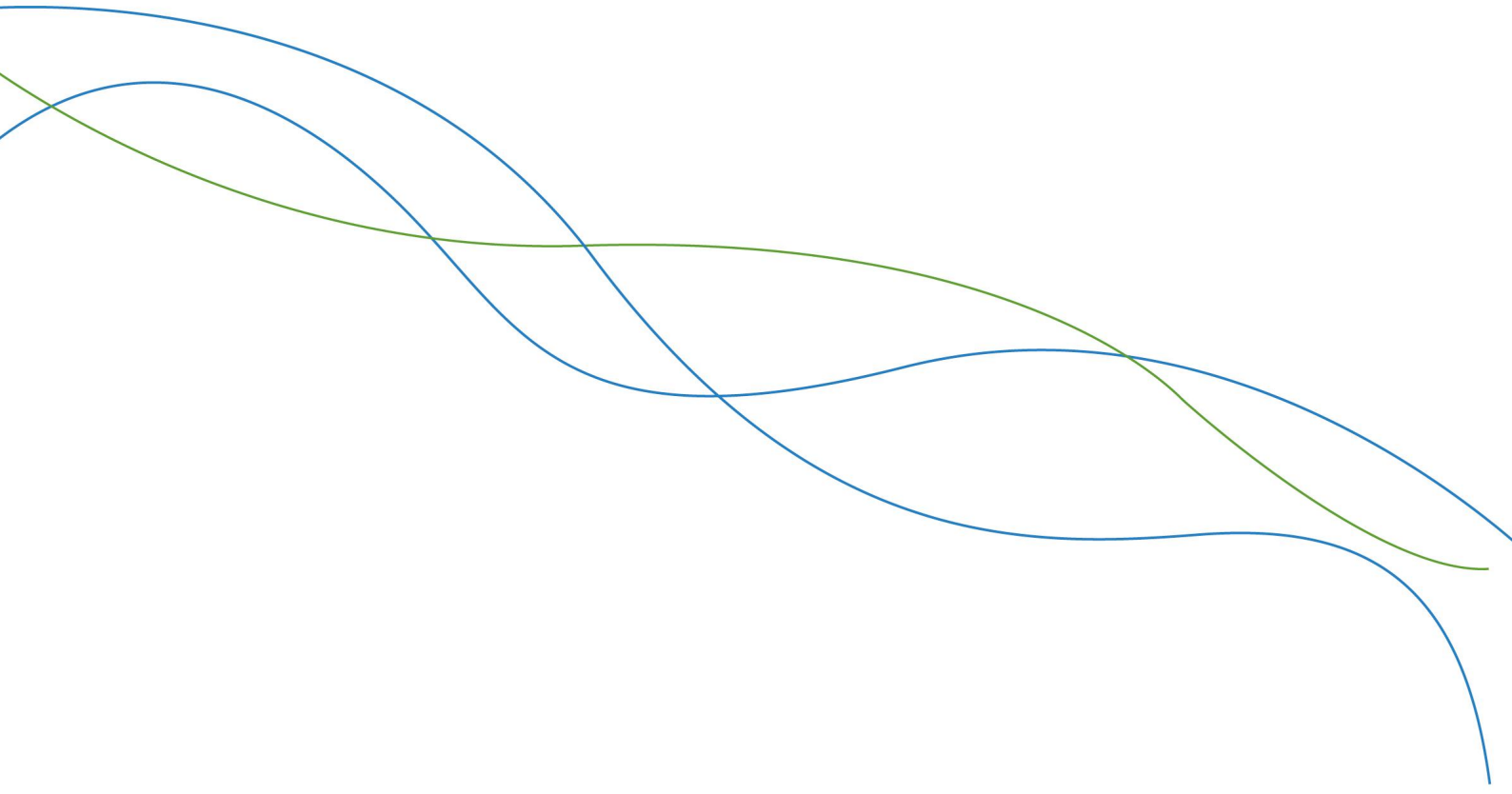
2025

ASHWAGANDHA WHITE PAPER

Natural Field Co., Ltd. / FTA



Natural FIELD × 食研汇 FTA —让食品创新更简单—

Recommendation

As a medicinal herb with a heritage of thousands of years in Ayurvedic medicine, Withania somnifera (Ashwagandha) has had its core active compounds — withanolides — extensively validated by modern research for their multi-target mechanisms of action. These compounds help regulate the hypothalamic-pituitary-adrenal (HPA) axis to balance stress hormones, thereby improving sleep and emotional well-being. They also exhibit neuroprotective, immunomodulatory, and metabolic regulatory effects, engaging multiple systems including the nervous, endocrine, and immune systems.

Furthermore, with advancements in extraction technology, the water solubility and safety profile of Ashwagandha extracts have been significantly enhanced, enabling cross-application in food, cosmetics, and pet health sectors. Ashwagandha extract thus serves as a vital bridge connecting ancient wisdom with modern health needs.

— Zhang Shengyong

Academician of the Chinese Academy of Engineering (CAE)

A positive emotional state plays a crucial role in disease treatment and recovery. In particular, alleviating anxiety and irritability in cancer patients remains a major clinical challenge. Withania somnifera

(Ashwagandha) is a dietary supplement known for its calming and anxiolytic effects and has been widely used in Europe and the United States for emotional regulation and immune support. Therefore, exploring its use as an adjuvant in cancer therapy and subsequent health recovery represents a promising and meaningful research direction.

— Liu Jie

Director of the Department of Gastroenterology,Huashan Hospital Affiliated to Fudan University

Against the backdrop of an aging population and rising health demands, the comprehensive value of Withania somnifera (Ashwagandha) has become increasingly prominent. As a representative adaptogenic ingredient, it differs from single-function compounds by emphasizing homeostatic regulation as its core mechanism. Ashwagandha helps alleviate chronic stress, improve age-related cognitive decline, and support metabolic and reproductive health. This aligns perfectly with the preventive, wellness-oriented approach to modern health management and represents an important direction for research and innovation in the field of nutritional supplements.

— Jiang Zhihong,

Vice President of Macau University of Science and Technology;

Director of the State Key Laboratory of Quality Research in Chinese Medicines

Withania somnifera (Ashwagandha) is driving a new trend in the functional food sector—evolving from traditional dietary supplements to snackable formats such as gummies and sparkling beverages. These innovations precisely address diverse consumption scenarios, including workplace energy support, relaxation before sleep, and post-exercise recovery. The combination of its natural adaptogenic properties with scenario-based applications not only meets consumers' growing demand for effortless, burden-free wellness but also expands the boundaries of functional food innovation. Ashwagandha thus stands out as a key high-potential ingredient amid the ongoing upgrade of health-oriented consumption.

——He Chunnian,

Researcher, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences

We adhere to a core belief: developing functional food ingredients with scientifically validated efficacy, guided by pharmaceutical principles and rigorous quality control standards. Sleep disorders affect hundreds of millions worldwide, creating an urgent need for solutions that excel in both safety and effectiveness. Our research focused on the herbal plant

Withania somnifera (Ashwagandha). Through systematic screening and pharmacological studies, we successfully identified and enriched its key bioactive compounds. Leveraging advanced liposomal encapsulation technology, we significantly enhanced their bioavailability and functional performance. We are confident that this integrated approach, combining modern separation techniques with cutting-edge biotechnology, provides a scientific, reliable, and highly effective solution to meet the growing global demand for sleep health.

Yang Haiying,

Chairman, Natural Field Co., Ltd.

Amid the upgrading of health consumption and the trend of naturalization, Withania somnifera (Ashwagandha) has broken the limitations of single-functional raw materials. It not only meets consumers' core needs for mood management, sleep improvement, and immune enhancement, but also adapts to the development of multiple scenarios such as functional foods, beauty products, and pet health. From the standardization of raw material cultivation to the innovation and optimization of extraction technology, its industrialization process has been accelerating. It is becoming a core link connecting the value of herbal resources with the upgrading of the big health industry, heralding a new development trend for natural functional ingredients".

--Food Technology Association (FTA)

catalogue

1.The Adaptogen Wave: Resolving the Imbalance Dilemma of Modern Life

The modern lifestyle is triggering a global crisis of homeostatic imbalance. The continuous elevation of cortisol levels caused by long-term stress has become an invisible threat to public health, with its negative impacts spreading across multiple physiological systems of the human body, such as the thyroid and adrenal glands. A survey conducted by the American Psychological Association (APA) reveals that over 70% of respondents have suffered health impairments due to stress, manifesting in symptoms like headaches, anxiety, and insomnia [1]. According to statistics from the World Health Organization (WHO), more than 322 million people worldwide are plagued by anxiety or depression. Among them, adolescents account for 3.5%, adults 5.7%, and the elderly 5.9%, indicating that emotional issues pose a universal risk across all age groups [2, 3]. Meanwhile, data from the *2025 China Healthy Sleep White Paper* shows that the rate of sleep disturbances among Chinese people aged 18 and above is as high as 65% [4]. This "modern disease" syndrome, characterized by stress, emotional problems, and sleep disorders, is not only the primary cause of declining quality of life but also a significant risk factor for chronic diseases such as cardiovascular diseases, metabolic syndrome, and immune dysfunction, presenting a severe health challenge.

In traditional health concepts, the synergistic effect of herbs and food has always been highly regarded for maintaining and promoting human health. Among them, adaptogens, with their unique advantages of "anti-stress, non-toxicity, and homeostatic regulation", precisely meet the urgent needs of contemporary people for emotional health and are gradually becoming the focus of attention in the health field. According to data from Grand View Research, the global adaptogen market size was estimated at $10.34 billion in 2023 and is projected to reach $16.32 billion by 2030, with a compound annual growth rate (CAGR) of 7.0% during the period. Notably, the Asia-Pacific region is expected to achieve a CAGR of 7.8%, reflecting the deep

recognition of the "balance concept" in Oriental health preservation culture.

Essentially,adaptogens are becoming the key to resolving the imbalance dilemma of modern life, driving the health consumer market into a new track of "homeostatic regulation".

1.1 Adaptogens: Natural "Regulators" of Human Homeostasis

Adaptogens are a class of natural herbal substances that can enhance the human body's resistance to external stressors such as stress in a non-specific manner. This concept was first proposed by the Soviet scientist Nikolai Lazarev in 1947 [5]. Such substances must meet three criteria [6]: 1) Adaptogens must be non-specific; 2) Adaptogens must maintain the body's internal balance; 3) Adaptogens must not impair the normal functions of the human body.

In 1969, the Soviet scientist Ilya Brekhman further expanded the definition and criteria of adaptogens, emphasizing that plant-derived adaptogens can help the human body maintain homeostasis under adverse environmental conditions [7]. In the 1990s, scholars such as Hildebert Wagner defined them clearly as natural biological regulators that can enhance the ability to adapt to the environment and reduce external harm. Their core advantage lies in alleviating the damage during the alarm phase of the stress response and delaying the onset of the exhaustion phase [8]. After more than half a century of continuous research, the concept of adaptogens has been constantly revised and improved. In 1998, the U.S. Food and Drug Administration (FDA) defined adaptogens as a new type of metabolic regulator that has been proven to be beneficial for environmental adaptation and prevention of external harm [9].

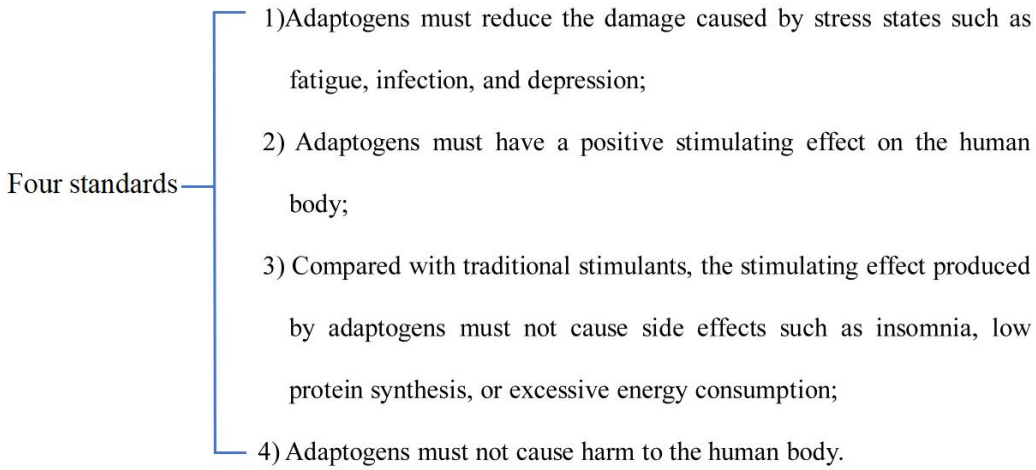


Figure 1 The Four standards for Plant Adaptogens [7]

Chronic stress leads to the overactivation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in elevated cortisol levels, which in turn have a negative impact on the nervous system. Studies have shown that adaptogens can normalize cortisol levels through a multi-target and multi-pathway network, thereby alleviating stress symptoms, improving mood, and enhancing cognitive function [10]. Yance and other researchers have classified adaptogens into three major categories: primary adaptogens, secondary adaptogens, and adaptogen companions [11]. The biological effects of plant adaptogens are associated with their contained bioactive compounds, such as triterpenoid saponins (ginseng contains ginsenosides, and Eleutherococcus senticosus contains eleutherosides), phytosterols and ecdysteroids (Rhaponticum carthamoides), lignans (Schisandra chinensis), as well as alkaloids, flavonoids, and vitamins.

Table 1 Overview of Mainstream Adaptogen Types

Type of Adaptogen	Core Active Ingredient	Main Efficacy
Ashwagandha	Withanolides and the derivatives	Alleviate anxiety and stress, reduce chronic inflammation, protect nerves and improve cognition, protect the heart, regulate immunity, improve sleep, and enhance muscle strength and recovery by regulating pathways such as inflammation,

Type of Adaptogen	Core Active Ingredient	Main Efficacy
		antioxidant, stress axis, and neurotransmitters [12].
Rhodiola Rosea	Rosavin, Tyrosol	Enhance the body's tolerance to various types of stress, resist fatigue, alleviate depression, exert antioxidant and anti-inflammatory effects, regulate immune function, prevent cardiovascular diseases, and protect the liver and skin [13].
Eleutherococcus Senticosus	Eleutherosides	Improve stress resistance, enhance mental and physical endurance, mainly affect the central nervous system and cardiovascular-cerebrovascular system, protect the nervous system, and also have the effects of regulating immunity, resisting oxidation, and lowering blood glucose [14].
Cordyceps Sinensis	Adenosine, Cordycepin	Possess multiple biological activities such as immune regulation, antioxidant, enhancing sexual and reproductive functions, lowering blood glucose, and resisting fatigue, and have protective effects on the kidneys and liver [15].
Schisandra Chinensis	Lignans	Mainly affect the central nervous system, sympathetic nervous system, cardiovascular system, endocrine system, and respiratory system, and have antioxidant, neuroprotective, and protective effects on the liver, cardiovascular system, and skin [16].
Ganoderma Lucidum	Ganoderma Polysaccharides, Ganoderic Acid	Help restore hormonal balance, enable the body to regain internal balance and regulate the immune system, and at the same time regulate various cellular functions and systems, including the endocrine (hormonal), immune, cardiovascular, central nervous, and digestive systems [17].
Ginseng	Ginsenosides	Enhance physical functions through immune, neural regulation, and vascular regulation effects, alleviate fatigue, promote vitality, protect the heart, resist oxidation, and alleviate menopausal symptoms [18].

Type of Adaptogen	Core Active Ingredient	Main Efficacy
Holy Basil	Eugenol, Ursolic Acid	Help the body internally manage and protect against damage caused by toxins, and have antibacterial, antioxidant, anti-inflammatory, liver-protective, neuroprotective, cardioprotective, anti-diabetic, immune regulatory, central nervous system inhibitory, memory-enhancing, and anti-stress effects [19].
Astragalus Membranaceus	Astragaloside IV	Promote resistance to exogenous pathogens, enhance overall vitality, and have immune regulatory, antioxidant, anti-aging, blood glucose-lowering, blood lipid-regulating, anti-fibrotic, antibacterial, and antiviral effects [20].

1.2 The Core Mechanism of Ashwagandha's Adaptogenic Effect

The HPA axis is the core endocrine pathway for the human body to respond to stress. When the body perceives stress, the hypothalamus secretes corticotropin-releasing hormone (CRH), which stimulates the pituitary gland to release adrenocorticotropic hormone (ACTH). Finally, the adrenal glands are prompted to secrete cortisol (the stress hormone), triggering the stress response [21].

Ashwagandha (Withania somnifera), a core herb in traditional Ayurvedic medicine, its core "adaptogenic" effect lies in helping the body actively adapt to various physiological or psychological stresses and maintain the homeostasis of the internal environment, rather than directly combating a specific stressor. The realization of this effect is closely related to the regulation of multi-system signaling pathways by its key active ingredients (such as withanolides).

The adaptogenic property of Ashwagandha is first reflected in the regulation of the HPA axis. Its active ingredient, withanolides, can directly bind to glucocorticoid receptors in the brain, block the transmission of stress signals, and reduce the

secretion of ACTH to lower cortisol levels. After the stress is relieved, cortisol inhibits the secretion of CRH and ACTH through negative feedback, restoring the balance of the HPA axis and forming a closed loop of "HPA axis negative feedback regulation" [22]. Secondly, it can increase the content of gamma-aminobutyric acid (GABA) in the brain and promote the expression of GABAA receptors, assisting in relieving the stress-induced excitement of the central nervous system [23]. In addition, Ashwagandha can inhibit the release of pro-inflammatory cytokines (such as IL-6 and TNF-α) by suppressing the NF-κB and MAPK signaling pathways, enhance the release of anti-inflammatory cytokines (such as TGF-β1), and at the same time up-regulate Nrf2 and HO-1 as well as reduce oxidative stress markers to enhance the body's antioxidant defense capability and improve the body's tolerance to stress [24, 25].

1.3 Market Size and Application Fields of Ashwagandha

1.3.1 Global Market Size of Ashwagandha

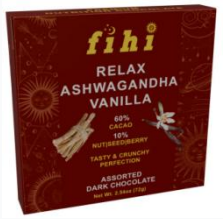
According to data from Mordor Intelligence, the global Ashwagandha market size will reach $760 million in 2025 and is expected to rise to $1.18 billion by 2030, with a compound annual growth rate (CAGR) of 9.2%. In terms of regional distribution, the North American market accounts for the highest proportion at 38.7%, and the Asia-Pacific market shows significant growth potential, with a projected CAGR of 9.4% by 2030. The driving forces behind this growth mainly include the expanding population suffering from anxiety and insomnia, the rising demand for functional foods, and the upgrading of cross-border health consumption. From the perspective of product composition, dietary supplements still dominated the market in 2024 (accounting for 62.5%), while functional foods and beverages achieved a remarkable growth rate, with a projected CAGR of 15.6% by 2030. This reflects the continuous improvement in consumers' acceptance of the application of Ashwagandha in ordinary food products.

1.3.2 Food Field

As consumers' demand for "functional snacks" continues to grow, the application scope of Ashwagandha in the food field is constantly expanding, and its product categories are rapidly evolving from dietary supplements to diversified forms. The application of Ashwagandha in the food field presents three major trends: Firstly, precise functional positioning, such as the development of dedicated formulas targeting segmented needs like mood regulation and sleep improvement. Secondly, dosage form innovation, extending from capsules to more acceptable forms such as gummies and beverages. Thirdly, scenario-based design, optimizing product forms and dosages in combination with scenarios such as sports nutrition supplementation, office refreshment, and pre-sleep relaxation. The market also witnesses the in-depth integration of flavor innovation and health attributes. To address the issue of acceptability of herbal flavors, brands generally adopt fruit flavor combinations, such as mango-flavored gummies and lime-flavored sparkling water. Meanwhile, in response to the trend of sugar reduction, most new products are labeled as "low-sugar" or "sugar-free", catering to consumers' pursuit of health value.

Table 2 Application of Ashwagandha in the Food Field (data from Amazon).

Brand	Product Name	Features	Product Image
Ascool	Ascool Ashwagandha Gummies	Relieve stress, improve sleep, aid in post-exercise recovery, and enhance immunity and energy levels	

Brand	Product Name	Features	Product Image
Life Extension	Life Extension Optimized Ashwagandha	Promote healthy energy levels, emotional balance, and memory health	
Juni	Juni Sparkling Adaptogen Beverage	Provide natural energy supplementation, low-sugar, and refreshing	
Unichi	Teddi Lab® Ashwagandha Gummy Bears	Lower cortisol levels, manage stress, improve sleep quality, and alleviate anxiety	
Brighfull	Focus Sugar-Free Beverage	Support mental clarity and calmness, and reduce fatigue	
Fihi Nutrition	Ashwagandha Vanilla Chocolate	Relieve stress and induce relaxation, and promote calmness and balance	

Brand	Product Name	Features	Product Image
Wise Bar	Adaptogenic Mushroom Nutrition Bar	Promote natural focus, provide energy, and support immunity	

1.3.3 Cosmetics Field

In the fast-paced modern life, the skin is exposed to stress challenges from various aspects such as the environment and emotions. Ingredients with regulatory and soothing effects are highly favored in the beauty and cosmetics field, and adaptogens are among the outstanding ones. As early as 2019, Mintel pointed out in the 2019 Global Beauty and Personal Care Trend Report that beauty and health care will be increasingly integrated in the future, and adaptogen ingredients with regulatory, restorative, and resistance-enhancing properties will become a hot topic.

Ashwagandha has been used in Ayurvedic medicine for centuries. It is reported that the paste made from steamed Ashwagandha roots has wound-healing properties [26]. A study on the efficacy of Ashwagandha toner on people with healthy photoaged skin shows that this plant can significantly reduce the signs of aging caused by ultraviolet radiation, including fine lines and wrinkles [27]. Thus, Ashwagandha is gradually becoming a powerful ingredient in the skincare and beauty field. In the trend report *Top 10 Natural Ingredients That Will Dominate the Food and Skincare Industry in 2022* released by Trendalytics, Ashwagandha ranks fifth. Additionally, in Canada, Ashwagandha leaf/root extract can be used as a skin conditioning agent.

Table 3 Application of Ashwagandha in the Cosmetics Field (data from Amazon)

Brand	Product Name	Application Type	Core Ingredient	Efficacy Claim
MARA	Volcanic Sea Clay Detox Masque	Face Mask	Spirulina, Ashwagandha, Volcanic Ash	Absorb excess oil, metabolize old cuticles to improve dullness, provide antioxidant and moisturizing effects, enhance skin elasticity and firmness, and create a plump and smooth texture.
82°E	Ashwagandha Bounce	Moisturizer	Ashwagandha, Sodium Hyaluronate	Reduce dark spots and shrink pores, brighten skin tone, promote collagen production to enhance elasticity, lock in moisture, and balance skin hydration.
Cleanse	Ayurvedic Face Toner	Toner	Centella Asiatica, Turmeric, Ashwagandha	Soothe and refresh the skin, revitalize and brighten the complexion, moisturize and firm the skin,

				balance oil secretion, and delay skin aging.
Grown Alchemist	Skin Renewal Toner Mist	Toner Spray	Ganoderma Lucidum, Ashwagandha, Echinacea	Improve skin tone, texture, and pores, reduce skin fatigue and resist environmental stress, and create balanced, radiant, and calm skin.
Kama Ayurveda	Vanasara Ashwagandha Invigorating Essence	Essence	Ashwagandha, Rose, Vetiver	Revitalize dull skin, strengthen the skin barrier, and immediately replenish moisture.
Mama Earth	Aloe Vera Face Wash	Facial Cleanser	Aloe Vera, Ashwagandha	Soothe and calm the skin, provide moisturization, fade pigmentation, and even out skin tone.
Modicare	Schloka Under Eye Cream	Eye Cream	Ashwagandha, Centella Asiatica	Help reduce dark circles, puffiness, and fine lines.

1.3.4 Pet Health Field

In modern society, a key trend is the transformation of pets' roles from companion animals to family members. A Marketplace survey shows that 48% of pet owners regard their pets as important members of the family, and 74% of consumers of pet supplements even believe that their pets "think they are human beings". This anthropomorphic perception has given rise to the concept of "humanized" pet nutrition and also reflects the upgrading of pet owners' needs from basic feeding to health management [28]. According to a report by Marketplace, 42% of consumers will look for specific health benefits when purchasing pet supplements for the first time. The supplements they purchase mainly target joint health, skin and coat health, and digestive health, followed by segmented needs such as anti-anxiety/sedation, allergy relief, immunity enhancement, and intestinal health [29].

A report titled *Pet Food Trends: June 2023* jointly released by Mintel and Tree Top Ingredients shows that 79% of U.S. pet food buyers are willing to pay a higher price for healthier products, and 60% of British consumers make purchasing decisions based on the healthiness of the formula [30]. Common healthy formulas contain vitamin D, omega-3 and omega-6, CBD, glucosamine, biotin, collagen, apple cider vinegar, prebiotics, Ashwagandha, elderberry, chondroitin, chlorophyll, and turmeric. Furthermore, a survey indicates that 35% of U.S. consumers of pet nutritional supplements are willing to consider purchasing products containing Ashwagandha [28].

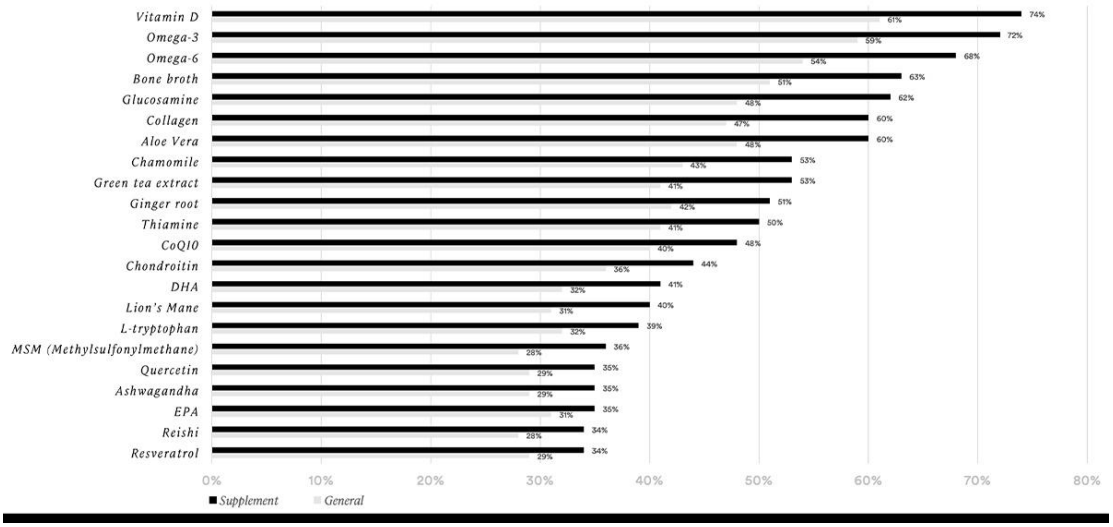


Figure 2 Functional Ingredients and Purchase Considerations: Likely or Very Likely to Consider [28]

The application of Ashwagandha in the pet health field mainly focuses on mood management and stress relief. In a randomized controlled trial involving 24 dogs with stress and anxiety, the test group was supplemented with standardized Ashwagandha root extract (withanolide content ≥5%) at a daily dose of 15mg/kg body weight. After 4 weeks, the urinary cortisol-to-creatinine ratio (UCCR) of the dogs in the test group significantly decreased. Meanwhile, as evaluated by the Canine Behavioral Assessment and Research Questionnaire (CBARQ) scale, the scores of fear and anxiety-related behaviors significantly reduced [31].

Table 4 Application of Ashwagandha in the Pet Health Field,Product (data from Amazon)

Product Name	Efficacy Features	Product Image
Ayush Herbs Ashwagandha Chewable Tab lets	Support energy levels, reduce stress, and enhance vitality in dogs and cats	

Product Name	Efficacy Features	Product Image
Petvitin Ashwagandha Fish Rice	Enhance energy, promote skin and coat health, and support a strong immune system	
Kradle Calming Bliss Bar	Maintain normal emotional balance and help pets stay calm and relaxed	
Qurea Pet Mood Regulation Drops	Help dogs stay calm and stable when facing various stressors such as fireworks and separation anxiety	

The application of Ashwagandha in pet health primarily targets emotion regulation and stress alleviation. In a randomized controlled trial involving 24 stressed and anxious dogs, daily supplementation with standardized Ashwagandha root extract (withanolides ≥5%) at a dose of 15 mg/kg body weight for 4 weeks resulted in a significant reduction of the urine cortisol-to-creatinine ratio (UCCR) in the treated group. Moreover, scores for fear and anxiety-related behaviors, as measured by the Canine Behavioral Assessment and Research Questionnaire (CBARQ), were significantly decreased. [31]

Table 4. Applications of Ashwagandha in the Post-Pet Health field (data from Amazon)

Product name	Functional Properties	Product Picture
Ayush Herbs	Support the energy of dogs and cats, relieve stress and enhance vitality	
Petvitin	Boost energy, promote fur and skin health, and support a strong immune system	
Kradle	Helps maintain normal emotional balance and help pets stay calm and relaxed	
Qurea	Helps dogs remain calm and stable when exposed to various stressors, such as fireworks or separation anxiety.	

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2. Withania somnifera — The “Herb of Life” from Ayurveda

2.1 Historical Origins of Withania somnifera

Withania somnifera (known as Ashwagandha), one of the oldest medicinal plants in the Ayurvedic system of medicine, belongs to the family Solanaceae. It is often referred to as “Indian ginseng” or “winter cherry.” Morphologically, Ashwagandha is an evergreen shrub that can grow up to approximately 1.5 meters in height. The leaves exhibit opposite phyllotaxis and are simple, ovate, glabrous, petiolate, with entire, glossy surfaces. Each leaf can reach up to about 10 cm in length. The branches are upright, extending roughly 60–120 cm. The flowers are bright yellow to pale green, measuring about 1 cm in length. The fruit is a small, berry-like drupe that turns orange-red when mature, with a diameter of approximately 6 mm. The seeds are yellow, small, and flattened, around 2.5 mm in diameter [1].

Descriptions of the Withania genus can also be found in classical Chinese botanical literature. The *Flora of China* (published in 1978) includes the genus Withania Pauquy and records the presence of one native species, Withania kansuensis Kuang et A.M. Lu, which is now recognized as a synonym of Withania somnifera and has been taxonomically unified. The *Flora of China* provides further morphological details of Withania genus: the stem is erect and often dichotomously branched; leaves are alternate on the lower stems and paired (unequal in size) on the upper branches; flowers occur in small clusters, nearly sessile; the calyx is campanulate (enlarging after flowering to enclose the berry); the corolla is narrowly bell-shaped; there are five stamens with longitudinally dehiscent anthers; the disk forms a ring surrounding the ovary base; the ovary is two-celled with numerous ovules; the fruit is a globose berry; and the seeds are flattened and reniform (kidney-shaped)

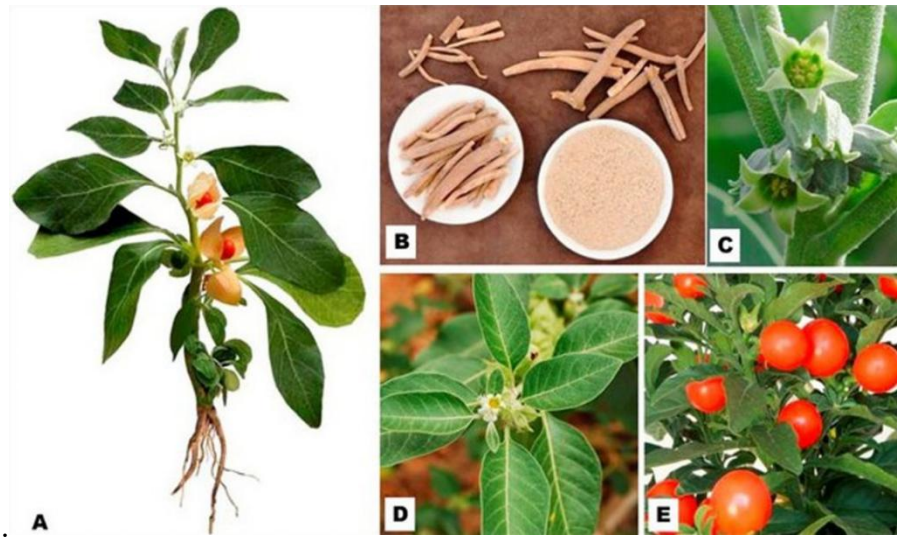

Figure 1. Withania somnifera and its parts [1]
(A) Whole herb; (B) Roots & their powdered form; (C) Flowers; (D) Leaves; (E) Fruits.

"Ashwagandha" originates from Sanskrit, derived from "ashwa" (meaning "horse") and "gandha" (meaning "odor"). This etymology not only refers to the distinct horse-like odor of its roots but also symbolizes the function of strength and vitality[2]. In the Ayurvedic medical system, Ashwagandha is classified as a Sattvic Kapha Rasayana, a rejuvenating herb that promotes longevity and vitality. Known as the "Herb of Life", it has been used for more than 3 millennia as an astringent, anti-inflammatory, adaptogenic, tonic, anesthetic, and diuretic agent. Traditionally, it has also been applied in the treatment of helminthic infections, hemorrhoids, goiter, leucoderma, neurasthenia, insomnia, and constipation. Furthermore, as early as 78 AD, Ashwagandha was documented in the Unani medical system within Kitab al-Hashaish by Dioscorides. The plant is also officially listed in the Indian Pharmacopoeia (1985 edition) as a recognized medicinal herb [3].

Ashwagandha originated from the Indian subcontinent, and as ancient Indian trade routes expanded, its use gradually spread to the Middle East, North Africa, and East Africa. Owing to its strong ecological adaptability, the plant established naturalized populations across arid and semi-arid regions of Africa, where it was subsequently incorporated into local traditional medical systems. By the 19th century, Western botanists and pharmacologists began to recognize its medicinal potential. In the mid-20th century, with the rise of research on natural and plant-based medicines, W. somnifera transitioned from a traditional herbal remedy to a subject of modern biomedical investigation, ultimately becoming one of the most representative adaptogenic plants worldwide.

2.2 Core bioactive compounds of Ashwagandha

Different plant parts of Ashwagandha have yielded a wide variety of chemical compounds belonging to distinct phytochemical classes, with notable variations in their total phenolic and flavonoid contents [4]. The root extract contains total phenolic compounds of 28.26 mg/g and total flavonoids of 17.32 mg/g, whereas the leaf extract contains only 5.4 mg/g of total phenolics and 5.1 mg/g of total flavonoids.

The concentration of withanolides also differs among plant parts: the roots contain 0.035–0.066%, the stems about 0.048%, and the leaves approximately 0.238%. Moreover, the withanolide levels in root extracts vary depending on the extract form—ranging from 0.003–0.051% in solid extracts to 0.027–0.065% in liquid preparations.

In both the Unani and Ayurvedic systems of medicine, the root of Ashwagandha is considered the primary medicinal part of the plant. Studies have shown that the roots contain 0.13–0.31% alkaloids, along with volatile oils, starch, amino acids, glycosides, reducing sugars, and steroidal compounds. Apart from the root, other parts of the plant also possess therapeutic value. The leaves are traditionally used for the treatment of fever and inflammation, the flowers act as astringents and diuretics, the fruits are employed in the management of skin ulcers, tumors, and abscesses, while the seeds are known to increase sperm count and promote testicular development [3].

Table 1. Phytochemical Constituents of Ashwagandha (Selected Data)[5]

Constituent Category	Compound name	Molecular Weight	Plant Parts
Withanolides	withaferin A	470.3	Roots, stems, leaves,whole herb
	withanolide A	470.6	Roots, stems, leaves
	withanolide B	454.6	Roots, stems, leaves
	withanolide C	523.1	Leaves
	withanolide E	486.6	Leaves, fruits, berries
	withanolide R	470.6	Leaves
	withanoside I	636.7	Roots

Constituent Category	Compound name	Molecular Weight	Plant Parts
	withanoside II	798.9	Roots
	withanoside III	652.8	Roots
	withanoside IV	782.9	Roots
	withanoside V	766.9	Roots
	withanoside VI	782.9	Roots, leaves
	withanoside VII	798.9	Roots, leaves
Alkaloids	choline	104.2	Roots, leaves
	anahygrine	224.3	Roots
	cuscohygrine	224.3	Roots
	caffeine	194.2	Fruits, Roots
	withanamide A	778.9	Fruits
	somniferine	184.2	Roots
	berberine	336.4	Roots
	noscapine	413.4	Roots
	papaverine	339.4	Roots
	isopelletierine	141.2	Roots
Flavonoids	quercetin	302.2	Whole herb
	rutin	610.5	Roots, stems, fruits, whole herb, leaves, berries
	catechin	290.3	Fruits, leaves, roots, berries

Constituent Category	Compound name	Molecular Weight	Plant Parts
	kaempferol	286.2	Berries, fruits, roots
	naringenin	272.3	Whole herb, fruit
	naringin	580.5	Whole herb
	hyperoside	464.4	Roots
	apigenin	270.2	Berries
Phenolic acids	gallic acid	170.1	Fruits, roots, leaves, berries, whole herb
	caffeic acid	180.2	Fruits, roots, leaves
	ferulic acid	194.2	Fruits, roots, leaves
	vanillic acid	168.1	Fruits, leaves
	p-coumaric acid	164.2	Fruits, roots, leaves
	quinic acid	192.2	Leaves
	vanillin	152.1	Fruits
Phytosterols	β-sitosterol	414.7	Roots, Fruits
	Stigmasterol	412.4	Roots, Fruits
	β-sitosterol glucoside	576.9	Roots, leaves
	Campesterol	400.7	Roots
	Cycloartenol	426.7	Fruits
	Stigmasterol glucoside	574.8	Roots
脂肪酸类 Fatty Acids	Linoleic acid	280.5	Roots, leaves
	Linolenic acid	278.4	Roots, leaves

Constituent Category	Compound name	Molecular Weight	Plant Parts
	Oleic acid	282.5	Roots, leaves
	Palmitic acid	256.4	Roots, leaves
	Stearic acid	284.5	Roots
	Arachidic acid	312.5	Fruits
Coumarins	Scopoletin	192.2	Fruits
	Aesculetin	178.1	Fruits
Trierponoides	Oleanolic acid	456.7	Roots
	β-amyrin	426.7	Fruits
Phenylpropyl esters	Withaninsams A）	294.3	Roots
	Withaninsams B）	294.3	Roots

Withanolides, a class of steroidal lactones unique to Ashwagandha, are characterized by a C28 steroidal backbone conjugated with a γ-lactone ring. Among them, Withanolide A is one of the most extensively studied and representative bioactive compound. Withanolide A exerts significant effects in regulating the stress response by interacting with the signaling pathways within the hypothalamic–pituitary–adrenal (HPA) axis. Moreover, due to its ability to promote neurogenesis and enhance cognitive function, it has been recognized for its potent neuroprotective properties [6].

Withanolides, a unique class of steroidal lactones found in Ashwagandha, characterized by a C28 steroidal backbone fused with a γ-lactone ring. Among them, withanolide A is one of the most extensively studied and representative constituents.
Drunk tomato lactone (withanolides) is A peculiar kind of steroid body South Africa drunk tomato ester compounds, its chemical structure in C28 steroids skeleton is characterized with gamma lactone ring, A of drunk tomato lactone (withanolide A) is the symbol of the most studied one of the ingredients.

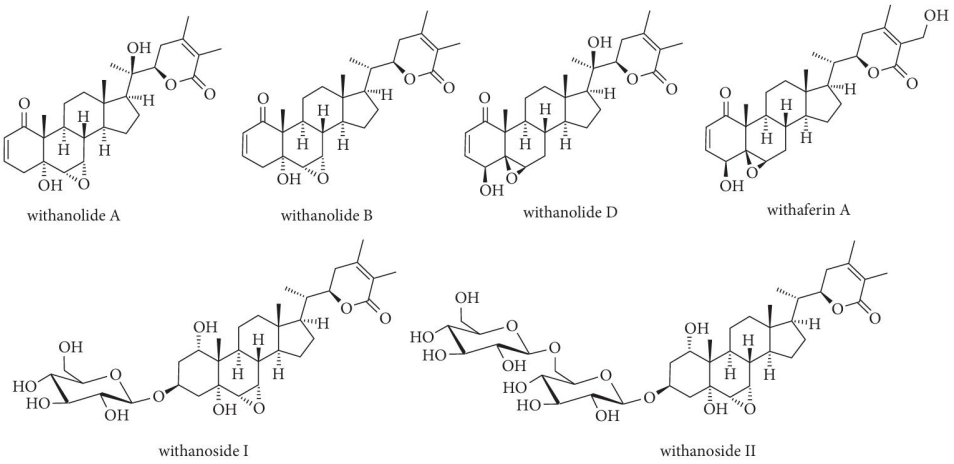

Figure 2. Chemical structures of withanolide derivatives from Ashwagandha[7]

Natural Field further investigated the differences in chemical compounds between Chinese and Indian Withania somnifera (Ashwagandha). The purity of major chemical compounds in the ethanol extracts of roots from both sources (root-to-extract mass ratio 10:1) was analyzed using the USP method. The results revealed significant differences in key compounds: the contents of withanolide B, RRT = 0.717, and RRT = 1.031 were relatively low in Chinese Ashwagandha, whereas withanolide A and withanosides V/VI were markedly higher compared to Indian Ashwagandha.

Table 2. Comparison of Core Compounds Detection in Ethanol Extract of Chinese and Indian Ashwagandha Roots (Data from Natural field)

Compound name	Chinese Ashwagandha	Indian Ashwagandha
RRT=0.099	10.86%	7.80%
RRT=0.111	18.23%	23.84%
RRT=0.188	3.06%	2.75%
RRT=0.717	0.61%	3.08%
RRT=0.749	2.13%	0.93%
Withanoside IV	1.19%	2.03%
Withanoside V/VI	9.31%	0.90%

Compound name	Chinese Ashwagandha	Indian Ashwagandha
Withaferin A	0.32%	4.65%
RRT=1.031	0.01%	4.26%
Withanolide A	14.44%	6.56%
Withanolide B	0.35%	2.93%
RRT=1.28	3.23%	5.29%
Total	100%	100%

2.3 Extraction Techniques of Ashwagandha

With the advancement of biopharmaceutical technologies, the extraction methods of Ashwagandha have undergone continuous evolution, from traditional solvent extraction to the integration of multiple modern techniques. The core objective is to maximize the preservation of the key bioactive functional compounds, such as withanolides and flavonoids. The process design should be optimized according to the chemical characteristics of the target compound, such as the difference between lipophilic withanolides and hydrophilic phenolic acids, as well as the distribution of these compounds across different plant parts.

Traditional methods for extracting phytochemicals from herbal materials include maceration extraction, percolation extraction, reflux extraction, and Soxhlet extraction. The core principle of these techniques lies in the penetration of the solvent into plant cells, dissolving phytochemicals within the plant matrix, followed by the diffusion of these compounds from the cells into the solvent. [4] Among these, maceration is considered one of the preferred techniques for Ashwagandha, as it operates under mild conditions that preserve delicate phytochemicals and antioxidant compounds. It is particularly suitable for sensitive herbs or heat-labile compounds.

Non-conventional extraction techniques include ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), supercritical fluid extraction (SFE), and subcritical water extraction (SWE). Among them, ultrasound-assisted solvent extraction is a non-thermal technology that uses high-frequency sound waves to disrupt plant cell walls, thereby enhancing the extraction efficiency of bioactive compounds. [5]

A comparative study using the leaves and roots of Ashwagandha evaluated two traditional extraction methods—(A) Maceration extraction and (B) Soxhlet extraction, against two modern techniques—(C) Microwave-assisted extraction and (D) Subcritical water extraction. The results demonstrated that modern techniques achieved significantly higher extraction yields and bioactive compound contents: (C)30.2% and (D)65.6%, compared to (A) 20.8% and (B) 25.7% for the traditional methods. [8]

Table 3. Extraction Methods of Ashwagandha[5]

Extraction Method	Core Principle	Core Advantage	Applicable Bioactive Compounds
Maceration Extraction（MC）	At room temperature, the plant materials are soaked in a suitable solvent, allowing the active constituents to be extracted through the processes of solvent penetration, dissolution, and diffusion.	Mild extraction process enables the preservation of thermolabile phytochemicals (e.g., antioxidant compounds) while offering advantages of low equipment requirements and cost efficiency.	Flavonoids, phenols, and heat-sensitive alkaloids
Reflux Extraction（RE）	The solvent is heated to boiling, and the vapor is condensed and returned to the extraction system. Through repeated reflux cycles, the dissolution and extraction of active compounds are enhanced.	Higher efficiency than maceration Extraction; Scalable for industrial use; Maximizes target compound yield and preserves bioactivity.	Withnalides, Alkaloids, phenols
Soxhlet Extr	Through a Soxhlet	Comprehensive extraction	Lipophilic

Extraction Method	Core Principle	Core Advantage	Applicable Bioactive Compounds
action（SE）	extractor, the solvent undergoes continuous reflux, repeatedly dissolving the active constituents from the plant material and thereby enhancing the completeness of extraction.	of diverse compounds. Well-established and reliable method with High reproductable	constituents (e.g., certain withanolides), Total phenolics, Flavonoids
Ultrasound-Assisted Solvent Extraction（UASE）	High-frequency ultrasonic vibrations disrupt plant cell walls, accelerating solvent penetration and promoting the rapid release and dissolution of active compounds.	Fast extraction (5–20 min); higher yield; The extraction rate is higher than that of traditional methods, and the total withanolides content is high (8.66 μg/mg).	Withnalides,Alkaloids, phenols
Microwave-Assisted Extraction（MAE）	Microwave energy is used to rapidly heat the solvent, enhancing the interfacial interaction between the solvent and plant materials and accelerating the dissolution of active ingredients.	High efficiency (13.74% vs. 9.51% yield); shorter extraction time; higher total phenolic content.	Lipophilic constituents (e.g., certain withanolides), Total phenolics, Flavonoids
Supercritical Fluid Extraction（SFE）	Using a supercritical fluid — which possesses both the diffusivity of a gas and the solvating power of a liquid — as the extraction solvent, selective extraction is	High-purity extract; solvent-free; eco-friendly with strong selectivity for lipophilic compounds.	Fatty acids, fat-soluble solanine, volatile components

Extraction Method	Core Principle	Core Advantage	Applicable Bioactive Compounds
	achieved by adjusting pressure and temperature parameters.		
Subcritical Water Extraction (SWE)	Using liquid water (with adjustable polarity) under high temperature (>100℃) and high pressure (>atmospheric pressure) as a solvent to enhance the extraction of active ingredients.	The extraction rate is extremely high (65.6%, which is much higher than 20.8%-25.7% of traditional methods); only water is used as the solvent, making it green and environmentally friendly.	Its polarity is adjustable, making it suitable for various components such as phenols and withanolides.

Although traditional and modern extraction technologies have significantly improved the extraction efficiency of active ingredients from Withania somnifera (such as withanolides and phenols), there are still two core bottlenecks in industrial application. First, there is a limitation in water solubility. The key active ingredients of Withania somnifera (such as some fat-soluble withanolides) have inherently poor water solubility and are difficult to stably disperse in water-based systems, which restricts their application in mainstream health product formulations such as functional beverages, oral liquids, and transparent drops. In addition, Withania somnifera extracts face a palatability barrier. The alkaloid components such as anahygrine and hyoscyamine contained in the extracts have an obvious natural bitter taste, which directly affects the taste experience of end products and reduces consumer acceptance, especially among populations sensitive to bitterness such as children and the elderly.

For the specific treatment of water solubility enhancement and bitterness removal, Natural Field's NF Ashwa® process can be adopted. This process uses imported whole Withania somnifera plants from India as the starting material, employs more professional modernized extraction technologies for traditional Chinese medicines, and integrates advanced modern nano-formulation technologies. It effectively reduces the bitter taste of traditional Withania somnifera extracts while retaining

core active substances such as withanolides. Additionally, it can directionally reduce the content of toxic components like withaferin A to below 0.1%, or even below 0.01%. Its series of products have improved the water solubility of withanolides, enabling them to disperse quickly and uniformly in aqueous systems, thus providing raw material solutions for innovative applications in fields such as functional beverages and health foods. When using Chinese Withania somnifera roots as raw materials, the water-soluble and bitterness-removed Withania somnifera produced through the same process mentioned above has stronger efficacy and an even lower content of withaferin A (all below 0.01%)

2.4 Safety Assessment of Ashwagandha

In vitro cytotoxicity research is a fundamental step in safety evaluation, which can directly reflect the killing effect of Withania somnifera components on cells. Natural Field used the MTT colorimetric method to investigate the cytotoxicity of the core single active ingredient (withaferin A) of Withania somnifera and extracts from different sources. Three representative cell lines were selected in the experiment to simulate different physiological environments, including the bEnd.3 mouse brain microvascular endothelial cell line (to simulate the vascular endothelial environment), the SH-SY5Y human neuroblastoma cell line (to simulate the nerve-related environment), and the RAW264.7 mouse monocyte-macrophage cell line (to simulate the immune cell environment). The test samples included three types of extracts: Ashwagandha root extract (Indian origin), Ashwagandha root extract (Chinese origin), and NF-Ashwa® instant debittered Ashwagandha (derived from Indian Ashwagandha raw material). The results showed a significant toxicity difference between the single active component of Ashwagandha and its extracts: Withaferin A exhibited certain toxicity to all three tested cell lines (with IC50 values ranging from 0.98 to 1.22 μM), while the three Ashwagandha extracts (covering different origins and processing forms) all showed "extremely low toxicity" within the experimental concentration range.

Table 4 Cytotoxicity Test Results of Ashwagandha's Core Components and Extracts (MTT

Colorimetric Assay, Data from Natural Field)

Compound name	bEnd.3 (Mouse Brain Microvascular Endothelial Cell Line)	SH-SY5Y(Human Neuroblastoma Cell Line)	RAW264.7(Mouse Monocyte-Macrophage Cell Line)
Withaferin A	1.12μM	1.22μM	0.98μM
Ashwagandha Root Extract (Indian Origin)	Extremely low toxicity (based on the maximum concentration in the experimental protocol, the half-lethal dose was not reached)		
Ashwagandha Root Extract (Chinese Origin)	Extremely low toxicity (based on the maximum concentration in the experimental protocol, the half-lethal dose was not reached)		
NF-Ashwa NF Ashwa Instant Debittered Ashwagandha (Derived from Indian Ashwagandha Raw Material)	Extremely low toxicity (based on the maximum concentration in the experimental protocol, the half-lethal dose was not reached)		

Meanwhile, studies have shown that Ashwagandha is safe when the single intake dose is no greater than 100 mg per kilogram of body weight (or an approximate daily intake of 21 g). In an animal model study, researchers administered Ashwagandha extract at oral doses of 0, 500, 1000, and 2000 mg/kg body weight for 28 consecutive days. The resulting data indicated that no adverse reactions were induced when the daily administration dose of Ashwagandha extract did not exceed 2000 mg/kg body weight [9].

Table 5 Safety Assessment of Ashwagandha

Safety	Experimental Results	Conclusions	References

Safety	Experimental Results	Conclusions	References
Genotoxicity	1.Bacterial Reverse Mutation Assay (relevant OECD guidelines): No increase in reverse mutant colonies at 0.156-5.00 mg/plate (±S9); 2. Chromosome Aberration Assay (OECD TG 474): No structural chromosome damage at 0.25-2.00 mg/ml (±S9); 3. In Vivo Micronucleus Assay (OECD TG 474): No increase in MNPCE or bone marrow toxicity at 500-2000 mg/kg (in mice).	No genotoxicity within the test range	[10]
Acute Toxicity Study	In the acute oral toxicity test (in compliance with OECD TG 423), female Wistar rats were used as the model. Ashwagandha was administered orally at doses of 500, 1000, and 2000 mg/kg bw, with a 15-day observation period. No animal death, morbidity, or abnormal clinical signs were observed; the animals showed normal weight gain, and no gross pathological damage to organs was found after dissection.	The acute oral toxicity is extremely low. The median lethal dose (LD_{50}) cutoff value is greater than 5000 mg/kg bw. According to GHS classification, it belongs to Category-5 or is unclassified, with no acute oral toxicity risk.	[10]
Subacute Toxicity Study	In accordance with the revised OECD 407 Guideline, 60 Wistar rats (30 males/30 females, divided into control group, 200/400/800 mg/kg bw groups, including a satellite group) were given Ashwagandha extract orally daily for 28	Ashwagandha root extract is safe for 28-day repeated oral administration in rats at doses of 200-800 mg/kg bw, with no	[11]

Safety	Experimental Results	Conclusions	References
	days, with the satellite group observed for an additional period until Day 43: ① No deaths occurred (only one female rat in the high-dose satellite group died on Day 29, suspected to be due to inter-animal cannibalism; another female rat in the high-dose satellite group showed abnormal posture on Day 30, which self-recovered within 6 days); ② All groups showed steady weight gain and normal food intake, with no differences compared to the control group; ③ Hematological parameters (Hb, platelets, coagulation time, etc.) and biochemical parameters (hepatic and renal function, blood lipids, electrolytes, etc.) were all within the normal range. Only the high-dose group showed a slight increase in ALP, total protein, and albumin (still in line with CPCSEA standards), and ALP in the satellite group returned to normal.	organ toxicity. Even at 800 mg/kg bw (approximately more than 5 times the recommended human dose), there were no significant adverse effects.	

Safety	Experimental Results	Conclusions	References
Subchronic Toxicity Study	In accordance with the OECD 408 Guideline, 100 Sprague-Dawley (SD) rats (50 males/50 females, divided into control group, 100/500/1000 mg/kg bw groups, including a satellite recovery group) were given PAE orally daily for 90 days, with the recovery group observed for an additional 1 month: ① No toxic symptoms or deaths occurred, and body weight/food intake were comparable to the control group; ② Hematological parameters (WBC, RBC, Hb, etc.) and biochemical parameters (CHO, TG, SGOT, SGPT, etc.) showed no significant differences from the control group; ③ The weights of major organs were normal, and no histopathological abnormalities were found.	Ashwagandha has good subchronic toxicity safety.The No-Observed-Adverse-Effect Level (NOAEL) for daily oral administration in rats is 1000 mg/kg bw, with no repeated-dose toxicity risk.	[12]
Reproductive and Developmental Toxicity	In accordance with the OECD 421 Guideline, 92 Wistar rats (40 males/52 females, divided into control group, 500/1000/2000 mg/kg bw groups) were given ARE orally: ① Parental generation: Males were dosed for 4 weeks, and females for 63 days (including pre-pregnancy to lactation period); There were no significant differences in parental	Oral administration of ARE has no reproductive and developmental toxicity in Wistar rats. The No-Observed-Adverse-Effect Level (NOAEL) is 2000 mg/kg bw, with no adverse effects on	[13]

Safety	Experimental Results	Conclusions	References
	body weight or reproductive organ weight compared to the control group; No dose-dependent abnormalities were observed in thyroid hormones (T4, TSH). ② Offspring: Litter size, number of surviving pups, and anogenital distance were normal; Offspring body weight ranged from 21.17 to 23.11 g (no difference from the control group); No external or pathological malformations were found. ③ At the highest dose of 2000 mg/kg bw, the parental pregnancy index was 92% (100% in the control group), which had no significant toxicological significance, and no abnormalities were observed in the pathology of reproductive organs.	parental reproductive function or offspring growth and development.	

2.5 Development of Ashwagandha Synergistic Formulations

Ashwagandha is often used in combination with other herbs and nutrients to maximize its health benefits while expanding its application scenarios in fields such as immune regulation, neurological health, and metabolic balance.

2.5.1 Ashwagandha and Magnesium

The synergistic formulation of Ashwagandha and magnesium holds significant value in the health sector. Ashwagandha can reduce cortisol levels by inhibiting the

overactivation of the hypothalamic-pituitary-adrenal axis and regulate neurotransmitters to improve sleep. Magnesium, on the other hand, modulates the activity of GABA receptors to assist in cortisol control, promotes melatonin synthesis to extend deep sleep duration, and participates in muscle cell metabolism to support muscle repair. A common formulation involves administering 300-600 mg of Ashwagandha extract (containing 30-40 mg of withanolides) daily, combined with 200-300 mg of magnesium glycinate or magnesium citrate (e.g., BIOGENA's Ashwagandha Formula, which contains 500 mg of Ashwagandha extract and 70 mg of magnesium per capsule). This formulation is suitable for scenarios such as relieving anxiety in high-stress populations, improving sleep in middle-aged and elderly individuals, and promoting recovery in athletes.

Natural Field Bio-technique Co., Ltd. has developed a series of novel trace element supplements (including Ashwagandha acid-calcium, -magnesium, -zinc, and -iron) using patented technology. Furthermore, Ashwagandha acid liposomes prepared from these supplements significantly enhance in vivo bioavailability, and relevant research is currently ongoing.

2.5.2 Ashwagandha and Chrysanthemum Extract

In a study on obese mice induced by a high-fat diet, the complex of Ashwagandha and Chrysanthemum (ASC) exhibited a significant synergistic anti-obesity effect. Its ability to reduce the final body weight of mice and decrease the accumulation of total white adipose tissue (including subcutaneous and visceral fat) was significantly stronger than that of Ashwagandha or Chrysanthemum alone. Meanwhile, in terms of improving lipid metabolism, activating energy metabolism-related pathways (e.g., AMPK and UCP1 pathways), and regulating the expression of key adipogenic proteins (e.g., PPARγ and C/EBPα), the effect of ASC also exceeded that of individual components. ASC exerts its anti-obesity effect by synergistically regulating metabolism-related processes [14].

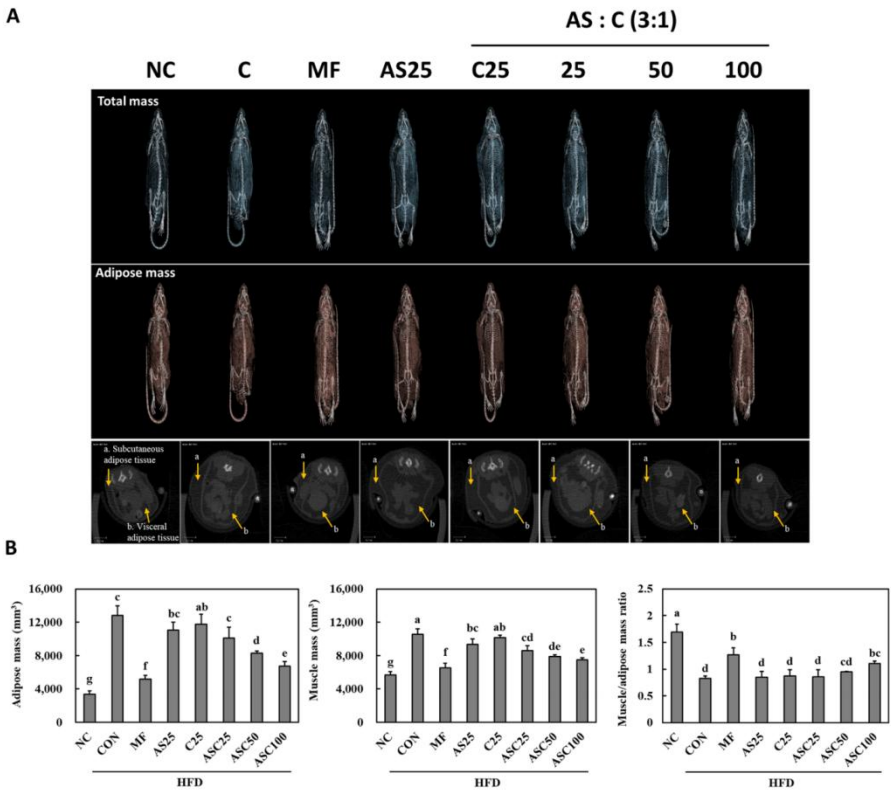

Figure 3 The effect of ASC complex on fat and muscle mass in HFD-induced obese mice [14]

2.5.3 Ashwagandha and Vitamin C

A study used juvenile Labeo rohita (rohu) as the research object and added different proportions of a mixture of Ashwagandha root extract and vitamin C to their diet. By detecting hematological indicators (e.g., increased hemoglobin content and red blood cell count), immunological indicators (e.g., improved nitroblue tetrazolium (NBT) reduction and lysozyme activity), biochemical indicators (e.g., decreased glucose content and alkaline phosphatase (ALP) activity), and the survival rate of juvenile fish under low pH and waterborne iron stress (e.g., the relative percentage survival under combined stress reached 65%, significantly higher than 0% in the control group), it was found that the mixture with a 1.0% addition amount could most effectively stimulate the immune response of juvenile fish, enhance their immune defense capabilities, and reduce damage caused by multiple stresses during a 15-day feeding period [15].

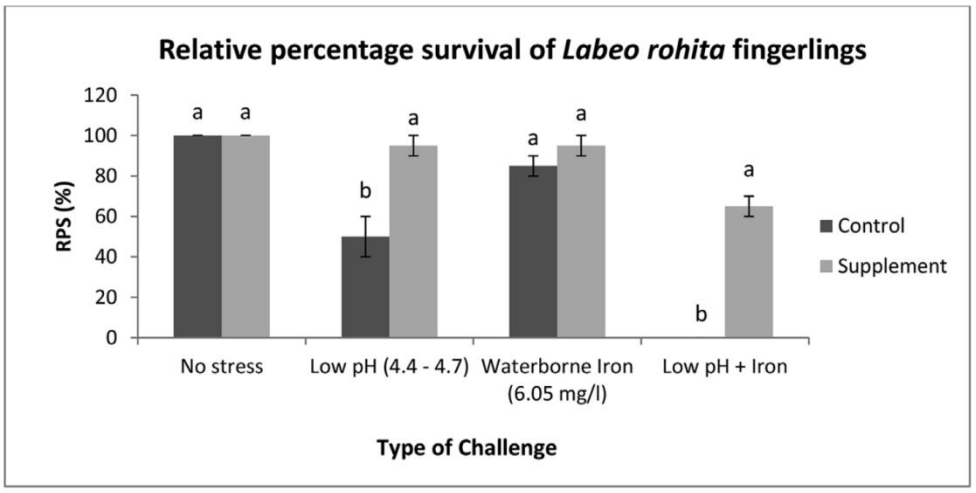

Figure 4 Relative survival rate of Labeo rohita fingerlings in the challenge experiment [15]

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3 Research on the Core Efficacy of Ashwagandha

3.1 Sleep-Improving Effect

Sleeping as a crucial physiological repair process of the human body, not only

supports cognitive function, immune defense, metabolic regulation, and emotional stability but also directly impacts overall health and quality of life. According to the recommendations of the National Sleep Foundation (USA), adults (18-64 years old) should sleep 7-9 hours per day, and the elderly (≥65 years old) should sleep 7-8 hours per day [1]. Sufficient sleep enhances memory consolidation, decision-making, and emotional recovery abilities, while long-term insufficient sleep is associated with an increased risk of cardiovascular diseases, obesity, diabetes, and mental health disorders.

According to statistics from Resmed's 2025 Global Sleep Survey, approximately one-third of respondents worldwide experience sleep issues such as difficulty falling asleep, frequent nighttime awakenings, or insufficient sleep duration. Among these, stress (57%), anxiety (46%), and financial pressure (31%) are the primary causes of poor sleep quality[2]. Notably, traditional sleep-aiding methods are often accompanied by side effects like next-day drowsiness and drug dependence; thus, finding safe and effective natural sleep regulation solutions is of great significance.

In a study on a caffeine-induced insomnia animal model, Ashwagandha demonstrated a significant sleep-improving effect compared to the placebo. Specifically, it not only effectively prolonged the total sleep duration of the animals but also shortened their sleep latency (i.e., the time required to transition from a waking state to a sleeping state). Additionally, Ashwagandha increased the duration of non-rapid eye movement (NREM) sleep and deep sleep in the animals. From the perspective of its mechanism of action, this active ingredient binds to GABAA receptors in the brain, thereby promoting changes in key molecules related to sleep regulation—including a significant increase in the expression levels of GABA and its receptors (GABAA and GABAB receptors), serotonin receptors (5-HT), and an overall increase in brain GABA content [3].

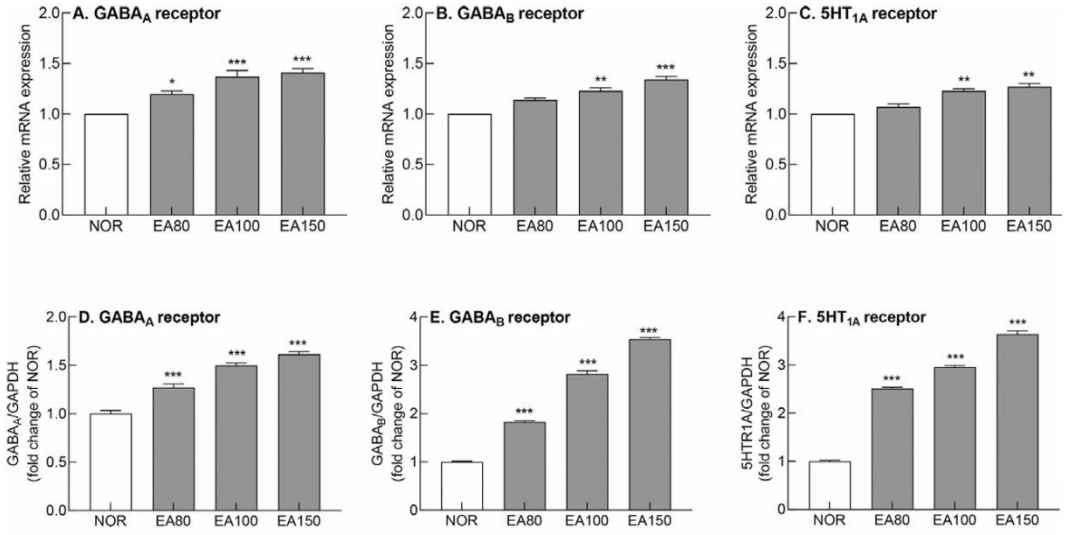

Figure 1 The effect of Ashwagandha on the mRNA and protein expression of GABAA, GABAB, and 5-HT1A receptors in the brains of mice [3]

A study by Kaushik et al. revealed that the core active ingredient in Ashwagandha extract responsible for its positive sleep-inducing effect is not the traditionally assumed withanolides, but triethylene glycol (TEG) present in its leaves. By orally administering the alcoholic extract (containing withanolides) and aqueous extract (containing TEG) of Ashwagandha leaves to mice, combined with electroencephalogram (EEG) and electromyogram (EMG) monitoring, it was found that withanolides had no effect on mouse sleep, while TEG significantly increased NREM sleep with minimal impact on rapid eye movement (REM) sleep. Meanwhile, the study found that commercial TEG also increased NREM sleep duration in mice in a dose-dependent manner (10-30 mg/mouse) (from 204.6±5.1 min to 287.9±5.4 min within 12 hours) and induced physiological sleep, providing a natural candidate for insomnia treatment [4].

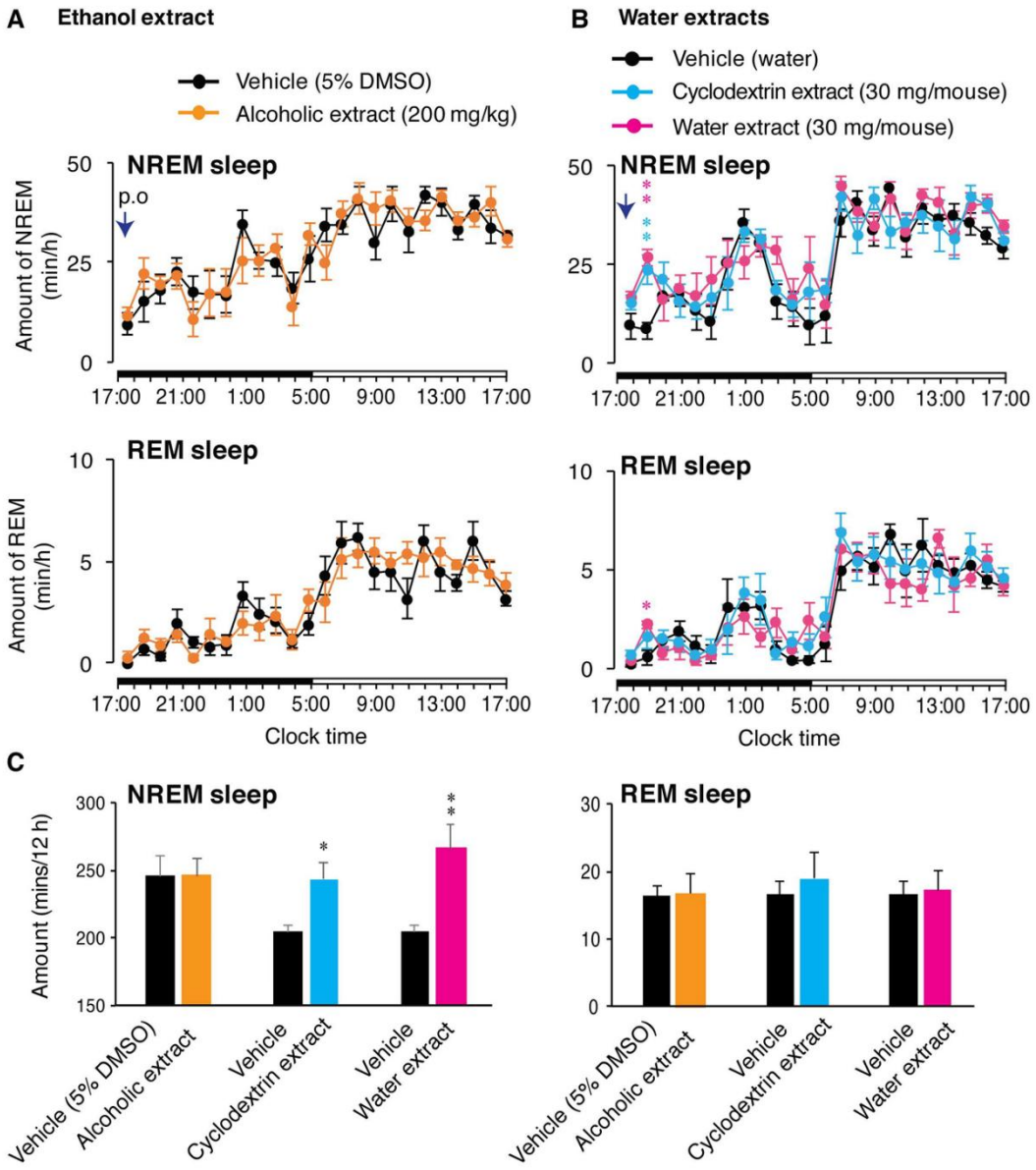

Figure 2 The effect of Ashwagandha extract on the sleep-wake state of mice [4]

In a randomized, double-blind, placebo-controlled trial involving 150 healthy adults with non-restorative sleep (NRS), participants took 120 mg of standardized Ashwagandha extract orally once daily for 6 weeks. After the trial, the participants' self-reported sleep quality significantly improved by 72% compared to the baseline, which was significantly higher than the 29% improvement in the placebo group. Additionally, activity monitoring data showed significant reductions in sleep onset latency (SOL) and wake after sleep onset (WASO), as well as significant improvements in sleep efficiency (SE) and total sleep time (TST) [5].

Table 1 Studies on Ashwagandha and Sleep Improvement

Study Subjects	Intervention Protocol	Core Results	Literature Source
52 healthy adults (＞18 years old)	1 control group: 1)Control group: 250 mL lactose-free skim milk 3 experimental groups: 1)Milk with 250 mg Ashwagandha 2)Milk with 250 mg Ashwagandha,175 mg tryptophan 3)Milk with 600 mg Ashwagandha Duration: 90 days	Compared to the control group, the three experimental groups showed significantly greater changes in the Sleep Quality Visual Analog Scale (VAS) scores; all groups exhibited improvements in the Pittsburgh Sleep Quality Index (PSQI) subscales; in terms of the Insomnia Severity Index, the experimental groups showed a greater decrease than the control group, with the most significant decrease in the 600 mg Ashwagandha group; daytime sleepiness also decreased in all experimental groups.	[6]
150 subjects with non-restorative sleep (NRS) (18-65 years old)	Control group: Placebo capsules Experimental group: Capsules with 300 mg Ashwagandha extract Duration: 50 days	Compared to the control group, the experimental group showed a significant improvement in the total score of the Richards-Campbell Sleep Questionnaire (RSQ-W) (average percentage change from baseline); significant reductions in sleep onset latency (SOL) and wake after sleep onset (WASO); significant increases in total sleep time (TST); a decreased micro-arousal index; and significant	[7]

Study Subjects	Intervention Protocol	Core Results	Literature Source
		improvements in the WHO Quality of Life-BREF (WHOQOL-BREF) score, Hospital Anxiety and Depression Scale (HADS) score, and serum C-reactive protein (CRP) level.	
39 healthy elderly individuals (60-85 years old)	Control group: Placebo capsules Experimental group: Capsules with 600 mg Ashwagandha root extract Duration: 12 weeks	Compared to the placebo group, the experimental group showed a significant improvement in quality of life (WHOQOL-BREF score increased from 140.53 to 161.84); additionally, sleep quality and mental alertness were significantly improved in the Ashwagandha-treated group.	[8]

3.2 Mood Regulation Effect

Emotional homeostasis is a core pillar of human mental health, which not only directly affects cognitive judgment, interpersonal interaction, and life satisfaction, but also is deeply associated with physical health. Prolonged exposure to negative emotional states such as stress, anxiety, or depression can lead to overactivation of the hypothalamic-pituitary-adrenal (HPA) axis, resulting in sustained elevation of cortisol levels. This disrupts the balance of the autonomic nervous system that maintains bodily equilibrium, triggering hormonal imbalances and brain changes [9].

Additionally, stress induces physiological alterations in the endocrine, autonomic, and immune systems, as well as psychological changes like memory and emotional dysfunction, further increasing the risk of conditions such as impaired immune function, metabolic disorders, cardiovascular diseases, and neurodegenerative diseases.

Globally, mood-related issues have become a major public health challenge in the 21st century. According to the 2024 Global Mental Health Report released by the World Health Organization (WHO), approximately 280 million people worldwide suffer from depression, and over 300 million people experience varying degrees of anxiety disorders. This figure continues to grow at an annual rate of 5%-8%. More notably, mood problems have shown trends of "rejuvenation" and "normalization". Data from the Anxiety and Depression Association of America (ADAA) indicates that 31.9% of adolescents aged 13-18 have experienced anxiety disorders. The 2023 Workplace Emotional Status Survey in China reveals that over half (56%) of workplace employees reported worse emotional states in 2023, with burnout issues; among them, 46% attributed their anxiety to work pressure and financial stress. Furthermore, the elderly face prominent mood problems due to declining physiological functions, narrowed social circles, and chronic illness-related distress. Research from the China National Committee on Aging shows that 26.4% of the elderly in China have depressive symptoms, including 20.2% with mild symptoms and 6.2% with moderate to severe symptoms.

The improving effect of Ashwagandha on stress and related emotions (anxiety, depression) is achieved through multi-targeted and multi-system synergistic regulation [10]. Firstly, as a core mechanism of an "adaptogen", Ashwagandha inhibits the overactivation of the HPA axis, significantly reducing stress-induced elevation of plasma cortisol to alleviate stress. Secondly, Ashwagandha extract can activate GABAA receptors, increase chloride ion influx, and inhibit neural excitation; certain components (e.g., withanolide B) can enhance inhibitory postsynaptic currents,

similar to GABAergic modulators. Meanwhile, it reduces oxidative stress in the brain (by decreasing lipid peroxidation and increasing glutathione levels) and inhibits neuroinflammation (by reducing glial activation markers such as GFAP and Iba1, and lowering pro-inflammatory factors like TNFα and IL-6) to alleviate anxiety-like behaviors. In terms of antidepressant effects, Ashwagandha primarily relies on mitigating oxidative stress-mediated neural damage and enhancing serotonergic neurotransmission. Studies have shown that withanolide A upregulates the mRNA expression of serotonin receptors 5-HT1A/2A and transporter SERT in a Caenorhabditis elegans model.

Table 2 Studies on Ashwagandha and Stress, Anxiety, and Depression

Study Subjects	Intervention Protocol	Key Results	Reference Source
86 patients aged 16-60 with Generalized Anxiety Disorder	Ashwagandha root powder + sucrose granules, 4g three times a day for 60 days	Scores of all symptoms on the Hamilton Anxiety Rating Scale (HAMA) significantly decreased; respiratory symptoms showed the highest remission rate, followed by muscle discomfort and depressive mood	[11]
54 adults aged 21-54 with mild to moderate stress-related anxiety (PSS 14-24, GAD-7 < 15)	Ashwagandha root extract capsules (containing 2.5% withanolides), 500mg once a day for 60 days	Scores on the Perceived Stress Scale (PSS) and Generalized Anxiety Disorder Scale (GAD-7) significantly decreased; scores on the World Health Organization Quality of Life-BREF (WHOQOL-BREF) significantly increased	[12]
40 adults with Generalized Anxiety Disorder (GAD)	70% ethanol extract of Ashwagandha root, 1g/day for 6 weeks	Scores on the Hamilton Anxiety Rating Scale (HAM-A) significantly decreased; combination with selective serotonin reuptake inhibitors (SSRIs) improved GAD symptoms	[13]

Study Subjects	Intervention Protocol	Key Results	Reference Source
Adult male Wistar rats (180-220g) with a chronic unpredictable foot shock stress (CS) model	Standardized Ashwagandha root extract, 25mg/kg or 50mg/kg once a day, administered 1 hour before stress for 21 consecutive days	Reduced stress-related hormone levels; significantly inhibited CS-induced elevation of plasma corticosterone in a dose-dependent manner; plasma corticosterone levels in the WS 50mg/kg group were close to those of the normal control group; alleviated stress-related depressive behaviors: shortened "behavioral despair" immobility time in forced swimming stress and reduced escape failure times in the "learned helplessness" test	[14]
28 male Wistar rats aged 35-49 days with a chronic unpredictable mild stress (CUMS) model	CUMS treatment (for 17 consecutive days) + Ashwagandha powder 50mg/kg/day, once a day via oral gavage (administered 1 hour before stress from Day 3 to Day 17)	Behavioral improvements (anti-stress and antidepressant effects): significantly increased sucrose preference rate, improving stress-induced anhedonia; significantly shortened immobility time and increased swimming time in the forced swimming test, alleviating despair-like behaviors; decreased anxiety index in the elevated plus maze (EPM), improving stress-induced anxiety	[15]

3.3 Immune Regulation Effect

The immune system is a complex defense network that protects the human body against pathogens, eliminates abnormal cells, and maintains internal environmental stability. The immune defense of mammals consists of three closely coordinated levels: physical barriers (e.g., skin, mucous membranes, tears, gastric acid), the innate immune system (a rapid, non-specific response involving various immune cells and inflammatory factors), and the adaptive immune system (a slow, specific response

primarily mediated by T lymphocytes and B lymphocytes). Typically, invading pathogens are eliminated by the innate immune system; when the innate immunity fails, the adaptive immune system is activated, responding precisely through two main pathways: cell-mediated (dominated by T cells) and humoral (dominated by antibodies produced by B cells) immunity [16].

Ashwagandha extract (withanolides) can significantly increase immunoglobulin levels, enhance the number of T cells, and elevate the expression of gamma-interferon (IFN-γ). It is an effective immunostimulant that can simultaneously enhance both humoral and cell-mediated immune responses. A study investigated the immunomodulatory properties of Ashwagandha on human neutrophils through a series of in vitro analyses. Researchers isolated target compounds from Ashwagandha roots and evaluated their potential to regulate immune function using three core experiments: the nitroblue tetrazolium (NBT) test, phagocytosis assay of inactivated Candida albicans, and assessment of neutrophil mobility and chemotaxis. These compounds were evaluated at different concentrations: 10, 20, 40, 100, and 1000 μg/mL. The results showed that the isolated compounds significantly affected the immune parameters of human neutrophils, confirming their prominent immunomodulatory effects [17].

In another randomized, double-blind, placebo-controlled trial involving healthy adults aged 45-72 years, 24 participants were randomly divided into two groups. The experimental group took capsules containing 60mg Ashwagandha extract (with 21mg withanolide glycosides) daily. After 30 days, the Ashwagandha group showed significant increases in immunoglobulins (IgA, IgM, IgG, and its subtypes IgG2/3/4), cytokines (IFN-γ, IL4), and TBNK cells (CD45+, CD3+, CD4+, CD8+, CD19+, NK cells), while the placebo group showed a significant decrease in TBNK cells. The study indicated that Ashwagandha extract can significantly improve the immune profile of healthy individuals by regulating the innate and adaptive immune systems, and is expected to be used to enhance immunity in populations at risk of infection [18].

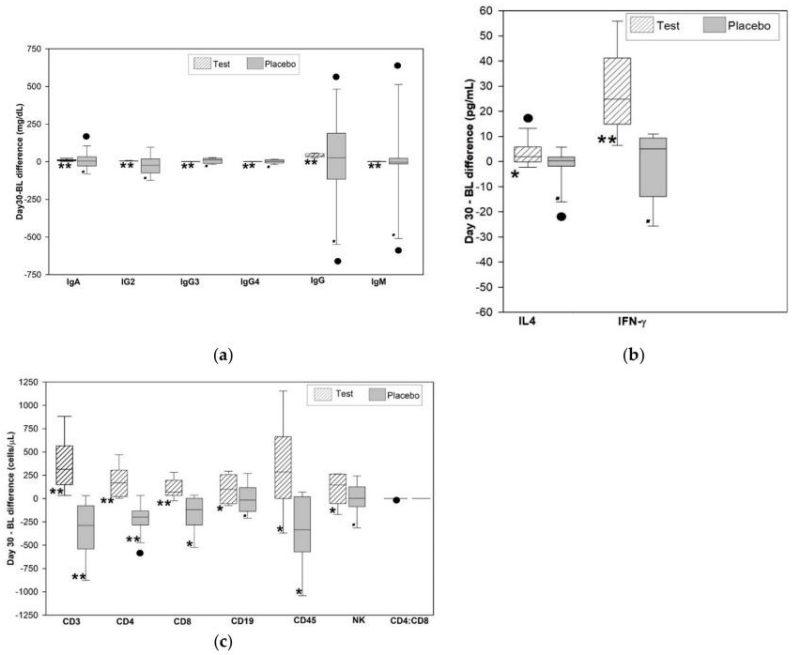

Figure 3 Effect of Ashwagandha on Immunoglobulins, Cytokines, and TBNK Cells [18]

3.4 Anti-Aging Effect

The world is experiencing an unprecedented demographic transition in both scale and speed. According to data from the United Nations' World Population Prospects 2019, the global proportion of the elderly population aged 65 and above is projected to rise from 1/11 in 2019 to 1/6 in 2050. Among them, the fastest-growing group is the elderly aged 80 and above, whose numbers are expected to triple, increasing from 143 million in 2019 to 426 million in 2050. The aging process is inherently associated with increased susceptibility to various non-communicable diseases (i.e., chronic diseases) and is gradually becoming the primary cause of morbidity and mortality worldwide. Studies have shown that cardiovascular diseases, cancer, chronic respiratory diseases, and neurological diseases are currently the main health burdens for the elderly population. A cross-sectional study involving 1.75 million patients found that only 23% of the total study population had multimorbidity (i.e., the coexistence of two or more chronic diseases in an individual), but this proportion rose significantly to 65% in the 65-84 age group and reached over 82% in the 85+ age group [19].

Aging and aging-related complexity (the multi-dimensional manifestations of aging, such as declined physiological function and increased disease susceptibility) are the result of the combined effects of external factors (environmental factors) and internal factors (gene expression regulation). In 2025, Kroemer et al. elaborated on the 14 hallmarks of aging in the article From Geroscience to Precision Geromedicine: Understanding and Managing Aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, altered intercellular communication, chronic inflammation, dysbiosis, extracellular matrix alterations, and psychosocial isolation [20]. In a Caenorhabditis elegans model, Ashwagandha root extract (withanolides) extended the average lifespan of the nematodes by 29.7% and regulated their insulin/insulin-like growth factor-1 signaling (IIS pathway) and neural activity [21], making it a natural product with potential anti-aging properties.

3.4.1 Ashwagandha and Telomere Protection

Telomeres are protective structures at the ends of eukaryotic chromosomes, composed of highly repetitive DNA sequences (human telomeres have the sequence "TTAGGG") and associated proteins (e.g., the telomere-protective protein complex Shelterin), resembling "caps" at the chromosome ends [22]. With each round of DNA replication, telomeres gradually shorten. When telomeres shorten to a critical length (the Hayflick limit), cells initiate a senescence program, which in turn affects tissue, organ, and overall aging [23]. Notably, telomerase is a ribonucleoprotein complex that participates in the protection and repair of chromosome (telomere) ends during DNA replication, preventing their shortening and promoting the maintenance of healthy cell function. However, telomerase activity is very low in human cells. A study by Iwama et al. pointed out that in the 4-39 age group, both telomere length and telomerase activity show a gradual decline; in individuals aged 40 and above, although telomere length continues to shorten progressively, 65% of them exhibit stable but low telomerase activity, while the remaining 35% show no detectable telomerase activity [24].

Therefore, low telomerase activity leads to the rapid appearance of short telomeres and reduced DNA repair efficiency, ultimately accelerating the aging process.

A 2016 study published in Advances in Bioscience and Biotechnology explored the effect of Ashwagandha root extract on telomerase activity using the human HeLa cell line as a model. In the experiment, HeLa cells were cultured in DMEM medium containing 10% fetal bovine serum under suitable conditions until 40%-60% confluency, then treated with the extract at 5 concentrations (10 μg, 50 μg, 100 μg, 500 μg, and 5 mg) for 72 hours. Detection via the Telomeric Repeat Amplification Protocol (TRAP assay) showed that telomerase activity was significantly increased by 45% compared to the control group at concentrations of 10-50 μg, while activity decreased when the concentration exceeded 50 μg [25]. This study was the first to confirm the anti-aging potential of Ashwagandha at the human cellular level.

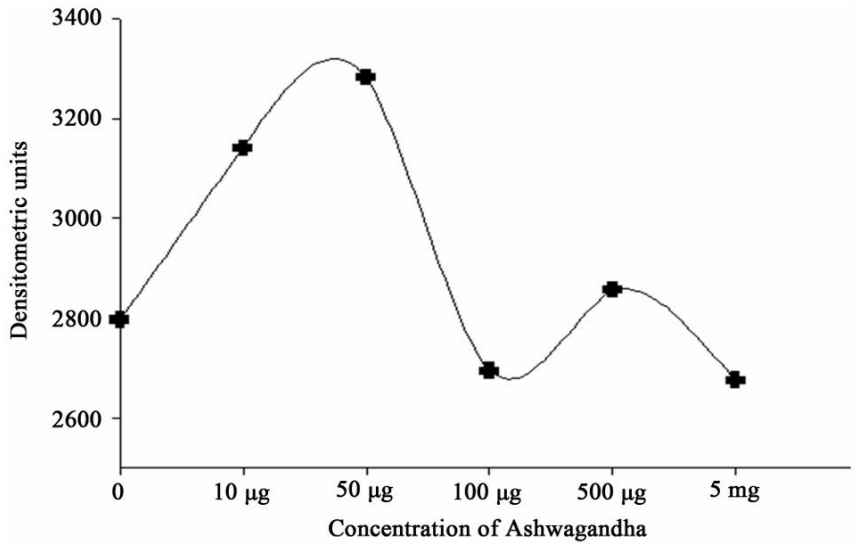

Figure 4 Effect of Different Concentrations of Ashwagandha Root Extract on Telomerase Activity in HeLa Cells [25]

3.4.2 Ashwagandha and Proteostasis

Proteostasis is a core process for maintaining cellular and organismal health, regulated by the proteostasis network (PN). This network includes molecular chaperones (heat

shock proteins), the ubiquitin-proteasome system, and autophagic mechanisms, which coordinate protein synthesis, folding, depolymerization, and degradation [26]. Stress response pathways (e.g., heat shock response, endoplasmic reticulum unfolded protein response, mitochondrial unfolded protein response) dynamically maintain proteostasis by regulating PN components. Impaired proteostasis is a common feature of aging and neurodegenerative diseases (Huntington's disease, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis).

Studies have shown that the proteostasis network (PN) exhibits significant age-related changes. Ben-Zvi et al. observed in Caenorhabditis elegans (with an average lifespan of approximately 21 days) that PN function declines as early as 2-5 days of adulthood, manifested by misfolding of temperature-sensitive proteins; meanwhile, the cellular protective stress response during this period is significantly weakened, and the ability to activate the heat shock response and unfolded protein response is severely reduced [27]. In humans, analysis of young and elderly brain tissues revealed that 101 core genes (including HSP70, HSP90, and TRiC subunits) among 332 PN-related genes were significantly downregulated, while 62 compensatory genes (e.g., small heat shock proteins) were upregulated. This change is consistent with PN gene changes in brain regions affected by Huntington's disease and Alzheimer's disease [28].

The regulation of cellular proteostasis by Ashwagandha (withaferin A) exhibits a concentration-dependent bidirectional effect [29]. Low concentrations of withaferin A can enhance proteostasis: by activating heat shock factor 1 (HSF1), it upregulates molecular chaperones such as HSP90α/β and HSP70, which assist in protein refolding, inhibit aggregation, and promote the degradation of abnormal proteins, forming a "pre-adaptive" protective effect. In contrast, high concentrations of withaferin A disrupt proteostasis: its electrophilic groups covalently bind to cysteine residues in a large number of proteins, inducing protein unfolding and aggregation. Meanwhile, it inhibits the proteasome, autophagy, and heat shock repair systems.

In addition, Ashwagandha can enhance the function of the ubiquitin-proteasome system. Withaferin A effectively inhibits chymotrypsin-like proteasome activity, leading to the accumulation of ubiquitinated proteins in breast cancer cell lines MCF-7 and MDA-MB-231. The ubiquitin-proteasome system is the main mechanism by which cells degrade and clear toxic proteins, thereby reducing the burden of cellular damage accumulated over time [30].

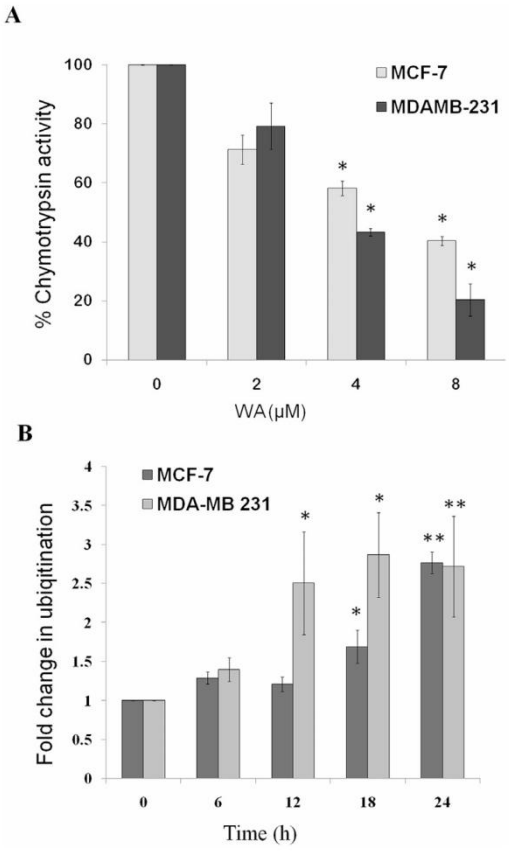

Figure 5 Determination of Ubiquitin-Proteasome Activity [30]

3.4.3 Ashwagandha and Chronic Inflammation

In 2000, Italian scholar Claudio Franceschi proposed the concept of "inflammaging", referring to the complex remodeling of the immune system during aging, characterized by both functional changes and adaptive adjustments. This process ultimately leads to altered immune responses and increased susceptibility to infections

and chronic non-communicable diseases (CNCDs). Importantly, the expression pattern of inflammatory mediators produced by immune cells gradually changes, leading to the formation of a persistent, chronic, low-grade, systemic, and sterile (no obvious infection) inflammatory state [31].

Studies have shown that Ashwagandha inhibits inflammation by suppressing two key enzymes in inflammatory pathways (cyclooxygenase and lipoxygenase); in addition, it reduces the transcription of pro-inflammatory cytokines by inhibiting the NF-κB pathway [32]. A study on aging rats aged 12-13 months (corresponding to 60-65 years in humans) found that Ashwagandha supplementation significantly reduced C-reactive protein (CRP, a key biomarker of systemic inflammation) and simultaneously decreased levels of the pro-inflammatory cytokines IL-6 and TNF-α, effectively alleviating the adverse effects of inflammation on aging muscles [33].

Table 3 Effects of Ashwagandha on Serum CRP, IL-6, and TNF-α [33]

Treatment Groups	CRP (mg/L)	Interleukin-6 (pg/mL)	TNF-α (pg/mL)
Young Control	5.83±0.65	7.82±0.56	2.63±0.42
Aging Control	19.83±1.30[a]	20.58±0.83[a]	10.27±0.40[a]
WSE	8.33±0.71[c]	12.54±1.31[c]	3.86±0.67[c]
WSE+Protein	5.83±1.07[c]	8.83±0.78[c]	3.16±0.42[c]
Protein	7.66±1.05[c]	11.13±1.28[c]	4.04±0.45[c]
RT	15.5±1.23	13.60±1.63[b]	5.75±0.23[c]
Reference	8.16±0.70[c]	9.29±1.12[c]	2.86±0.50[c]

Ashwagandha also demonstrates efficacy in regulating specific inflammatory conditions. In a randomized controlled trial involving patients with knee pain, Ashwagandha significantly reduced Visual Analog Scale (VAS) pain scores and improved physical function in patients [34]. In terms of relieving skin inflammation, a study on the HaCaT human keratinocyte cell line showed that the aqueous extract of Ashwagandha roots can reduce the expression of pro-inflammatory cytokines such as IL-8, IL-6, TNF-α, IL-1β, and IL-12 by inhibiting the nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, while increasing the levels of

anti-inflammatory cytokines [35]. In the field of neuroinflammation, preclinical studies have confirmed that an aqueous extract of Ashwagandha (Withania somnifera) counteracts lipopolysaccharide-induced systemic neuroinflammation. Specifically, it suppresses reactive gliosis, reduces the production of inflammatory cytokines such as TNF-α, IL-1β, and IL-6, and downregulates the expression of enzymes associated with nitro-oxidative stress. Furthermore, Ashwagandha has demonstrated regulatory effects in interventions for pulmonary, renal, and hepatic inflammation [36]

3.5 Metabolic Modulatory Effects

Metabolic Syndrome (MetS) is a multifactorial pathological condition characterized by core features including insulin resistance, dyslipidemia, obesity, and elevated blood glucose. It significantly increases an individual's risk of developing type 2 diabetes and cardiovascular diseases. Insulin resistance is a core feature of MetS, which prevents insulin from effectively promoting the transport of glucose into target cells. According to the diagnostic criteria established by the International Diabetes Federation (IDF) in 2006, a diagnosis of MetS is confirmed when an individual meets two or more of the specified conditions [37]. Statistics indicate that approximately one-quarter of the global population is affected by MetS, making it a major public health concern [38].

Table 4. Diagnostic Criteria for Metabolic Syndrome (IDF, 2006) [37]

Diagnostic Criteria	Diagnostic Components
Waist Circumference	> 80 cm in women, > 90 cm in men
Fasting Blood Glucose	>100mg/dL(5.6mmol/L)
Blood Pressure	Systolic blood pressure > 135 mmHg or diastolic blood pressure > 85 mmHg, or a history of hypertension treatment
High-Density Lipoprotein(HDL)	Men< 40 mg/dL , women< 50 mg/dL , or a history of using HDL-raising medications
Triglycerides(TG)	> 150 mg/dL, or a history of dyslipidemia

Diagnostic Criteria	Diagnostic Components
	treatment

3.5.1 Improves insulin sensitivity and regulate blood glucose levels

Metabolic syndrome is a key predictive factor for the development of type 2 diabetes, and patients with metabolic syndrome have a 5-fold higher risk of developing diabetes than the general population [38].

When the human body fails to secrete insulin normally or use insulin effectively, the glucose level in the body rises. A long-term state of hyperglycemia can trigger a variety of complications, including diabetic retinopathy, diabetic nephropathy, and diabetic ketoacidosis [39].

Active constituents in Ashwagandha (e.g., withanolide A, withanolide B, and withanoside IV) demonstrate the ability to modulate glucose metabolism and delay the progression of diabetes. In a 30-day clinical study, patients with type 2 diabetes took ashwagandha in capsule form daily at a dose of 3 grams per day. The results showed that daily ashwagandha intake produced a potassium-sparing diuretic effect similar to that of antidiabetic drugs; meanwhile, a significant increase was observed in their urinary sodium content and urine output, and a significant decrease in the levels of serum cholesterol, triglycerides, low-density lipoprotein (LDL) cholesterol, and very low-density lipoprotein (VLDL) cholesterol [40].

Research indicates that Ashwagandha exerts its hypoglycemic effects through multiple mechanisms [41]:
1)Protection of Pancreatic β-Cells: It mitigates inflammatory responses in β-cells and reduces cytokine-induced damage, thereby preserving normal insulin secretion.
2)Modulation of Insulin Signaling Pathways: Ashwagandha upregulates the expression of key genes involved in insulin signaling, including the insulin receptor (INSR), insulin receptor substrate 1 (IRS1), and glucose transporter 4 (SLC2A4), consequently enhancing systemic insulin sensitivity.
3)Inhibition of Key Carbohydrate-Metabolizing Enzymes: It suppresses the activity of α-glucosidase and α-amylase, thereby delaying the breakdown and absorption of carbohydrates.
4)Amelioration of Oxidative Stress and Inflammation: reduces lipid peroxidation (LPO), enhances the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione (GSH), and decreases the levels of pro-inflammatory cytokines including TNF-α and IL-6.

3.5.2 Lipid-Level Modulation

Lipid metabolism disorder is a condition characterized by abnormalities in the synthesis, breakdown, and transport of lipids in the human body. This disorder can lead to elevated levels of cholesterol and triglycerides in the blood, subsequently triggering serious health issues such as cardiovascular disease, diabetes, and metabolic syndrome. Common lipid metabolism disorders include hyperlipidemia, atherosclerosis, and obesity. Among these, hyperlipidemia (HLP) refers to abnormally elevated lipid levels in the blood, significantly increasing an individual's susceptibility to atherosclerosis. In the pathogenesis of atherosclerosis, lipid plaques gradually accumulate within the arterial walls, not only causing luminal narrowing and reduced blood flow but also substantially increasing the risk of heart attack and ischemic stroke [42].

In an in vitro experiment, Ashwagandha root extract demonstrated a significant cholesterol-lowering effect. When the extract—administered via two distinct preparations (distilled water and cow urine immersion)—was added to a pooled serum sample from hyperlipidemic patients with a baseline total cholesterol of 189 mg/dL, it reduced total cholesterol levels to 112 mg/dL and 110 mg/dL, respectively.

The study further indicated that the cholesterol-lowering effect of Ashwagandha root extract is associated with its active constituent, withanolide A, and its dietary fiber content. The former promotes the fecal excretion of cholesterol and bile acids, while the latter reduces the intestinal absorption of exogenous cholesterol and inhibits the activity of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, thereby suppressing cholesterol synthesis. This establishes a triple regulatory mechanism targeting "excretion, absorption, and synthesis" [43].

3.5.3 Modulation of Body Weight and Fat Metabolism

Excessive fat accumulation in adipose tissue leads to adipocyte hypertrophy and hyperplasia. Inadequate blood supply then creates a hypoxic environment, which stimulates the production of pro-inflammatory mediators—such as TNF-α, IL-6, PAI-1, leptin, and resistin—from the adipose tissue. This cascade triggers inflammatory responses, reduces antioxidant capacity, and contributes to the development of metabolic diseases including diabetes, dyslipidemia, cardiovascular disease, and hypertension.

Ashwagandha helps prevent obesity by improving mitochondrial function in both adipose tissue and skeletal muscle, thereby enhancing energy expenditure. In one study, rats fed a high-fat diet were administered Ashwagandha extract at varying concentrations (0.25%, 0.5%, 0.70%) for 10 weeks. Results demonstrated that the extract increased energy expenditure through the modulation of mitochondrial activity, while concurrently suppressing hepatic lipid accumulation, reducing serum lipid levels, and decreasing body weight. Furthermore, ashwagandha was found to elevate oxygen consumption in adipocytes and skeletal muscle, and upregulate the expression of mitochondrial uncoupling protein 1 (UCP-1), thereby promoting the browning of subcutaneous white adipose tissue [44].

Ashwagandha further contributes to obesity prevention by activating key signaling pathways—specifically AMP-activated protein kinase (AMPK) and extracellular signal-regulated kinase 1/2/mitogen-activated protein kinase (ERK/MAPK)—thereby enhancing energy expenditure. In a related study, oral administration of withanolide A to rats for 7 days demonstrated that this active component elevates energy expenditure in both brown adipose tissue (BAT) and white adipose tissue (WAT) through the activation of AMPK, MAPK, and ERK/MAPK pathways, consequently mitigating obesity [45].

Table 5. Research on the Association Between Ashwagandha and Improvement of Metabolic Syndrome

Study Subject	Intervention Protocol	Primary Outcomes	Reference(s)
Streptozotocin (STZ)-Induced Type 2 Diabetic Rats	Administration of 200/400 mg/kg Ashwagandha Root Extract for 5 Weeks	Reduces Blood Glucose and Glycated Hemoglobin (HbA1c) Levels, and Improves Insulin Sensitivity	[46]
Prediabetic Dogs	Combination Therapy of Ashwagandha (100 mg/kg body weight) and Terminalia chebula for 30 Days	Improvement in diabetic biomarkers (including 8.2% reduction in random blood glucose and 8.1% decrease in fasting blood glucose) with concomitant reduction in oxidative stress.	[47]
Fructose-fed insulin-resistant rats	Administration of 62.5 mg/g Ashwagandha Root Extract for 8 Weeks	Reduces blood glucose and HOMA-IR (Homeostatic Model Assessment of Insulin Resistance), suppresses the NF-κB pathway, decreases inflammatory factors such as TNF-α and IL-6, and	[48]

Study Subject	Intervention Protocol	Primary Outcomes	Reference(s)
		improves insulin sensitivity.	
Hyperlipidemic Rats	Administration of 400 mg/kg Ashwagandha Root Aqueous Extract for 5 Weeks	Reduces TG, LDL, VLDL, and lactate dehydrogenase (LDH) levels, increases HDL, diminishes lipid peroxidation (LPO), and enhances antioxidant enzyme activity.	[49]
Hyperlipidemic Rats	75% Ethanolic Extract of Ashwagandha Root, Administered as Needed	Inhibits HMG-CoA reductase activity, and reduces LDL, TG, and cholesterol levels.	[50]
Hyperlipidemic Rabbits	Administration of 0.5 g/kg Ashwagandha Root Extract for 90 Days	Improves lipid profile while elevating antioxidant levels (e.g., glutathione, GSH).	[51]
High-Fat Diet-Induced Obese Rats	Administration of 0.25%/0.5%/0.70% Ashwagandha Methanolic Extract for 10 Weeks	Enhances oxygen consumption in adipocytes/skeletal muscle, upregulates mitochondrial UCP-1 expression, promotes subcutaneous fat browning, and reduces body weight and hepatic lipid accumulation.	[44]
High-Fat Diet (HFD)-Induced Obese Rats	Administration of 0.75/1.5 mg Withanolide A for 7 Days	Activates AMPK and ERK/MAPK pathways, promotes the transformation of white adipose tissue (WAT) into brown adipose tissue (BAT), upregulates UCP-1 expression, and enhances energy expenditure.	[45]
3T3 Cell Line (Adipocyte Model)	Treatment with 25 µM Ashwagandha Root-Derived Withanolides (1-6)	Upregulates lipolytic genes (HSL, ATGL), downregulates lipogenic genes (SREBP1), suppresses lipid droplet	[52]

Study Subject	Intervention Protocol	Primary Outcomes	Reference(s)
	for 24 Hours	expansion, and promotes lipid metabolism.	

3.6 Neuroprotection and Cognitive Enhancement

The nervous system serves as the central regulatory network for physiological functions and cognitive processes in the human body. It is responsible not only for nerve signal transmission, motor control, and sensory processing but also supports cognitive functions such as learning, memory, attention, and decision-making. However, under the influence of aging, chronic stress accumulation, or pathological factors (e.g., protein misfolding, oxidative stress), the nervous system becomes vulnerable to damage. This manifests clinically as a high incidence of neurodegenerative diseases (such as Alzheimer's disease and Parkinson's disease) and cognitive decline (including memory impairment and attention deficits). According to data from the World Health Organization (WHO), approximately 57 million people worldwide live with dementia, of which Alzheimer's disease (AD) accounts for 60%–70%, with 10 million new cases reported annually. Additionally, over 8.5 million people are affected by Parkinson's disease (PD). The mortality and disability burden caused by neurodegenerative diseases represents a significant global public health challenge, which is projected to intensify in the coming decades due to population aging.

Alzheimer's disease (AD) is a neurodegenerative disorder primarily leading to progressive dementia, characterized by memory loss and progressive neurocognitive dysfunction [53]. In the brain tissue of AD patients, abnormal deposition of β-amyloid protein (Aβ) occurs. The fibrillar form of this protein exhibits confirmed neurotoxicity, which can induce free radical generation and impair neuronal glucose transport, ultimately resulting in neuronal damage and even cell death. In vitro studies on human neuronal cells have demonstrated that Ashwagandha effectively neutralizes the toxicity of β-amyloid protein. Further in vivo experiments in rats revealed that oral administration of Ashwagandha extract significantly improved cognitive function in test subjects. The underlying mechanisms include inhibition of amyloid-beta 42 (Aβ42) generation, reduction in pro-inflammatory cytokines (TNF-α, IL-1β, IL-6, MCP-1), nitric oxide, and lipid peroxidation levels, accompanied by significant downregulation of β-secretase and γ-secretase activity—key enzymes responsible for the formation of insoluble neurotoxic aggregates of β-amyloid [54].

In patients with Parkinson's disease (PD), degeneration of dopaminergic neurons in the nigrostriatal system is observed, leading to an imbalance between the inhibitory effects of dopamine and the excitatory effects of acetylcholine and glutamate. Research indicates that an ethanolic root extract of Ashwagandha can mitigate the pathological progression of PD. In a study utilized an MB-PQ-induced mouse model of PD, co-treatment with the Ashwagandha extract resulted in downregulated expression of inducible nitric oxide synthase (iNOS) in the mouse brain. Concurrently, it significantly ameliorated the MB-PQ-induced pro-apoptotic state by downregulating the pro-apoptotic protein Bax and upregulating the anti-apoptotic protein Bcl-2. Furthermore, the extract reduced the expression of glial fibrillary acidic protein (GFAP), a marker of astrocyte activation and neuroinflammation, ultimately conferring protection to nigrostriatal dopaminergic neurons [55].

Cognitive function and memory form the foundation for daily functioning, learning, and decision-making processes. Impairments in these domains can severely impact an individual's quality of life and adversely affect overall mental health. In a randomized, double-blind, placebo-controlled trial involving adults with mild cognitive impairment (MCI), the group receiving Ashwagandha intervention demonstrated significant improvements in immediate memory, general memory, working memory, and visuospatial processing abilities after 30 and 60 days compared to the placebo group. Furthermore, their performance assessed using the Wechsler Memory Scale-III (WMS-III) showed superior outcomes relative to the placebo controls [56].

3.7 Other Potential Effects

3.7.1 Reproductive Health

Reproductive health is a crucial component of overall human well-being. According to the World Health Organization (WHO), it encompasses not only the absence of disease or dysfunction in the reproductive system but also includes optimal fertility, balanced hormonal levels, and psychological stability related to reproductive matters. Globally, reproductive health issues represent a significant public health challenge. Datas indicate that approximately 15% of couples of reproductive age (15–49 years) are affected by infertility worldwide [57]. Studies have found that male factors (e.g., oligoasthenospermia, low testosterone levels) account for 20%–50% of cases, female factors (e.g., polycystic ovary syndrome (PCOS), ovulation disorders, inadequate endometrial receptivity) account for 40%, and the cause remains unknown in approximately 25% of cases [58].

Ashwagandha exerts significant effects on the male reproductive system and fertility,

primarily through oxidative and non-oxidative mechanisms [59]. The oxidative mechanisms involve the modulation of antioxidant activity, as well as the regulation of antioxidant enzymes themselves and their essential cofactors. The non-oxidative mechanisms operate mainly via two pathways: first, by influencing the hypothalamic-pituitary-gonadal (HPG) axis; and second, through its anti-stress effects mediated by the hypothalamic-pituitary-adrenal (HPA) axis. In a triple-blind, randomized controlled trial comparing Ashwagandha, pentoxifylline(PTX), and a placebo group, results demonstrated that the group receiving Ashwagandha exhibited an average increase of 12.5% in sperm count, along with 21% improvements in both sperm motility and morphology. Consequently, Ashwagandha is considered a viable alternative to pentoxifylline [60].

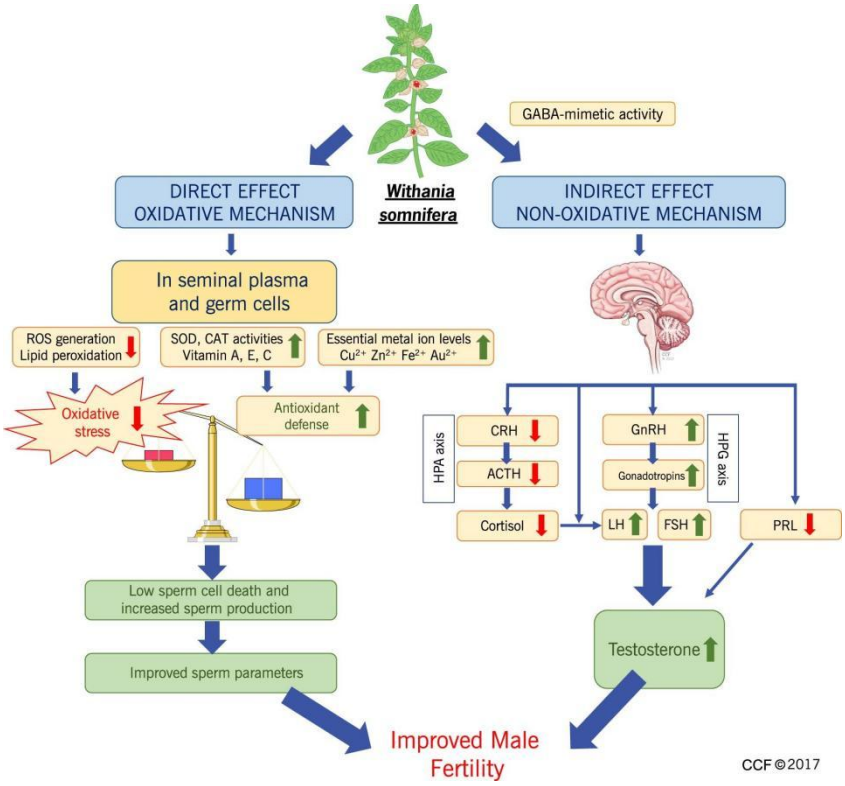

图 6 南非醉茄对男性生殖系统的作用机制[60]

Figure 6. Mechanisms of Action of Ashwagandha on the Male Reproductive System [60]

Ashwagandha contributes to the improvement of female fertility primarily by promoting hormonal balance. In a clinical study comparing an Ashwagandha emulsion with clomiphene citrate (the standard medication for anovulation), Ashwagandha was found to increase levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which are crucial for folliculogenesis. The study also observed that Ashwagandha effectively increased follicular volume and endometrial thickness [61]. Furthermore, a randomized placebo-controlled study, using the Female Sexual Function Index (FSFI) and the Female Sexual Distress Scale

(FSDS) as assessment tools, demonstrated that women supplemented with Ashwagandha root extract showed significant improvements in sexual function, including desire, arousal, lubrication, orgasm, and satisfaction [62].

3.7.2 Skin Health

The skin, being the largest organ of the human body, performs essential functions such as providing a physical barrier, immune defense, sensory transmission, and thermoregulation. Its condition directly reflects overall physiological balance and external health. Exposure to environmental pollutants, ultraviolet (UV) radiation, psychological stress, aging, and poor lifestyle habits can lead to varying degrees of skin health issues. UV radiation increases the risk of long-term damage including photoaging, photoimmunosuppression, and photocarcinogenesis. Adverse effects primarily manifest as sunburn, photosensitive dermatitis, hyperpigmentation, photoaging, as well as precancerous lesions and skin cancer.

A randomized, double-blind, placebo-controlled trial involving subjects aged 18–60 with photoaged facial skin (Fitzpatrick skin types III–VI) demonstrated that topical application of Ashwagandha significantly improved wrinkles, pore appearance, skin hydration, brightness/complexion, and pigmentation. Furthermore, it produced more substantial improvements in transepidermal water loss (decreased by 15.12%), skin hydration (increased by 20.66%), and skin elasticity (R2 ratio increased by 16.34%). The Ashwagandha group also showed a greater enhancement in quality of life, as measured by the SF-12 questionnaire, with a comparable incidence of adverse events—mostly mild local reactions—between the two groups. These results indicate that topical Ashwagandha emulsion can effectively ameliorate signs of photoaging and improve the quality of life in affected individuals [63].

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4. Global Regulatory Status of Ashwagandha

4.1 China

In China, Ashwagandha has not yet been approved as a food ingredient or dietary supplement component. It is currently permitted for sale only through cross-border e-commerce channels, requiring a Certificate of Free Sale from the country of origin and a safety assessment report. Furthermore, according to reports, as of July 2025, the General Administration of Customs has included Ashwagandha in the list of ingredients subject to enhanced supervision. Cross-border products containing Ashwagandha and similar components must submit a Certificate of Origin and technical specifications; otherwise, they may face rejection or return shipment.

4.2 USA

Unlike pharmaceutical drugs, Ashwagandha products are marketed as dietary supplements and do not require pre-market approval from the FDA. Manufacturers bear the responsibility for ensuring their Ashwagandha products are safe, accurately labeled, and compliant with FDA regulations, including Good Manufacturing Practices (GMPs). The FDA monitors the marketplace and may take action—such as issuing warning letters or removing products from the market—if public health concerns arise regarding safety, or if products make unauthorized drug claims [1]. Currently, commercially available Ashwagandha supplements are primarily used for conditions such as stress and anxiety relief, sleep support, male infertility, and athletic performance.

4.3 Canada

Ashwagandha leaf/root extract is classified as a natural health product ingredient in Canada and may be used in pharmaceuticals, non-pharmaceuticals, and for homeopathic treatments. In non-pharmaceutical applications, it primarily functions as a skin conditioning agent (emollient/moisturizer) [2]. On March 25, 2025, Shoden®, an Ashwagandha extract manufactured by Arjuna Natural, was approved by Health Canada as a Natural Health Product (NHP). Shoden®, which contains over 35% withanolide glycosides, is authorized for use in tablets, capsules, and powders, and may be incorporated into soft drinks, energy shots, and other sports nutrition products [3].

4.4 India

In India, the use and regulation of ashwagandha are governed by the Food Safety and Standards (Health Supplements, Nutraceuticals, Foods for Special Dietary Use, Foods for Special Medical Purpose, Functional Foods and Novel Foods) Regulations, 2016, as amended in 2021, under the oversight of the Food Safety and Standards Authority of India (FSSAI). Ashwagandha is explicitly listed in the "Schedule of Plant or Herbal Ingredients", with the following conditions: Only the root may be used; It must be appropriately processed or formulated as an extract;Light processing (e.g., cleaning and drying) is not permitted for standalone or combined use. Dosage limits are as follows: Adults: 3–6 g/day (powder form) or 0.5–1 g/day (extract form); Children aged 5–16: half the adult dose; Children aged 2–5: one-quarter the adult dose, only under the supervision of a physician or certified nutritionist [4].
In 2021, the Indian government issued guidance explicitly discouraging the use of ashwagandha leaves, further reinforcing restrictions on plant parts [5].

4.5 South Korea

In South Korea, ashwagandha extract is regulated under the Health Functional Food Act by the Ministry of Food and Drug Safety (MFDS). It must undergo Health Functional Food Ingredient Certification to be legally used. On November 19, 2024, Sensoril®—an ashwagandha extract produced by Natreon—received certification as a health functional food ingredient for its ability to alleviate tension caused by stress [6].

4.6 Poland

On February 7, 2020, the Polish Committee on Dietary Supplements, under the National Sanitary Inspection Act, issued Resolution No. 7/2020, providing an official

opinion on the use of ashwagandha in dietary supplements.

The resolution stipulates:Maximum daily intake of powdered root: 3g; Maximum daily intake of withanolides: 10 mg.

Table 1 Conditions and Limits for Ashwagandha Use [7]

Restriction Category	Specific Requirement	Applicable Scope
Raw Material Form	Powdered root only	All dietary supplements containing this ingredient
Daily Dosage	Single or daily dose ≤ 3 g	Adults only; excluded: pregnant and lactating women
Active Ingredient Content	Withanolide content in recommended daily dose ≤ 10 mg	All dietary supplements containing this ingredient
Product Labeling Requirement	Marketed products must include quantitative specifications clearly stating total withanolide content per recommended daily dose	All dietary supplements containing this ingredient

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5 Annex

Quality Standards and Testing Specifications for Water-soluble Debittered Ashwagandha Extract

1.Purpose
To ensure effective quality control of Water-soluble Debittered Ashwagandha Extract
2. Scope
Applicable to quality testing of Water-soluble Debittered Ashwagandha Extract
3. Quality Standards

No.	Item		Standard
1	Assay	Total Withanolides	≥5.0%
		Withaferin A	≤0.1%
2	Appearance		Off-white to light yellow powder
3	Taste		600 mg in 150 mL water: no bitterness,with a mild herbal aroma600mg
4	Solubility		1 g in 100 mL water yields a transparent suspension1g
5	Size(80mesh)		≥95%
6	Loss on Drying		≤5.0%

No.	Item		Standard
7	Ash Content	≤8.0%	
8	pH	4.5-7.0	
9	Bulk Density	≥0.25 g/mL	
	Tapped Density	≥0.30 g/mL	
10	Solvent Residual	Conforms with USP/ICH	
11	Heavy Metals	Arsenic	≤1.0 ppm
		Cadmium	≤0.3 ppm
		Lead	≤1.0 ppm
		Mercury	≤0.1 ppm
	Microbial Limits	Total Aerobic Count	≤10,000 CFU/g
		Total Yeast & Mold	≤1,000 CFU/g
		Escherichia coli	Negative
		Salmonella spp.	Negative / 25 g
		Staphylococcus spp.	Negative

4. Testing Specifications

4.1 Assay

Chromatographic Conditions

Column: C18, 4.6 mm × 250 mm

Mobile Phase A: 0.14 g potassium dihydrogen phosphate + 0.5 mL phosphoric acid in 900 mL water, diluted to 1000 mL

Mobile Phase B: Acetonitrile

Column Temperature: 27°C

Detection Wavelength: 257 nm

Flow Rate: 1.5 mL/min

Injection Volume: 20 μL

Gradient Program：

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	95	5
18	55	45
25	20	80
28	20	80
30	95	5
40	95	5

4.1.1 Test Method

Sample Solution: Accurately weigh 500 mg into a 10-mL volumetric flask. Add 5 mL of 50% isopropanol, sonicate for 10 min, and dilute to volume with 50% isopropanol.

Reference Standard Solution: Accurately weigh 10 mg of withaferin A reference standard into a 10-mL flask. Add 5 mL of 50% isopropanol, sonicate for 10 min, and dilute to volume.

Inject blank (50% isopropanol), reference standard, and sample solutions into HPLC. Record chromatograms. Calculate total withanolides using external standard method, integrating all peaks with retention time >5 min.

4.1.2 Calculation Formulas:

$$\text{Total Withanolides (\%)} = \frac{A_{供}}{m_{供}} \times \frac{m_{对} \times P \times V_{供}}{\overline{A_{对}} \times V_{对} \times (1 - W)} \times 100\%$$

$$\text{Withaferin A (\%)} = \frac{A_{供A}}{m_{供}} \times \frac{m_{对} \times P \times V_{供}}{\overline{A_{对}} \times V_{对} \times (1 - W)} \times 100\%$$

$A_{供}$– Total peak area of the test sample；

$A_{供A}$– Peak area of withaferin A in the test sample；

$A_{对}$– Peak area of the withaferin A reference standard solution；

$m_{供}$– Weight of the test sample, mg；

$V_{供}$– Volume of the test sample solution, ml；

$m_{对}$–Weight of the withaferin A reference standard, mg；

$V_{对}$– Volume of the reference standard solution, ml；

P – Withaferin A reference standard content, %；

W– loss on drying,%；

4.2 Appearance

Place an appropriate amount of sample on clean white paper, spread evenly, and inspect visually.

4.3 Taste

Dissolve 600 mg of sample in 150 mL water with shaking. The solution should be non-bitter and exhibit a mild herbal aroma.

4.4 Water solubility

Dissolve 1 g of sample in 100 mL water with shaking. The solution should be a transparent suspension.

4.4 Particle Size

Weigh 10 g of sample into an 80-mesh sieve with a tightly fitted receiver and lid. Rotate horizontally for 5 minutes, periodically tapping vertically. Weigh the material passing through the sieve and calculate the percentage.

4.6 Loss on Drying

Accurately weigh 1.0 g of sample into a pre-weighed, tared dish. Spread evenly and dry in an oven at 105 ± 2°C to constant weight. Calculate percentage loss on drying as:

Loss on Drying (%) ：

$$\frac{\text{The mass of the weighing bottle and the sample - The mass of the weighing bottle and the sample after drying}}{\text{The weight of the sample}} \times 100\%$$

4.7 Ash Content

Accurately weigh 2.0 g of sample into a pre-weighed crucible. Char gently without flaming, then incinerate in a muffle furnace at 675 ± 25°C to constant weight. Calculate total ash as:

Total Ash (%) = $(W_a / W_s) \times 100$

Where W_a = ash weight, W_s = sample weight.

4.8 pH

Dissolve 1 g of sample in 100 mL water with shaking. Measure pH using a calibrated pH meter.

4.9 Bulk Density

4.9.1 Bulk Density

Weigh approximately 20 g of powder accurately. Gently pour into a graduated glass cylinder. Level the top without compacting. Record the apparent volume (V_1) to the nearest graduation. Calculate bulk density as:

$$\text{Bulk Density} = M / V_1$$

$$\text{Computational formula:} \quad = \frac{M}{V_1} \times 100\%$$

In the formula: M - Mass of the powder sample to be tested, g; V_1 - Apparent volume of the powder sample to be tested, ml

4.9.2 Tapped Density

The operation was carried out according to the bulk density measurement method. The graduated cylinder filled with loose powder was compacted 50 times in the vertical direction at 3mm. The apparent volume was recorded with the nearest scale line, and the compaction density was calculated according to the following formula.

$$\text{Computational formula:} \quad = \frac{M}{V_1} \times 100\%$$

4.10 Solvents of Residual

Do the testing according to the method of USP<565>

4.11 Heavy Metals

Tested semi-annually by a third-party laboratory using USP <730> methods.

4.12 Microbial Limits

4.12.1 Microbial Enumeration

4.12.1.1 Sample Preparation:

Weigh 10 g into a conical flask, add 100 mL of sterile pH 7.0 sodium chloride–peptone buffer, and mix to obtain a 1:10 dilution.

4.12.1.2 Aerobic Count:

Take 1 ml of the test solution, place it in a sterile petri dish with a diameter of 90 mm, pour 15-20 ml of melted tryptic soy agar medium at a temperature not exceeding 45°C, and prepare two parallel petri dishes. Separately, take the diluent used in the test and operate in the same manner as a negative control. Incubate at 30-35°C for 2-3 days.

4.12.1.3 Yeast & Mold Count:

Take 1 mL of the test solution, place it in a sterile petri dish with a diameter of 90 mm, then pour 15-20 mL of melted Sabouraud Dextrose Agar (SDA) medium at a temperature not exceeding 45°C, and prepare two parallel petri dishes. Separately, take the diluent used in the test and perform the same operation to serve as the negative control. Incubate at 20-25°C for 5-7 days.

4.12.2 Pathogen Testing

4.12.2.1 Escherichia coli

Take 10 mL of the test solution, add it to 100 mL of tryptic soy broth (TSB) medium, and mix well. Prepare another sample in the same manner, add less than 100 CFU of Escherichia coli to it, and use it as the positive control. Separately, take 10 mL of the pH 7.0 sterile sodium chloride-peptone buffer solution used in the test, add it to 100 mL of tryptic soy broth (TSB) medium, and use it as the negative control. Place all the above samples in a constant temperature environment at 30-35°C for incubation for 18-24 hours.

Take 1 mL of the enriched culture, transfer it to 100 mL of MacConkey broth medium, and incubate it at a constant temperature of 42-44°C for 24-48 hours.

Dip a loop into the culture from the MacConkey broth medium respectively, streak it onto the MacConkey agar medium plates, and incubate them at a constant temperature of 30-35°C for 18-72 hours.

4.12.2.2 Salmonella spp.

Weigh 10 g of the test sample, inoculate it into 100 ml of tryptic soy broth (TSB) medium, and mix well. Prepare another sample in the same manner, and add less than 100 CFU of Salmonella bacterial suspension to one of them as the positive control. Separately, take 100 ml of tryptic soy broth (TSB) medium as the negative control.

Incubate all of them at a constant temperature of 30-35°C for 18-24 hours.

Take 0.1 mL of the enriched culture respectively, transfer it to 10 mL of Rappaport-Vassiliadis (RV) Salmonella enrichment broth medium, and incubate it at a constant temperature of 30-35°C for 18-24 hours.

Dip a inoculating loop into the cultures from the Rappaport-Vassiliadis (RV) Salmonella enrichment broth medium respectively, streak the cultures onto Xylose Lysine Desoxycholate (XLD) agar medium plates, and incubate the plates at a constant temperature of 30-35°C for 24-48 hours.

If suspected colonies grow on the Xylose Lysine Desoxycholate (XLD) agar medium used for the test sample inspection, pick the suspected colonies with an inoculating needle, and perform slant and deep stab inoculation on the slant of triple sugar iron (TSI) agar medium, then incubate for 24-48 hours.

4.12.2.3 Staphylococcus spp.

Weigh 10 mL of the test solution, transfer it to 100 mL of tryptic soy broth (TSB) medium, mix well, and use this as the test sample for inspection. Prepare another sample following the same method, add less than 100 CFU of Staphylococcus aureus bacterial suspension to it, and use this as the positive control (which can be tested periodically). Separately, take 10 mL of the pH 7.0 sterile sodium chloride-peptone buffer solution used in the test, transfer it to 100 mL of tryptic soy broth (TSB) medium, and use this as the negative control. Place all the above samples in a constant temperature environment at 30-35°C for incubation for 18-24 hours.

Then, use an inoculating loop to take the enriched cultures respectively, streak them onto mannitol salt agar (MSA) medium plates, and incubate the plates at a constant temperature of 30-35°C for 24-48 hours.

6 Disclaimer

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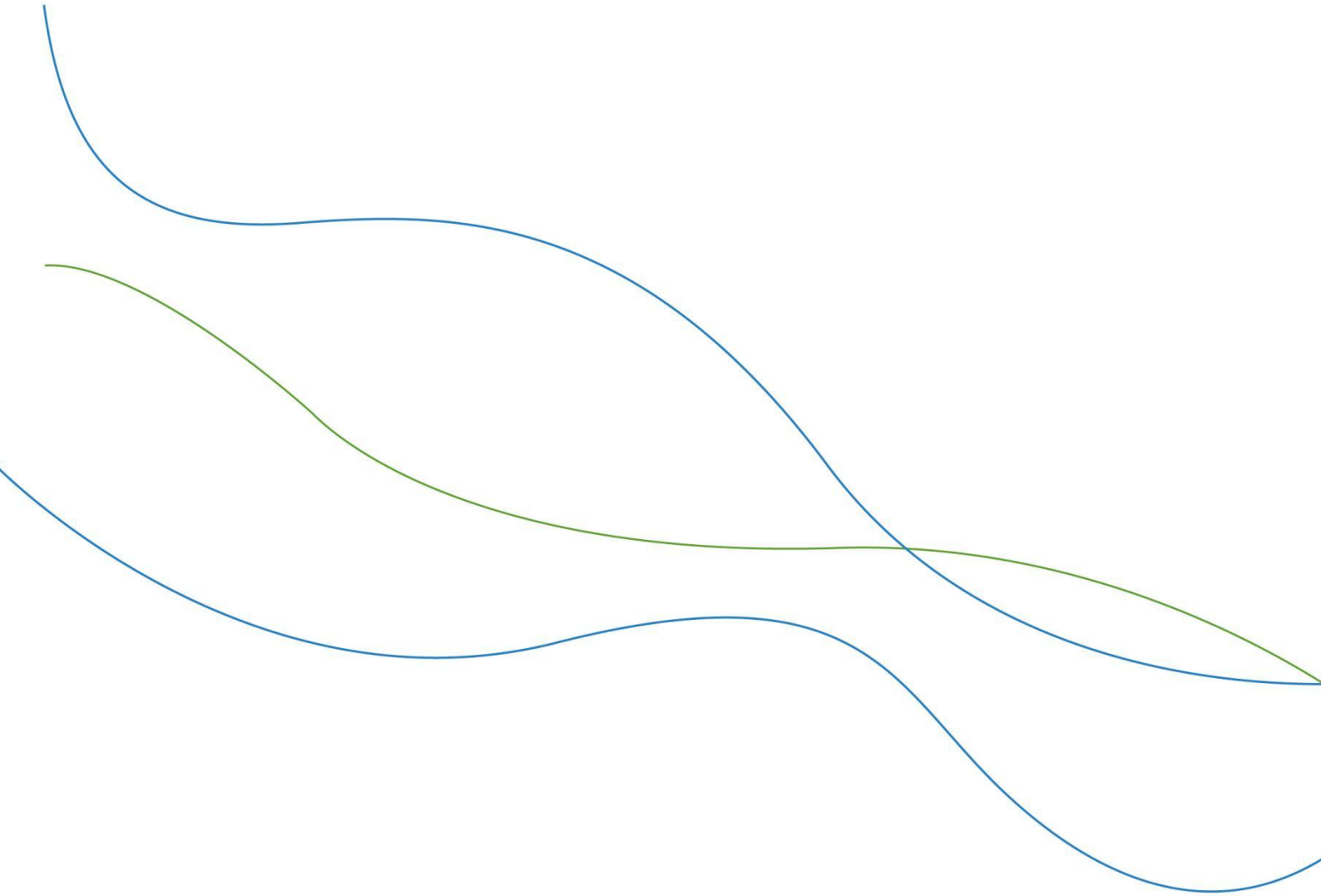

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