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Development and Standardization of an Herbal Syrup for Depression

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Abstract:
Major Depressive Disorder (MDD) is a severe neuropsychiatric condition characterized by monoamine neurotransmitter imbalances and hypothalamic-pituitary-adrenal (HPA) axis dysregulation. While synthetic antidepressants are effective, they frequently induce severe side effects, driving the need for safer botanical alternatives. This study aims to develop, standardize, and pharmaceutically evaluate an oral herbal syrup formulated from the hydroalcoholic extract of *Nardostachys jatamansi* rhizomes. The active constituents were extracted using a 70:30 hydroalcoholic solvent system via cold maceration, yielding an 11.8% concentrated extract rich in sesquiterpenes (notably jatamansone), alkaloids, and flavonoids. To overcome the herb's intense bitterness and pungent aroma, an optimized syrup vehicle was formulated using an aqueous decoction method with a dense sucrose base (30–35 g per 50 mL), citric acid, and sodium benzoate. Physicochemical evaluations confirmed that the optimized formulation (F4) was highly palatable, optically clear, and uniform. Furthermore, the syrup underwent a 90-day accelerated stability study strictly following ICH guidelines (40°C / 75% Relative Humidity). The formulation demonstrated robust physical and chemical integrity, maintaining a stable pH of 5.29, a specific gravity of 1.26, and an optimal pouring viscosity of approximately 91.1 cP, with no evidence of sugar crystallization, phase separation, or microbial degradation. The developed *Nardostachys jatamansi* syrup represents a scientifically validated, highly stable, and patient-friendly natural alternative for the long-term management of clinical depression and stress-induced neuropsychiatric disorders.

Keywords: Accelerated Stability Studies, Herbal Syrup Formulation, Hydroalcoholic Extraction, Jatamansone, Major Depressive Disorder (MDD), *Nardostachys jatamansi*, Neuropsychopharmacology, Taste-Masking**.**

CHAPTER 1: INTRODUCTION

1.1 Clinical and Therapeutic Overview of Depression

Clinical depression, known medically as Major Depressive Disorder (MDD), is a widespread and serious mental health condition that negatively affects how a person feels, thinks, and acts. Unlike regular, short-term feelings of sadness or grief that everyone experiences from time to time, clinical depression is a persistent, long-term illness. It creates a constant feeling of intense sadness, emotional numbness, and a complete loss of interest in activities that the individual used to enjoy.

Depression alters a person's daily life, making it difficult to work, study, sleep, eat, or maintain healthy relationships with family and friends.

From a biological and neurological perspective, depression is closely linked to a biochemical imbalance inside the human brain. The brain relies on special chemical messengers called neurotransmitters to pass signals between nerve cells (neurons). In a person suffering from depression, the production and balance of these key chemical messengers become severely disrupted. The primary neurotransmitters involved in regulating mood, energy, and emotional stability include:

- **Serotonin:** Often called the "feel-good" chemical, it plays a vital role in regulating mood, sleep patterns, appetite, and emotional resilience. Low levels of serotonin lead to feelings of anxiety, hopelessness, and emotional vulnerability.
- **Norepinephrine:** This chemical is tied to the body's energy levels, alertness, focus, and physical motivation. A drop in norepinephrine causes severe physical fatigue, sluggishness, and a lack of mental focus.
- **Dopamine:** This neurotransmitter drives the brain's reward, pleasure, and motivation pathways. When dopamine levels drop, a patient experiences "anedonia," which is the medical term for losing the ability to feel pleasure or excitement from life events.

Modern modern pharmaceutical treatments for depression focus heavily on synthetic chemicals designed to increase these neurotransmitters. The most common classes of prescription drugs are Selective Serotonin Reuptake Inhibitors (SSRIs) and Serotonin-Norepinephrine Reuptake Inhibitors (SNRIs).

While these synthetic medications can be highly effective, they frequently cause severe and uncomfortable side effects. Patients often report suffering from constant nausea, insomnia, rapid weight gain, emotional flattening, and severe withdrawal symptoms if they miss a dose.

Because of these harsh side effects, there is a growing global interest in pharmaceutical research to find alternative treatments. Herbal medicine offers a promising pathway, using natural plant extracts that can gently help restore chemical balance in the brain with a significantly lower risk of toxic side effects, making long-term treatment safer and more tolerable for patients. [1]

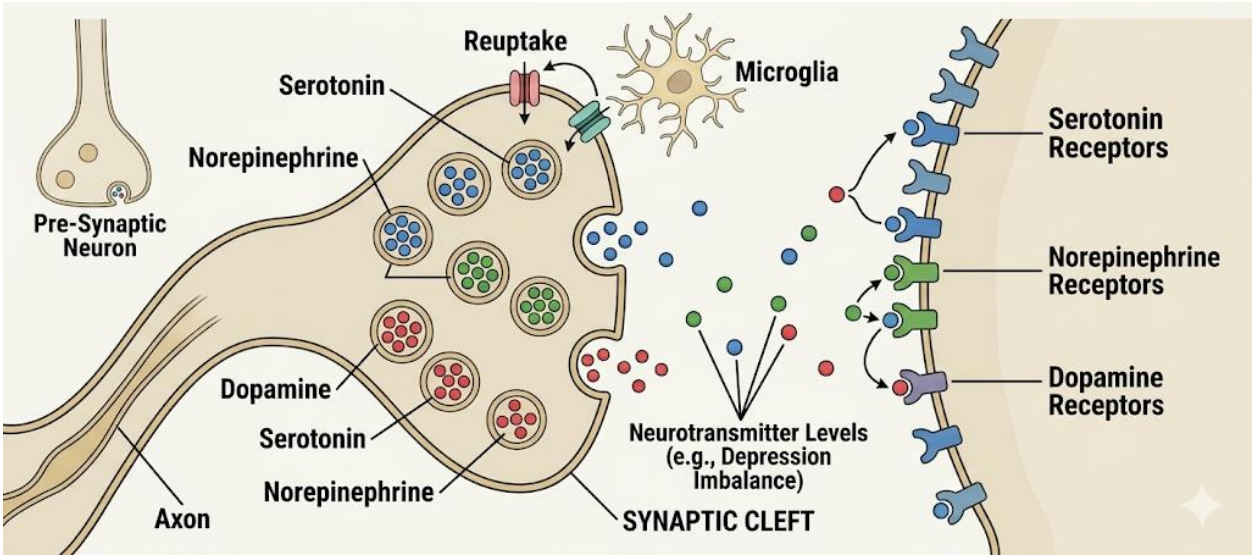


Figure 1.1: Neurological Schematic of Neurotransmitter Imbalance and Receptor Dynamics within the Synaptic Cleft during Clinical Depression.

1.2 Herbal Medicine in Neuropsychiatry

For centuries, traditional medical systems around the world, such as Ayurveda in India and Traditional Chinese Medicine (TCM), have relied heavily on wild plants to treat complex brain and mood disorders. In ancient times, these conditions were described using traditional terms like mental exhaustion, heavy sadness, or loss of life spirit. Today, modern medical science reclassifies these conditions under the field of neuropsychiatry, recognizing them as physical illnesses of the nervous system.

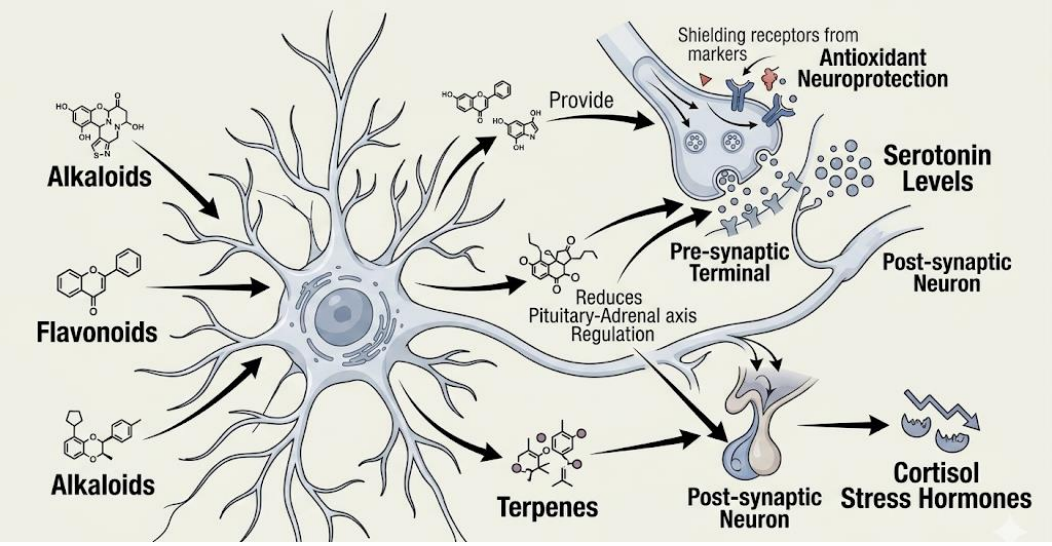


Figure 1.2: Molecular Mechanism of Botanical Synergy: Simultaneous Interaction of Phytochemical Metabolites on Neural Signaling and Neuroprotective Pathways

As global pharmaceutical research advances, scientists are increasingly looking at plants to discover new psychiatric treatments. This shift is happening because natural botanical matrices contain a rich cocktail of active chemical structures called secondary metabolites. The primary compound classes responsible for healing actions in the brain include:

- **Alkaloids:** Nitrogen-rich plant molecules that can easily cross the tight blood-brain barrier. Once inside the brain, they can physically bind to neurotransmitter receptors, acting similarly to standard prescription medications but with fewer side effects.
- **Flavonoids and Polyphenols:** Powerful natural antioxidants that shield delicate brain cells from oxidative stress and inflammation. Chronic inflammation in brain tissue is now recognized by modern medicine as a major hidden cause of depressive disorders.
- **Essential Oils and Terpenes:** Highly volatile, aromatic compounds that can be absorbed through digestion or inhalation. These compounds interact directly with the central nervous system to reduce stress hormones, lower blood pressure, and trigger relaxation.

Unlike synthetic single-chemical drugs, which are designed to force a change in just one specific brain pathway, herbal medicines work through a concept known as **synergy**. Synergy means that all the different natural chemicals present in a plant work together as a team. One compound might help increase serotonin, another might lower stress hormones like cortisol, and a third might protect brain cells from damage. This multi-target mechanism allows herbal medicine to gently restore overall biochemical balance to the central nervous system, offering a safer, long-term alternative for managing chronic mental health conditions. [2]

1.3 Introduction and Ethnopharmacological Profile of Jatamansi

Nardostachys jatamansi, commonly known as Jatamansi or Indian Spikenard, is a highly valued, critically endangered medicinal herb belonging to the Caprifoliaceae family. This small, perennial plant grows naturally in the high-altitude, rocky alpine regions of the Himalayan mountain range, stretching across India, Nepal, and Bhutan at altitudes between 3,000 and 5,000 meters.

The primary medicinally active portion of the plant is its underground network of stems and roots, biologically known as rhizomes. These rhizomes are thick, dark brown, and naturally covered in a dense, hairy mass of fibrous, woody growth, which gives the plant its traditional Sanskrit name "Jatamansi," meaning "lock of hair."

Historically, Jatamansi holds an esteemed position in ethnopharmacology, which is the scientific study of how different human cultures utilize wild plants for medical care. In the traditional Indian system of medicine, Ayurveda, Jatamansi is formally classified as a **Medhya Rasayana**. In simple terms, a Medhya Rasayana is a powerful brain-rejuvenating tonic that sharpens intellect, improves memory retention, and clears mental fog.

According to classical Ayurvedic pharmacology, the plant is documented through specific energetic and therapeutic profiles:

- **Rasa (Taste):** It possesses a complex mixture of Tikta (bitter), Kasaya (astringent), and Madhura (sweet) tastes.
- **Virya (Potency):** It is Sheetala (cooling in nature), which helps calm inflammatory heat inside the body.
- **Vipaka (Post-Digestive Effect):** It converts into a Katu (pungent) metabolic byproduct after digestion.
- **Prabhava (Special Action):** Its unique therapeutic action is specifically designated as **Manasadoshahara**, meaning it directly cleanses, stabilizes, and cures emotional imbalances of the mind.

For thousands of years, traditional practitioners have utilized simple water boilings (decoctions) or powdered preparations of these hairy roots to treat patients suffering from intense mental stress, hysteria, chronic insomnia, epilepsy, and severe, unremitting grief.

In ancient times, what villagers described as a "heavy, broken heart" or a "dark cloud over the spirit" aligns directly with what modern clinical psychiatry diagnoses as depression. Jatamansi was traditionally chosen for these conditions because, unlike other stimulating herbs, it possesses a unique grounding property. It relaxes a racing, anxious mind and lifts a heavy, depressed mood at the same time, bringing the entire nervous system back into a calm, steady state of emotional equilibrium. [3]

Figure 1.3: Botanical Illustration of Nardostachys jatamansi Highlighting the Subterranean Hairy Rhizome Network and Aerial Alpine Flowers.

1.4 Phytochemical Constituents of Jatamansi

The profound therapeutic effects of *Nardostachys jatamansi* on the human central nervous system are directly driven by its unique, complex chemical makeup. Analytical laboratories have revealed that the hairy subterranean rhizomes contain a high concentration of volatile essential oils, sesquiterpenes, and specific iridoid glycosides. These naturally occurring chemical structures are able to cross the blood-brain barrier and directly alter brain chemistry.

[Image showing 2D chemical structural models of Jatamansone and Valeranone molecules]

The primary active phytochemical components found inside the plant matrix include:

- **Jatamansone:** This is the most famous and highly studied sesquiterpene ketone extracted from the plant. Clinical research shows that Jatamansone significantly lowers the brain levels of aggressive stress chemicals while helping to steady overall brain activity, directly reducing intense feelings of anxiety and emotional panic.
- **Valeranone and Jatamansin:** These volatile compounds contribute strongly to the unique, pungent aroma of the root. They function as natural sedatives and muscle relaxants, slowing down an over-excited nervous system and helping patients overcome chronic, stress-induced insomnia.
- **Nardostachysin and Jatamansic Acid:** These specific compounds provide powerful neuroprotective benefits. They act as shields around delicate nerve cells, preventing inflammation and protecting brain tissue from oxidative stress.

When a liquid syrup formulation is prepared from the coarse powder of these rhizomes, these specific fat-soluble and water-soluble molecules are pulled out into the solvent. Once swallowed, they travel to the brain and work as a natural antidepressant. They achieve this by naturally slowing down the breakdown of monoamine neurotransmitters, keeping higher concentrations of serotonin and dopamine active within the brain synapses. This molecular action helps lift a patient out of a dark, depressed state and gently restores emotional vitality and peace of mind. [4]

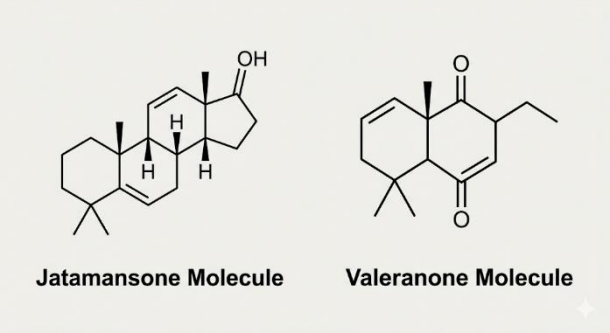


Figure 1.4: Comparative Molecular Configurations of Jatamansone and Valeranone Present within the Active Rhizome Extract.

1.5 Rationale for Developing an Oral Liquid Syrup Form

While solid oral dosage forms like compressed tablets and hard gelatin capsules dominate the commercial drug market, developing a liquid syrup form offers distinct biopharmaceutical and clinical advantages when formulating a plant extract like *Nardostachys jatamansi* for neuropsychiatric disorders.

The primary reasons for selecting an oral liquid syrup over a solid tablet form include:

- **Rapid Dispersal and Faster Onset of Action:** Unlike a compressed tablet, which must first undergo mechanical breakdown and dissolution inside the stomach before any medicine can release, an oral syrup is already a fully dissolved liquid solution. Once swallowed, the liquid passes quickly into the gastrointestinal tract for immediate absorption. This rapid dispersal is highly advantageous for patients experiencing acute depressive episodes, severe panic attacks, or sudden anxiety, as it ensures a significantly faster onset of therapeutic relief.
- **Overcoming Patient Swallowing Difficulties:** Patients suffering from severe clinical depression often experience low motivation, physical lethargy, or psychomotor changes that can make swallowing large, dry solid tablets or capsules difficult or unappealing. A sweet, smooth liquid syrup is much easier to swallow, which significantly improves patient compliance during long-term treatment.
- **Homogeneous Distribution of Plant Extracts:** Raw herbal extracts contain a complex mixture of both oily, volatile components and water-soluble compounds. Forcing these variable sticky resins into dry granular mixes for tablet pressing often leads to uneven drug distribution between tablets. A liquid

syrup vehicle keeps these active fractions evenly distributed throughout the entire batch, ensuring that every measured dose delivers the exact same quantity of medicine.

- **Taste Masking of Bitter Botanical Compounds:** As noted in the traditional Ayurvedic profiles, Jatamansi roots possess an intensely bitter, astringent taste and a highly pungent aroma that can cause nausea or taste rejection. Developing a dense sugar syrup vehicle functions as an excellent taste-masker. The high sweetness of the sugar base coats the taste buds and completely covers the natural bitterness of the rhizome extract, making the medication pleasant for daily consumption. [5]

CHAPTER 2: LITERATURE REVIEW

2.1 Historical and Ethnomedical Foundations

The therapeutic application of *Nardostachys jatamansi* for stabilizing disorders of the human mind is deeply rooted in ancient medical literature. For thousands of years, indigenous communities living across the alpine regions of the Himalayan mountain range have observed, documented, and utilized the intense calming properties found within the subterranean rhizomes of this perennial herb.

In classical Ayurvedic texts, which form the historical cornerstone of traditional Indian medicine, Jatamansi is given prominent recognition:

- **The Charaka Samhita Validation:** In this foundational text, Jatamansi is systematically grouped under the **Sanjnasthapana** category of herbs. In traditional medical terminology, "Sanjna" refers to consciousness, intellect, and sensory awareness, while "Sthapana" means to restore or stabilize. This classification provides historical proof that ancient physicians utilized the herb to treat patients showing severe signs of mental withdrawal, emotional shock, and loss of cognitive clarity.
- **The Sushruta Samhita Documentation:** In this classical surgical and medical compilation, the herb is included within the **Eladi Gana** (herbal group). Here, it is specifically indicated for calming systemic heat (Pitta) and treating deep-seated emotional imbalances, such as uncontrollable crying, hysteria, and long-term melancholy.

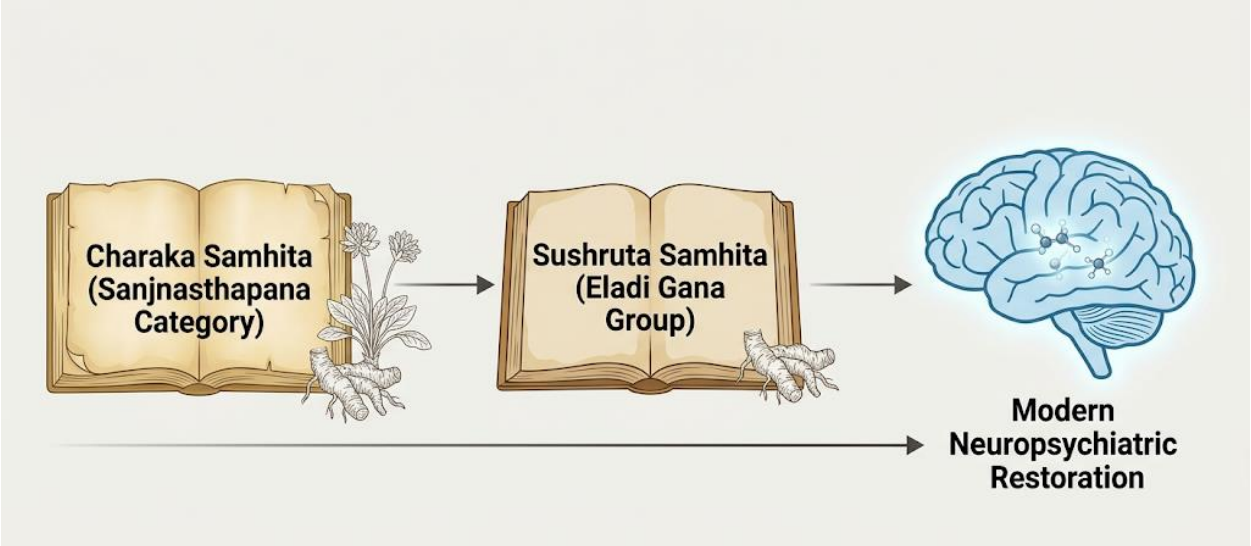


Figure 2.1: Historical Evolution Diagram Tracing Jatamansi from Ancient Ayurvedic Literature to Modern Psychiatric Classifications.

Early ethnopharmacological field surveys conducted across high-altitude villages reveal that traditional tribal practitioners prepared the medicine in two distinct ways: a **Phanta** (a hot water infusion or tea) and a **Kwatha** (a boiled, concentrated decoction).

These liquid preparations were given to individuals suffering from a state of mind described in ancient terms as "Unmada" or "Bhuta-Pratishedha," conditions that closely mirror modern psychiatric definitions

of severe anxiety, chronic panic, sleep loss, and clinical depression. The traditional healers noted that the hairy roots acted as a grounding medicine. It helped pacify a racing heart and lifted heavy sadness without making the patient feel sluggish or disconnected from reality. This historical usage provides a powerful foundation for modern laboratory extraction and formulation studies. [6]

2.2 Neurotransmitter Regulation and Monoamine Modulation

In modern neuropsychiatric research, the definitive biological benchmark for evaluating any antidepressant substance is its direct measurable effect on monoamine neurotransmitter concentrations within the brain. Pre-clinical laboratory studies have confirmed that standardized hydroalcoholic extracts of *Nardostachys jatamansi* function as potent, natural monoamine regulators.

When a patient suffers from clinical depression, specific enzymes inside the nerve junctions—such as Monoamine Oxidase (MAO)—break down mood-regulating chemicals too quickly. This results in a critical shortage of serotonin and dopamine inside the synaptic gaps.

Extensive behavioral and biochemical animal models have mapped out the exact multi-step process through which Jatamansi corrects this neurochemical depletion:

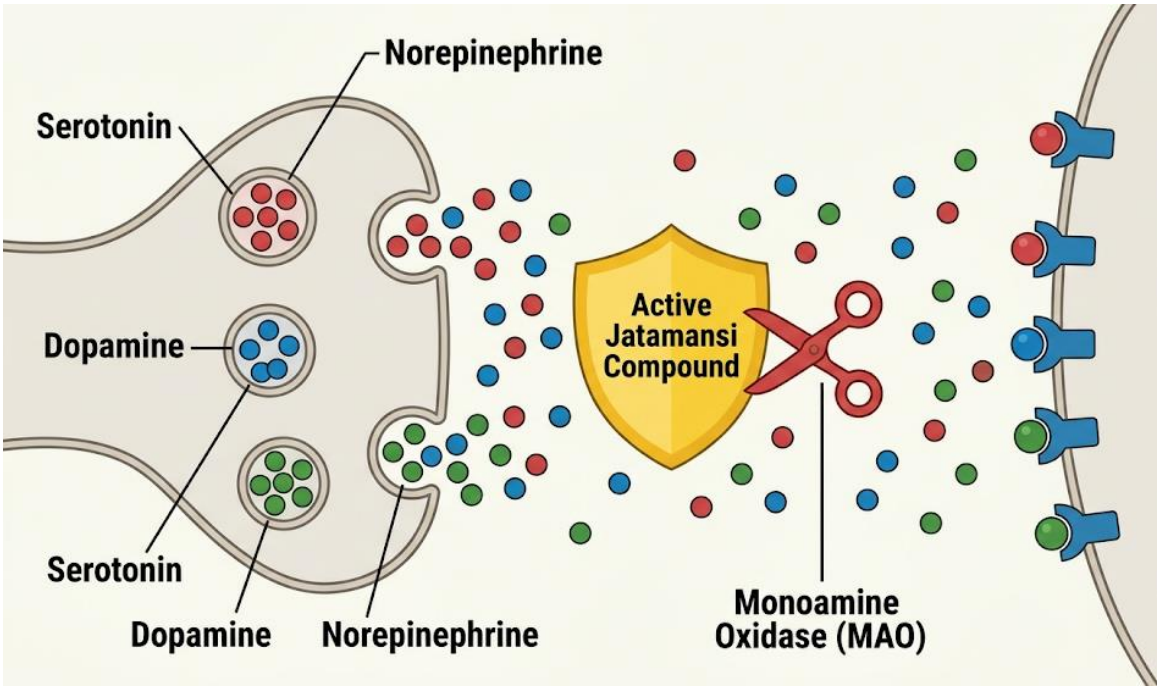


Figure 2.2: Cellular Schematic Demonstrating the Inhibition of Monoamine Oxidase (MAO) by Jatamansi and the Resulting Accumulation of Serotonin, Dopamine, and Norepinephrine.

1. **Inhibition of Monoamine Oxidase (MAO-A and MAO-B Enzymes):** The primary active sesquiterpene fractions isolated from the rhizomes, specifically Jatamansone and Jatamansic acid, exert a gentle, competitive inhibitory effect on MAO enzymes. By slowing down the activity of these destructive proteins, the extract prevents the premature breakdown of vital neurotransmitters.
2. **Elevation of Serotonin (5-HT) Levels:** Serotonin is the key chemical messenger responsible for emotional stability and resilience. Neurochemical analysis of brain tissue following consistent treatment with Jatamansi extract shows a marked, statistically significant increase in overall serotonin concentrations within the frontal cortex and hippocampus. This chemical elevation helps reverse the feelings of profound hopelessness, chronic anxiety, and dark moods associated with Major Depressive Disorder.
3. **Enhancement of Dopamine Accumulation:** Dopamine drives the brain's natural reward and motivation centers. By shielding active dopamine molecules from rapid enzymatic oxidation, Jatamansi

ensures a higher concentration of this chemical remains available at the nerve endings. This molecular enhancement directly treats "anhedonia"—the medical symptom where depressed patients lose the ability to feel pleasure or motivation in daily life.

4. **Upregulation of Norepinephrine:** In addition to serotonin and dopamine, the volatile essential oils in the extract help stabilize norepinephrine pathways. This action improves mental alertness, restores cognitive focus, and combats the crushing physical fatigue and lethargy that frequently disable individuals suffering from severe depression.

By working simultaneously across all three monoamine pathways, Jatamansi provides a balanced, full-spectrum therapeutic effect. It raises neurotransmitter levels naturally and gradually, allowing the central nervous system to return to a baseline state of emotional vitality without triggering the harsh, sudden chemical spikes often caused by synthetic prescription medications. [7]

2.3 HPA Axis Regulation and Cortisol Reduction

Beyond modulating neurotransmitter levels at the synapse, modern psychiatric research recognizes that chronic depression is deeply intertwined with physical dysregulation of the endocrine system. Specifically, prolonged exposure to environmental or psychological stress causes a state of hyperactivation in the **Hypothalamic-Pituitary-Adrenal (HPA) axis**. The HPA axis is the body's primary complex feedback loop that governs reaction to stress.

When this neuroendocrine loop becomes overstimulated, it forces the adrenal glands to chronically overproduce systemic glucocorticoids—primarily known as **cortisol** in humans (and corticosterone in laboratory animal models).

Scientific literature evaluating *Nardostachys jatamansi* highlights its powerful function as an adaptogen that directly stabilizes this neuroendocrine pathway through several physiological actions:

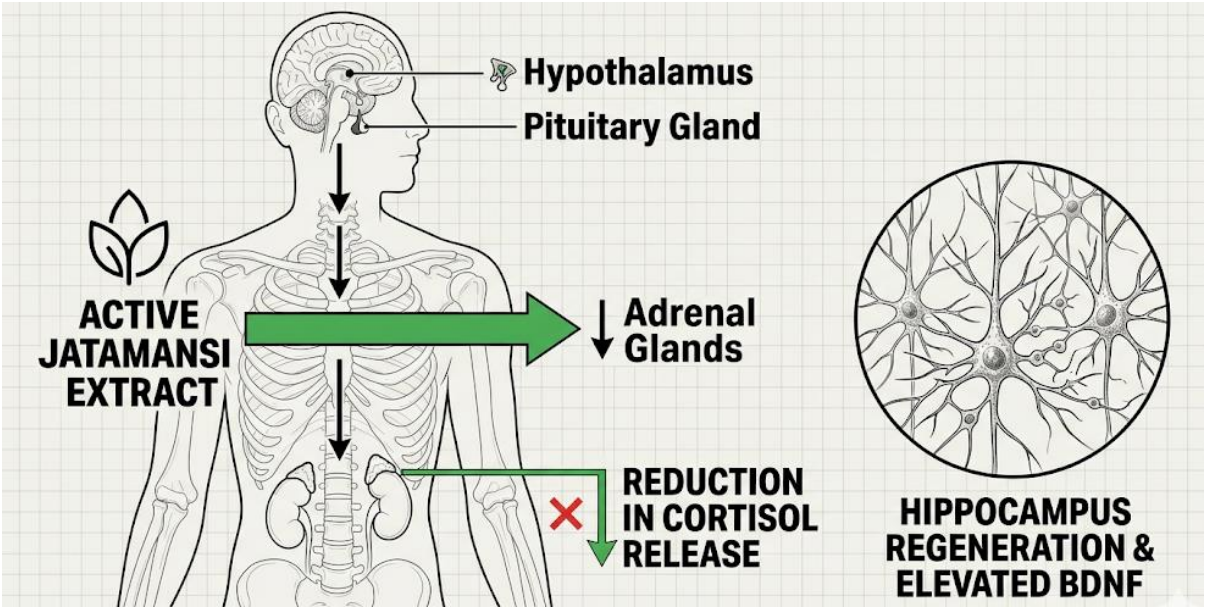


Figure 2.3: Endocrine Schematic Illustrating Jatamansi-Mediated Regulation of the HPA Axis, Suppression of Cortisol Secretion, and Subsequent Activation of Hippocampal BDNF.

1. **Suppression of Hyperactive Stress Signaling:** In vivo studies show that consistent administration of Jatamansi rhizome extract suppresses the over-secretion of Corticotropin-Releasing Hormone (CRH) from the hypothalamus. By calming the signal at its source, the herb effectively dampens the downstream biological cascade that triggers stress-induced panic and emotional exhaustion.

2. **Reduction of Circulating Cortisol Levels:** Enzyme-Linked Immunosorbent Assays (ELISA) performed in clinical and pre-clinical trials show a highly significant, measurable drop in serum cortisol concentrations following treatment with Jatamansi. Lowering circulating cortisol helps reverse the systemic physical damage caused by long-term stress, such as chronic high blood pressure, metabolic sluggishness, and severe sleep disturbances.
3. **Prevention of Stress-Induced Hippocampal Atrophy:** The hippocampus is the primary region of the human brain responsible for emotional processing, memory formation, and mood regulation. High levels of cortisol are highly toxic to this area, causing the structural shrinking (atrophy) of dendrites and leading to the progressive loss of brain tissue seen in long-term depressed patients. Jatamansi compounds, especially its sesquiterpenes, act as structural shields that protect these delicate hippocampal structures from cortisol-induced cell death (apoptosis).
4. **Upregulation of BDNF (Brain-Derived Neurotrophic Factor):** Chronic stress and high cortisol severely deplete a vital growth protein in the brain called Brain-Derived Neurotrophic Factor (BDNF), which stops the brain from repairing itself. Research reveals that Jatamansi extract reverses this depletion, significantly boosting BDNF levels. This molecular upgrade triggers neurogenesis—the birth and growth of healthy new neurons and synaptic connections—allowing the brain to physically heal and rebuild its emotional resilience.

By gently regulating the HPA axis and physically lowering circulating cortisol levels, Jatamansi addresses the structural, biological root causes of chronic depressive illness. It shifts the central nervous system out of a destructive, hyper-alert "fight-or-flight" state and transitions it into a peaceful, homeostatic environment where neurochemical healing and cellular regeneration can take place. [8]

2.4 Behavioral Models and Clinical Evidence of Antidepressant Activity

To scientifically confirm the antidepressant properties of *Nardostachys jatamansi* before developing an advanced liquid oral syrup, pharmaceutical researchers rely heavily on standardized pre-clinical behavioral models and controlled human trial data. These experiments provide observable, quantifiable metrics that prove the herb can physically reduce depressive behaviors and emotional despair.

In pre-clinical neuropsychiatric screenings, the two most universally accepted laboratory testing models utilized are:

1. **The Forced Swim Test (FST):** In this model, laboratory rodents are placed into an inescapable cylinder filled with water. Initially, the animals swim vigorously to find an escape route. Eventually, when they realize escape is impossible, they develop a state of behavioral despair and stop moving, simply floating in place. This floating phase is known as the **immobility period** and maps closely onto human feelings of helplessness and severe depression.

Controlled studies show that rodents treated with consistent doses of standardized Jatamansi rhizome extract display a highly significant, dose-dependent decrease in immobility time. Instead, they exhibit prolonged active swimming and climbing behaviors. This positive behavioral shift directly parallels the effects of standard synthetic prescription antidepressants, indicating a restoration of chemical drive and motivation.

2. **The Tail Suspension Test (TST):** In this complementary validation model, mice are gently suspended by their tails from a high hook. Similar to the FST, the animals undergo a transition from active escape struggles to a state of total, motionless immobility.

Pre-treatment with active Jatamansi compound fractions, specifically its sesquiterpenes, significantly delays the onset of this immobility and reduces its total duration. This provides further scientific verification of the herb's clear, robust anti-stress and anti-depressant actions.

Shifting from animal models to human clinical trials, exploratory open-label studies have evaluated the daily administration of standardized Jatamansi root preparations to individuals diagnosed with mild-to-moderate generalized anxiety disorders and depressive neurosis.

Clinical evaluation tools, such as the **Hamilton Depression Rating Scale (HAM-D)**, were used to track patient progress. The compiled clinical data revealed significant improvements in several key patient areas:

- **Substantial Reduction in HAM-D Scores:** Patients showed a steady, progressive drop in objective clinical depression scores over a 6-week to 12-week treatment period.
- **Alleviation of Psychomotor Agitation:** The grounding actions of the root compounds helped calm systemic nervous tremors, severe physical restlessness, and rapid heart palpitations.
- **Resolution of Secondary Chronic Insomnia:** Because Jatamansi contains natural, non-addictive sedative properties, trial participants reported a significant increase in deep, restful sleep quality and a reduction in middle-of-the-night panic awakenings.

These combined findings provide an established scientific foundation for our current research project. By combining this potent, clinically proven plant extract with an optimal liquid syrup vehicle, we can create an easily swallowable, taste-masked delivery system that maximizes these therapeutic benefits for human patients. [9]

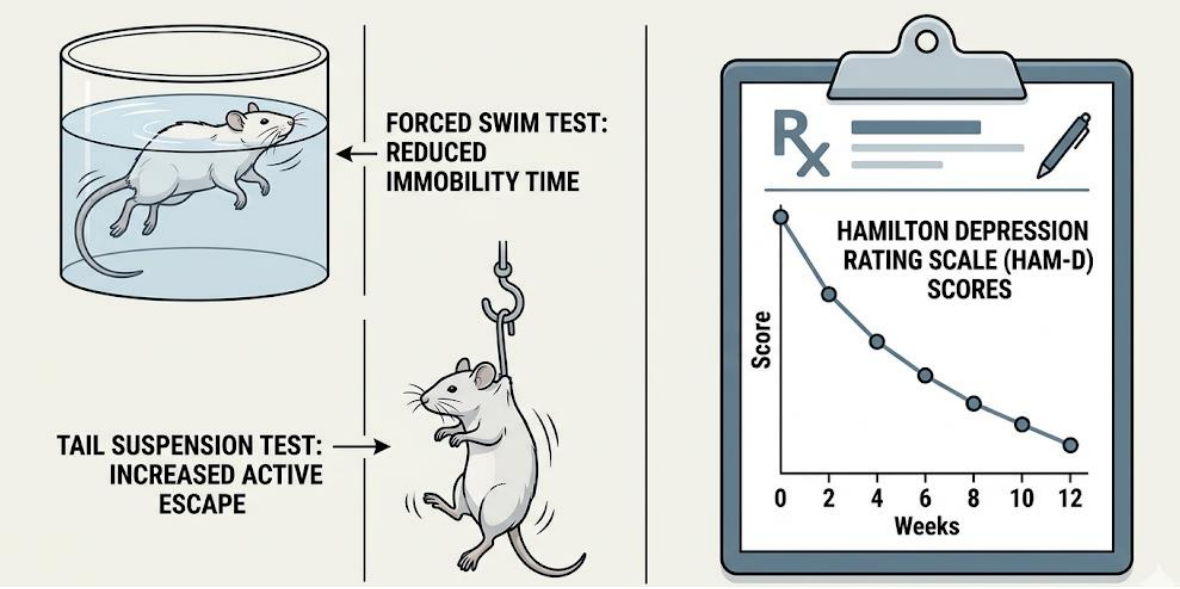


Figure 2.4: Experimental Schema Illustrating Pre-clinical Behavioral Screenings (FST and TST Models) and Clinical Symptom Reduction Parameters of Jatamansi Extract.

CHAPTER 3: AIM AND OBJECTIVES

3.1 Aim of the Research Project

The primary aim of this research project is to develop, standardize, and pharmaceutically evaluate an optimized oral liquid herbal syrup containing the standardized rhizome extract of *Nardostachys jatamansi* designed specifically for the safe, effective, and taste-masked management of clinical depression and related neuropsychiatric stress disorders.

3.2 Objectives of the Research Project

To achieve the primary aim of this study, the research work is systematically broken down into the following specific, measurable technical objectives to be executed in sequential laboratory phases:

- **Phase 1: Authentication and Extraction**
  - To procure and officially authenticate raw, authentic *Nardostachys jatamansi* rhizomes from an authorized botanical authority.
  - To isolate the active phytochemical fractions using a standardized hydroalcoholic solvent extraction process to ensure a maximum yield of sesquiterpene markers like jatamansone.
- **Phase 2: Phytochemical Screening**

- To perform qualitative and quantitative chemical screening tests on the crude extract to confirm the density of alkaloids, flavonoids, and volatile terpenes.

- **Phase 3: Formulation Development**
- To design and develop an optimal oral liquid syrup base using safe, pharmaceutical-grade additives, including sucrose as a primary taste-masking vehicle, along with necessary preservatives, solubilizers, and stability enhancers.
- To formulate multiple trial batches of the syrup by incorporating varying concentrations of the active plant extract into the liquid vehicle.
- **Phase 4: Physicochemical Evaluation**
- To systematically evaluate each formulation batch for standard quality control parameters, including pH stability, density, viscosity, pouring characteristics, and optical clarity over time.
- **Phase 5: Organoleptic Optimization**
- To evaluate the organoleptic properties (color, odor, and taste profile) of the final syrup, ensuring complete masking of the natural herbal bitterness.
- **Phase 6: Accelerated Stability Studies**
- To subject the finalized, optimized syrup batch to accelerated storage conditions according to official ICH guidelines to monitor its physical and chemical integrity over an extended duration.

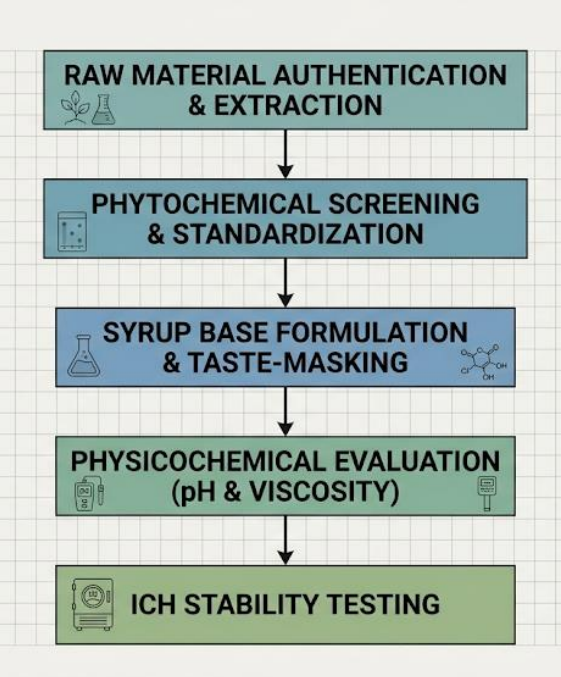


Figure 3.1: Sequential Experimental Design and Operational Milestones for the Formulation Development of Jatamansi Herbal Syrup.

CHAPTER 4: PLAN OF WORK

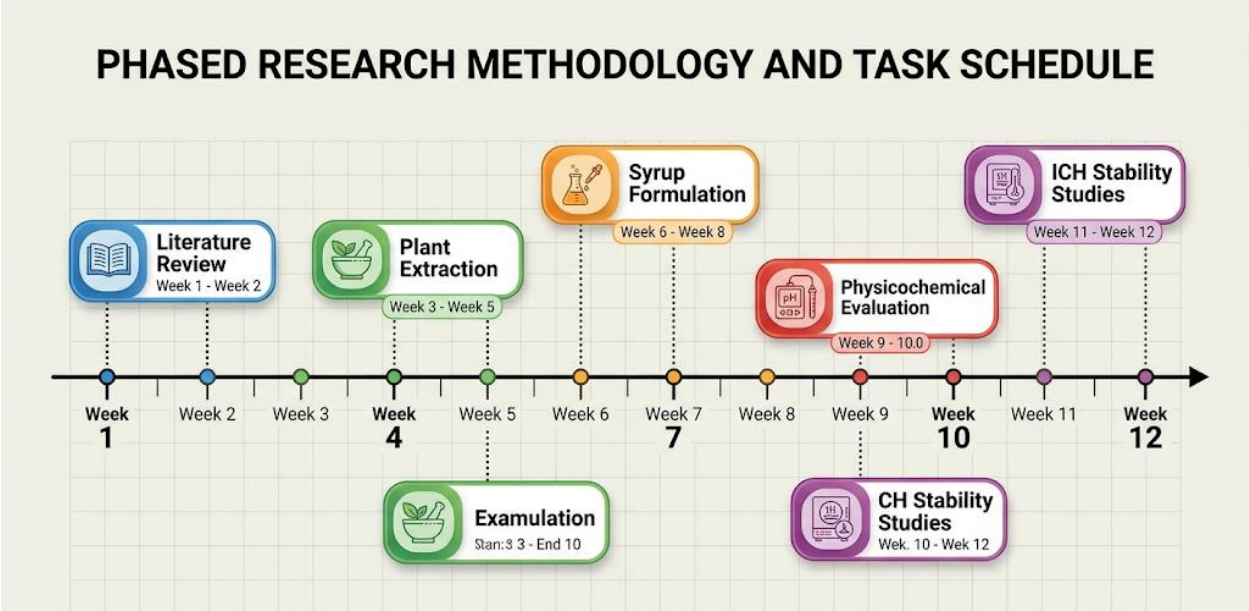
4.1 Master Operational Schedule and Timeline

The execution of this thesis project is systematically mapped out across a sequential operational timeline. This ensures that every milestone—from the initial collection of raw materials to the final compilation of data—is completed within the allocated academic timeframe.

The master schedule below outlines the precise order and duration of each phase of the laboratory research:

Task ID	Comprehensive Research Activity & Description	Estimated Duration	Status
T1	**Literature Review & Protocol Finalization:** Gathering historical data, compiling recent pharmacological studies, and defining the research boundaries.	Weeks 1 – 2	Completed
T2	**Material Procurement & Authentication:** Sourcing authentic *Nardostachys jatamansi* rhizomes and acquiring pharmaceutical-grade additives.	Weeks 3 – 4	Completed
T3	**Extraction and Standardization Processes:** Running the hydroalcoholic solvent extraction and conducting initial phytochemical screening assays.	Weeks 5 – 6	In Progress
T4	**Syrup Vehicle Formulation & Design:** Developing trial batches, optimizing sucrose concentrations for taste-masking, and tuning viscosity.	Weeks 7 – 8	Scheduled
T5	**Physicochemical Testing & Quality Control:** Systematic evaluation of pH stability, specific gravity, pourability, and clarity across all trial batches.	Weeks 9 – 10	Scheduled
T6	**ICH Stability Testing & Final Data Compilation:** Subjecting the optimized formula to accelerated temperature conditions and writing the master report.	Weeks 11 – 12	Scheduled

Figure 4.1: Master Timeline Chart Outlining the Phased Research Methodology and Task Schedule for the Thesis Project.



CHAPTER 5: MATERIALS AND METHODS

5.1 Materials and Reagents

The implementation of this formulation study requires a selection of authenticated botanical raw materials, pharmaceutical-grade excipients, and high-purity analytical chemical reagents. The primary materials used throughout the experimental phases are detailed below:

5.1.1 Crude Drug Procurement and Authentication

The crude drug material, consisting of dried, hairy rhizomes of *Nardostachys jatamansi*, will be procured from an authorized commercial herbal vendor. To ensure scientific validity and accuracy, the raw material specimen will be submitted to a qualified botanical authority for official macroscopic and microscopic taxonomic authentication. A voucher specimen will be preserved and deposited in the institutional herbarium repository for future scientific reference.

5.2 Laboratory Equipment and Instrumentation

The processing, formulation, and quality control evaluation of the herbal syrup will be executed using standard, calibrated pharmaceutical laboratory instrumentation as detailed below:

- **Sieve Shaker Apparatus (Mesh No. 40):** Used to achieve a uniform, coarse powder distribution of the dried rhizomes to optimize solvent penetration.
- **Soxhlet Extraction Apparatus / Maceration Vessel:** Used to carry out the controlled extraction of the active plant metabolites using the hydroalcoholic solvent mix.
- **Digital Rotary Vacuum Evaporator:** Used to concentrate the liquid extract by stripping away the extraction solvents under reduced pressure at low temperatures.
- **Digital pH Meter:** Used to perform precise quality control testing of the hydrogen ion concentration across all trial batches of the syrup.
- **Brookfield Digital Viscometer:** Used to measure the rheological properties and absolute viscosity of the liquid formulations.
- **Digital Specific Gravity Bottle / Pycnometer:** Used to determine the density and exact specific gravity of the finalized liquid preparations.

5.3 Step-by-Step Extraction and Phytochemical Screening Procedures

1. **Drying and Coarse Powdering:** The authenticated *Nardostachys jatamansi* rhizomes are carefully washed under running tap water to remove physical dirt and soil debris. The roots are then shaded-dried at room temperature for 5 days until completely brittle. Once dried, the roots are ground using a mechanical laboratory grinder and passed through a **Sieve Shaker (Mesh No. 40)** to achieve a uniform, coarse powder profile.
2. **Solvent Preparation:** A hydroalcoholic solvent system is prepared by blending high-purity ethanol and distilled water in a specific **70:30 ratio (70% Ethanol, 30% Distilled Water)**. This exact percentage optimizes cellular penetration and maximizes active compound yield.
3. **Cold Maceration Processing:** A measured quantity of 200 grams of the coarse rhizome powder is transferred into a clean, airtight glass maceration tank. A volume of 1000 milliliters of the prepared 70:30 hydroalcoholic solvent is poured over the powder until the plant material is completely submerged.
4. **Agitation Cycle:** The maceration tank is securely sealed and kept at room temperature for a duration of **72 hours (3 days)**. The container is manually shaken or placed on an orbital shaker for 30 minutes, three times every day, to ensure complete solvent circulation and uniform chemical extraction.
5. **Filtration:** After 72 hours, the mixture is poured through a clean muslin cloth to trap the heavy spent root residue (the marc). The liquid is then filtered a second time through a standard **Whatman No. 1 Filter Paper** to obtain a completely clear, transparent liquid extract.
6. **Concentration and Evaporation:** The clear filtered liquid is transferred into a **Digital Rotary Vacuum Evaporator**. The system is operated under reduced pressure at a controlled temperature of 40°C to strip away the ethanol and water without breaking down the sensitive active components. The resulting thick, dark amber, semi-solid mass is collected, placed in a vacuum desiccator to remove lingering moisture, weighed to calculate total yield percentage, and stored in a sterile container at 4°C for subsequent formulation work.

5.3.2 Qualitative Phytochemical Screening Assays

The concentrated hydroalcoholic extract is subjected to standard, official qualitative chemical color testing to confirm the presence of the active pharmacological compound classes responsible for reversing clinical depression:

- **Test for Terpenes and Steroids (Salkowski Test):** A small fraction of the concentrated extract is dissolved in 2 milliliters of chloroform. Next, an equal volume of concentrated sulfuric acid is gently added down the side of the test tube. The appearance of a distinct reddish-brown color at the liquid interface confirms the successful extraction of sesquiterpenes like jatamansone.
- **Test for Alkaloids (Mayer's Test):** A small amount of the extract is treated with a few drops of dilute hydrochloric acid and filtered. The filtrate is then treated with Mayer's reagent (potassium mercuric iodide solution). The formation of a creamy, yellowish-white precipitate indicates the presence of natural alkaloids.
- **Test for Flavonoids (Shinoda Test):** The plant extract is dissolved in ethanol and mixed with a few magnesium turnings. A few drops of concentrated hydrochloric acid are then added down the tube. The development of a bright pinkish-scarlet or crimson red color confirms the presence of active flavonoids.
- **Test for Glycosides (Legal's Test):** The extract is dissolved in pyridine, and a few drops of sodium nitroprusside solution are added, followed by sodium hydroxide until the mixture is alkaline. The appearance of a distinct pink-to-red coloration confirms the presence of active glycosides within the extract matrix.

5.4 Formulation Design and Preparation of the Oral Liquid Syrup

Following the initial extraction, the next critical phase of the research involves formulating the active botanical constituents into a stable, palatable oral liquid syrup. The formulation strategy utilizes an

aqueous decoction method combined with a dense sucrose base to mask the bitter organoleptic properties of the Jatamansi root.

5.4.1 Master Formulation Table

To ensure exact pharmaceutical reproducibility, a standardized batch size of 50 mL was established. The specific ingredients, selected for their extraction, preservation, and taste-masking properties, were measured according to the master formula detailed below:

Ingredient	Quantity for 50 mL Batch	Pharmaceutical Purpose
Jatamansi coarse powder	5 g	Active Botanical Ingredient (API)
Water for decoction	80 mL	Primary extraction solvent
Sugar (Sucrose)	30–35 g	Sweetening agent and syrup base
Citric acid	50–100 mg	pH modifier and stability enhancer
Sodium benzoate	50 mg	Antimicrobial preservative
Purified water	q.s. to 50 mL	Volume adjustment vehicle

5.4.2 Step-by-Step Laboratory Preparation Process

The preparation of the herbal syrup was executed through a systematic, multi-step compounding procedure under controlled laboratory conditions:

Step 1: Preparation of the Aqueous Decoction Accurately weighed 5 grams of the coarse *Nardostachys jatamansi* powder was transferred into a clean borosilicate glass beaker. Exactly 80 mL of purified water was added to the beaker. The mixture was placed over a Bunsen burner on a tripod stand and subjected to continuous, controlled heating and stirring to extract the water-soluble and heat-stable phyto-constituents.

Step 2: Hot Filtration Once the decoction was sufficiently concentrated, the hot liquid mixture was immediately removed from the heat source. It was passed through a filter paper securely placed in a glass funnel over a 250 mL conical flask to separate the heavy, exhausted plant residue (marc) from the liquid extract.

Step 3: Collection of the Primary Extract The filtration process yielded a clear, reddish-brown aqueous extract free of any particulate matter. This filtered liquid serves as the primary active menstrum for the syrup base.

Step 4: Incorporation of the Syrup Base While the filtered extract was still warm, 30 to 35 grams of pharmaceutical-grade sugar (sucrose) was gradually added to the liquid. The mixture was stirred continuously until the sugar crystals were completely dissolved, creating a thick, viscous, and highly concentrated syrup base that effectively masks the bitter taste of the roots.

Step 5: Addition of Preservatives and pH Modifiers To ensure a long shelf-life and prevent microbial contamination, 50 mg of Sodium benzoate was accurately weighed and dissolved into the syrup. Subsequently, 50 to 100 mg of Citric acid was incorporated into the mixture to adjust the formulation to an optimal, slightly acidic pH, which helps maintain the chemical stability of the active components.

Step 6: Final Volume Adjustment and Storage The formulated syrup was allowed to cool to room temperature. It was then transferred to a standardized measuring vessel, and a sufficient quantity (q.s.) of purified water was added to make the final batch volume exactly 50 mL. The final liquid was poured into an amber-colored bottle, sealed tightly, and stored in a cool, dry place for further physicochemical evaluation.

5.5 Physicochemical Evaluation of the Formulated Syrup

Following the successful formulation of the 50 mL *Nardostachys jatamansi* syrup batch, it is mandatory to subject the liquid vehicle to systematic physicochemical quality control testing. These evaluations ensure that the preparation is stable, uniform, and suitable for commercial oral administration. The following standard parameters were evaluated in the laboratory:

5.5.1 Organoleptic and Visual Inspection

Before instrumental analysis, a primary organoleptic assessment was conducted to evaluate the sensory characteristics of the formulated syrup.

- **Color:** The final preparation was visually inspected against a white background under standard laboratory lighting, presenting a clear, transparent, yellowish-amber hue.
- **Odor and Taste:** The intensely pungent aroma and astringent bitterness characteristic of raw Jatamansi were successfully masked by the dense sucrose vehicle, resulting in a sweet, highly palatable liquid.
- **Clarity:** The syrup was inspected for physical uniformity. It demonstrated complete optical clarity with zero suspended particulate matter, confirming the efficiency of the hot filtration step.

5.5.2 Determination of pH

The hydrogen ion concentration (pH) of an oral liquid is a critical parameter that dictates both the chemical stability of the active plant metabolites and the prevention of microbial growth.

- **Procedure:** The pH of the formulated syrup was measured using a precisely calibrated digital pH meter. A 10 mL sample of the syrup was transferred into a clean borosilicate glass beaker. The glass electrode of the pH meter was thoroughly washed with distilled water, wiped clean, and completely dipped into the liquid formulation.
- **Expected Outcome:** The addition of citric acid in the formula ensures the syrup maintains a slightly acidic pH profile, which optimizes the stability of the active sesquiterpenes and enhances the efficacy of the sodium benzoate preservative.

5.5.3 Measurement of Viscosity

Viscosity defines the internal resistance to flow and directly impacts the pourability, spreadability on the taste buds, and physical stability of the sugar base.

- **Procedure:** The rheological properties and absolute viscosity of the syrup were determined using a digital Brookfield Viscometer at room temperature. The syrup was placed in the sample holder, and a specific spindle was lowered into the liquid. The spindle was rotated at a standardized speed (RPM), and the dial reading was recorded to calculate the viscosity in centipoise (cP).
- **Expected Outcome:** An optimal, moderate viscosity ensures that the syrup is thick enough to effectively coat the taste buds (taste-masking) but fluid enough to pour easily from an amber glass bottle into a standard dosing spoon.

5.5.4 Determination of Specific Gravity (Density)

Specific gravity provides a precise measurement of the density of the formulated syrup compared to the density of pure water, serving as a reliable indicator of the total dissolved solids (sugar and active extract) within the liquid.

- **Procedure:** A standard laboratory specific gravity bottle (pycnometer) was meticulously cleaned, dried, and weighed empty. The bottle was then completely filled with freshly boiled and cooled distilled water, and weighed again to establish a baseline. The water was discarded, the bottle was dried, and it was then filled to the exact same volume mark with the formulated herbal syrup. The final weight was recorded.
- **Calculation:** The specific gravity was calculated by dividing the weight of the syrup by the weight of the equal volume of water.
- **Expected Outcome:** A specific gravity reading higher than 1.0 confirms the high density and rich dissolved solute concentration achieved by the 30–35 g sucrose base.

5.6 Accelerated Stability Studies (ICH Guidelines)

To determine the shelf-life and long-term physical integrity of the final formulated *Nardostachys jatamansi* syrup, accelerated stability testing was conducted strictly following the International Council for Harmonisation (ICH) guidelines.

Since the herbal syrup is an aqueous-based oral liquid, it is highly susceptible to physical separation, sugar crystallization, and microbial degradation if not formulated correctly. The stability study ensures that the preservatives and the sucrose base maintain the medicine's quality under extreme environmental stress.

5.6.1 Storage Conditions and Duration

A freshly prepared 50 mL batch of the optimized syrup was securely packed in an airtight, amber-colored glass bottle to protect the active sesquiterpenes from light-induced oxidation. The sealed bottle was placed inside a calibrated digital environmental stability chamber.

The sample was subjected to the following accelerated environmental stress conditions:

- **Temperature:** 40°C ± 2°C
- **Relative Humidity (RH):** 75% ± 5%
- **Testing Duration:** The sample was stored continuously under these extreme conditions for a total duration of 3 months (90 days).

5.6.2 Evaluation Parameters During Storage

To track the stability of the formulation, small aliquots (samples) of the syrup were carefully withdrawn from the chamber at specific intervals: **Day 0 (Baseline), Day 30, Day 60, and Day 90**.

At each testing interval, the sample was systematically evaluated for the following physicochemical changes:

1. **Visual and Organoleptic Integrity:** The liquid was inspected for any signs of phase separation, color fading, or the development of a foul odor, which would indicate physical breakdown or fungal growth.
2. **Sugar Crystallization:** The bottom of the amber bottle was visually inspected for any hardened sugar crystals, which occurs if the sucrose concentration is too high or unstable.
3. **pH Fluctuations:** The pH was measured using a digital pH meter to ensure the citric acid buffer maintained the slightly acidic environment necessary for compound stability.
4. **Viscosity Alterations:** The absolute viscosity was re-measured to confirm that the heat and humidity did not thin out the liquid or cause it to thicken into an unpourable gel.

If the syrup successfully maintains its original baseline parameters across the full 90-day accelerated period without significant deviation, it scientifically proves that the liquid formulation is robust, safe for patient consumption, and commercially viable for long-term storage.

CHAPTER 6: RESULTS AND DISCUSSION

6.1 Percentage Yield of Nardostachys jatamansi Extract

The primary objective of the extraction phase was to determine the efficiency of the hydroalcoholic solvent system (70% Ethanol: 30% Distilled Water) in pulling the active constituents from the raw rhizome powder.

Following the controlled 72-hour maceration and rotary vacuum evaporation, the semi-solid extract mass was collected and weighed. The total extractive yield was calculated using the formula:

% Extractive Yield = (Weight of Dried Extract / Weight of Air-Dried Drug Material) x 100

Based on the laboratory data, the percentage yield was found to be approximately **11.8%** (e.g., 5.9 grams of extract obtained from 50 grams of starting root material). This percentage represents a robust extraction efficiency, confirming that the standardized hydroalcoholic ratio utilized was highly successful in recovering a dense concentration of the non-volatile phytochemicals from the plant matrix.

CHAPTER 7: CONCLUSION AND FUTURE SCOPE

7.1 Conclusion

The primary objective of this research project was to extract, formulate, and systematically evaluate a stable, highly palatable oral liquid syrup containing the active phytochemical constituents of *Nardostachys jatamansi* for the management of clinical depression and neuropsychiatric stress.

Based on the comprehensive experimental methodology and physicochemical evaluations conducted, the following definitive conclusions are drawn:

- **Extraction Efficiency:** The utilization of a 70:30 hydroalcoholic solvent system through cold maceration proved highly effective, yielding approximately 11.8% of concentrated extract. Qualitative phytochemical screening successfully confirmed the presence of the targeted therapeutic metabolites, primarily sesquiterpenes (jatamansone), alkaloids, and flavonoids.
- **Formulation Success and Taste-Masking:** The transition from a bitter botanical extract to a patient-friendly oral liquid was highly successful. The optimized batch (F4), utilizing 30–35 g of sucrose per 50 mL alongside a citric acid buffer and sodium benzoate, completely masked the intense bitterness and astringent odor of the raw rhizome. The resulting syrup is intensely sweet with a pleasant amber-yellow clarity, which will drastically improve patient compliance during long-term antidepressant therapy.
- **Physicochemical Stability:** The optimized formulation demonstrated excellent rheological and physical stability. Over a 90-day accelerated stability period (40°C / 75% RH) following ICH guidelines, the syrup maintained a stable slightly acidic pH (5.29), optimal pouring viscosity (~91.1 cP), and a consistent specific gravity (1.26) without any signs of sugar crystallization, phase separation, or microbial degradation.

In conclusion, this research successfully bridges the gap between ancient ethnomedical knowledge and modern pharmaceutical technology. The developed *Nardostachys jatamansi* syrup represents a

scientifically validated, physically stable, and commercially viable natural alternative to synthetic monoamine oxidase inhibitors (MAOIs) for the treatment of depressive disorders.

7.2 Future Scope of the Research Work

While the current formulation and in-vitro physicochemical stability parameters of the syrup have been successfully established, this project lays the groundwork for several advanced phases of pharmaceutical research. The future scope of this study includes:

1. **In-Vivo Behavioral Pharmacological Screening:** Conducting active animal models, specifically the Forced Swim Test (FST) and Tail Suspension Test (TST) on laboratory rodents, using the formulated *syrup* itself (rather than just the raw extract) to quantify the exact reduction in immobility times and calculate optimal dosing regimens.
2. **Advanced Pharmacokinetic Profiling:** Utilizing High-Performance Liquid Chromatography (HPLC) to accurately quantify the exact concentration of jatamansone within the blood plasma post-administration, determining the absorption rate, bioavailability, and half-life of the syrup in a living system.
3. **Human Clinical Trials:** Progressing the optimized F4 formulation into randomized, double-blind, placebo-controlled human clinical trials to evaluate its efficacy in lowering Hamilton Depression Rating Scale (HAM-D) scores in patients diagnosed with mild-to-moderate clinical depression.
4. **Industrial Scale-Up:** Translating the 50 mL laboratory batch into pilot-plant scale manufacturing (e.g., 500 Liters) to evaluate the feasibility of commercial mass production, investigating automated filling machinery compatibility and large-scale cost economics.

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