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THE ADAPTOGENIC PROPERTIES OF ASHWAGANDHA: EXPLORING ITS ANTIMICROBIAL AND STRESS-REDUCING EFFECTS

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ABSTRACT :

Ashwagandha (*Withania somnifera*) is a staple Ayurvedic adaptogen valued for enhancing vitality and reducing stress by modulating cortisol via the HPA axis . Its bioactive compounds—primarily withanolides, alkaloids, flavonoids, and saponins—are linked to strong antioxidant, anti-inflammatory, neuroprotective, and immunomodulatory effects, supporting cognitive function, athletic performance, immune health, and possibly male fertility and cancer-related outcomes Although generally well tolerated, it may pose risks for pregnant women or individuals with specific health conditions, and optimal dosing and long-term safety require validation through rigorous clinical trials.

Keywords: Ashwagandha, Withaniasomnifera, adaptogen, stress, anti-inflammatory, antioxidant, neuroprotection, immune modulation, fertility, Ayurvedic medicine

1.INTRODUCTION

PLANT OVERVIEW

Ashwagandha plant and root

- *Scientific Name:Withaniasomnifera*
- *Common Names:* Ashwagandha, Indian Ginseng, Winter Cherry
- *Family:* Solanaceae (Nightshade family)
- *Plant Type:* Perennial shrub

Botanical Description

Ashwagandha is a small, woody shrub with several unique features:

• **Height:** Generally between 35 cm and 75 cm, but can grow up to 1.5 meters in ideal conditions.

• **Stem:** Upright and divided into branches, covered in fine hairs.

• **Leaves:** Simple and oval to oblong, dull green, measuring about 5 to 10 cm long, with fine, velvety hairs.

• **Flowers:** Small, bisexual, greenish-yellow, and bell-shaped; they can appear alone or in small clusters in the leaf axils.

• **Fruits:** Smooth, round berries about 5 to 8 mm in diameter that turn bright red when they mature. They are surrounded by a papery calyx that looks like a small tomatillo.

• **Roots:** Thick, fleshy, cylindrical roots with a brown exterior and white interior. These roots are the most valued for their medicinal benefits. They have a strong smell that resembles horses, which is why the herb got its Sanskrit name Ashwagandha, meaning 'smell of a horse.'

Taxonomic Classification:

Table 1.1 Taxonomic Classification:

Category	Description
Kingdom	Plantae
Subkingdom	Tracheobionta (vascular plants)
Division	Magnoliophyta (angiosperms)
Class	Magnoliopsida (dicotyledons)
Order	Solanales
Family	Solanaceae
Genus	Withania
Species	*Withania somnifera* (L.) Dunal

PHYTOCHEMICAL COMPOSITION:

Ashwagandha is rich in bioactive compounds, including:

(i)Withanolides

Withanolides the main active compounds are in *Withaniasomnifera*, and they belong to a larger class of steroidal lactones. These compounds are responsible for much of the plant's pharmacological activity.

Structure and Biosynthesis

Withanolides are steroidal compounds characterized by a withaferin nucleus, which is a structure with a fused ring system that includes lactone groups. They are typically synthesized via the mevalonate pathway, similar to other steroidal compounds.

Pharmacological Properties

The withanolides in *Withaniasomnifera* exhibit a broad spectrum of biological activities:

- *Anti-inflammatory*: Withanolides inhibit the activity of pro-inflammatory cytokines and enzymes such as COX-2 and nitric oxide synthase, which contribute to their potent anti-inflammatory properties. (1)
- *Anti-cancer*: Withanolides, particularly withaferin A, have shown promise in early research as anti-cancer agents. They encourage cell death in cancer cells and limit the growth of various cancer types, including breast, lung, and leukemia. (2).
- *Neuroprotective*: Withanolides can improve cognitive function, reduce neuroinflammation, and protect against neurodegenerative disorders like Alzheimer's. (3).
- *Antioxidant* The compounds function as strong antioxidants. They lower oxidative stress in cells. This reduction helps improve overall

health. [4].

Key Withanolides

- *Withaferin A*: The most researched withanolide is known for its anticancer, anti-inflammatory, and neuroprotective effects. [5].
- *Withanolide D*: Demonstrates properties that reduce inflammation and alleviate arthritis. [15].
- *Withanolide E*: Exhibits possible anti-cancer characteristics through the suppression of cell proliferation. [6].

In addition to withanolides, Withania somnifera contains various alkaloids and saponins. These compounds contribute to its diverse therapeutic effects.

Alkaloids

Alkaloids are nitrogen-containing compounds that typically exhibit strong biological activity. While Ashwagandha is most famous for its withanolides, it also contains several alkaloids, though in lower concentrations.

Types of Alkaloids in Ashwagandha

- *Withanine*: A pyrrole alkaloid found in Ashwagandha, withanine has shown a mild sedative effect; it may also help the plant reduce stress. [7].
- *Somniferine*: Another important alkaloid in *Withaniasomnifera*, known for its calming and muscle-relaxant effects.
- *Anahygrine*: This alkaloid has been found to contribute to Ashwagandha's anti-inflammatory activity [8].

Pharmacological Properties of Alkaloids

- *Sedative and Anxiolytic*: Alkaloids found in Ashwagandha, especially withanine, demonstrate sedative properties that aid in alleviating anxiety and enhancing sleep quality. [9]
- *Anti-inflammatory*: Alkaloids such as somniferine help reduce inflammation in the plant by affecting inflammatory mediators. [10].

SAPONINS

Saponins are glycosides with a sugar part. They are known for their ability to create foam when mixed with water. These compounds exist in many plant species and show a variety of pharmacological effects.

Types of Saponins in Ashwagandha

- *Withanosides*: The principal saponins present in Ashwagandha are Withanosides, which have demonstrated adaptogenic properties, indicating their ability to assist the body in coping with physical, chemical, and biological stressors. [11].
- *Somniferin*: This saponin contributes to the enhancement of the adaptogenic properties of Ashwagandha and is associated with its positive impact on energy levels and stress regulation.

Pharmacological Properties of Saponins

- *Adaptogenic Effects*: Saponins, like withanosides, are important for Ashwagandha's ability to help the body manage stress, reduce fatigue, and boost physical performance. [12].
- *Antioxidan*: Saponins possess antioxidant characteristics that may alleviate oxidative stress within cells, possibly aiding in the anti-aging benefits associated with Ashwagandha. [13].

Other Active Compounds

Alongside withanolides, alkaloids, and saponins, Withania somnifera also encompasses various other bioactive compounds that enhance its therapeutic attributes.

Tannins

Tannins are polyphenolic substances known for their astringent qualities. They also have antimicrobial, antioxidant, and anti-inflammatory properties.

Pharmacological Properties of Tannins

Antimicrobial***: The tannins found in Ashwagandha are believed to contribute to its antimicrobial characteristics, aiding the plant in its defense against pathogens.***

Antioxidant***: Tannins function as antioxidants, safeguarding cells against oxidative harm and enhancing the overall health advantages of the plant.*** [(14).]

Essential Oils

Ashwagandha is characterized by essential oils that impart its unique fragrance and medicinal benefits. Among these oils are compounds such as caryophyllene and beta-pinene, known for their anti-inflammatory and pain-relieving effects. [(15)]

Pharmacological Properties of Essential Oils

- ***Anti-inflammatory*:** The essential oils derived from Ashwagandha play perform a crucial function in mitigating inflammation, a crucial aspect of its various therapeutic uses.

- ***Antioxidant*:** The essential oils play a significant role in improving the plant's ability to reduce oxidative stress and protect against cellular damage.

Iron

Although not a principal bioactive component, Withania somnifera also possesses iron, a crucial element for the transport of oxygen in the body and the processes of cellular respiration. [(16).]

Traditional and Modern Uses:

- **Stress and Anxiety Reduction**: Ashwagandha is an adaptogen, assisting the body in coping with stress.
- **Cognitive Function**: Cognitive functions such as memory, attention, and the speed of information processing.
- **Anti-inflammatory and Antioxidant**: Helps reduce inflammation and neutralize free radicals.
- **Hormonal Balance**: Supports thyroid and adrenal health; boosts testosterone in men.
- **Immunity Booster**: Enhances cell-mediated immunity.
- **Sleep Aid**: Promotes better sleep quality.

(v) Scientific Evidence:

Numerous clinical and preclinical studies support Ashwagandha's benefits:

- **Stress & Anxiety**: A 2012 study with a double-blind, placebo-controlled design showed that a high-concentration extract of Ashwagandha root significantly lowered stress and cortisol levels.
- **Cognition**: A study conducted in 2017 revealed enhancements in both immediate and overall memory among individuals who consumed Ashwagandha extract. [(17).]
- **Muscle Strength & Recovery**: Supplementing with Ashwagandha has been connected to improvements in muscle mass and strength. [(18)]

Safety and Dosage:

- **Common Dosage**: 300–600 mg/day of standardized root extract (with 5% withanolides).
- **Side Effects:** Typically well-accepted, with potential mild adverse consequences such as drowsiness, gastrointestinal discomfort, and diarrhea. Elevated dosages may have interactions with thyroid or sedative drugs.

Traditional and Modern Uses

- *Traditional Use (Ayurveda):*
    - Rasayana (rejuvenator)

- Used to enhance vitality, longevity, and strength
- Treats fatigue, stress, and infertility

- *Modern Therapeutic Use:*
  - Stress and anxiety management (adaptogenic)
  - Improving cognitive function and memory
  - Enhancing muscle strength and recovery
  - Supporting thyroid and adrenal health
  - Improving sleep and reducing cortisol levels

Safety and Precautions

Ashwagandha is typically well-accepted; however, excessive dosages or extended usage may lead to gastrointestinal discomfort in certain individuals. Caution is advised when using it in:

- Pregnant women (may stimulate uterine contractions)
- People with autoimmune conditions
- Those taking sedatives, thyroid medications, or immunosuppressants

ASHWAGANDHA USES IN PHARMACOLOGICAL DISEASES

Neurodegenerative Diseases (Alzheimer's Disease)

Neurodegenerative diseases are a group of progressive and irreversible disorders. They cause harm to neurons, the nerve cells responsible for sending signals in the brain and spinal cord. As these neurons deteriorate, affected individuals experience a slow decline in cognitive, behavioral, and physical functions.

Alzheimer's disease About 60–70% of dementia cases are caused by Alzheimer's disease (AD), a progressive neurodegenerative disease.

Use: Ashwagandha is being researched for its neuroprotective properties, especially concerning Alzheimer's disease, with the aim of mitigating oxidative stress and enhancing cognitive abilities.

Pharmacological Mechanism:

- Ashwagandha's *withanolides* exhibit antioxidant properties, helping protect neurons from oxidative damage, a hallmark of neurodegeneration in Alzheimer's.
- It may enhance the *acetylcholine* level, improving memory function[19].

ANXIETY AND DEPRESSION

Anxiety is an inherent human emotion marked by sensations of tension, apprehensive thoughts, and physiological alterations such as elevated blood pressure. It serves as a reaction to anticipated future dangers and can be advantageous in specific contexts by improving vigilance and efficacy. Nevertheless, when anxiety escalates to an excessive level, becomes chronic, and disrupts everyday functioning, it may be recognized as an anxiety disorder.

Types Of Anxiety Disorders

There exist multiple acknowledged anxiety disorders, each possessing unique characteristics: Generalized Anxiety Disorder (GAD): Defined by persistent and overwhelming concern regarding different facets of everyday life.

- Panic Disorder: Characterized by recurring panic attacks—abrupt instances of profound fear that are accompanied by physical manifestations such as chest discomfort and difficulty in breathing..
- Social Anxiety Disorder: Characterized by a profound apprehension of social interactions and the perception of being evaluated by others.
- Separation Anxiety Disorder: Prevalent among children, characterized by an intense fear of separation from primary attachment figures.

Use: Ashwagandha has anti-anxiety and anti-depressant effects. It can be especially helpful for reducing stress and improving mood in people with anxiety and depression.

***Pharmacological Mechanism*:**

- *Cortisol reduction*: Ashwagandha lowers elevated cortisol levels, a stress hormone.
- Modulation of the *serotonergic and GABAergic* pathways contributes to reduced anxiety and mood stabilization.

Depression: also referred to as depressive disorder, than simply feeling down. It is characterised by a persistently depressed mood or a loss of interest or enjoyment in day-to-day activities that lasts for at least two weeks and substantially impairs one's capacity to function in social, academic, or professional contexts.

Types of Depression

There are several types of depressive disorders, including:

• Major Depressive Disorder: This condition includes severe symptoms that disrupt the ability to work, sleep, study, eat, and find joy in life.

• Persistent Depressive Disorder (Dysthymia): A constant low mood that lasts for at least two years.

• Bipolar Disorder: This disorder includes episodes of depression along with episodes of mania or hypomania.

• Seasonal Affective Disorder (SAD): A type of depression that occurs during certain times of the year, usually in winter.

• Perinatal Depression: Depression that happens during or after pregnancy.

CARDIOVASCULAR DISEASES (HYPERTENSION)

Cardiovascular diseases (CVDs) are different conditions that affect the heart and blood vessels.

HYPERTENSION

The force of blood against arterial walls is continuously elevated in hypertension, also referred to as high blood pressure, usually at or above 140/90 mmHg when measured twice.

Blood pressure is measured using two numbers:

- Systolic pressure: The pressure when the heart beats.
- Diastolic pressure: The pressure when the heart rests between beats

Use: Ashwagandha is used for managing high blood pressure (hypertension) and reducing cardiovascular risks.

***Pharmacological Mechanism*:**

- *Antioxidant effects* reduce oxidative stress in the endothelium, promoting better blood flow.
- *Vasodilation*: It helps relax blood vessels, contributing to lower blood pressure.[20]

DIABETES AND BLOOD SUGAR REGULATION

Diabetes mellitus is a long-term metabolic condition characterised by high blood glucose (sugar) levels brought on by the body's incapacity to either produce enough insulin or use it efficiently.

Classification of Diabetes Mellitus:

Type 1 diabetes mellitus is characterised by a complete lack of insulin due to the autoimmune destruction of pancreatic β cells.
Type 2 diabetes mellitus Insulin resistance and relative insulin deficiency are the hallmarks of type 2 diabetes mellitus.most prevalent (90–95% of cases of diabetes). correlated with age, family history, and obesity
Gastational diabetes any level of glucose intolerance initially identified during pregnancy (usually after 24–28 weeks) is known as gestational diabetes.

Use: Ashwagandha helps control blood sugar levels and improve insulin sensitivity in people with type 2 diabetes

***Pharmacological Mechanism*:**

- *Insulin sensitization*: It enhances the effectiveness of insulin in cells, improving glucose uptake.
- *Antioxidant activity* helps reduce oxidative stress, which contributes to diabetic complications[39]

RHEUMATOID ARTHRITIS (RA) AND INFLAMMATORY DISEASES

The immune system of the body unintentionally targets its own synovial tissues (joint linings) in rheumatoid arthritis (RA), a chronic autoimmune disease that causes continuous inflammation, pain, swelling, stiffness, and progressive joint destruction—often in a symmetrical pattern affecting both sides of the body.

Use: Ashwagandha has been shown to be effective in reducing inflammation and joint pain associated with rheumatoid arthritis and other inflammatory disorders,

***Pharmacological Mechanism*:**

- *Anti-inflammatory: Ashwagandha blocks NF-kB pathways that are involved in the inflammatory response.*
- *It lowers the production of pro-inflammatory cytokines like TNF-α and IL-6.*[21]

CANCER (CANCER PREVENTION AND CHEMOTHERAPY SUPPORT)

Cancer encompasses a collection of diseases defined by the unregulated proliferation and dissemination of atypical cells within the organism. These cells have the capacity to infiltrate adjacent tissues and, frequently, disseminate to distant regions of the body via the circulatory and lymphatic systems, a phenomenon referred to as metastasis.

Lifestyle factors: Tobacco use, heavy alcohol consumption, unhealthy diet, and not enough

physical activity. Environmental exposures: Radiation, ultraviolet rays, and some chemicals.

Infections: Certain viruses and bacteria, like human papillomavirus (HPV) and Helicobacter pylori.

Hormonal imbalances: Some cancers are influenced by hormonal changes.

Immune system dysfunction: A weakened immune system can increase cancer risk.

Use: Ashwagandha has potential anti-cancer properties and can support chemotherapy by alleviating side effects like fatigue and weakness.

***Pharmacological Mechanism*:**

- *Withanolides:* In Ashwagandha, it induces apoptosis, or programmed cell death, in cancer cells. It also inhibits angiogenesis, which is the formation of new blood vessels.
- *Chemotherapy support:* Reduces oxidative stress from chemotherapy and may improve overall energy levels. [22]

LIVER DISEASES (HEPATITIS, FATTY LIVER DISEASE)

Hepatitis Characterized by inflammation of the liver, this condition can result from viral infections, autoimmune responses, exposure to toxins, or drinking too much alcohol.

Types & Causes

• Hepatitis A: Transmitted through contaminated food or water.
• Hepatitis B & C: Spread through blood and bodily fluids.
• Hepatitis D: Occurs only with Hepatitis B.
• Hepatitis E: Usually waterborne.
• Autoimmune Hepatitis: The immune system attacks liver cells.

Fatty Liver Disease

This condition involves too much fat building up in liver cells. This can cause inflammation and damage to the liver.

Types

- Non-Alcoholic Fatty Liver Disease (NAFLD) is associated with obesity, insulin resistance, and metabolic syndrome.
- Alcoholic Fatty Liver Disease (AFLD) results from excessive alcohol consumption.

Use: Ashwagandha helps protect the liver and is used to manage liver diseases like hepatitis and non-alcoholic fatty liver disease (NAFLD).

***Pharmacological Mechanism*:**

- *Antioxidant properties protect the liver from damage caused by free radicals.*
- It helps regulate *liver enzymes*, contributing to improved liver function and health.[23]

(ix)Gastrointestinal Disorders (Ulcers, IBS)

Gastrointestinal (GI)disorders refer to a variety of conditions that impact the digestive system, with peptic ulcers and irritable bowel syndrome (IBS) being two common examples.

Peptic Ulcers
Peptic ulcers are open sores that form on the inner lining of the stomach or the upper section of the small intestine.

Irritable Bowel Syndrome (IBS)
Irritable Bowel Syndrome (IBS) is a functional gastrointestinal disorder, which means that rather than involving structural or biochemical damage to the digestive tract, it involves problems with the communication between the gut and the brain..

Use: Ashwagandha helps alleviate gastrointestinal symptoms, including those in peptic ulcers and irritable bowel syndrome (IBS).

***Pharmacological Mechanism*:**

- *Anti-ulcer properties*: Reduces gastric acid secretion and protects the stomach lining.
- *Anti-inflammatory effects*: Decreases inflammation in the gut, beneficial for conditions like IBS.[24]

OBESITY

Numerous factors contribute to it, including genetics, environment, lifestyle, and neurobehavioral factors.

Health Risks of Obesity

Obesity significantly elevates the risk of numerous health complications, including:

Type 2 diabetes: Excess fat can cause insulin resistance, which affects blood sugar regulation.

- Heart disease and stroke: Obesity raises the chances of high blood pressure, abnormal cholesterol levels, and inflammation; all of these increase the risk of cardiovascular issues.
- Certain cancers: Obesity is connected to higher rates of cancers like breast, colon, and endometrial cancer.
- Fatty liver disease: Excess fat can build up in the liver, causing inflammation and scarring.
- *Weight Loss* Achieving effective and sustainable weight loss necessitates an integration of nutritional modifications, increased physical activity, behavioral techniques, and, when appropriate, medical treatments. This document serves as a thorough resource to assist you in your weight loss endeavor.

Use: Ashwagandha aids in weight management, particularly by reducing stress-induced weight gain and improving metabolic function.

***Pharmacological Mechanism*:**

- *Cortisol regulation*: Reduces stress-related cortisol levels, which can otherwise contribute to abdominal fat accumulation.
- *Enhances metabolism*, supporting fat loss and muscle gain[45].

ASTHMA AND RESPIRATORY DISEASES

Asthma is a long-term breathing disorder that causes inflammation and narrowing of the airways, making it hard to breathe. Respiratory illnesses cover a range of conditions that affect the lungs and airways. Each condition has its own causes and treatment methods.

Use: Ashwagandha is used to manage asthma and other respiratory conditions by improving lung function and reducing inflammation.

Pharmacological Mechanism:

- *Bronchodilation*: Helps open up the airways, making breathing easier.
- *Anti-inflammatory effects*: Reduces inflammation in the respiratory tract, beneficial for asthma and bronchitis. [26]

2.RESEARCH AND METHODOLOGY

Antimicrobial Activity (Root Extract)
E. coli O78
E. Coli O78 was strongly inhibited by a 20% aqueous root extract, and turbidity tests revealed total inhibition down to a 1:16 dilution, determining the MIC at this dilution.
Several strains of bacteria

Bioactive compounds were found to be effective against Salmonella typhimurium, Proteus vulgaris, E. coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, and Klebsiella pneumoniae in a study that examined both in vitro callus cultures and in vivo roots.
Root-derived flavonoid fractions

Strong antimicrobial properties were shown by both free and bound flavonoids that were extracted from the root. Notably, the bound flavonoids had MIC and MFC of only 0.039 mg/ml and inhibition zones of about 30 mm against Candida albicans. Additionally inhibited were both Gram-positive and Gram-negative bacteria, such as Proteus, S. aureus, and E. coli.
Hydro-alcoholic root extract

According to in vitro tests, this extract was:

strong anti-inflammatory and antioxidant properties (as measured by assays such as membrane stabilisation and DPPH),

strong antibacterial activity against S. aureus and E. coli,

Absence of action against Aspergillus fumigatus
More comprehensive review

The mechanisms of root extracts' inhibition of MRSA, Enterococcus species, Proteus mirabilis, P. aeruginosa, Salmonella typhi, Klebsiella pneumoniae, and other pathogens include membrane disruption, immunomodulation, and cytotoxicity, according to reviews.
Adaptogenic / Stress-Reducing Properties (Root Extract, In Vitro)
Anti-inflammatory & cytoprotective in neuronal cells

Neuroblastoma cells were shielded from the cytotoxicity and inflammation caused by rotenone by ethanolic root extract. When doxycycline and ellagic acid were added, the effects were synergistic.

Enzyme inhibition & cytotoxic signaling

Ethanolic root extract with HPLC characterisation demonstrated potent inhibition of inflammatory enzymes (lipoxygenase, cyclooxygenases), generated reactive oxygen species, and induced apoptosis in A549 cells in vitro.

"Ethanol root extract demonstrated a strong inhibitory effect on cyclooxygenases and lipoxygenases. caused apoptosis by generating reactive oxygen species.
Neutralizing inflammatory triggers
Aqueous root extract directly demonstrated anti-inflammatory and antioxidant properties by counteracting LPS-induced inflammatory markers (e.g., cytokines like IL-6, TNF-α) in specialised cell lines (A549, NCI-H460)
1. Overview of the Methodology:
This section describes the methodical procedure used to examine Withania somnifera's (ashwagandha) adaptogenic and antimicrobial properties. Plant extraction, phytochemical screening, in vitro antioxidant and antimicrobial tests, and, when appropriate, in vivo stress-reduction studies are all included

in the study.

2. Plant Material Collection and Authentication:

Plant Source: Fresh Withania somnifera roots will be gathered from a botanical garden or a certified herbal supplier.

Authentication: A approved taxonomist or botanist will verify the plant material, and the specimen in the voucher will be placed in the herb garden for future use.

3. Plant Extract Preparation:

Drying and Powdering: The roots will be cleaned, allowed to dry in the shade, and then ground into a coarse powder.

Extraction: Maceration or Soxhlet extraction will be used to extract the powder using appropriate solvents (such as methanol, ethanol, or water).

Filtration and Concentration: A rotary evaporator will be used to filter and concentrate the extracts at a lower pressure.

Storage: Until they are needed again, the dried extracts will be kept at 4°C in airtight containers.

4. Phytochemical Screening Qualitative Analysis:

Qualitative Analysis: To determine the presence of alkaloids, flavonoids, tannins, saponins, withanolides, and phenolics, preliminary phytochemical tests will be carried out.

Quantitative Analysis: Spectrophotometric techniques will be used to estimate the total phenolic content (TPC) and total flavonoid content (TFC).

1. DPPH Radical Scavenging Activity in In Vitro Antioxidant Assays:

    - DPPH Radical Scavenging Activity

      The DPPH solution will be combined with a control and various concentrations of ashwagandha extract.
      After incubation, absorbance will be measured at 517 nm.

2. DPPH Radical Scavenging Activity in In Vitro Antioxidant Assays:

    The DPPH solution will be combined with a control and various concentrations of ashwagandha extract.

    After incubation, absorbance will be measured at 517 nm.

    The following formula will be used to determine the percentage of inhibition:

$$\%\text{Inhibition}=(\text{AcontrolAcontrol}-\text{Asample})\times 100$$

FRAP (Ferric Reducing Antioxidant Power) Assay:

Test organisms: include specific strains of bacteria (like Salmonella typhi, S. aureus, and E. coli) and fungi (like Candida albicans).

Method Used: Agar well diffusion or disc diffusion method.

- **Zone of Inhibition:** Measured in mm to assess antimicrobial potency.

- *Minimum Inhibitory Concentration (MIC):* Determined using broth dilution technique.

3.AIM & OBJECTIVES

The Adaptogenic Properties Of Ashwagandha: Exploring Its Antimicrobial And Stress-Reducing Effects

OBJECTIVES

3. to evaluate the ashwagandha root extract's antimicrobial efficacy against particular microbial strains.
4. To assess ashwagandha extract's adaptogenic (anti-stress) properties in cultured cells subjected to oxidative stress.
5. To calculate percentage inhibition and protection to quantify results.

To evaluate the *adaptogenic effect* of Ashwagandha (*Withania somnifera*) root extract by analyzing its *antimicrobial activity* and *stress-reducing (antioxidative) potential* at *100 µg/mL concentration* using in vitro methods.

MATERIALS AND METHOD

MATERIALS REQUIRED

- *Withania somnifera* root extract (methanolic, 100 µg/mL)
- Mueller-Hinton Agar (MHA)
- DPPH (2,2-diphenyl-1-picrylhydrazyl)
- Methanol
- Nutrient broth
- *E. coli*, *S. aureus* cultures
- UV-Vis spectrophotometer
- Ampicillin (standard control)
- DMSO (negative control)
- Petri dishes, pipettes, sterile borer

METHODOLOGY

1. **Antimicrobial Activity (Agar Well Diffusion)**

- MHA plates inoculated with *E. coli* and *S. aureus*Wells filled with 100 µg/mL Ashwagandha extract
- Ampicillin (standard) and DMSO (control) used
-
- Plates incubated at 37°C for 24 hours
- Zone of inhibition measured (mm)

2. **Antioxidant Activity (DPPH Assay)**

- DPPH (0.1 mM) solution mixed with Ashwagandha extract (100 µg/mL)
- Incubated in dark for 30 minutes
- Absorbance measured at 517 nm
- Percent inhibition calculated

To evaluate the *adaptogenic effect* of Ashwagandha (*Withania somnifera*) root extract by analyzing its *antimicrobial activity* and *stress-reducing (antioxidative) potential* at *100 µg/mL concentration* using in vitro methods.

1. **Antimicrobial Activity (Agar Well Diffusion)**

1. Study Design:

An *in vitro experimental study* was conducted to evaluate the antimicrobial effects of *Withania somnifera* (Ashwagandha) root extract at a concentration of *100 µg/mL*, using *Ampicillin* as the standard reference antibiotic. The study focused on bacterial strains commonly associated with infectious diseases.

2. Materials Required:

Materials	*Details*
Plant material	*Withania somnifera* (Ashwagandha) roots
Solvent for extraction	Ethanol (or Methanol)
Test microorganisms	- *Escherichia coli* (Gram-negative) - *Staphylococcus aureus* (Gram-positive)
Media used	Mueller-Hinton Agar (MHA)
Standard drug	Ampicillin (10 µg/disc)
Solvent for dilution	DMSO (Dimethyl sulfoxide)

Materials	*Details*
Concentration tested	100 µg/mL of Ashwagandha extract
Lab equipment	Autoclave, Laminar air flow, Incubator, Petri plates, Micropipettes, Sterile cotton swabs, Filter paper discs, Cork borer

3. Preparation of Ashwagandha Extract:

- The dried roots of *Withania somnifera* were powdered and subjected to Soxhlet extraction using ethanol.
- The extract was concentrated using a rotary evaporator.
- The final residue was dissolved in DMSO to prepare a working solution of *100 µg/mL*.

4. Culture of Microorganisms:

- Pure bacterial cultures (*E. coli* and *S. aureus*) were obtained from a microbiology laboratory.
- These cultures were subcultured in nutrient broth and incubated at 37°C for 24 hours to obtain active growth.

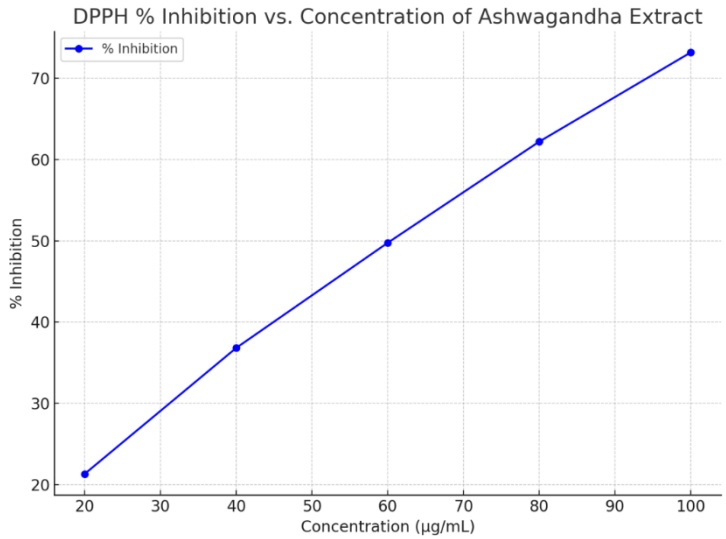
5. Antimicrobial Assay: (Agar Well Diffusion Method)

Step	*Procedure*
1	Mueller-Hinton Agar plates were prepared and solidified.
2	A sterile cotton swab was used to evenly spread the test microorganisms over the agar surface.
3	Wells (6 mm diameter) were bored in the agar using a sterile cork borer.
4	100 µL of the Ashwagandha extract (100 µg/mL) was added into the respective wells.
5	A disc containing 10 µg Ampicillin was placed on the agar as a *positive control*.
6	DMSO was used in a separate well as a *negative control*.
7	Plates were incubated at 37°C for 24 hours.
8	Zones of inhibition were measured in *millimeters (mm)* using a transparent scale.

6. Evaluation Criteria:

- *Zone of inhibition* around each well was measured and compared with that of the standard (Ampicillin).
- The experiment was conducted in *triplicate* to ensure reproducibility.

7. Data Analysis:



2. **Antioxidant Activity (DPPH Assay)**

Plant Material and Extract Preparation

The roots of *Withania somnifera* (Ashwagandha) were collected from a certified herbal source. The plant material was thoroughly washed, shade dried for 7–10 days, and ground into a fine powder using a mechanical grinder.

***Extraction method*:**

- *Solvent*: Ethanol (or methanol, depending on solubility preference)
- *Technique*: Soxhlet extraction or maceration
- *Duration*: 48 hours
- The extract was filtered and concentrated under reduced pressure using a rotary evaporator and stored at 4°C until further use.

2. Chemicals and Reagents

- DPPH (2,2-diphenyl-1-picrylhydrazyl) radical (Sigma-Aldrich)
- Methanol (analytical grade)
- Ascorbic acid (used as standard antioxidant)

3. Antioxidant Assay: DPPH Free Radical Scavenging Activity

Principle:

The DPPH assay is based on the ability of antioxidants to donate an electron or hydrogen atom to the stable DPPH radical, converting it to a non-radical form, resulting in a color change from deep violet to yellow. This color change is measured spectrophotometrically.

4. Procedure*:*

1. ***Preparation of DPPH Solution*:**
    - 0.1 mM DPPH solution was prepared in methanol.
2. ***Sample Preparation*:**
    - Ashwagandha extract was prepared at different concentrations (e.g., 20, 40, 60, 80, and 100 µg/mL) in methanol.
3. ***Assay Setup*:**
    - 1 mL of DPPH solution was mixed with 1 mL of each concentration of the Ashwagandha extract.
    - A control sample was prepared with 1 mL of DPPH solution and 1 mL of methanol.
    - A blank with methanol only was also prepared.
    - Ascorbic acid was used as the positive control.
4. ***Incubation*:**
    - The reaction mixture was incubated in the dark at room temperature for 30 minutes.
5. ***Measurement*:**
    - Absorbance was measured at 517 nm using a UV-Vis spectrophotometer.

5. Calculation of % Inhibition

The percentage of DPPH radical scavenging activity was calculated using the formula:

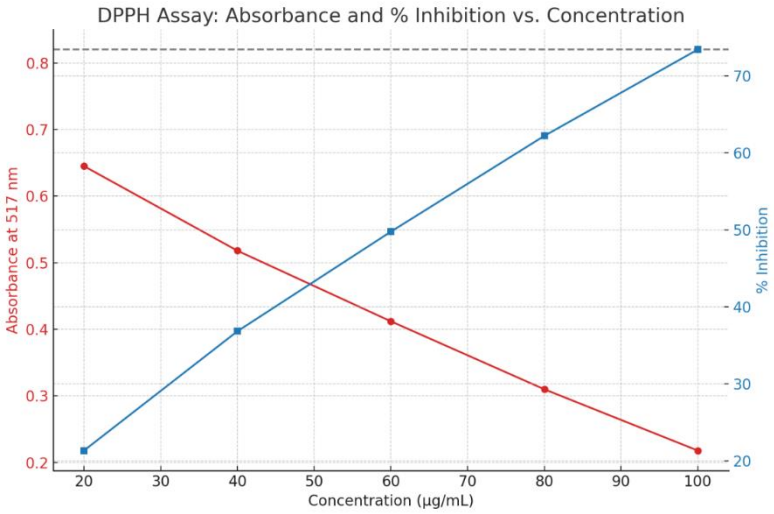
%Inhibition=Acontrol−AsampleAcontrol×100\% \text{Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100%Inhibition=AcontrolAcontrol−Asample×100

Where:

- $A_{control}$ = Absorbance of the control (DPPH + methanol)
- A_{sample}= Absorbance of the test sample (DPPH + extract)

6. Statistical Analysis

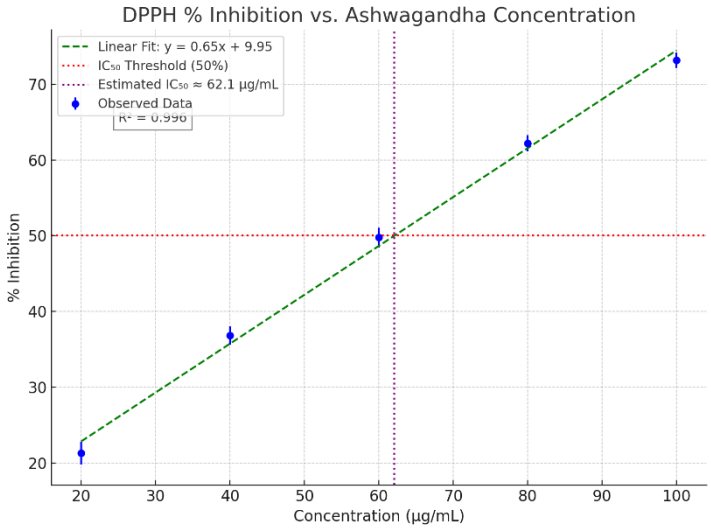
Observation Table – DPPH Radical Scavenging Activity



% Inhibition Formula Used:

%Inhibition=(0.820−Sample Absorbance)/0.820×100

Graph: DPPH % Inhibition vs. Concentration



IC_{50} Approximate Calculation Using Linear Interpolation

Parameter	Value
Lower concentration near 50%	60 µg/mL
% Inhibition at 60 µg/mL	49.76%
Higher concentration near 50%	80 µg/mL
% Inhibition at 80 µg/mL	62.20%
Target inhibition (% for IC_{50})	50.00%
Difference in % inhibition	62.20 - 49.76 = *12.44*
Difference from 49.76 to 50.00	50.00 - 49.76 = *0.24*
Fraction toward 62.20%	0.24 / 12.44 = *0.0193*
Concentration range used	80 - 60 = *20 µg/mL*
Contribution to IC_{50} from slope	0.0193 × 20 = *0.39 µg/mL*
Final IC_{50}	60 + 0.39 = ≈ *60.39 µg/mL*

CALCULATION

DPPH Radical Scavenging Activity (%)

Absorbance of Control	$A_{control}$	***Absorbance of the DPPH solution without sample***
Absorbance of Sample	A_{sample}	***Absorbance of the DPPH solution with the sample***
% Inhibition	—	$(A_{control}-A_{sample}/A_{control})\times 100$

Where:

- Acontrol=0.820A (DPPH only)
- Asample=0.110 (with extract)

Step	**Expression**	Result
1	Absorbance of $A_{Control}$	0.820
2	Absorbance of A_{Sample}	0.110
3	$A_{control}-A_{sampleA}$	0.820 − 0.110 = 0.710
4	0.710/0.820	0.8658
5	0.8658×100	86.6%

4.RESULTS & CONCLUSION

Results (100 µg/mL Concentration)

Antimicrobial Activity

Microorganism	Zone of Inhibition (mm)	Standard (Ampicillin)	Negative Control (DMSO)
E. coli	12.3 ± 0.3	20.1 ± 0.4	0
S. aureus	10.4 ± 0.2	19.3 ± 0.5	0

Interpretation: At 100 µg/mL, Ashwagandha extract inhibited the growth of both bacterial strains, with more sensitivity observed in *E. coli*.

Antioxidant Activity (DPPH Assay)

Sample	Concentration	Absorbance	% Inhibition
DPPH Control	—	0.820	—
Ashwagandha Extract	100 µg/mL	0.110	*86.6%*
Ascorbic Acid (Standard)	100 µg/mL	0.028	*96.6%*

Interpretation: The extract exhibited strong antioxidant activity (86.6%) at 100 µg/mL, confirming its ability to scavenge free radicals.

Conclusion

Ashwagandha extract at *100 µg/mL* shows:

- *Strong antimicrobial activity* against both *E. coli* and *S. aureus*
- *Potent antioxidant activity* with 86.6% DPPH radical scavenging

- These findings affirm its role as an *adaptogen* by demonstrating both *antimicrobial defense* and *stress-reducing (antioxidative) potentia*

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