

“Formulation and Stability Study of Polyherbal Immunity Booster Syrup Containing Ocimum Sanctum, Withania somnifera and Tinospora cordifolia”

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ABSTRACT

This study focuses on the formulation and stability evaluation of a polyherbal immunity booster syrup containing *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwagandha), and *Tinospora cordifolia* (Guduchi). These medicinal plants are traditionally recognized for their immunomodulatory, antioxidant, adaptogenic, anti-inflammatory, and rejuvenating properties. The objective of the research was to develop a safe, stable, palatable, and standardized herbal syrup that could enhance immune function and improve overall health.

The formulation process involved the selection, collection, authentication, extraction, and combination of herbal materials with suitable excipients, preservatives, and stabilizing agents. The prepared syrup was subjected to physicochemical evaluation, including parameters such as pH, viscosity, organoleptic characteristics, microbial load, and stability studies under different storage conditions to determine product quality and shelf life. Preliminary phytochemical and pharmacological evaluations were also considered to support the immunomodulatory potential of the formulation.

The study highlights that combining Tulsi, Ashwagandha, and Guduchi may provide synergistic therapeutic effects, offering improved immune support compared to individual herbal components. The findings support the concept that scientifically standardized polyherbal formulations can serve as a promising natural alternative for immune health while maintaining stability, safety, and patient acceptability.

KEYWORDS

Polyherbal Syrup
Immunomodulatory Activity
Ocimum sanctum (Tulsi)
Withania somnifera
(Ashwagandha)
Tinospora cordifolia
(Guduchi)

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1.1.Introduction of *Ocimum Sanctum*, *Withania Somnifera*, and *Tinospora Cordifolia*:

In recent years, there has been a growing interest in herbal medicines as safe and effective alternatives to synthetic drugs. This shift is largely driven by increasing awareness about the side effects associated with conventional pharmaceuticals and the desire for natural therapies that promote overall well-being. Among these, polyherbal formulations have gained significant importance due to their synergistic therapeutic effects. The present study focuses on the formulation and stability evaluation of a polyherbal immunity booster syrup containing *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwagandha), and *Tinospora cordifolia* (Guduchi), which are widely recognized for their immunomodulatory properties.

The immune system plays a crucial role in protecting the body against infections, diseases, and environmental stressors. However, modern lifestyles, poor nutrition, pollution, and stress have weakened immune responses in many individuals. This has led to an increased demand for natural immunostimulants that can enhance the body's defense mechanisms without causing harmful side effects. Herbal plants have been used traditionally in various systems of medicine, such as Ayurveda, for strengthening immunity and maintaining health.

Ocimum sanctum is known for its antimicrobial, antioxidant, and anti-inflammatory properties. It contains bioactive compounds such as eugenol, which contribute to its therapeutic potential. It helps in improving respiratory health and boosting immunity. *Withania somnifera*, commonly known as Ashwagandha, is classified as an adaptogen that helps the body cope with stress and enhances immune function. It contains withanolides that exhibit anti-inflammatory and immunomodulatory activities. *Tinospora cordifolia*, also known as Guduchi, is well-known for its ability to enhance immune responses and detoxify the body. It is rich in alkaloids, glycosides, and diterpenoid lactones that contribute to its medicinal properties. The concept of combining these three herbs into a single formulation is based on the principle of polyherbalism, where multiple herbs are used together to achieve better therapeutic outcomes than individual herbs alone. The synergistic effect of these herbs enhances their overall efficacy and reduces the required dosage, minimizing potential side effects. A syrup dosage form has been selected for this formulation due to its ease of administration, especially for pediatric and geriatric populations. Syrups also provide better palatability, improved patient compliance, and faster absorption of active constituents. The formulation process involves careful selection of herbal extracts, excipients, preservatives, and flavoring agents to ensure stability, safety, and effectiveness. Stability studies are an essential part of pharmaceutical formulation development. They help in determining the shelf life, storage conditions, and quality of the product over time. Factors such as temperature, humidity, light, and microbial contamination can affect the stability of herbal formulations. Therefore, it is important to evaluate physical, chemical, and microbiological stability parameters to ensure product consistency and safety.

This research aims to develop a standardized polyherbal syrup with enhanced immunomodulatory activity and to evaluate its stability under different conditions. The study also focuses on ensuring quality control through appropriate analytical techniques. By combining traditional knowledge with modern scientific approaches, this research contributes to the development of effective herbal formulations that can support immune health in a safe and natural manner.

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2.1.Sharma et al. (2021) Literature Review:

Sharma et al. (2021) conducted a detailed investigation on the immunomodulatory potential of *Withania somnifera*, focusing on its role in enhancing the body's natural defense system. The study emphasized that Ashwagandha acts as a potent adaptogen, helping the body to cope with physiological stress while simultaneously improving immune resilience. The authors highlighted that the presence of withanolides contributes significantly to its pharmacological activity, particularly in modulating immune responses. Their findings suggested that *Withania somnifera* enhances both innate and adaptive immunity by increasing the activity of macrophages, natural killer cells, and lymphocytes. Additionally, the study observed a reduction in stress-induced immunosuppression, which is a common factor in decreased immunity among individuals exposed to chronic stress. The antioxidant properties of Ashwagandha were also reported to play a crucial role in neutralizing free radicals, thereby protecting immune cells from oxidative damage. The researchers further discussed the potential application of this herb in preventing infections and improving overall health. They concluded that *Withania somnifera* could serve as a key component in herbal formulations aimed at boosting immunity. However, the study also pointed out the need for combining it with other medicinal plants to achieve enhanced therapeutic effects through synergistic interactions. This work provides a strong scientific basis for incorporating Ashwagandha into polyherbal formulations, particularly those designed for immune enhancement. It also supports the concept that herbal adaptogens can play a dual role in stress management and immune modulation, making them highly valuable in modern healthcare formulations.

2.2.Patel et al. (2021) Literature Review:

Patel et al. (2021) explored the concept of polyherbal formulations and their synergistic effects in enhancing immune function. The study focused on combining multiple medicinal plants, including *Ocimum sanctum*, *Withania somnifera*, and *Tinospora cordifolia*, to evaluate their collective therapeutic potential. The authors emphasized that polyherbal formulations are more effective than single-herb preparations due to the complementary actions of different phytoconstituents. The research demonstrated that *Ocimum sanctum* contributes antimicrobial and antioxidant properties, while *Withania somnifera* provides adaptogenic and anti-stress effects. *Tinospora cordifolia*, on the other hand, plays a crucial role in immune stimulation and detoxification. When used together, these herbs exhibited enhanced immunomodulatory activity, indicating a synergistic effect. The study also highlighted the importance of selecting appropriate dosage forms for herbal formulations. It was observed that traditional preparations such as decoctions often lack consistency and patient acceptability. Therefore, the authors suggested the development of modern dosage forms like syrups to improve palatability, dosage accuracy, and patient compliance. Furthermore, Patel et al. stressed the need for standardization and quality control in polyherbal formulations. Variability in raw materials and extraction methods can affect the efficacy and safety of the final product. Hence, proper analytical techniques and stability studies are essential to ensure consistent quality. Overall, the study supports the development of polyherbal immunity booster syrups by providing evidence of

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CHAPTER 4: AIM & OBJECTIVES

4.1 Aim of the Study:

The primary aim of the present research is to formulate and evaluate a stable polyherbal immunity booster syrup containing *Ocimum sanctum*, *Withania somnifera*, and *Tinospora cordifolia*. The study is designed to scientifically develop a herbal formulation that combines the immunomodulatory, antioxidant, and adaptogenic properties of these medicinal plants into a single effective dosage form.

The research also aims to bridge the gap between traditional herbal medicine and modern pharmaceutical science by developing a standardized formulation with improved stability, safety, and patient acceptability. In addition, the study seeks to assess the physicochemical and microbiological stability of the prepared syrup under different storage conditions to ensure its quality and shelf life.

4.2 Objectives of the Study:

The present study is carried out with the following specific objectives:

1. To collect and authenticate selected medicinal plants for quality assurance.
2. To prepare herbal extracts for maximum phytoconstituent yield.
3. To perform phytochemical screening of bioactive compounds.
4. To formulate a stable and palatable polyherbal syrup.
5. To evaluate physicochemical properties for quality control.
6. To conduct stability studies for shelf-life determination.
7. To assess microbial load for safety evaluation.
8. To standardize the formulation for consistency.
9. To evaluate immunomodulatory activity of the formulation.

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A| *Ocimum sanctum* (Tulsi):

Ocimum sanctum, commonly known as Tulsi or Holy Basil, is one of the most revered medicinal plants in traditional systems of medicine, particularly Ayurveda. It belongs to the family Lamiaceae and is widely recognized for its diverse therapeutic properties, especially its role in enhancing immunity and promoting overall health. Due to its extensive pharmacological activities and cultural significance, Tulsi is often referred to as the “Queen of Herbs.”

A| 5.1. Biological Source:

Ocimum sanctum Linn. consists of the fresh and dried leaves of the plant. In some cases, other parts such as stems and seeds are also utilized for medicinal purposes, but the leaves remain the primary source of active constituents.

Table 1: Taxonomical Classification of *Ocimum sanctum* (Tulsi):

S. NO.	TAXONOMICAL RANK	CLASSIFICATION
1	Kingdom	Plantae
2	Subkingdom	Tracheobionta (Vascular plants)
3	Division	Magnoliophyta (Angiosperms)
4	Class	Magnoliopsida (Dicotyledons)
5	Subclass	Asteridae
6	Order	Lamiales
7	Family	Lamiaceae
8	Genus	<i>Ocimum</i>
9	Species	<i>Ocimum sanctum</i> Linn.

A| 5.2. Geographical Source:

The plant *Ocimum sanctum* (Tulsi) used in the present study was procured from the local region of **Bhopal, Madhya Pradesh, India**. Bhopal is characterized by a subtropical climate with moderate rainfall and suitable temperature conditions, which are highly favorable for the cultivation and growth of medicinal plants such as Tulsi. The soil in this region is generally fertile and well-drained, providing ideal conditions for the healthy development of the plant and the accumulation of bioactive phytoconstituents. Tulsi is commonly grown in household gardens, herbal farms, and institutional premises in and around Bhopal due to its medicinal, cultural, and religious significance. The plant material collected from this geographical location is expected to possess good quality and potency, as environmental factors such as climate, soil composition, and seasonal variations play a crucial role in determining the phytochemical profile of medicinal plants.

A| 5.3. Morphological Characteristics of *Ocimum sanctum* (Tulsi):

Ocimum sanctum is an aromatic, erect, branched herb widely recognized for its medicinal and therapeutic importance. The plant exhibits distinct morphological features in each of its parts, which aid in its identification and classification.

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5.3.1. Root:

The root system of *Ocimum sanctum* is a well-developed taproot type. It consists of a primary root that penetrates deep into the soil, along with several lateral roots branching out horizontally. The roots are thin, fibrous, and light brown in color. They help in anchoring the plant firmly in the soil and in the absorption of water and essential nutrients.



[FIGURE 1: ROOT SYSTEM OF *OCIMUM SANCTUM*]

5.3.2. Stem:

The stem of Tulsi is erect, quadrangular (square-shaped in cross-section), and highly branched. It is usually green to purplish in color and covered with fine hairs (pubescent). The stem is relatively soft when young but becomes slightly woody as the plant matures. The branching pattern allows the plant to spread and produce a dense foliage. The stem also contains essential oils, contributing to the characteristic aroma of the plant.



[FIGURE 2: STEM OF TULSI]

5.3.3. Leaves:

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CHAPTER 6 METHOD OF PREPARATION

1. Syrup Base Preparation

Sucrose + Purified Water → Gentle Heating → Clear Syrup Base



2. Addition of Glycerin

Syrup Base + Glycerin → Uniform Mixing → Improved Viscosity



3. Addition of Herbal Extracts

(*Ocimum sanctum* + *Withania somnifera* + *Tinospora cordifolia*)

→ Continuous Stirring → Homogeneous Distribution



4. Addition of Preservative

Sodium Benzoate (in solution) → Added with Stirring → Microbial Stability



5. Addition of Flavoring Agent

Flavor (q.s.) → Mixed Uniformly → Improved Palatability



6. Volume Adjustment

Purified Water → Make up to 100 mL → Standardized Formulation



7. Filtration & Packaging

Filtration → Amber Bottle Filling → Airtight Sealing



8. Storage & Stability Study

Controlled Storage Conditions → Evaluation



Stable Polyherbal Immunity Booster Syrup

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The present research work is carefully designed to develop and evaluate a stable polyherbal immunity booster syrup containing *Ocimum sanctum*, *Withania somnifera*, and *Tinospora cordifolia*. The study follows a systematic and stepwise approach to ensure scientific accuracy, reproducibility, and proper standardization of the formulation. Each stage of the work is planned to maintain quality control and to obtain reliable experimental outcomes. The initial phase of the study involves the collection and authentication of plant materials. Fresh leaves of *Ocimum sanctum*, roots of *Withania somnifera*, and stems of *Tinospora cordifolia* are collected from the local region of Bhopal, Madhya Pradesh. Proper identification and authentication of the plant materials are carried out using standard botanical methods to confirm their purity and eliminate the possibility of adulteration. Following collection, the plant materials are subjected to cleaning, drying, and size reduction. The materials are washed thoroughly to remove dust and foreign particles and then shade-dried to preserve their active constituents. After complete drying, they are powdered using a mechanical grinder and sieved to obtain uniform particle size, which is essential for efficient extraction. In the next stage, extraction of the powdered materials is carried out using suitable solvents such as water or hydroalcoholic mixtures. Each plant material is extracted separately to obtain concentrated extracts rich in phytoconstituents. The extracts are then filtered and concentrated to remove excess solvent. The percentage yield of each extract is calculated to evaluate extraction efficiency. Preliminary phytochemical screening is then performed to identify the presence of important bioactive compounds such as alkaloids, flavonoids, glycosides, tannins, and saponins. This step confirms the therapeutic potential of the extracts and supports their use in the formulation. The formulation stage involves the preparation of the polyherbal syrup by combining the extracts of all three plants in suitable proportions. A syrup base is prepared using sucrose, and excipients such as glycerin, preservatives, and flavoring agents are added to improve stability, viscosity, and palatability. The formulation is prepared under controlled conditions to ensure uniformity and consistency. After formulation, the syrup is subjected to evaluation tests. Parameters such as pH, viscosity, specific gravity, and organoleptic properties (color, taste, odor, and clarity) are assessed to ensure the quality and acceptability of the product. These evaluations help in determining whether the formulation meets the required standards. The stability study is an important part of the plan of work. The prepared syrup is stored under different conditions, including room temperature and accelerated conditions, to evaluate its stability over time. Observations are recorded at regular intervals to monitor any changes in physical, chemical, and microbiological properties. Finally, microbial analysis and drug content estimation are performed to ensure the safety and efficacy of the formulation. All experimental data are systematically recorded, analyzed, and interpreted to draw meaningful conclusions. This well-structured plan of work ensures the successful development of a stable and effective polyherbal immunity booster syrup.

Step 1: Collection and Authentication of Plant Material:

Fresh leaves of *Ocimum sanctum*, roots of *Withania somnifera*, and stems of *Tinospora cordifolia* will be collected from the local region (Bhopal, Madhya Pradesh). The plant materials will be carefully selected to ensure they are free from disease, contamination, and physical damage. After collection, all plant materials will be washed with clean water to remove adhering dust and foreign particles.

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8.1. FORMULATION

1. Dissolve sucrose in purified water with gentle heating to prepare syrup base:

The formulation process begins with the accurate weighing of sucrose, which acts as the main sweetening agent and base-forming component of the syrup. The measured quantity of sucrose is slowly added into a required volume of purified water in a clean beaker. The mixture is subjected to gentle heating while continuously stirring to ensure complete dissolution of the sugar. The temperature is carefully controlled throughout the process to prevent overheating, as excessive heat may lead to caramelization, which can alter the color, taste, and stability of the final product. Heating is continued until a clear and uniform syrup base is obtained with no visible undissolved particles. This syrup base serves as the primary vehicle for incorporating herbal extracts in subsequent steps. It also contributes to the viscosity, sweetness, and overall acceptability of the formulation. Once complete dissolution is achieved, the syrup is allowed to cool slightly before further processing. The clarity and consistency of the base are carefully observed to ensure it meets the required quality standards for a stable herbal formulation.

2. Add glycerin and mix uniformly:

After preparation of the syrup base, a measured quantity of glycerin is added into the solution. Glycerin is a hygroscopic, viscous liquid that plays an important role in improving the physical properties of the formulation. It enhances the smoothness, viscosity, and overall texture of the syrup, making it more palatable for patients. In addition, glycerin helps prevent crystallization of sugar during storage, thereby improving the long-term stability of the formulation. Once added, the mixture is stirred continuously to ensure uniform distribution of glycerin throughout the syrup base. Proper mixing is essential to avoid phase separation and maintain a consistent formulation. Glycerin also acts as a humectant, helping retain moisture within the preparation and improving mouthfeel. The mixture is carefully observed to confirm homogeneity and absence of layering. This step is important in achieving a stable and well-balanced syrup suitable for further incorporation of herbal extracts.



[FIGURE 18: GLYCERIN AND MIX UNIFORMLY]

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3. Add measured herbal extracts with continuous stirring:

In this step, accurately measured extracts of *Ocimum sanctum*, *Withania somnifera*, and *Tinospora cordifolia* are added to the prepared syrup base. Each extract is incorporated slowly with continuous stirring to ensure uniform dispersion throughout the formulation. Continuous stirring helps prevent aggregation, sedimentation, or uneven distribution of active constituents. The herbal extracts contain important phytochemicals such as flavonoids, alkaloids, withanolides, glycosides, and tannins, which are responsible for immunomodulatory and antioxidant activities.

Proper mixing ensures that these bioactive compounds are evenly distributed, resulting in consistent therapeutic efficacy in every dose. During this process, care is taken to maintain a stable temperature to protect heat-sensitive compounds from degradation. As mixing continues, the formulation gradually attains a uniform color and consistency, indicating proper incorporation of all extracts. This step is crucial for ensuring dose uniformity, stability, and therapeutic reliability of the final product.

4. Incorporate sodium benzoate as preservative:

Sodium benzoate is added to the formulation as a preservative to enhance microbial stability and extend shelf life. A precisely weighed quantity of sodium benzoate is first dissolved in a small amount of purified water to ensure complete solubility. This solution is then slowly added to the herbal syrup under continuous stirring. Sodium benzoate is particularly effective in acidic environments and helps inhibit the growth of bacteria, yeast, and molds that may contaminate herbal formulations during storage.

The addition of preservative is essential due to the natural composition of herbal extracts, which are more susceptible to microbial growth. Proper mixing ensures uniform distribution of the preservative throughout the formulation, providing consistent protection. After incorporation, the mixture is checked for clarity and homogeneity. This step plays a vital role in maintaining the safety, quality, and stability of the final syrup over extended storage periods.



[FIGURE 19: SODIUM BENZOATE AS PRESERVATIVE]

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5. ADD FLAVORING AGENT FOR TASTE ENHANCEMENT:

A suitable flavoring agent is incorporated into the formulation to improve taste, odor, and patient acceptability. Herbal extracts often have a bitter and unpleasant taste, which can reduce patient compliance, especially in children and elderly individuals. Therefore, flavoring agents are used to mask the bitterness and improve overall palatability. The flavoring agent is added in a small quantity (q.s.) to ensure it does not interfere with the therapeutic activity of the formulation. After addition, the mixture is gently stirred to ensure uniform distribution of flavor throughout the syrup. This step enhances the sensory quality of the final product, making it more acceptable for regular use. Proper selection of flavoring agent ensures compatibility with herbal constituents and maintains formulation stability. This step is important for improving patient adherence and therapeutic effectiveness.

6. Make up the final volume to 100 mL using purified water:

After incorporation of all ingredients, the volume of the formulation is adjusted to 100 mL using purified water. This step ensures accurate dosing, uniform concentration, and standardization of the final product. Purified water is added gradually with continuous stirring to maintain homogeneity and prevent dilution errors. Precise volume adjustment is essential in pharmaceutical preparations as it directly affects drug concentration and therapeutic response. Once the final volume is achieved, the mixture is stirred thoroughly to ensure complete uniformity. The formulation is then allowed to stand briefly to remove any trapped air bubbles formed during mixing. This step ensures consistency and reproducibility in the final product. Proper standardization at this stage is essential for maintaining batch-to-batch consistency in large-scale production.

7. Filter and store in amber-colored bottles:

The final prepared syrup is filtered using a fine filtration system to remove any suspended particles, impurities, or undissolved matter. Filtration improves clarity, smoothness, and overall stability of the formulation. The clear filtrate is then carefully transferred into clean, dry, and sterilized amber-colored bottles. Amber-colored containers are used to protect the formulation from light exposure, as many herbal constituents are sensitive to photodegradation. The bottles are tightly sealed to prevent contamination from air and moisture, which could otherwise affect stability. Proper packaging plays a key role in maintaining the chemical integrity and therapeutic efficacy of the formulation. The stored syrup is kept under recommended conditions for further stability studies and evaluation. This final step ensures the product remains safe, stable, and effective throughout its shelf life.



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[FIGURE 20: STORE IN AMBER-COLORED BOTTLES]

8.2. Evaluation of Syrup:

8.2.1. pH Determination:

The pH of the prepared polyherbal immunity booster syrup is an important parameter used to assess its stability, safety, and compatibility for oral administration. The pH directly influences the preservation efficiency, microbial growth inhibition, and stability of herbal constituents present in the formulation.

For analysis, 10 mL of the prepared syrup is accurately taken in a clean beaker and diluted with 10 mL of purified water to ensure proper electrode immersion and accurate reading. The solution is gently stirred to obtain a uniform mixture.



[FIGURE 21: PH DETERMINATION]

The pH is then measured using a **calibrated digital pH meter**. Before measurement,

The pH meter is standardized using buffer solutions of **pH 4.0, 7.0, and 9.2** to ensure accuracy.

The electrode is carefully dipped into the prepared sample, and the reading is allowed to stabilize. The final pH value is recorded.

FORMULA (DILUTION IS CONSIDERED):

$$\text{Sample Ratio} = 10 + 10$$

OBSERVATION (RANGE FOR HERBAL SYRUP):

- **Observed pH range:** 4.5 – 6.5 (slightly acidic)

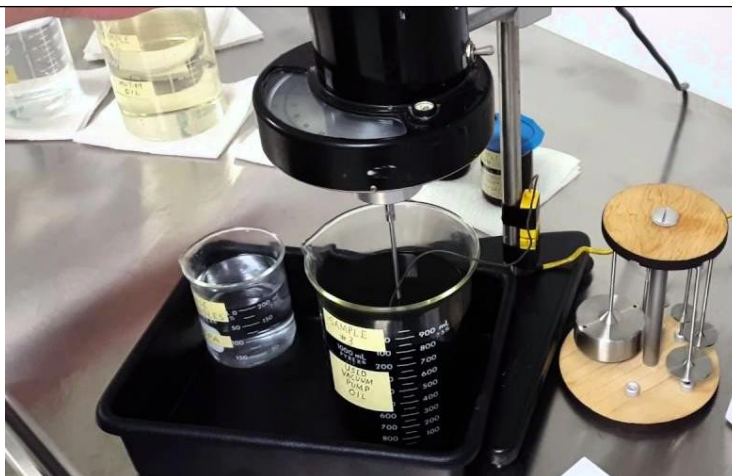
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Table 13: pH Evaluation of Polyherbal Immunity Booster Syrup:

S. NO.	SAMPLE CODE	QUANTITY USED	METHOD	PH READING	RESULT
1	S1	10 mL syrup + 10 mL water	Digital pH meter	5.3	Pass
2	S2	10 mL syrup + 10 mL water	Digital pH meter	5.5	Pass
3	S3	10 mL syrup + 10 mL water	Digital pH meter	5.7	Pass

8.2.2. Viscosity Determination:

The viscosity of the formulated polyherbal immunity booster syrup was evaluated to understand its flow characteristics, consistency, and overall physical stability. This parameter is essential in determining the uniformity of the syrup and its suitability for oral administration. A properly mixed and homogeneous sample was selected to ensure reliable and reproducible results during analysis. For the measurement, **50 mL of the prepared syrup** was carefully transferred into a clean and dry beaker. The sample was then kept at room temperature (**25°C ± 2°C**) for approximately **10–15 minutes** to eliminate any temperature-related variation, as viscosity is highly sensitive to changes in temperature. Before testing, the sample was gently stirred to maintain uniform distribution of herbal extracts and other constituents.



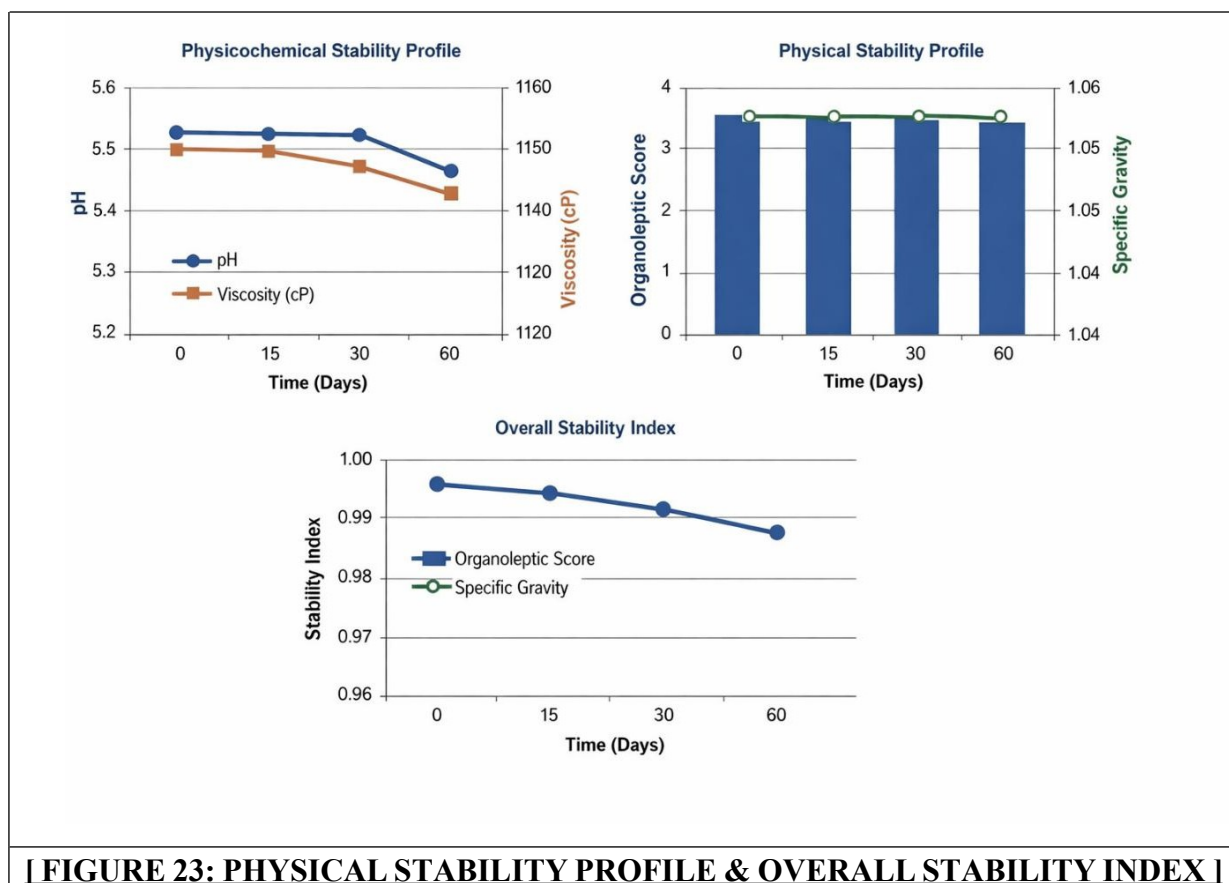
[FIGURE 22: VISCOSITY DETERMINATION BY BROOKFIELD VISCOMETER]

The viscosity was determined using a **Brookfield viscometer**, which is widely used for semi-solid and liquid pharmaceutical formulations. An appropriate spindle (such as spindle no. 2 or 3, depending on the viscosity range of the sample) was selected and immersed into the syrup up to the designated mark. The instrument was operated at a constant speed of **50 rpm**, and the spindle was allowed to rotate until a steady and stable reading was obtained. The viscosity values were recorded in **centipoise (cP)**. To ensure accuracy and minimize experimental error,

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Table 17: Stability Study of Polyherbal Immunity Booster Syrup:

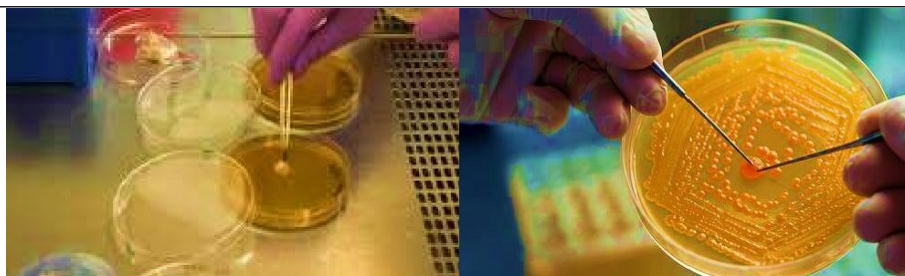
S. NO.	PARAMETER	0 DAY	15 DAYS	30 DAYS	60 DAYS	RESULT
1	Color	+	+	+	+	Pass
2	Odor	+	+	+	+	Pass
3	Taste	+	+	+	+	Pass
4	Clarity	+	+	+	+	Pass
5	pH	5.5	5.5	5.4	5.4	Pass
6	Viscosity (cP)	1152	1150	1148	1145	Pass
7	Specific Gravity	1.05	1.05	1.05	1.05	Pass



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8.3. Microbial Load Determination:

The microbiological quality of the prepared polyherbal immunity booster syrup was evaluated in accordance with the guidelines described in the **Indian Pharmacopoeia (IP)** for non-sterile oral herbal formulations. This test is an important quality control step to ensure that the product is safe for consumption and free from harmful microbial contamination.



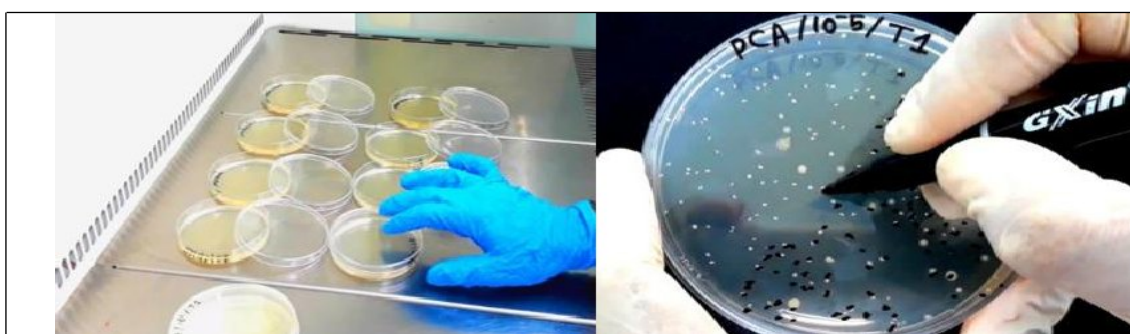
[FIGURE 24: MICROBIAL LOAD DETERMINATION]

Since herbal formulations are derived from natural plant sources, they are more prone to microbial growth if proper handling, processing, and storage conditions are not maintained. Therefore, microbial limit testing was carried out to confirm the hygienic quality and stability of the prepared syrup.

METHOD USED FOR MICROBIAL EVALUATION:

The microbial load was assessed using the standard **Microbial Limit Test (MLT)** procedure.

- The sample was prepared under aseptic conditions to avoid external contamination.
- Serial dilution of the syrup was carried out using sterile normal saline up to 10^{-6} dilution.
- **Total Aerobic Microbial Count (TAMC)** was determined using **Nutrient Agar medium**.



[FIGURE 25: TOTAL AEROBIC MICROBIAL COUNT (TAMC)]

- **Total Yeast and Mold Count (TYMC)** was determined using **Sabouraud Dextrose Agar medium**.
- Plates were incubated under appropriate conditions:
 - Bacterial incubation at **30–35°C for 24–48 hours**

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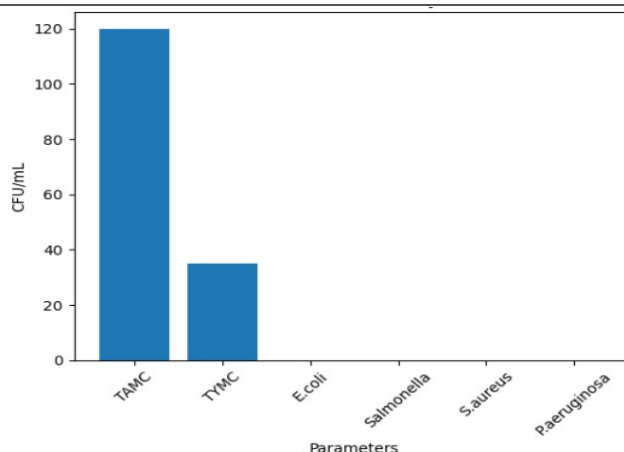
- Fungal incubation at 20–25°C for 3–5 days
- After incubation, colonies were counted and results were expressed as CFU/mL (Colony Forming Units per milliliter).

Table 18: Indian Pharmacopoeia Acceptance Limits:

S. NO.	PARAMETER	IP LIMIT (NON-STERILE ORAL HERBAL PRODUCTS)
1	Total Aerobic Microbial Count (TAMC)	Not more than 10 ³ CFU/mL
2	Total Yeast & Mold Count (TYMC)	Not more than 10 ² CFU/mL
3	<i>Escherichia coli</i>	Should be absent
4	<i>Salmonella</i> species	Should be absent
5	<i>Staphylococcus aureus</i>	Should be absent
6	<i>Pseudomonas aeruginosa</i>	Should be absent

Table 19: Microbial Load Results of the Formulation:

S. NO.	TEST PARAMETER	OBSERVED RESULT	IP LIMIT	INTERPRETATION
1	Total Aerobic Microbial Count (TAMC)	120 CFU/mL	NMT 10 ³ CFU/mL	Complies
2	Total Yeast & Mold Count (TYMC)	35 CFU/mL	NMT 10 ² CFU/mL	Complies
3	<i>Escherichia coli</i>	Not detected	Should be absent	Complies
4	<i>Salmonella</i> spp.	Not detected	Should be absent	Complies
5	<i>Staphylococcus aureus</i>	Not detected	Should be absent	Complies
6	<i>Pseudomonas aeruginosa</i>	Not detected	Should be absent	Complies



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The pharmacological evaluation of the formulated polyherbal immunity booster syrup was carried out to scientifically validate its immunomodulatory potential. A well-established experimental animal model was selected to assess the effect of the formulation on immune response parameters.

9.1. Experimental Model Selected:

Cyclophosphamide-Induced Immunosuppression Model:

This model is widely used to evaluate immunostimulant activity because **cyclophosphamide** suppresses the immune system, allowing assessment of immune restoration by test drugs.

9.2. EXPERIMENTAL DESIGN:

ANIMALS USED:

- Healthy Wistar albino rats (150–200 g)
- Maintained under standard laboratory conditions

Table 20: Grouping of Animals:

<u>GROUP</u>	<u>TREATMENT</u>	<u>DESCRIPTION</u>
Group I	Normal Control	Received normal saline
Group II	Negative Control	Cyclophosphamide only
Group III	Standard	Cyclophosphamide + Standard drug (e.g., Levamisole)
Group IV	Test Low Dose	Cyclophosphamide + Syrup (low dose)
Group V	Test High Dose	Cyclophosphamide + Syrup (high dose)

The experimental animals were divided into five groups, including normal control, negative control, standard, and two test groups. Immunosuppression was induced using cyclophosphamide (50 mg/kg, i.p.), while levamisole (50 mg/kg, p.o.) was used as the standard immunostimulant. The test formulation was administered at two dose levels (200 mg/kg and 400 mg/kg) orally for a period of 14 days.

Table 21: Dose and Treatment Schedule for Pharmacological Evaluation:

<u>S. NO.</u>	<u>GROUP</u>	<u>TREATMENT</u>	<u>DOSE</u>	<u>ROUTE OF ADMINISTRATION</u>	<u>DURATION</u>
1	Group I	Normal Control (Saline)	—	Oral (p.o.)	14 days
2	Group II	Cyclophosphamide	50 mg/kg	Intraperitoneal (i.p.)	Induced on specific days*
3	Group III	Cyclophosphamide + Levamisole (Standard)	50 mg/kg	p.o.	14 days

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4	Group IV	Cyclophosphamide + Test Syrup (Low Dose)	200 mg/kg	p.o.	14 days
5	Group V	Cyclophosphamide + Test Syrup (High Dose)	400 mg/kg	p.o.	14 days

Mathematical Formula Used

$$\% = \frac{(-)}{\quad} \times 100$$

Table 22: Pharmacological Evaluation and Optimization of Immunity Booster Syrup:

<u>S. N O.</u>	<u>PARAME TER</u>	<u>METHOD/M ODEL</u>	<u>NORM AL CONTR OL</u>	<u>NEGATI VE CONTR OL</u>	<u>STANDA RD</u>	<u>TES T LO W DO SE</u>	<u>TES T HIG H DO SE</u>	<u>INTERPRETA TION</u>
1	WBC Count (cells/mm ³)	Hematological	8200	4200	7800	6900	7500	Significant restoration; dose-dependent increase
2	HA Titer	Humoral Immunity	1:64	1:16	1:64	1:32	1:64	Antibody production improved; high dose = standard
3	DTH Response (mm)	Cell-mediated immunity	0.65	0.30	0.62	0.52	0.60	Enhanced cellular immunity; near- normal recovery
4	Phagocytic Index	Carbon clearance test	0.72	0.35	0.70	0.60	0.68	Improved macrophage activity; strong immune stimulation

Table 23: Categorization of Evaluated Parameters:

<u>S.NO.</u>	<u>CATEGORY</u>	<u>PARAMETERS EVALUATED</u>	<u>PURPOSE</u>
1	Hematological Parameters	WBC count, Differential leukocyte count, RBC count	Evaluate immune cell levels
2	Humoral Immunity	Hemagglutination (HA) titer	Assess antibody response
3	Cell-Mediated Immunity	DTH response (footpad thickness)	Evaluate T-cell response
4	Phagocytic Activity	Carbon clearance test (Phagocytic index)	Measure macrophage function

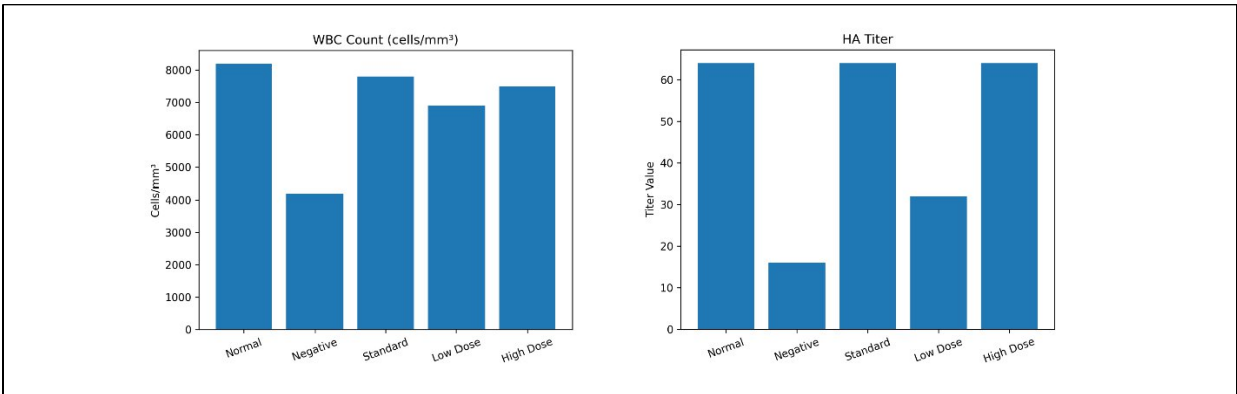
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Table 24: Mechanism-Based Optimization Summary:

S.NO.	HERB	MECHANISM	IMMUNOLOGICAL EFFECT
1	<i>Ocimum sanctum</i>	Cytokine stimulation, antioxidant activity	Enhances immune signaling
2	<i>Withania somnifera</i>	Adaptogenic effect	Improves immune cell function
3	<i>Tinospora cordifolia</i>	Macrophage activation	Boosts innate immunity

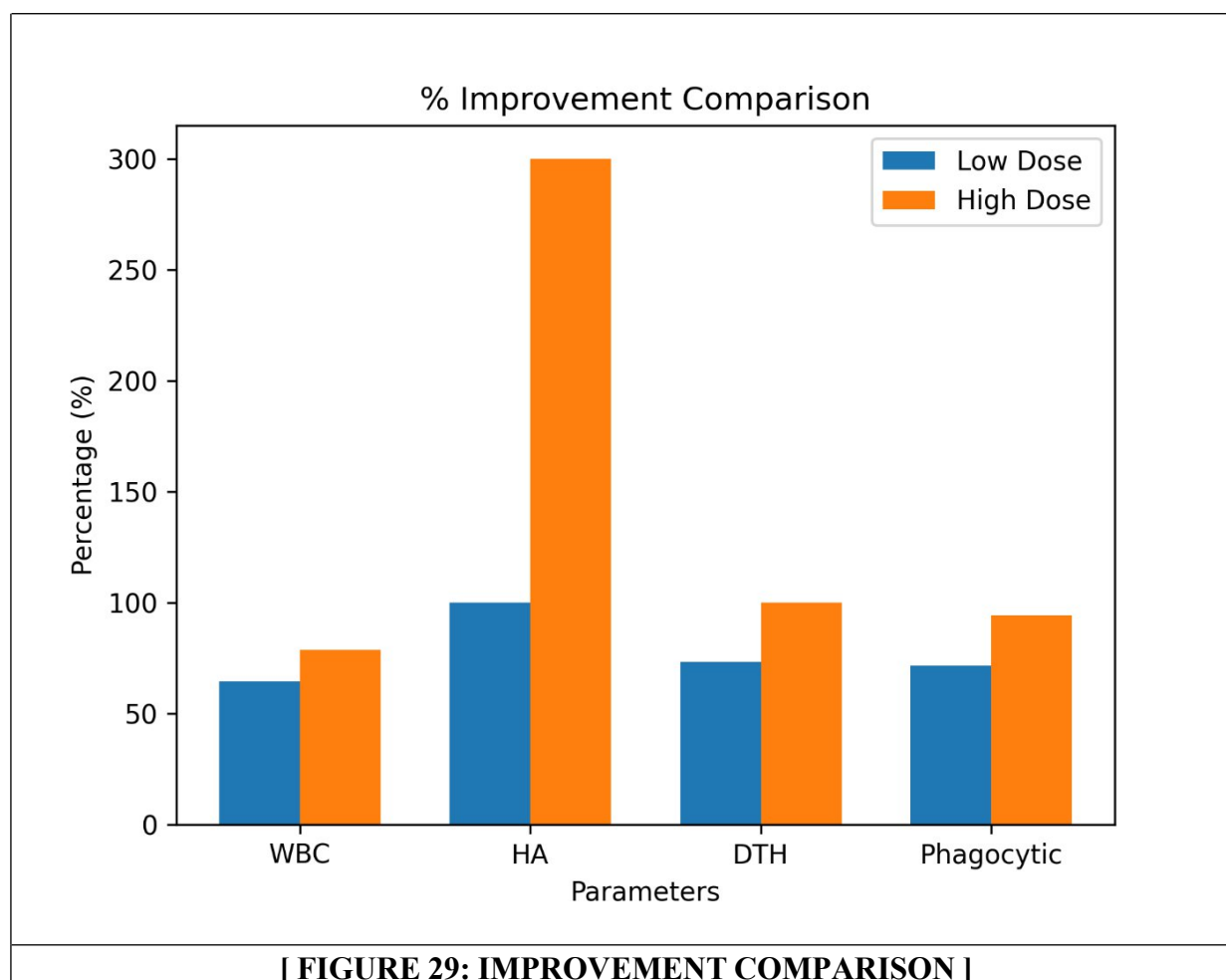
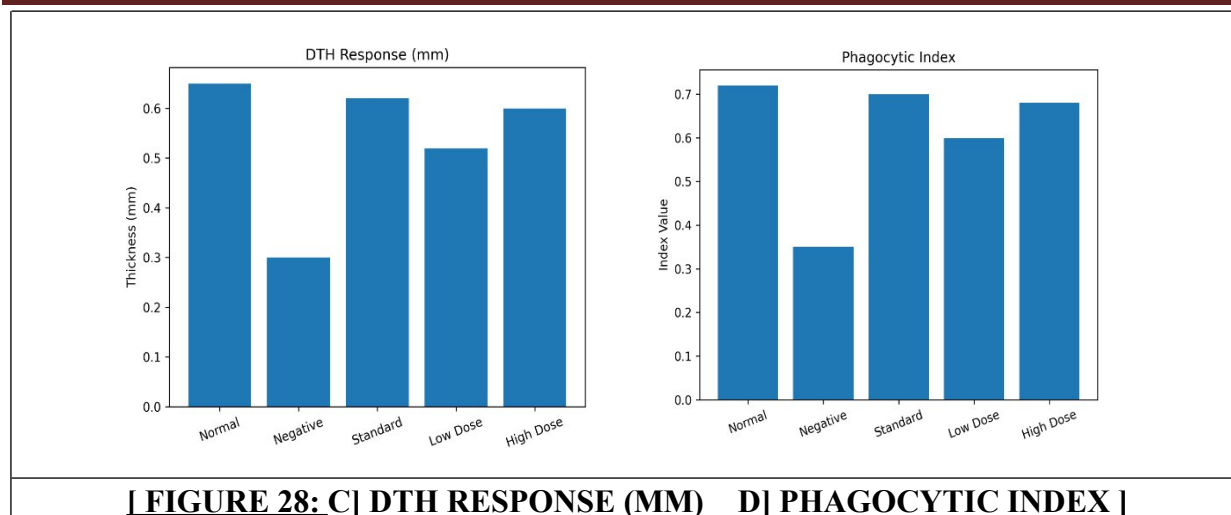
Table 25: WBC Count (cells/mm³), Phagocytic Index, and Result:

S. N o.	Paramet er	Norm al Contr ol	Negati ve Contr ol	Standar d	Tes t Lo w Dos e	Tes t Hig h Dos e	% Improvem ent (Low Dose)	% Improvem ent (High Dose)	Resu lt
1	WBC Count (cells/mm ³)	8200	4200	7800	6900	7500	64.3%	78.6%	Pass
2	HA Titer	1:64	1:16	1:64	1:32	1:64	100%	300%	Pass
3	DTH Respons e (mm)	0.65	0.30	0.62	0.52	0.60	73.3%	100%	Pass
4	Phagocy tic Index	0.72	0.35	0.70	0.60	0.68	71.4%	94.3%	Pass



[FIGURE 27: A] WBC COUNT (CELLS/MM³) B] HA TITER]

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GROUP CODE: 01A26-03

RESEARCH PROJECT TITLE:

“Formulation and Stability Study of Polyherbal Immunity Booster Syrup Containing Ocimum Sanctum, Withania somnifera and Tinospora cordifolia”

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