Ashwa.30™

ADVANCING ASHWAGANDHA TO A NEW LEVEL

Natural Remedies combines proprietary technology and a commitment to innovation to yield a low dose, high efficacy extract

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Used in traditional Indian medicine since 1000 BC, ashwagandha root is a powerful *Rasayana*—an adaptogen that helps the body navigate physical, biological and chemical stressors.[1] This evergreen shrub, which is cultivated in tropical and subtropical areas of Asia, Africa and Europe, is officially known as *Withania somnifera* (L.) Dunal; the term *ashwagandha* comes from the Sanskrit, combining 'ashwa' (horse) and 'gandha' (smell) to describe the wet horse smell of roots of the plant.

The U.S. National Institutes of Health (NIH) notes that ashwagandha is rich in phytochemicals, including steroidal lactones that are known as withanolides, as well as other phytoactives and alkaloid compounds.[2] It further states that results of clinical trials suggest ashwagandha extracts may help reduce stress and anxiety, help with sleep, and appear to be safe and well tolerated for up to three months of use.

Whatever its historic use, it's clear that consumers are turning to ashwagandha—and other natural stress-relieving solutions—in record numbers. In fact, the global market for stress relief supplements is expected to grow from US$605 million in 2024 to more than US$1.03 billion by 2034, surging at a CAGR (compound annual growth rate) of 5.5%.[3] And what is leading the way, according to Future Market Insights' (FMI) principal consultant Nandini Roy Choudhury? In the report, she states: "Ashwagandha is expected to dominate the stress relief supplement market. This herb has been in the thick of things lately owing to its various uses in Ayurvedic treatments. Ashwagandha is quite a significant herb, with an ancient historical presence, coupled with the capability of delivering adaptogenic attributes helpful in managing stress."

In fact, new findings from FMI suggest the global market for ashwagandha extract will top US$1.78 billion by 2035, rising at a CAGR of 8.2% from its current US$815.7 million share.[4] In the latest report, Choudhury observes: "More consumers are turning to ashwagandha-based supplements to combat stress and anxiety, enhance cognitive function, and boost their immune systems." However, she also cites a need to ensure the consistency and purity of ashwagandha extracts, suggesting a whitespace market opportunity.

EXPLORING THE ASHWAGANDHA SPACE

Known internationally as a leader in proprietary ingredients, Natural Remedies seeks to deliver truly unique offerings that meet a market need. Dr. Suresh Lakshmikanthan, PhD, chief business officer, notes the company uses a Stage Gate process to improve product outcomes and prevent risk by adding gates, or areas for review, throughout a project plan. "By utilizing multiple review mechanisms and cross functional teams, we ensure that we can optimize the outcome and business investment," he says. As an example, the innovation and bioresource teams may screen botanicals not only for the scientific potential but also the identification and sourcing considerations. Starting from market trend data and financial viability and moving through the full product development process can take three to four years but ensures there is truly a whitespace opportunity that Natural Remedies can fill.

As noted above, the ashwagandha market has been growing incrementally and there doesn't appear to be any slowdown projected. "Ashwagandha has gained significant popularity due to increasing consumer demand for natural health supplements, rising awareness of stress management, and the growing adoption of Ayurvedic medicine," according to a new Data Bridge report, which added, "The market is primarily driven by expanding applications in nutraceuticals, increasing investments in organic farming, and advancements in extraction technology".[5]

"Traditionally, everyone in India knows the benefits of ashwagandha because of the Ayurvedic science and historical usage," Dr. Lakshmikanthan says. "But in the last

"OUR APPROACH ENCOMPASSES FULL SPECTRUM ACTIVITY, ENSURING THE DELIVERY OF AN INGREDIENT WITH ENHANCED EFFICACY, ROBUST SAFETY PROFILE AND WITH SIGNIFICANT IMPACT."

ADAPTOGENS AND STRESS

ENHANCING ENDURANCE & SUSTAINED PERFORMANCE WITH ASHWA.30™

Adaptogens are natural substances that help the body become more resilient to stress by reducing its sensitivity to stressful stimuli. This enhanced adaptability allows the body to maintain a stronger, more balanced physiological state for longer periods. Instead of quickly succumbing to exhaustion, the body enters a more advanced adaptive phase known as heterostasis—a heightened state of balance. This response is known as the State of Nonspecific Resistance (SNSR), a core mechanism by which adaptogens exert their protective effects.

Ashwa.30™, developed using the B.O.T.™ (Bio-Optimized Technology), contains an ATP-active fraction and withanolides that support optimal adaptogenic activity. This formulation is designed to help the body deal with stress, sustain energy and improve performance under stress.

MAST Protocol and Mental Stress

The Maastricht Acute Stress Test (MAST) is a standardized laboratory procedure designed to reliably induce stress in a controlled setting. It is a combination of cold pressor test (hand immersion in ice water) & psychological stressor (Unpredictable mental arithmetic tasks under time pressure and observation). This stress induces an increase in cortisol.

The Bruce Protocol and Physical Stress

The Bruce Protocol is a standardized treadmill test used to evaluate cardiovascular endurance and the body's ability to manage increasing physical stress. It involves progressive increases in treadmill speed and incline every 3 minutes, challenging the heart and lungs as the workload intensifies.

Physical stress from such protocols can negatively impact ATP production, reducing overall performance and endurance. This decline is primarily due to:

- Inhibition of mitochondrial respiration and glycolysis, which impairs aerobic energy production.
- Activation of anaerobic respiration, leading to lactic acid buildup, reduced ATP output, and early fatigue.

Energy Pathways and Performance

- Aerobic Glycolysis:
  - 1 glucose molecule → ~34 ATP
  - Supports long-term, sustained performance
- Anaerobic Glycolysis:
  - 1 glucose molecule → ~4 ATP
  - Leads to fatigue and reduced performance

Role of Ashwa.30™ in Stress Resilience & Performance Optimization

Adaptogens like Ashwa.30™ help in modulating cortisol levels and supporting physical performance; these effects have been demonstrated in clinical studies and further validated by preclinical research.

Ashwa.30™ helps counteract the negative effects of the stress by:

- Inducing the State of Nonspecific Resistance (SNSR)
  - Modulating cortisol levels – preventing harmful overproduction
- Enhancing aerobic glycolysis, supporting efficient ATP production evident from lower lactic acid build up
- Reducing lactic acid accumulation, delaying fatigue
- Promoting sustained, long-term performance even under physical stress

Ashwa.30™ enhances cellular energy dynamics and strengthens stress resilience, providing a powerful, natural solution for improving endurance and accelerating recovery in both physically and mentally demanding contexts.

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"ASHWAGANDHA HAS GAINED SIGNIFICANT POPULARITY DUE TO INCREASING CONSUMER DEMAND FOR NATURAL HEALTH SUPPLEMENTS, RISING AWARENESS OF STRESS MANAGEMENT, AND THE GROWING ADOPTION OF AYURVEDIC MEDICINE."

few years, we're seeing a move to combine traditional knowledge with modern science." He references the investment in clinical validation, extraction techniques, safety profiling and much more, which is all designed to deliver ever-more efficacious natural solutions to consumers.

Because of the Natural Remedies's deep expertise and understanding of phytochemistry as well as traditional use, Natural Remedies strategically chose to focus exclusively on ashwagandha root. The decision aligns with Ayurvedic medicine's emphasis on the root for internal applications, deliberately excluding the aerial parts. Further, the company's approach extended beyond mere phytochemical analysis, aiming to develop a product that was not only phytochemically optimized but also well-defined in terms of its pharmacodynamics and mechanism of action.

B.O.T.™ OPTIMIZES BIOACTIVITY

Natural Remedies leverages its proprietary, Bioactive Optimization Technology (B.O.T.)™ This state-of-the-art technology combines the principles of chemistry and biological sciences to deliver a bioactive optimized extract, characterized by high bioactivity at low dose. "Through this integrated strategy, we are able to unlock the potential of bioactive compounds, enabling us to identify the target phytocompounds and implementation of a tailored extraction process to yield the optimized ingredient," says Dr. Deepak Mundkinajaddu, PhD, head of R&D at Natural Remedies. "Our approach encompasses full spectrum activity, ensuring the delivery of an ingredient with enhanced efficacy, robust safety profile and with significant impact."

Natural Remedies follows a two phase process in this endeavour. The initial discovery phase involves designing multiple fractions, guided by a new product need. These fractions may encompass diverse characteristics, such as polar or nonpolar properties or hydro actives components, etc. Approximately 50 to 60 leads are generated, subsequently subject to rigorous screening, utilizing mathematical models, predictive screening methodologies and biological studies, to generate a comprehensive dataset. This

dataset comprises information pertaining to the effects of individual fractions and their combinations, predictive effects of these combinations, and the interactive effects of specific phytoactive fractions. The subsequent optimization phase focuses on refining the identified lead. This is achieved through an iterative process involving the integration of phytochemical and biological investigations, culminating in a bioactive-optimized extract that exhibits high and targeted bioactivity at a reduced dose. This process has yielded Ashwa.30™, a standardized ashwagandha ingredient demonstrating clinical efficacy at a dose of only 30 mg.

ASHWA.30™: VALIDATING THE IMPACT

To establish the efficacy of Ashwa.30™, five pre-clinical trials were conducted, evaluating its impact (at a dose equivalent to 30 mg in humans) on key health parameters, including anxiety, stress, sleep, and endurance. These trials yielded positive results across all areas. In the assessment of Ashwa.30's anxiolytic effect using the light/dark box test, mice administered the test dose for three days exhibited a 32% increase in time spent in the light chamber, indicative of reduced anxiety. The forced swim test revealed that a single dose of Ashwa.30™ more than halved corticosterone levels compared to the stressed control group. In the Diazepam-induced sleep model, a three-day administration of the test dose resulted in a 40% decrease in sleep latency time and a 35% increase in sleep duration.

Furthermore, two pre-clinical trials investigated physical endurance. In the first, a swim endurance test, mice receiving a single dose of Ashwa.30™ prior to the assessment of swim duration until exhaustion demonstrated a 1.51-fold increase in swim time compared to control animals, indicating improved endurance capacity. Similarly, in a rodent treadmill model, a three-day treatment with Ashwa.30™ resulted in a 2.05-fold increase in distance travelled on the treadmill compared to the control group, corroborating its positive effect on physical endurance and energy.

In addition to the efficacy studies, the safety profile of this innovative ingredient was examined through three pre-clinical investigations. The results of an in vitro AMES study indicated that Ashwa.30™, at concentrations up to 5000 mcg/plate, exhibited no mutagenic effects. An in vivo acute oral toxicity study determined the LD50 cut-off value of Ashwa.30™ in rats to be >5000 mg/kg bw, effectively demonstrating a lack of acute toxicity. Finally, treatment with Ashwa.30™ resulted in a significant decrease in serum AST and ALT levels compared to the carbon tetrachloride-treated control group, demonstrating a significant hepatoprotective effect.

To elucidate the probable mechanism of action of Ashwa.30™, the Natural Remedies team employed *in silico* systems biology testing. Dr. Mundkinajaddu elaborates: "Once a product is developed, it remains crucial to elucidate the roles of the various phytoactives that contribute to its efficacy. Given that the extract contains a multitude of these compounds, *In silico* testing provides an invaluable platform for understanding their actions on cellular and energy pathways. This approach facilitates a comprehensive understanding of the phytoactives, overcoming the cumbersome process of isolating each compound and investigating its mechanisms individually using *in vitro* and *in vivo* systems." The presence of both withanolides and other

phytoactive fractions in ashwagandha prompted the team to investigate the specific contributions of these fractions to various biological pathways.

The initial phase of this investigation focused on predictive transcriptomics, specifically predicting gene expression profiles in relation to chemical structures. This analysis centered on two core areas of claims—stress and endurance—examining the biological processes and bioinformatics tools relevant to the associated pathways. The targeted pathways included cellular response to stress, cortisol biosynthesis and secretion, mitochondrial biogenesis, and organelle biogenesis and maintenance. Ultimately, this work yielded a map of the genes modulated by each phytocompound, revealing that new ATP Active fractions, distinct from withanolides, significantly modulated the energy pathway, whereas withanolides predominantly modulated the cellular response to the stress pathway.

The second phase of this work explored the molecular docking of the Hypothalamic-Pituitary-Adrenal (HPA) axis pathway receptors with Ashwa.30™ phytoconstituents. Using experimental methodology, the team examined the effect of Ashwa.30™ on the genes of the HPA axis pathway, which plays a crucial role in regulating the body's response to stress. Consistent with the findings of the first phase, the new ATP Active fractions significantly modulated the energy pathways, while withanolides modulated the stress pathways, including receptor binding within the HPA axis.

An in vivo study further validated the pro-endurance effects of the Ashwa.30™ fraction, demonstrating an impressive 80% improvement in treadmill test performance. Collectively, these findings offered robust scientific support for establishing a specification as the ATP-active fraction. Having gained a thorough understanding of the correct composition, standardization, and effective dosages, Natural Remedies was ready to proceed with clinical trials for Ashwa.30™.

ASHWA.30™ CLINICAL STUDIES

The impact of Ashwa.30™ has been investigated in several clinical studies. The first study is a randomized, prospective, double-blind, placebo-controlled trial explored its effect on cardiovascular endurance and fatigue in 63 healthy adults. Participants were divided, with 42 receiving a daily 30 mg dose of Ashwa.30™ and 21 receiving a placebo over 56 days. The primary outcome was the change in cardiovascular endurance, quantified by VO2 max using the Modified Bruce Protocol Treadmill Test (MBPTT) at the beginning and end of the study. Notably, the Ashwa.30™ group exhibited an 8-fold increase in VO2 max compared to the placebo group at the study's conclusion. This finding contrasts with other popular ashwagandha products, which have typically shown only a 3% to 4% improvement over placebo. According to Dr. Mundkinajaddu, "Ashwa.30™ significantly increases VO2 max levels in healthy individuals and helps them perform intense exercises for a longer period."

A subsequent clinical study focused on Ashwa.30™'s influence on stress levels. In this seven-day trial, 40 healthy adults were equally randomized to receive either Ashwa.30™ (30 mg/d) or a placebo. Salivary cortisol levels were measured at

baseline, on day 7 before treatment, and on day 7 following the Maastricht Acute Stress Test (MAST). The MAST, a validated experimental stressor, involves participants immersing their hand in cold water while completing arithmetic tasks for alternating intervals. The results indicated that participants in the Ashwa.30™ group experienced almost 40% reduction in cortisol levels compared to the placebo group by day 7.

Further, a third clinical trial into the stress-relieving properties of Ashwa.30™ is currently underway. This ongoing randomized, double-blind, placebo-controlled trial is examining the anti-stress and anti-fatigue effects of Ashwa.30™ in 60 generally healthy adults aged 18 to 65 experiencing high stress. Over a 28-day period, subjects will receive either Ashwa.30™ or a placebo, and changes in self-reported stress, mood, and fatigue will be assessed using the Depression Anxiety Stress Scales (DASS-21), the Chalder Fatigue Scale (CFS), and Single-item Mood Rating. The findings from this study are anticipated by late 2025.

ASHWA.30™: PHYTOCHEMISTRY AND STANDARDIZATION

Ashwa.30™ is a 100% root extract, characterized by two primary standardized fractions:

- **Withanolide Fraction:** Standardized to > 15% w/w by HPLC (USP analytical method).
- **ATP Active Fraction:** Standardized to a >15% w/w using a validated proprietary analytical method. This fraction contains a complex array of secondary metabolites and primary biomolecules.

Rationale for Dual Standardization and Synergistic Effects:

- **Withanolides** These steroidal lactones significantly modulated the hypothalamic-pituitary-adrenal (HPA) axis/cellular response to stress, contributing significantly to antistress activity.
- **ATP Active fraction** contributes significantly to the endurance/anti-fatigue effects through mechanisms involving enhanced mitochondrial bioenergetics, and mitigation of exercise induced oxidative stress and oxidative phosphorylation.

SUSTAINABILITY IN THE SUPPLY CHAIN

In a new report on the ashwagandha market,[7] Future Market Insights' Nandini Roy Choudhury observes: "As consumers become more environmentally conscious, the demand for sustainably sourced and ethically produced products is on the rise. Brands that prioritize responsible sourcing of ashwagandha and adhere to environmentally friendly production practices are gaining a competitive edge in the market."

Natural Remedies has a network of contracted farmers with whom it has buy guarantee programs. The company works collaboratively with the farmers around sustainable practices to optimally grow its ashwagandha. It intends to expand its network of farmers to meet market needs, ensuring that it can meet demand while focusing on sustainability across the supply chain. This means, for example, paying optimal prices and giving back to farming communities.

Further, Natural Remedies' *Withania somnifera* extract is certified 100% Fair Trade under ECOCERT's Fair for Life program. The certification goes beyond fair pricing and focuses on acting responsibly throughout the supply chain. Certified companies have implemented good economic, social and environmental practices, which may include long-term contracts with fixed pricing and volumes, and establishing a partnership among supply chain members.

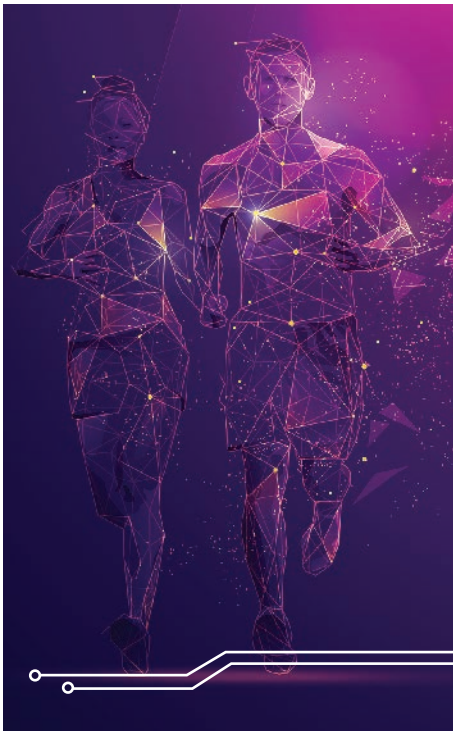
The synergistic interaction between the standardized withanolide fraction and the ATP active fraction may benefit both antistress and endurance effects.

As it relates to analytical rigor, the withanolide fraction can be tested using the established US Pharmacopeial (USP) method; the ATP active fractions can be tested using a validated proprietary analytical method. This demonstrates commitment to precise and consistent quantification of these complex bioactive matrix.

ASHWA.30™: BREAKTHROUGH FOR FORMULATORS

Awareness of Ayurveda and other traditional medicine systems, as well as of the adaptogen category is boosting interest in ashwagandha as a safe and effective natural remedy. At the same time, market watchers observe: "Innovation in product development is paramount, particularly the introduction of novel delivery formats and formulations. This includes the development of convenient and palatable options like gummies, capsules, and powders, catering to diverse consumer preferences. The focus is on creating unique blends combining ashwagandha with other adaptogens and complementary ingredients, such as turmeric, L-Theanine, Rhodiola, magnesium, to address specific health concerns and maximize efficacy."[6]

However, there are often challenges in developing multi-ingredient blends, including daily dosing requirements and constraints on product format. Ashwa.30™ is the solution for formulators seeking to deliver synergistic health effects around key areas such as stress management and physical endurance.



As Dr. Lakshmikanthan notes, "Because the ingredient is standardized to a 30 mg dose, it is groundbreaking for companies to incorporate into complex proprietary formulations." Because the health benefits of Ashwa.30™ are so foundational to general wellness, it affords companies the chance to expand into multiple categories, ranging from sports nutrition and cognitive/mental health to men's and women's health.

Natural Remedies' formulation team is further equipped to collaborate with companies around the globe to optimize the use of Ashwa.30™ in unique formulations. The ingredient's natural herbal taste can be easily masked as needed and it can be made dispersible in liquids. This versatility allows for its incorporation not only into traditional capsules or tablets but also into gummies, beverages, and food products. As noted previously, the low 30 mg dose facilitates its integration into more complex formulations while maintaining efficacy.

Furthermore, Natural Remedies is committed to being fully compliant with regulatory requirements around the globe. For example, in the United States, Natural Remedies planned to have Ashwa 30™ self-affirmed GRAS status with FDA notification, and WADA certified for its compliance with anti-doping standards. It is in communication with European and Canadian authorities, as well as the Australian TGA and into Korea and Japan. As Dr. Lakshmikanthan states: "We will ensure that in every region our product is approved and qualified for sale." ■

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