it abundant in bioactive compounds. Withanolides and alkaloids are the primary components responsible for its pharmacological actions. It can be used in combination with other bioactive compounds or supplements that help to improve the pharmacological effects. Instead of various pharmacological effects, it has limited clinical use. There are various reasons such as lack of documentation at the modern scientific platforms as well as the requirement of standardization of different qualitative and quantitative parameters like dosage form, amount of drug, route of administration, frequency of dosage, interaction with the drugs and need to explore the *in-vivo* studies. Its commercialization at global platform is equally needs to explore for enhancing its utilization. Therefore, we need to explore the use of medicinal plants for the treatment of illness which opens up a new frontier in the field of medicine and anticipates good health. This review will help the researchers to scrutinize the different pharmacological activities of Ashwagandha and optimization at different parameters.

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