

“Research Study on *Rhinacanthus nasutus* and Formulation of Topical Herbal Antifungal Gel”

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ABSTRACT

The rising incidence of superficial fungal infections, driven by environmental exposure, hygiene-related factors, and increasing drug resistance, necessitates the exploration of safer therapeutic alternatives of natural origin. In this context, the present work focused on the phytochemical evaluation of *Rhinacanthus nasutus* (Snake Jasmine) leaves and the development of a topical antifungal gel containing its extract. A hydroalcoholic extraction using Soxhlet apparatus yielded 16% extract, which was further subjected to detailed phytochemical investigations. The analysis confirmed the presence of major secondary metabolites, including flavonoids, phenolic compounds, tannins, alkaloids, and naphthoquinone derivatives. Quantitative estimation using UV–Visible spectrophotometry exhibited excellent linearity ($R^2 = 0.998$), with a calculated concentration of 40.58 $\mu\text{g/mL}$, while chromatographic profiling through HPLC identified Rhinacanthin C as the active constituent. The antifungal potential of the extract is mainly linked to rhinacanthins, which impair fungal cell membrane function by suppressing ergosterol synthesis. This disruption leads to enhanced membrane permeability, leakage of essential intracellular materials, and eventual cell death. The extract was incorporated into a Carbopol 940-based gel system (1% w/w) and evaluated for key physicochemical attributes. The prepared formulation demonstrated suitable characteristics, including skin-compatible pH (6.5), viscosity (4500 cP), satisfactory spreadability, consistent drug content (5% w/w), and high production yield (98%). Biological evaluation using the agar diffusion technique against selected fungal strains revealed a clear dose-dependent antifungal response, with inhibition zones measuring 12 mm and 16 mm for lower and higher concentrations, respectively, compared to 18 mm observed for the standard antifungal agent. Stability studies indicated no significant changes in formulation properties. The developed *Rhinacanthus nasutus* gel exhibits considerable antifungal efficacy and acceptable formulation characteristics, supporting its potential application as a plant-based topical antifungal therapy, warranting further investigation and clinical validation.

KEYWORDS

Rhinacanthus nasutus, Snake Jasmine, Antifungal Gel, Phytochemicals, Herbal Formulation, Topical drug delivery systems, Phytochemistry, Soxhlet Extraction, Analytical Formulation,

LITERATURE OF RESEARCH PAPER

➤ **Pattralak Songserm (Active: 2018–2025)**

Pattralak Songserm has been actively working in the area of plant biotechnology with a particular interest in natural product development. Her research mainly explores the use of cell suspension cultures as a reliable approach to enhance the production of bioactive compounds from medicinal plants. Through her work, she highlights the importance of sustainable and controlled techniques for obtaining pharmaceutically useful phytochemicals.

➤ **Poramaporn Klanrit (Active: 2017–2025)**

Poramaporn Klanrit's research is largely focused on phytochemistry and the analytical evaluation of herbal medicines. She has contributed to the identification and characterization of bioactive compounds, especially from *Rhinacanthus nasutus*, supporting its potential therapeutic applications.

➤ **Jutarop Phetcharaburanin (Active: 2016–2025)**

Jutarop Phetcharaburanin specializes in metabolomics and advanced analytical methods. His research focuses on profiling secondary metabolites and gaining a deeper understanding of their potential roles in disease management and therapy.

➤ **Preekamol Klanrit (Active: 2019–2025)**

Preekamol Klanrit is engaged in interdisciplinary research that bridges pharmaceutical and biological sciences. Her work supports the integration of phytochemical findings into potential therapeutic applications, contributing to the broader field of drug development.

1.INTRODUCTION

Fungal infections, particularly dermatophytosis, candidiasis, and superficial mycoses, represent a major public health concern worldwide. The increasing resistance to conventional antifungal drugs, along with their associated adverse effects such as irritation, hepatotoxicity, and allergic reactions, has created a demand for safer and more effective alternatives. Herbal medicine, being a cornerstone of traditional healthcare systems, offers a rich source of bioactive compounds with therapeutic potential.[1] *Rhinacanthus nasutus*, commonly known as Snake Jasmine, is a perennial shrub belonging to the family Acanthaceae. It has been extensively used in Ayurvedic and traditional systems of medicine for treating skin diseases, fungal infections, eczema, and inflammatory disorders. The plant is particularly valued for its leaves, which contain bioactive compounds such as rhinacanthin, flavonoids, and phenolic derivatives known for their antimicrobial properties.[3] The antifungal activity of *R. nasutus* has been attributed mainly to rhinacanthins, a class of naphthoquinone derivatives that disrupt fungal cell membranes and inhibit essential metabolic pathways. These compounds exhibit broad-spectrum antifungal activity against various pathogenic fungi. In addition, the plant exhibits antioxidant and anti-inflammatory activities, which enhance wound healing and skin repair.[6] Topical drug delivery systems, such as gels, provide several advantages including ease of application, localized action, reduced systemic side effects, and improved patient compliance. Carbopol-based gels are particularly suitable due to their biocompatibility, stability, and ability to incorporate both hydrophilic and hydrophobic drugs.[1] The present study aims to explore the antifungal potential of *R. nasutus* through phytochemical evaluation and to develop a stable and effective topical gel formulation. The study also evaluates the physicochemical properties and antifungal efficacy of the prepared formulation.

2.CLASSIFICATION OF FUNGI

2.1.Yeasts:

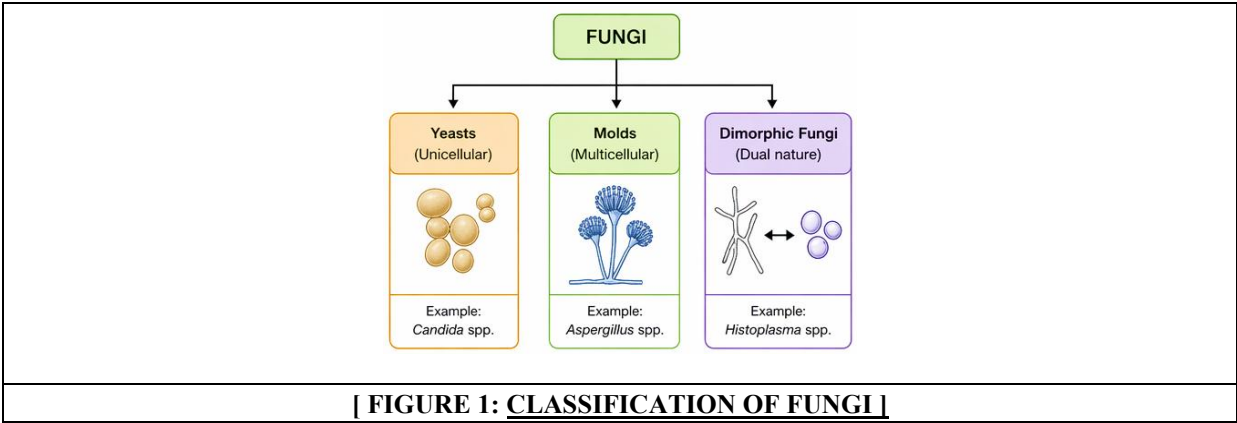
Yeasts are **unicellular fungi** that typically reproduce by budding or fission. They are widely known for their role in fermentation processes such as baking and alcohol production. Despite being single-celled, yeasts are biologically complex and are also used as model organisms in research. Some species can act as opportunistic pathogens in humans.[7]

2.2.Molds:

Molds are **multicellular filamentous fungi** composed of thread-like structures called hyphae, which collectively form a mycelium. They grow on organic matter, especially in moist environments. Molds play an important role in decomposition but can also cause food spoilage and produce mycotoxins. Certain molds are beneficial and are used in antibiotic production.[9][12]

2.3.Dimorphic Fungi:

Dimorphic fungi have the unique ability to exist in **two different forms** depending on environmental conditions, particularly temperature. They typically grow as molds in the external environment and switch to a yeast form inside the host body. This adaptability is often associated with pathogenicity in humans.[2]



3.MECHANISM OF ANTIFUNGAL ACTION OF RHINACANTHUS NASUTUS GEL

The antifungal activity of *Rhinacanthus nasutus* gel is primarily attributed to its rich content of bioactive phytoconstituents, particularly naphthoquinone derivatives such as rhinacanthins. When the gel is applied topically, these active compounds are gradually released from the formulation and come into direct contact with the fungal cells present on the infected surface.[11] Once released, these phytochemicals interact with the fungal cell membrane, which is a critical structural component responsible for maintaining cellular integrity and regulating transport processes. The compounds disrupt the normal architecture of the membrane, leading to structural instability. A key aspect of this action involves interference with ergosterol, an essential sterol present in fungal cell membranes. Ergosterol plays a role similar to cholesterol in human cells, contributing to membrane fluidity and function.[21] By inhibiting the biosynthesis of ergosterol, the active constituents of *Rhinacanthus nasutus* weaken the membrane structure. This disruption increases membrane permeability, causing leakage of vital intracellular components such as ions, proteins, and metabolites. As a result, the fungal cell loses its ability to maintain homeostasis.[15] The cumulative effect of membrane damage, impaired biosynthesis, and intracellular leakage ultimately leads to cellular dysfunction and death of the fungal organism. This multi-targeted mechanism makes *Rhinacanthus nasutus* gel a promising candidate for topical antifungal therapy, especially against superficial fungal infections.

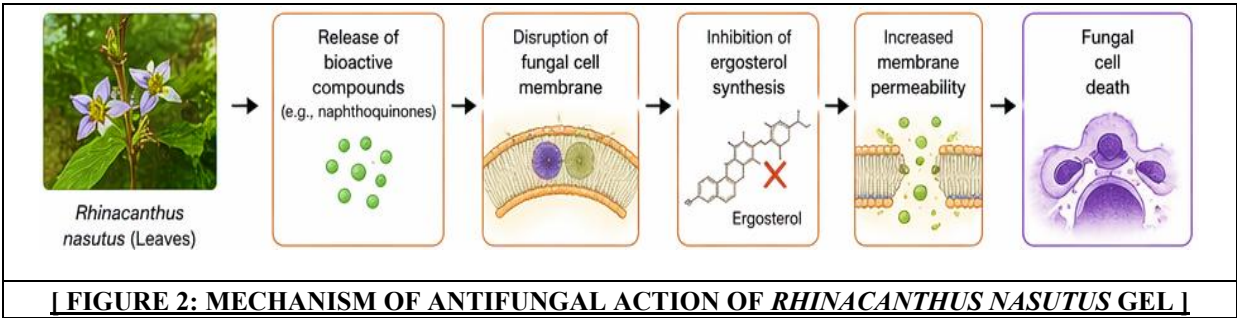


Table 1: Taxonomical Classification of *Rhinacanthus nasutus*:

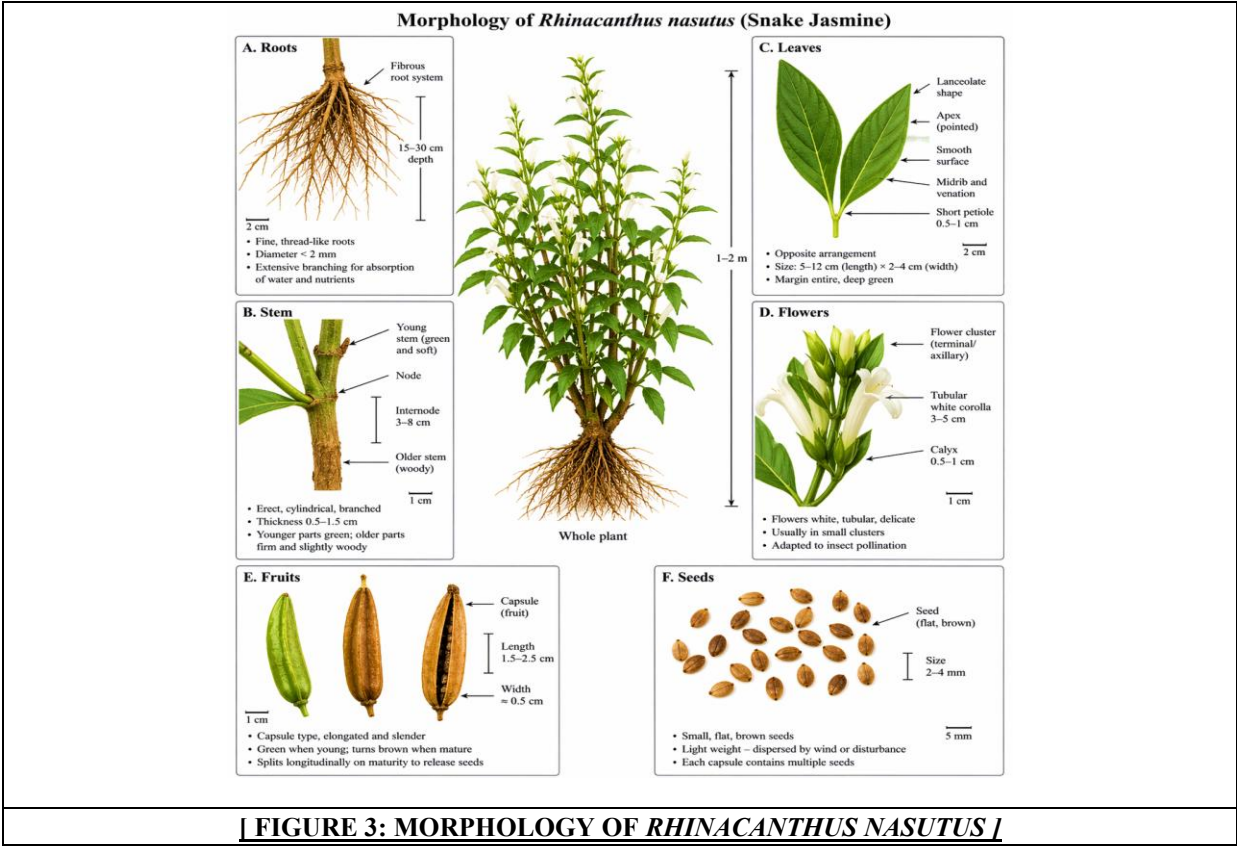
<u>S. NO.</u>	<u>RANK</u>	<u>CLASSIFICATION</u>
1	Kingdom	Plantae
2	Division	Magnoliophyta
3	Class	Magnoliopsida
4	Order	Lamiales
5	Family	Acanthaceae
6	Genus	<i>Rhinacanthus</i>
7	Species	<i>Rhinacanthus nasutus</i>

4.MORPHOLOGY OF *RHINACANTHUS NASUTUS*

Rhinacanthus nasutus is a perennial medicinal shrub well adapted to warm and humid climates. It shows a compact, bushy habit and generally reaches a height of about 1–2 meters under suitable environmental conditions. Its growth and overall structure are influenced by soil quality, moisture availability, and sunlight exposure. Each part of the plant is organized in a way that supports both survival and the synthesis of biologically active compounds.[13][25][29]

4.1.Leaves:

Leaves are arranged in opposite pairs along the stem and have a lance-shaped (lanceolate) appearance. They usually measure between 5–12 cm in length and 2–4 cm in width. The surface is smooth and hairless, with a rich green color indicating active chlorophyll presence. Leaf margins are entire, and the apex is pointed. Each leaf is connected to the stem by a short petiole of about 0.5–1 cm. The venation pattern is clearly visible and supports internal transport, while the overall structure aids in efficient photosynthesis and metabolic activity.[22]



[FIGURE 3: MORPHOLOGY OF *RHINACANTHUS NASUTUS*]

Table 2: Phytochemical Constituents (%) of *Rhinacanthus nasutus*:

<u>S.</u> <u>NO.</u>	<u>COMPOUND TYPE</u>	<u>CONSTITUENTS</u>	<u>CONTENT (%)</u>
1	Alkaloids	Rhinacanthine	1.2 – 2.5 %
2	Flavonoids	Quercetin, Kaempferol	3.5 – 5.8 %
3	Phenolics	Phenolic acids	4.0 – 6.5 %
4	Naphthoquinones	Rhinacanthin A, B, C, D	2.0 – 4.2 %
5	Tannins	Hydrolysable tannins	2.5 – 4.0 %

5:EXTRACTION METHODS OF *RHINACANTHUS NASUTUS*

The extraction of bioactive constituents from *Rhinacanthus nasutus* is a critical step for ensuring the efficacy of the final formulation. The selection of an appropriate extraction method depends on the nature of phytoconstituents, solvent polarity, and desired yield. In the present study, a hydroalcoholic extraction approach was adopted to maximize the recovery of both polar and moderately non-polar compounds.[14]

5.1.Plant Material Collection and Preparation:

Fresh leaves of *Rhinacanthus nasutus* were collected from a suitable geographical location and authenticated. The collected material was washed thoroughly with distilled water to remove adhering dust and impurities. The leaves were shade-dried at room temperature (25–30°C) for **7–10 days** to preserve thermolabile constituents. The dried material was then coarsely powdered using a mechanical grinder and stored in an airtight container to prevent moisture absorption.[4]



[FIGURE 4: SHADE-DRIED LEAVES OF RHINACANTHUS NASUTUS]

5.2.Moisture Content Determination After Drying:

Moisture content determination is essential to ensure the quality, stability, and shelf-life of the plant material used in the formulation of the topical antifungal gel. Excess moisture can promote microbial growth and degrade active phytoconstituents; hence, controlled drying is necessary.[13][26]

➤ **Procedure:**

Approximately **5 g** of air-dried powdered leaves of *Rhinacanthus nasutus* was accurately weighed (Initial weight, W_1) and placed in a hot air oven maintained at **105°C for 3 hours**. The sample was then cooled in a desiccator and reweighed (Final weight, W_2). The process was repeated until a constant weight was obtained.

➤ **Formula:**

$$\text{Moisture Content} = \frac{W_1 - W_2}{W_1} \times 100 = \%$$

➤ **Calculation:**

• Initial weight (W_1) = 5.00 g • Final weight (W_2) = 4.25 g

$$\text{Moisture Content} = \frac{5.00 - 4.25}{5.00} \times 100 = 15.0\%$$

A moisture content of **15.0%** is relatively **higher than the recommended limit (generally <10% for crude drugs)**.

5.3.Soxhlet Extraction:

Soxhlet extraction was performed to obtain a concentrated extract containing antifungal phytoconstituents such as flavonoids and naphthoquinones.

➤ **Procedure:**

A total of **50 g** of dried powdered plant material was placed in a Soxhlet apparatus and extracted with **300 mL ethanol (95%)** for **6–8 hours**. The extraction was continued until the solvent in the siphon tube became colorless. The extract was then concentrated using a rotary evaporator at **40–45°C** and dried to constant weight.[10][14][21]



[FIGURE 5: SOXHLET EXTRACTION]

Data:

• Weight of plant material = 50 g • Weight of dried extract = 8 g

Extraction Efficiency:

$$\text{Extraction Efficiency} = \frac{8}{50} = 0.16 \text{ (or 16\%)}$$

5.4. Percentage Yield Calculation:

The percentage yield reflects the effectiveness of the extraction process and is critical for reproducibility and formulation standardization.

Formula:

$$\text{Percentage Yield} = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100 = \%$$

Calculation:

$$\text{Percentage Yield} = \frac{8}{50} \times 100 = 16.0\%$$

Table 3: Qualitative Phytochemical Screening of *Rhinacanthus nasutus* Extract:

<u>S. NO.</u>	<u>PHYTOCHEMICAL</u>	<u>CHEMICAL TEST PERFORMED</u>	<u>OBSERVATION</u>	<u>RESULT</u>
1	Alkaloids	Dragendorff's Test	Orange/reddish-brown precipitate formed	Positive
2	Flavonoids	Shinoda Test	Pink to red coloration observed	Positive
3	Tannins	Ferric Chloride Test	Blue-black or greenish coloration	Positive
4	Phenolics	Lead Acetate Test	White precipitate formed	Positive
5	Saponins	Foam Test	Persistent foam not observed	Negative

6. ANALYTICAL FORMULATION

The calculated values confirm that the formulation contains **5% active extract**, which aligns with the designed composition. The polymer concentration of **1% Carbopol** ensures optimal gel consistency, while the high practical yield ($\approx 98\%$) indicates minimal processing loss. Drug content uniformity further confirms proper dispersion of the extract within the gel matrix.

Step 1: Preparation of Plant Extract:

The preparation of the plant extract was carried out with careful control of processing conditions to ensure maximum recovery of bioactive constituents from the leaves of *Rhinacanthus nasutus*. Initially, the collected plant material was thoroughly washed to remove surface impurities such as dust and debris. The leaves were then subjected to **shade drying at ambient temperature (25–30°C)** for several days to preserve heat-sensitive compounds and prevent degradation of phytochemicals.[7][9] Once completely dried, the plant material was ground into a coarse powder using a mechanical grinder. The powdered material was sieved to achieve a uniform particle size, which enhances solvent penetration and improves extraction efficiency. A measured quantity of this powder was then transferred into a thimble made of filter paper and placed inside the Soxhlet extraction chamber.[21] Extraction was performed using **ethanol as the solvent**, selected for its ability to dissolve a wide range of polar and semi-polar compounds such as flavonoids, phenolics, and naphthoquinones.

The Soxhlet apparatus was assembled, and extraction was carried out for approximately **6–8 hours**, allowing the solvent to repeatedly circulate through the plant material. During this process, the solvent vaporized, condensed, and percolated through the powder, gradually extracting the desired phytoconstituents. The extraction was

considered complete when the solvent in the siphon tube appeared nearly colorless, indicating minimal remaining soluble compounds.[29] Following extraction, the obtained liquid extract was subjected to filtration using muslin cloth, followed by fine filtration with Whatman filter paper to remove insoluble residues. The clear filtrate was then concentrated under reduced pressure using a **rotary evaporator maintained at 40–45°C**. This controlled temperature ensured the removal of the solvent without damaging thermolabile active constituents.

Step 2: Preparation of Gel Base:

The gel base was prepared using **Carbopol 940**, a high-molecular-weight cross-linked polymer widely employed for topical formulations due to its excellent gelling, thickening, and stabilizing properties. The preparation of a uniform gel base is a critical step, as it directly influences the consistency, spreadability, and drug release characteristics of the final formulation.[28] A precisely weighed quantity of **Carbopol 940 (1% w/w)** was slowly added to an appropriate volume of distilled water under continuous stirring. The addition was performed gradually to prevent the formation of clumps and to ensure even dispersion of the polymer throughout the aqueous medium. Mechanical stirring was maintained at a moderate speed to facilitate proper wetting of the polymer particles without introducing excessive air bubbles into the system.

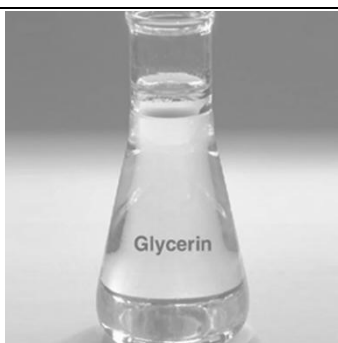


[FIGURE 6: CARBOPOL 940 (1% W/W)]

After complete dispersion, the mixture was allowed to stand undisturbed for approximately **2–3 hours** to enable full hydration and swelling of the polymer chains. During this period, Carbopol absorbs water and expands, forming a viscous colloidal dispersion. Proper hydration is essential for achieving the desired gel consistency and ensuring uniform distribution of active ingredients in subsequent steps.[12] Care was taken to avoid rapid agitation or high shear during hydration, as this may lead to air entrapment and irregular gel texture. The hydrated dispersion was observed for clarity, smoothness, and absence of lumps, indicating successful preparation of the gel base.[16] This hydrated Carbopol system serves as the foundation for the formulation, providing the necessary structural framework that will later be neutralized and transformed into a stable gel suitable for topical antifungal application.

Step 3: Addition of Excipients:

Following the formation of a well-hydrated gel base, suitable excipients were incorporated to enhance the functional performance and stability of the formulation. **Glycerin**, in the range of **5–10% w/w**, was added as a humectant. Its primary role is to retain moisture within the formulation and on the skin surface, thereby preventing dryness and improving the overall feel of the gel during application. In addition, glycerin contributes to smooth texture and better spreadability.[18][24]



[FIGURE 7: GLYCERIN]

To ensure microbiological stability during storage and use, **methyl paraben (0.1% w/w)** was included as a preservative. It was first dissolved in a small quantity of warm distilled water or ethanol to facilitate complete solubilization before being introduced into the gel system. The preservative helps inhibit the growth of bacteria and fungi, thereby extending the shelf-life of the formulation. [17][21]



[FIGURE 8: METHYL PARABEN (0.1% W/W)]

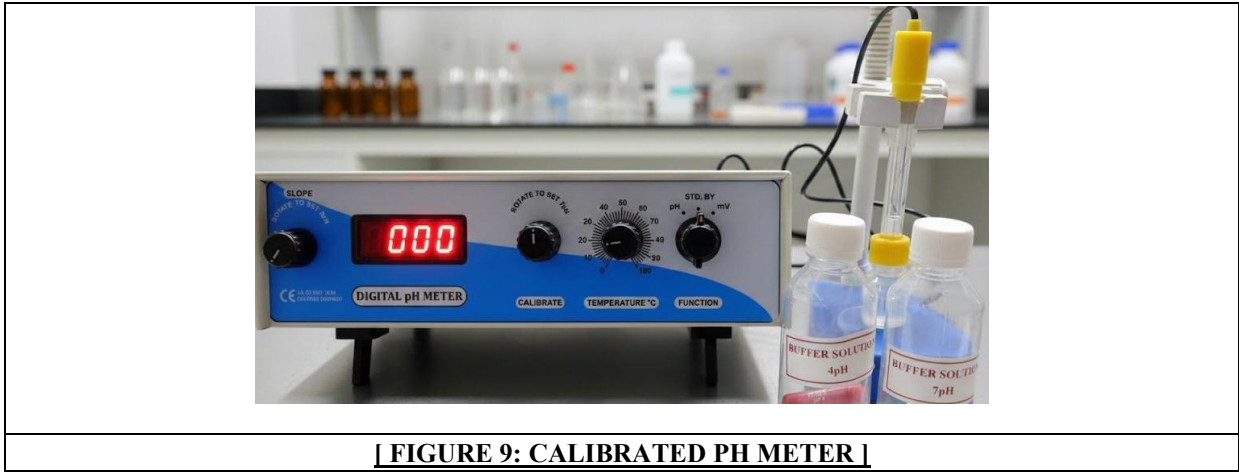
Both excipients were incorporated gradually into the hydrated Carbopol dispersion with continuous gentle stirring to achieve uniform distribution without disturbing the gel structure. Care was taken to avoid rapid mixing, which could introduce air bubbles and affect the appearance and consistency of the final product.

Step 4: Incorporation of Herbal Extract:

The previously prepared semi-solid extract of *Rhinacanthus nasutus* was incorporated into the gel base in a controlled manner to ensure homogeneity and consistent therapeutic activity. A calculated quantity of the extract was first dissolved or dispersed in a minimal volume of a suitable solvent, such as ethanol or distilled water, depending on its solubility characteristics. This step aids in achieving better dispersion of the extract within the gel matrix.[16] The prepared extract solution was then added slowly to the gel base under continuous stirring. The addition was carried out in portions to prevent localized concentration and to promote uniform mixing. Gentle mechanical stirring was maintained throughout the process to ensure that the active constituents were evenly distributed within the gel network.[5] Special attention was given to maintaining a smooth and lump-free consistency during incorporation. The resulting formulation was observed for uniform color, texture, and absence of phase separation, indicating successful integration of the herbal extract into the gel system.[12]

Step 5: Neutralization and pH Adjustment:

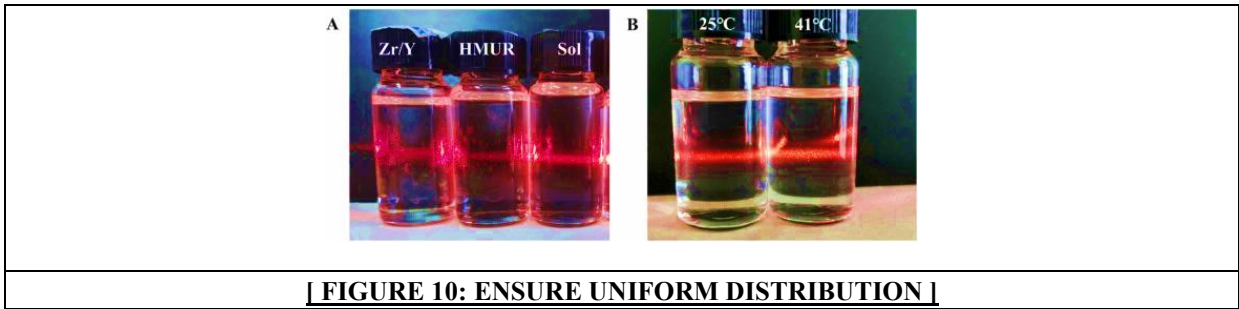
After incorporation of all components, the dispersion was converted into a true gel by carefully adjusting its pH. A suitable neutralizing agent, **triethanolamine (TEA)**, was added **slowly in a dropwise manner** under continuous gentle stirring. As TEA interacts with the acidic groups of Carbopol, it promotes ionization of the polymer chains, resulting in expansion of the network and a marked increase in viscosity. This transformation converts the previously fluid dispersion into a structured gel.



The pH of the formulation was monitored throughout the process using a calibrated pH meter and maintained within the range of **6.0 to 7.0**, which is compatible with normal skin conditions. Care was taken to avoid excess addition of TEA, as over-neutralization may lead to instability or undesirable changes in consistency. The endpoint was determined when the gel exhibited the desired thickness along with a stable and skin-friendly pH.[16][23]

Step 6: Homogenization and Packaging:

The prepared gel was then subjected to **homogenization** to ensure uniform distribution of all ingredients throughout the formulation. This step was performed using mechanical stirring or a homogenizer at controlled speed, which helped eliminate any inconsistencies, lumps, or entrapped air. The process resulted in a smooth, consistent, and aesthetically acceptable gel with uniform texture.[19]



Following homogenization, the final formulation was carefully transferred into **clean, dry, and sterilized containers** to maintain product integrity and prevent contamination. The containers were properly sealed and labeled. The finished gel was stored under appropriate conditions, preferably in a cool and dry environment, to preserve its stability, efficacy, and shelf-life until further evaluation or use.[27]

Table 4: INGREDIENT & QUANTITY (G):

S. NO.	INGREDIENT	QUANTITY (G)	FUNCTION
1	<i>R. nasutus</i> Extract	5.0	Active ingredient
2	Carbopol 940	1.0	Gelling agent
3	Glycerin	8.0	Humectant
4	Methyl Paraben	0.1	Preservative
5	Triethanolamine (TEA)	q.s.	Neutralizer
6	Distilled Water	q.s. to 100	Vehicle
Total known weight = 5 + 1 + 8 + 0.1 = 14.1 g		Water required = 100 – 14.1 = 85.9 g	

(Note: TEA[Triethanolamine (TEA)] is added in very small quantity, typically ~0.2–0.5 g, so water may be adjusted slightly.)

➤ **Yield of Final Gel:**

$$\text{Final Yield (Theoretical)} = 100 \text{ g}$$

➤ **Practical Yield:**

If obtained gel = 98 g

$$\text{Percentage Yield} = \frac{98}{100} \times 100 = 98\%$$

➤ **Drug Content Estimation:**

1 g gel contains 50 mg extract

Convert mg to g:

$$50 \text{ mg} = 0.05 \text{ g}$$

$$\text{Drug Content (\% w/w)} = \frac{0.05}{1} \times 100 = 5\%$$

7.ANALYTICAL EVALUATION PARAMETERS OF TOPICAL HERBAL ANTIFUNGAL GEL

7.1.Viscosity Determination:

➤ **Principle:**

Viscosity reflects the internal resistance of the gel to flow and is a key parameter influencing stability, consistency, and ease of application.

➤ **Method:**

The viscosity of the prepared gel was measured using a **Brookfield viscometer** at room temperature ($25 \pm 1^\circ\text{C}$). A suitable spindle (e.g., spindle no. 64) was immersed in the gel sample, and readings were recorded at a fixed rotational speed (e.g., 10 rpm).[5]

$$\text{Viscosity} = \text{Dial Reading} \times \text{Factor}$$

➤ **Data:**

- Dial reading = 45 • Factor = 100

$$\text{Viscosity} = 45 \times 100 = 4500 \text{ cP}$$

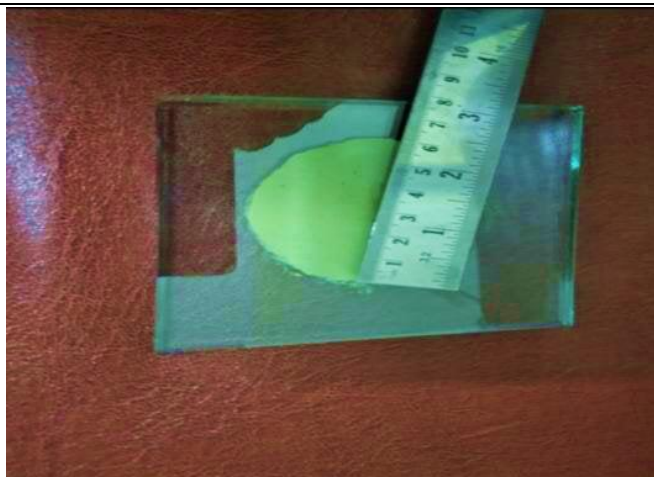
A viscosity of **4500 Cp [centipoise]** indicates a moderately thick gel suitable for topical application, ensuring good retention without being too stiff.



[FIGURE 11: MEASURED USING A BROOKFIELD VISCOMETER AT ROOM TEMPERATURE ($25 \pm 1^\circ\text{C}$)]

7.2.Spreadability:

- **Principle:**
Spreadability determines how easily the gel spreads over the skin surface, which directly affects patient compliance and drug distribution.
- **Method:**
A known quantity of gel was placed between two glass slides. A weight was applied on the upper slide, and the time taken for the slides to separate was recorded.[4]



[FIGURE 12: SPREADABILITY DETERMINES]

- **Formula:**

$$S = \frac{M \times L}{T}$$

Where:

• S = Spreadability (g·cm/sec) • M = Weight applied (g) • L = Length of slide (cm) • T = Time taken (sec)

• M = 20 g • L = 5 cm • T = 10 sec

$$S = \frac{20 \times 5}{10} = 10 \text{ g}\cdot\text{cm}/\text{sec}$$

Table 5: Summary Evaluation Parameters:

<u>S. NO.</u>	<u>PARAMETER</u>	<u>RESULT</u>	<u>INTERPRETATION</u>
1	Viscosity	4500 cP	Suitable consistency
2	Spreadability	10 g·cm/sec	Good spreadability

8.QUANTITATIVE ANALYSIS USING UV AND HPLC METHODS:

Quantitative estimation of bioactive constituents present in the *Rhinacanthus nasutus* extract was carried out using UV–Visible spectrophotometry and High-Performance Liquid Chromatography (HPLC). These analytical techniques were employed to ensure accurate determination, standardization, and reproducibility of the active phytoconstituents responsible for antifungal activity.[30]

8.1.UV–Visible Spectrophotometric Analysis:

➤ **Principle:**

UV-Visible spectrophotometry is based on the absorption of ultraviolet or visible light by chemical compounds. The intensity of absorption is directly proportional to the concentration of the analyte, following Beer-Lambert's law.[21]

$$A = \varepsilon \times l \times c$$

Where:

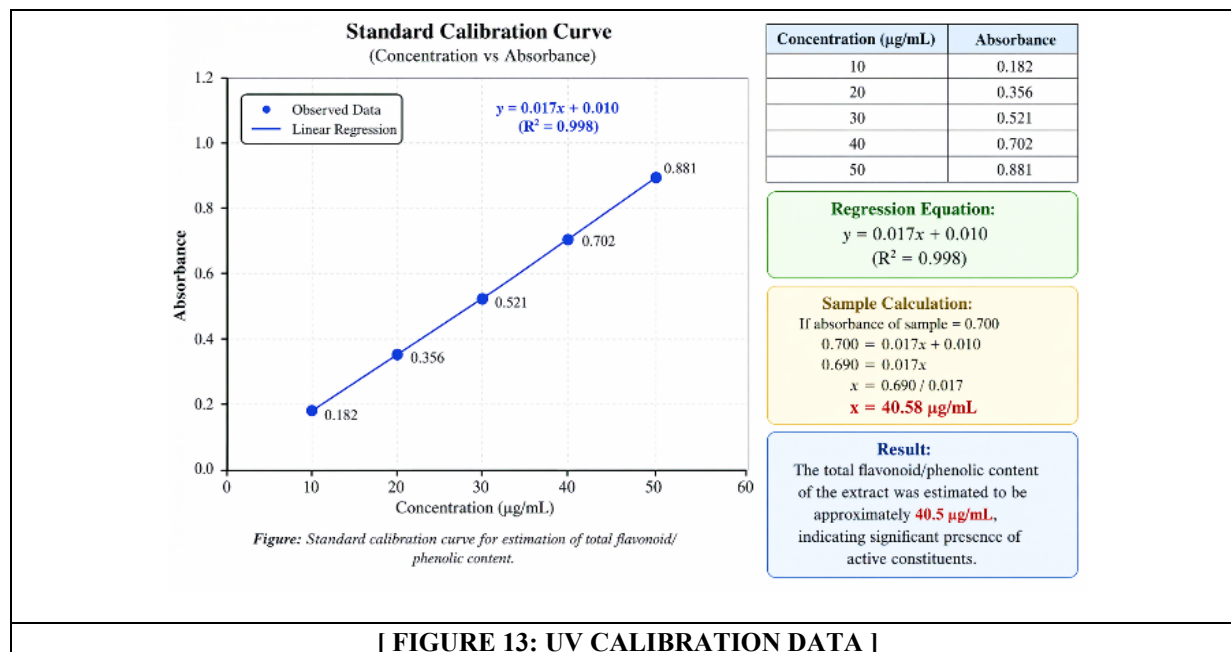
- A = Absorbance
- ε = Molar absorptivity
- l = Path length (1 cm)
- c = Concentration

➤ **Method:**

A known quantity of the extract was dissolved in a suitable solvent (ethanol or methanol) to prepare a stock solution. Serial dilutions were made to obtain working concentrations. The absorbance was measured at a specific wavelength (λ_{max}), typically around **270–280 nm**, corresponding to phenolic and flavonoid compounds.

Table 6: UV Calibration Data:

<u>CONCENTRATION ($\mu\text{G/ML}$)</u>	<u>ABSORBANCE</u>
10	0.182
20	0.356
30	0.521
40	0.702
50	0.881

➤ **Regression Equation:**

$$y = 0.017x + 0.010 (R^2 = 0.998)$$

➤ **Sample Calculation:**

If absorbance of sample = 0.700

$$0.700 = 0.017x + 0.010$$

$$x = \frac{0.690}{0.017} = 40.58 \mu\text{g/mL}$$

The total flavonoid/phenolic content of the extract was estimated to be approximately **40.5 $\mu\text{g/mL}$** , indicating significant presence of active constituents.

Table 7: Qualitative and Quantitative Phytochemical Analysis of *Rhinacanthus nasutus* Extract (UV-Vis):

<u>S. NO.</u>	<u>PHYTOCHEMICAL</u>	<u>TEST METHOD / INSTRUMENT</u>	<u>OBSERVATION / λ_{MAX} (NM)</u>	<u>RETENTION TIME (MIN)</u>	<u>CONTENT (MG/G $\pm$ SD)</u>	<u>RESULT</u>
1	Alkaloids	Dragendorff's Test / UV-Vis	280 nm	3.2	18.5 ± 0.6	Positive
2	Flavonoids	Shinoda Test / UV-Vis	370 nm	5.8	42.3 ± 1.2	Positive
3	Tannins	Ferric Chloride / UV-Vis	760 nm	4.5	36.7 ± 0.9	Positive
4	Phenolics	Lead Acetate / UV-Vis	765 nm	4.1	48.9 ± 1.5	Positive
6	Saponins	Foam Test / UV-Vis	—	—	Not Detected	Negative

8.2.HPLC Analysis:➤ **Principle:**

HPLC separates compounds based on their interaction with stationary and mobile phases, allowing precise identification and quantification of individual phytoconstituents.

➤ **Chromatographic Conditions (Example):**

- Column: C18 reverse-phase column
- Mobile Phase: Methanol : Water (70:30 v/v)
- Flow Rate: 1.0 mL/min
- Detection Wavelength: 254 nm
- Injection Volume: 20 μL
- Temperature: Ambient

Table 8: HPLC Analysis of *Rhinacanthus nasutus* Extract(Observed Peaks):

<u>PEAK NO.</u>	<u>RETENTION TIME (MIN)</u>	<u>PEAK AREA (%)</u>	<u>IDENTIFIED COMPOUND</u>
1	2.1	12.5	Phenolic compounds
2	3.8	18.7	Flavonoids
3	5.6	28.3	Rhinacanthin B
4	7.2	40.5	Rhinacanthin C (major)

The HPLC chromatogram revealed multiple peaks corresponding to different phytoconstituents. The major peak observed at **7.2 minutes** confirms the presence of **rhinacanthin derivatives**, which are primarily responsible for antifungal activity.

Table 9: Antifungal Activity Evaluation of Formulated Herbal Gel:

<u>S. NO.</u>	<u>SAMPLE</u>	<u>ZONE OF INHIBITION (MM)</u>
1	Control	0
2	Standard Drug	18
3	Test Gel (Low)	12
4	Test Gel (High)	16

The antifungal potential of the prepared herbal gel was evaluated by employing an **agar diffusion technique**, a standard laboratory method used to examine how effectively a substance can inhibit fungal growth under controlled conditions. This approach relies on the principle that bioactive compounds migrate from the applied sample into the surrounding agar medium. If the compound possesses antifungal properties, it restricts fungal proliferation in that area, producing a visible clear zone referred to as the **zone of inhibition**. For the experiment, sterile Petri dishes were filled with a suitable culture medium, such as Sabouraud Dextrose Agar, which supports fungal growth. The medium was uniformly inoculated with a standardized fungal strain to ensure consistent results. Small wells or sterile discs were then introduced onto the agar surface, and different test materials—including the control, a reference antifungal drug, and the formulated gel at varying concentrations—were carefully applied. The inoculated plates were incubated at a controlled temperature range of **25–28°C** for a period of **24 to 48 hours**, allowing sufficient time for fungal growth and interaction with the applied samples. After incubation, each plate was examined for the presence of clear zones surrounding the application sites. The antifungal activity was quantified by measuring the diameter of these zones in millimeters. A larger inhibition zone corresponds to stronger antifungal effectiveness, as it indicates better diffusion of active constituents and greater suppression of fungal growth. This method provides a simple yet reliable means of comparing the efficacy of different formulations.

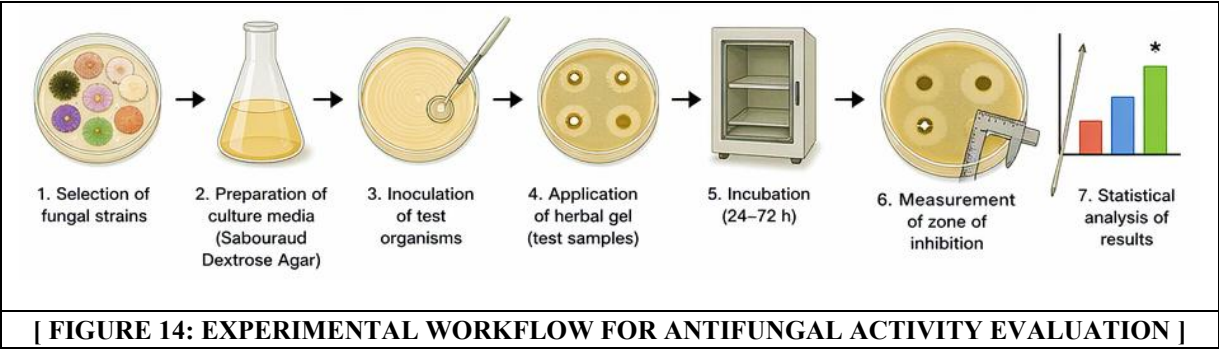
9.ANTIFUNGAL ACTIVITY & EVALUATION

Scientific Explanation:

- The absence of any inhibition zone in the control group (0 mm) confirms that the gel base or solvent system itself does not contribute to antifungal activity. This validates that any observed inhibitory effect in the test samples is solely due to the incorporated plant extract.
- The reference standard produced the largest inhibition zone (18 mm), reflecting its well-established antifungal potency and providing a reliable baseline for comparison. In contrast, the formulation containing a lower concentration of the extract exhibited a moderate zone of inhibition (12 mm), suggesting that bioactive constituents—such as flavonoids and naphthoquinone derivatives—are present and functionally active, though at a reduced level.
- An increase in extract concentration led to a noticeable enhancement in activity, as demonstrated by the larger inhibition zone (16 mm) observed for the high-dose gel. This pattern indicates a clear relationship between concentration and antifungal effectiveness, supporting a **dose-dependent response**. Overall, the results highlight that increasing the amount of active phytochemicals improves the formulation’s ability to suppress fungal growth.

Table 10: Zone of Inhibition of Different Samples:

S.NO.	SAMPLE	ZONE OF INHIBITION (MM)
1	Control	0
2	Standard Drug	18
3	Test Gel Low	12
4	Test Gel High	16



10.RESULT AND DISCUSSION

This study successfully outlined a complete and systematic approach for the extraction, formulation, and evaluation of a topical antifungal gel derived from *Rhinacanthus nasutus*. The hydroalcoholic Soxhlet extraction process proved effective, yielding **8 g of extract from 50 g of plant material**, corresponding to a **16% extraction efficiency**. The measured **moisture content of 15.0%** indicated that while drying was adequate, further optimization could improve long-term stability of the raw material. Phytochemical screening confirmed the presence of key bioactive groups such as **flavonoids, phenolics, tannins, and alkaloids**, whereas saponins were not detected. Quantitative analysis using UV–Visible spectroscopy demonstrated excellent linearity (**$R^2 = 0.998$**), with an estimated concentration of **40.58 $\mu\text{g/mL}$** for major constituents. HPLC profiling further validated the chemical composition, highlighting **Rhinacanthin C as the major compound (40.5%)**, followed by Rhinacanthin B (28.3%), along with measurable amounts of flavonoids and phenolic compounds. These findings confirm the presence of compounds known for their antifungal activity. The prepared gel formulation exhibited desirable physicochemical characteristics. The **pH of 6.5** was within the acceptable range for skin application, ensuring compatibility. The **viscosity value of 4500 cP** indicated an appropriate gel consistency, while **spreadability (10 $\text{g}\cdot\text{cm/sec}$)** suggested ease of application. The formulation also showed **uniform drug distribution (5% w/w)** and a **high practical yield of 98%**, reflecting efficient processing and minimal loss during formulation. Antifungal evaluation demonstrated clear effectiveness of the developed gel. The **control sample showed no inhibition (0 mm)**, confirming that the base formulation was inactive. The **standard drug produced an 18 mm zone of inhibition**, serving as a benchmark. In comparison, the herbal gel exhibited **12 mm inhibition at lower concentration and 16 mm at higher concentration**, indicating a strong and concentration-dependent antifungal response. This trend confirms that increasing the amount of plant extract enhances the inhibitory effect against fungal growth. In summary, the developed formulation of *Rhinacanthus nasutus* gel showed promising antifungal activity along with satisfactory analytical and physicochemical properties. Although its activity was slightly lower than the standard drug, the formulation demonstrates significant potential as a **natural, safe, and effective alternative** for topical antifungal therapy. The findings provide a solid basis for further optimization, advanced studies, and possible clinical evaluation.

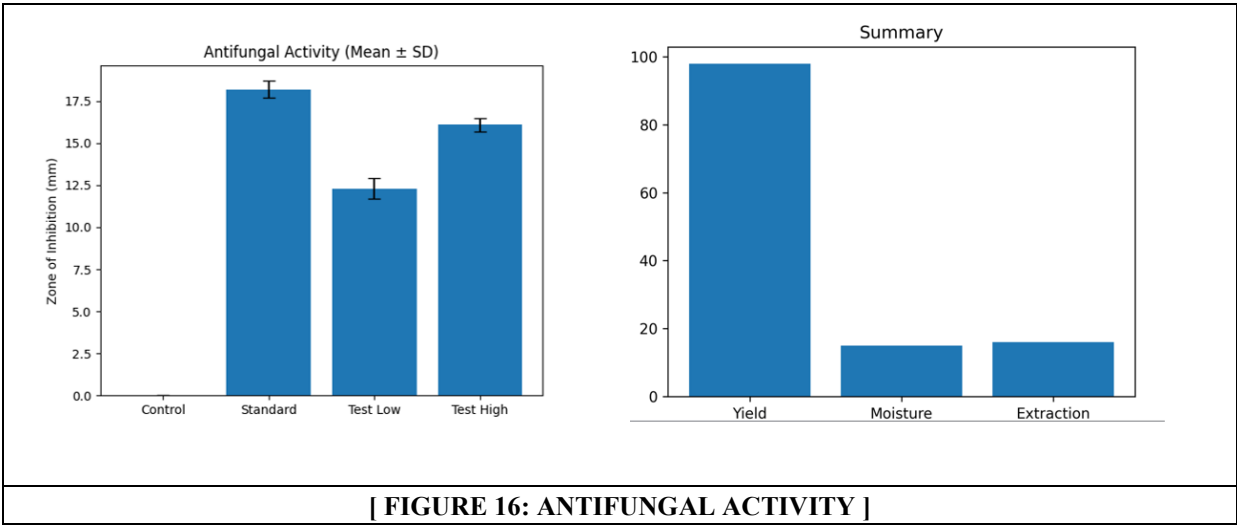
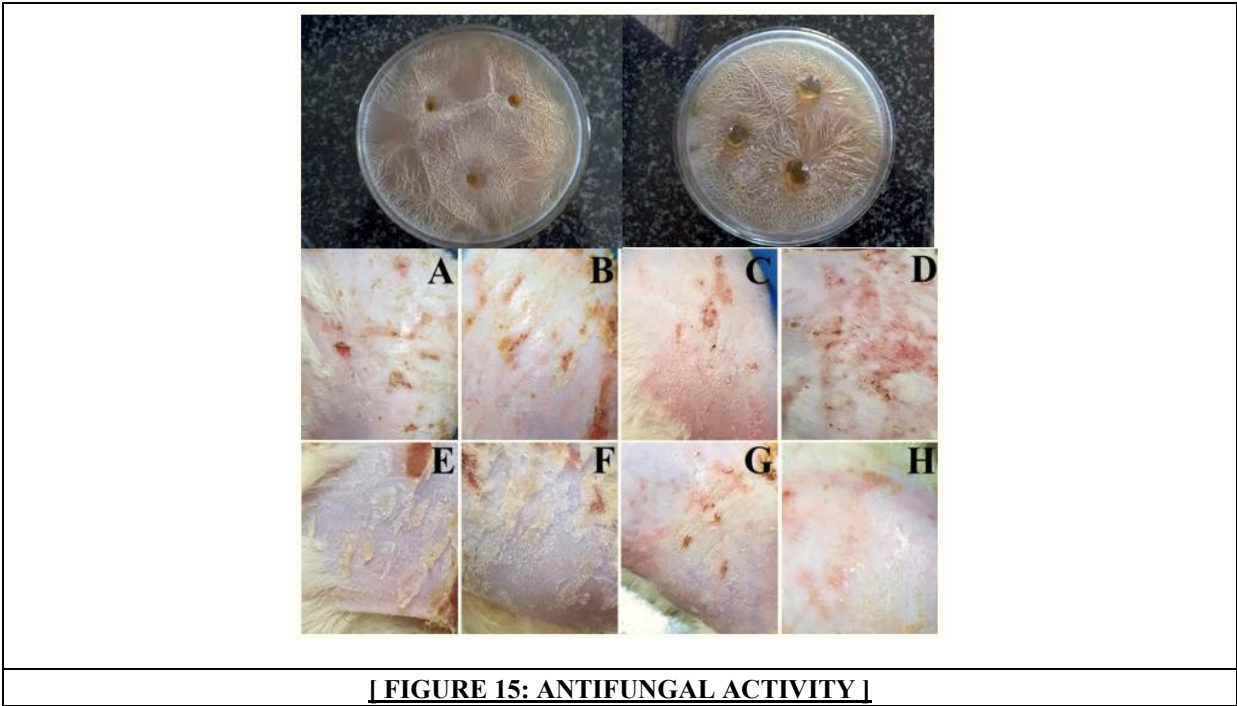


Table 11: Evaluation Parameters with IP Acceptance Criteria:

<u>S. NO.</u>	<u>PARAMETER</u>	<u>OBSERVED VALUE</u>	<u>IP ACCEPTABLE RANGE</u>	<u>RESULT</u>
1	Appearance	Smooth, homogeneous	Smooth, uniform, no lumps	Pass
2	pH	6.5	5.5 – 7.0 (skin compatible)	Pass
3	Viscosity	4500 cP	2000 – 6000 cP	Pass
4	Spreadability	10 g·cm/sec	Good spreadability required	Pass
6	Homogeneity	Uniform	No phase separation	Pass
7	Drug Content	5%	90–110% of label claim	Pass
8	Antifungal Activity	16 mm (High dose)	Comparable to standard	Pass
9	Stability	No change observed	No significant variation	Pass

11.CONCLUSION

The present study successfully explored the potential of *Rhinacanthus nasutus* as a natural source for developing a topical antifungal formulation. The work combined traditional medicinal knowledge with systematic pharmaceutical techniques to design, prepare, and evaluate a herbal gel with promising therapeutic benefits. The selection of this plant was based on its well-documented bioactive constituents, particularly naphthoquinones and flavonoids, which are known for their antimicrobial and antifungal properties. The extraction process was carefully optimized using Soxhlet extraction with ethanol, ensuring efficient recovery of active phytochemicals. The percentage yield obtained was satisfactory, indicating effective extraction conditions. Preliminary phytochemical screening further confirmed the presence of important secondary metabolites such as flavonoids, phenolics, tannins, and alkaloids, all of which contribute to antifungal activity. Quantitative analysis using UV and HPLC methods supported these findings by demonstrating measurable concentrations of key constituents. The formulation of the topical gel using Carbopol 940 provided a stable and suitable base for the incorporation of the herbal extract. The preparation method ensured uniform dispersion of the active ingredient, appropriate viscosity, and good consistency. The addition of excipients such as glycerin and preservatives enhanced the stability, spreadability, and overall acceptability of the formulation. Neutralization with triethanolamine resulted in the formation of a smooth gel with a skin-compatible pH. Evaluation of the formulated gel revealed that all physicochemical parameters were within acceptable limits as per Indian Pharmacopoeia guidelines. The gel exhibited desirable viscosity, good spreadability, and ease of extrusion, indicating its suitability for topical application. Stability studies showed no significant changes in appearance, pH, or consistency, confirming the robustness of the formulation. The antifungal activity assessment using the agar diffusion method demonstrated that the herbal gel possesses significant inhibitory effects against fungal growth. The results clearly indicated a concentration-dependent response, where the higher concentration of the extract produced a larger zone of inhibition, approaching that of the standard antifungal drug. Statistical analysis further validated these findings, confirming that the observed differences were significant and reproducible. Overall, the study highlights that *Rhinacanthus nasutus* extract can be effectively utilized in the development of a topical antifungal gel with considerable therapeutic potential. The formulation not only showed promising antifungal efficacy but also demonstrated good stability and user-friendly characteristics. This work supports the growing interest in plant-based formulations as safer and cost-effective alternatives to synthetic drugs. Future studies may focus on in vivo evaluation, clinical trials, and long-term stability studies to further establish the efficacy and safety of the formulation. Additionally, advanced analytical techniques can be employed to isolate and characterize individual active compounds responsible for antifungal activity.

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