

FORMULATION AND EVALUATION OF POLYHERBAL ANTIFUNGAL NAIL LACQUER

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Article Received on 15 Feb. 2026,
Article Revised on 05 March 2026,
Article Published on 16 March 2026,

<https://doi.org/10.5281/zenodo.19081885>

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How to cite this Article: Ms. Nivedhita K. Shaine*¹, Mr. Febin Sheen², Mr. Mathew Chacko³, Mr. Muhammad Shiyad M. P.⁴, Ms. Aneena Aliyar⁵, Mrs. Reshma B.⁶, Dr. Sabu M. C.⁷ (2026). Formulation And Evaluation Of Polyherbal Antifungal Nail Lacquer. World Journal of Pharmaceutical Research, 15(6), 1398-1414.

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ABSTRACT

Onychomycosis, a fungal infection of the nails, is a common dermatological condition that requires effective treatment. Conventional antifungal therapies often come with side effects, highlighting the need for safer, more natural alternatives. The objective of this study was to formulate and evaluate a herbal antifungal nail lacquer using Henna (*Lawsonia inermis*) and Neem (*Azadirachta indica*) leaf extracts as active ingredients, known for their potent antifungal properties. The herbal nail lacquer was formulated by incorporating Henna and Neem extracts into a nitrocellulose-based lacquer system. The lacquer was prepared using a combination of ethyl acetate as a solvent, castor oil as a plasticizer, glycerin as a humectant, and phenoxyethanol as a preservative. The formulation process involved blending the active ingredients with the film-forming agent, followed by thorough stirring and solvent evaporation to form a stable lacquer. The prepared formulation was evaluated

for various physicochemical properties, including color, transparency, gloss, drying time, film thickness, water resistance, and adhesion properties. The antifungal efficacy of the lacquer was tested against common nail pathogen *Aspergillus niger* using standard antifungal tests.

The results indicated that the herbal nail lacquer demonstrated excellent gloss and smooth flow, with a quick drying time and satisfactory film thickness. The antifungal activity was significant, with both Henna and Neem extracts showing promising results against fungal strains. Additionally, the lacquer exhibited good water resistance and adhesion, making it suitable for long-lasting application on nails. This study concludes that the formulated herbal antifungal nail lacquer using Henna and Neem extracts offers a safe, effective, and natural alternative for the treatment of onychomycosis, providing both therapeutic benefits and cosmetic appeal. The formulation holds potential for future development and use as a topical treatment for fungal nail infections.

KEYWORDS: The lacquer was prepared using a combination of ethyl acetate as a solvent, castor oil as a plasticizer, glycerin as a humectant, and phenoxyethanol as a preservative.

INTRODUCTION

Nail lacquer, commonly known as nail polish, is a cosmetic product applied to fingernails and toenails to enhance their appearance, protect the nail plate, and serve as a fashion statement. nail lacquers are available in a wide array of colours, finishes, and formulations, catering to diverse preferences and needs.

ADVANTAGES

- Nail health: Nail polish can help nails grow longer and stronger, and protect them from damage and infection.
- Mental health: Nail polish can boost your mood and make you feel more confident.
- Nail biting: Nail polish can help stop nail biting.
- Self-care: Applying nail polish can be a relaxing and therapeutic experience.
- Manicure longevity: Breathable nail polish can increase the longevity of your manicure.
- Nail protection: A top coat can protect your nail polish from chipping and damage, and strengthen your nails.
- Blood circulation: A manicure can improve blood circulation in your hands, which can reduce inflammation, swelling, and pain.

IDEAL PROPERTIES OF NAIL LACQUER

- Nail lacquer should adhere well to the nail plate.
- It must form a proper film on nail bed.
- It must be flexible enough not to crack or become brittle.

- It must provide a good gloss.
- It must have a uniform colour.
- It must be long lasting and should provide a tough finish.

DISEASES AFFECTING NAILS^[6]

Onychomycosis: also known as tinea unguium, is a fungal infection of the nail that can cause discoloration, thickening, and separation from the nail bed. It's more common in toenails than fingernails.

CAUSATIVE ORGANISMS^[7]

- Dermatophytes

The most common cause of onychomycosis, accounting for 60–70% of infections. The most common dermatophytes are *Trichophyton rubrum* and *Trichophyton mentagrophytes*.

- Yeasts

Candida albicans and *Candida parapsilosis* are the most common yeasts that cause onychomycosis. *Candida* is more common in fingernail infections.

- Non-dermatophyte molds

Less common in the general population, but more common in patients with HIV.

SYMPTOMS

- Nail may appear white, yellow, green or black in colour
- May have chalky or cloudy appearances in some spots.
- Maybe seen as thickened, misshaped or deformed.
- Nails may separate from nail bed, leaving space between nail and skin.
- Nail maybe painful especially when pressure is applied.
- The nail may smelly.

RISK FACTORS

- Being older.
- Having a weak immune system.
- Wearing shoes that make your feet hot and sweaty.
- Walking barefoot in places where fungus spreads.
- Having athlete's foot.
- Recently having an injury or surgery on your nail.

TYPES OF ONYCHOMYCOSIS^[8]

- i. **Distal subungual onychomycosis (DSO):** The most common type, where the fungus spreads from the plantar skin and invades the nail bed through the hyponychium.
- ii. **White superficial onychomycosis (WSO):** A rarer type where the fungus directly invades the nail plate's surface.
- iii. **Proximal subungual onychomycosis (PSO):** The least common type, where the fungus penetrates the nail matrix through the proximal nail fold.

1.2 ACTIVE PHARMACEUTICAL INGREDIENT (API)

APIs are the biologically active ingredients in drugs that are responsible for their therapeutic effect. This Antifungal nail lacquer is a polyherbal formulation containing Henna and Neem as active pharmaceutical ingredients. Lawsone from Henna and *Azadirachtin* from neem extracts having anti-fungal properties. Henna is a plant-based dye that has many properties including Anti-oxidant, Anti-bacterial, Anti-fungal, Anti-inflammatory, Anti-viral, Insecticidal, etc. Neem (*Azadirachta Indica*) is a tree that has been used for thousands of years for its medicinal properties. It has many potential uses, Including treating Skin conditions, infections and wounds. It also used for certain skin condition like acne, skin ulcer, dandruff, psoriasis and infections like Fungal, Gastrointestinal, Tooth plaques, Gingivitis.

CHEMICAL CONSTITUENTS^{[12][13]}

Henna possesses active components including carbohydrates, phenolic compounds (lawsoniaside, lalioside, and syringinoside), flavonoids (apigenin, luteolin, kampferol, quercetin, and catechin), saponins, proteins, alkaloids (harmine and harmaline), terpenoids, quinones (arbutin, juglone, lawsone, alizarin, emodin.

Henna contains the active ingredient called lawsone (2- hydroxy-1,4-naphthoquinone), which is an orange-red pigment responsible for the typical coloration. Lawsone interacts directly with the amine or sulfur functional groups of keratins.

due to its strong affinity. Flavonoids (e.g., quercetin, kaempferol) has Synergistic antifungal effects and Tannins (e.g., gallic acid, ellagic acid) also exhibit Antifungal and antimicrobial properties.

ANTIFUNGAL MECHANISM^{[15][16]}

- Cell Membrane Disruption: Lawsone interacts with fungal cell membranes, disrupting integrity and function.
- Inhibition of Fungal Enzymes: Lawsone inhibits essential fungal enzymes, such as: Chitin synthase (cell wall formation) , Ergosterol synthesis (membrane component) ,Proteases (protein degradation)
- Oxidative Stress: Henna's flavonoids and tannins induce oxidative stress, damaging fungal cells.
- DNA Interference: Lawsone intercalates into fungal DNA, disrupting replication and transcription.
- Immunomodulation: Henna stimulates host immune response, enhancing antifungal activity.

NEEM^[18]**CHEMICAL CONSTITUENTS^[19]**

The most important active constituent is azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinat, gedunin, salannin, and quercetin.

ACTIVE INGREDIENTS

1. Azadirachtin: Main antifungal compound.
2. Nimbin: Sesquiterpene with antifungal properties.
3. Nimbolide: Furanocoumarin with antifungal and anti-inflammatory effects.
4. Quercetin: Flavonoid with antifungal and antioxidant properties.

ANTIFUNGAL MECHANISMS OF NEEM

- Inhibits fungal growth: Disrupts cell membrane integrity and mitochondrial function.
- Interferes with fungal enzymes: Inhibits enzymes essential for fungal growth.
- Induces oxidative stress: Increases reactive oxygen species (ROS) production.
- Modulates immune response: Stimulates host immune system to combat fungal infections.

PLANT PROFILE HENNA**Source of plant**

Scientific name: Lawsonia inermis

Kingdom: Plantae. **Division:** Magnoliophyta. **Class:** Magnoliopsida.

Order: Myrtales. **Family:** Lythraceae. **Species:** Inermis

NEEM

Source of plant

Scientific name: Azadirachtaindica

Kingdom: plantae **Division:** Magnoliophyta **Class:** Magnoliopsied

Order: Sapindales **Family:** Meliaceae **Genus:** Azadirachta

Species: Azadirachtaindica

EXCIPIENT PROFILE

Excipients are substances other than the active pharmaceutical ingredient that have been appropriately evaluated for safety. These are the Substances that are not active pharmaceutical ingredients, but are included in the formulation. Various excipient used in the formulation of antifungal nail lacquer includes.

ETHYL ACETATE^[20]

Formula: C₄H₈O₂ Melting point: -83°C Boiling point: 77.1°C

Density: 902 kg/m³

Solubility: It is slightly soluble in water, but soluble in most organic solvents

Storage: Keep ethyl acetate away from heat, hot surfaces, sparks, open flames, and other ignition sources. Keep away from incompatible materials such as oxidizing agents, acids, and alkalis.

Use: used as a solvent.

NITROCELLULOSE^{[21][22]}

Formula: C₁₂H₁₆N₄O₁₈ Boiling point: less than 100°C Melting point: 160 °C Density: 0.765–0.775 g/cm³

Solubility: not soluble in water but soluble in organic solvents such as amyl acetate, glacial acetic acid, methanol etc.

Storage conditions: Nitrocellulose should be stored in a cool, dry, well-ventilated area away from heat and ignition sources. Always keep damped and Keep away from chemical vapours, which can partially dissolve nitrocellulose.

Use: used as a film former to create a shiny, flexible film that adheres to the nail.

POLYVINYL ACETATE^[23]

Boiling point: 72.5 °C Melting point: 30–50°C Density: 1.18 g/mL

Solubility: Soluble in benzene, chloroform, methanol, acetone, butyl acetate. Insoluble in ligroin, diethyl ether, butanol, turpentine, water, oils

Storage conditions: stored in a cool, dry, well-ventilated place in a tightly closed container.

The recommended storage temperature is between 15–25°C.

Use: used as a resin to improve adhesion and flexibility, which can reduce chipping and wear.

GLYCERIN^[24]

Formula: C₃H₈O₃ Boiling point: 290°C

Melting point: 17.8-18.6°C Density: 1.26g/cm³

Solubility: highly soluble in water and ethanol, but insoluble in hydrocarbons. Storage conditions: Store glycerin in a cool, dry, well-ventilated area. Avoid storing it indirect sunlight or freezing it.

Use: Glycerin retains moisture (moisturizer) and protects nails from damage and breakage during the removal process.

CASTOR OIL^[30]

Formula: C₅₇H₁₀₄O₉ Boiling point: 313°C

Melting point: -10°C to -18°C Density: 0.95-0.96 g/cm³

Solubility: Soluble in ethanol, chloroform, di ethyl ether, Glacial acetic acid, and Methanol

Storage conditions: Stored in a dry, cool and well-ventilated place.

Use: used as a laxative, in medicine, and in industry.

AIM AND OBJECTIVE

To formulate and evaluate antifungal nail lacquer.

OBJECTIVES

- Selection of herbal ingredients with antifungal properties.
- Selection of an herbal antifungal agent with maximum nail benefits.
- Preparation of herbal extract.
- Formulation of antifungal nail lacquer.
- Evaluation of antifungal nail lacquer.

PLAN OF WORK

- Literature review.
- Selection of herbal ingredients.
- Formulation of Anti-fungal nail lacquer.
- Evaluation of anti-fungal nail lacquer.
- Optimization of best formula based on evaluation parameters.
- Comparative evaluation with a marketed Nail lacquer.

MATERIALS AND METHODS**RAW MATERIALS**

SL.NO	NAME OF THE RAW MATERIALS	NAME OF SUPPLIERS
1	Neem Leaves	Local Market
2	Henna Leaves	Local Market
3	Ethyl acetate	Isochem laboratories
4	Polyvinyl acetate	Spectrum Reagents and Chemical Pvt Ltd
5	Nitro Cellulose	Nice chemicals pvt ltd
6	Castor oil	Isochem laboratories

EQUIPMENT

SL NO.	NAME OF EQUIPMENT	COMPANY
1	Electronic balance	Smart impact
2	Heating mantle	Rotek

METHODOLOGY**EXTRACTION OF HENNA: SOXHLET EXTRACTION**

- Collection and Preparation: Henna leaves were collected and shade-dried to remove moisture.
- Loading Thimble: 50g of the dried henna powder was placed in a thimble (which is a small container used in the Soxhlet extraction process).
- Setting up Soxhlet Apparatus: The thimble was placed into the Soxhlet extractor.
- Solvent Mixture: A solvent mixture of ethanol and water in a 70:30 ratio was prepared for the extraction.
- Extraction: The Soxhlet extraction was carried out at 50°C to extract the active compounds from the henna powder.
- Solvent Removal: After extraction, excess solvent was removed from the extract, leaving behind the concentrated components of henna.
- Storage: The obtained extract was stored in a tightly closed container for further use.

EXTRACTION OF NEEM: COLD MACERATION

- Collection and Preparation: Fresh neem leaves were collected and shade-dried to reduce moisture content.
- Powdering: The dried neem leaves were finely powdered to increase the surface area for extraction.
- Cold Maceration: The crude powder was macerated (soaked) with 70% ethanol. This process was done at room temperature for 7 days, allowing the ethanol to extract the active compounds from the neem powder.
- Solvent Removal: After the 7-day maceration period, the excess solvent (ethanol) was removed from the extract.
- Storage: The final neem extract was stored in a tightly closed container to preserve its properties.

INGREDIENTS AND FORMULATION CATEGORY

SL NO.	INGREDIENT	USE
1	Neem leaf extract	Active ingredient
2	Henna leaf extract	Active ingredient
3	Nitrocellulose	Film former
4	Ethyl acetate	Solvent
5	Polyvinyl acetate	Resin
6	Castor oil	Plasticizer
7	Glycerine	Humectant
8	Phenoxy ethanol	Preservative

FORMULATION TABLE

SL No	NAME OF INGREDIENT	F1	F2	F3
1	Henna leaf extract	-----	1.2ml	0.6ml
2	Neem leaf extract	1.2ml	-----	0.6ml
3	Nitrocellulose	3g	3g	3g
4	Polyvinyl acetate	1ml	1ml	1ml
5	Glycerin	0.5ml	0.5ml	0.5ml
6	Castor oil	1.2ml	1.2ml	1.2ml
7	Phenoxy ethanol	0.1ml	0.1ml	0.1ml
8	Ethyl acetate	qs to 10 ml	qs to 10 ml	qs to 10 ml

METHOD OF PREPARATION OF HERBAL NAIL LAQUER

Phase 1: Preparation of Nitrocellulose powder

Triturate the nitrocellulose film former to a fine powder. This helps to increase the surface area and allows for easier dissolution in the solvent.

Phase 2: Dissolving Nitrocellulose

Dissolve the powdered nitrocellulose in ethyl acetate. Ethyl acetate is a common solvent used to dissolve nitrocellulose due to its ability to break down the polymer. Stir the mixture until the nitrocellulose is completely dissolved.

Phase 3: Dissolving Plant Extract

In a separate beaker, dissolve the plant extract using a solvent. The solvent used here is ethyl acetate.

Phase 4: Mixing Extract with Nitrocellulose Mixture

Add the plant extract solution to the nitrocellulose mixture. Stir the two mixtures together magnetically to ensure even distribution of the extract throughout the nitrocellulose solution.

Phase 5: Adding Other Ingredients

Add the remaining ingredients to the above solution. Continue stirring to ensure uniform mixing and dispersion of all components.

Phase 6: Final Adjustment to required Volume

Transfer the final mixture into a bottle or container. Adjust the volume of the solution to the desired quantity by adding more solvent, if necessary, to reach the required consistency or concentration.

EVALUATION OF HERBAL NAIL LACQUER**1. PHYSICAL APPEARANCE****Color**

- Evaluation: Visually examine the formulation for its color. This is important because the color can indicate the presence of active compounds, the concentration of ingredients, and the quality of the formulation. For example, the presence of plant extracts like neem or henna could influence the color, and consistency in the color of the formulation can suggest uniformity in the mixing process.

Application Quality

- o Evaluation: Evaluate the ease of application and the spread ability of the formulation. Consider factors such as:

- Viscosity: The formulation should not be too thick or too runny; it should have an ideal viscosity for the intended use.

- Smoothness: The formulation should spread evenly without clumping, streaking, or uneven patches.
- Drying/Setting Time: Evaluate how quickly the film dries and whether it forms a smooth, solid layer.

2. SMOOTHNESS OF FLOW AND GLOSS

- Spreading on Glass Slide

Apply formulations F1, F2 and F3 over a 1.5-inch area on a clean glass slide. Use a tilted glass slide to spread the formulations evenly. This step is intended to simulate how the formulation would spread and apply when used in real-world conditions, ensuring even distribution of the product.

- Assessing Smoothness:

Visually evaluate how smoothly the formulations spread across the glass slide. This could include checking for uniformity, ease of application, and the absence of streaks or uneven areas.

A smooth, consistent application is important for products like coatings or films. The goal is to determine if the formulations have the same smooth application as a professional product (nail lacquer).

- Grading Glossiness:

Visually inspect the glossiness of the formulations after they have been spread out. Glossiness is usually evaluated based on the surface sheen and how reflective the product is once it has dried or set.

- Rating Scale:

- Good (++) : The formulation has a noticeable but moderate glossiness.
- Very Good (+++) : The formulation has a noticeable shine, approaching that of a high-quality commercial product.
- Excellent (++++): The formulation has a high, +
- gloss, similar to that of premium nail lacquers.

Glossiness is an important visual attribute, especially for cosmetic or aesthetic products, and provides an indication of the formulation's overall appearance and quality.

3. DRYING TIME

The drying time is the primary test used for the nail lacquer. Briefly a brush applicator was used to spread the lacquer on a 2 cm area on a glass slide at room temperature and let to dry. After every 15 sec, a gloved finger was used to touch the film. The time required to obtain dry to touch condition was recorded.

4. WATER RESISTANCE TEST

- A continuous layer of the nail lacquer (formulation) is applied to the petri dish. The film is allowed to dry completely, forming a solid, uniform layer on the surface.
- This ensures that the film is fully dried before exposure to water, as drying is a critical factor in assessing the film's performance and durability.
- Once the film has dried, the petri dish with the lacquer film is submerged in water for a certain period. The exact duration of submersion can vary, but it is typically done for a set time.
- This simulates exposure to moisture or water, such as in daily use scenarios such as hand washing, rain, or exposure to humid environments.
- After the specified time submerged in water, the petri dish is taken out and allowed to dry again at room temperature.
- The drying process after submersion helps to assess if the film can recover its original properties after contact with water.
- Perform a visual inspection of the film after it has dried post-submersion. Look for specific signs of damage or degradation such as.
 - Blisters: Raised areas or bubbles on the surface of the film, indicating that the lacquer has absorbed water or that the adhesion of the film to the surface has been compromised.
 - Discoloration: Any change in the colour of the film, which could indicate that the formulation is not stable or that it reacts negatively when exposed to water.
- This evaluation helps determine how well the film resists the effects of water. If blisters or discoloration appear, it suggests that the lacquer may not be water-resistant and could degrade or lose its appearance when exposed to moisture.

5. DETERMINATION OF ANTIFUNGAL ACTIVITY

Materials Required

1. Test microorganism: *Aspergillus niger*
2. Herbal formulation: antifungal nail lacquer

3. Standard antifungal agent: clotrimazole
4. Medium: Potato dextrose agar medium
5. Cotton swabs: For inoculation
6. Sterile Borer: For creating wells
7. Incubator: For incubating the plates

Well Diffusion Method

1. Prepare PDA plates and allow them to solidify.
2. Inoculate the *Aspergillus niger* spore suspension onto the SDA plates using a cotton swab.
3. Allow the inoculum to dry for 10-15 minutes.
4. Using a borer, create three wells on each plate:
 - Well 1: Standard antifungal agent (positive control)
 - Well 2: Herbal antifungal nail lacquer (test)
 - Well 3: DMSO (negative control)
5. Fill each well with 50-100 μ L of the respective solution.
6. Incubate the plates at 25°C for 3 days.
7. Observe the plates for zones of inhibition around each well.
8. Measure the diameter of the zones of inhibition using a ruler.

RESULTS AND DISCUSSION

Evaluation results of all the formulation are given below.

PHYSICAL APPEARANCE

SL.NO	FORMULATION	ODOUR	COLOUR
1	F1	Pleasant	Pale brown
2	F2	Pleasant	Brown
3	F3	Pleasant	Dark brown



F1



F2



F3

DRYING TIME**F1****F2****F3**

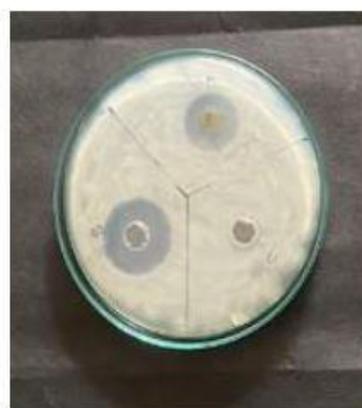
SL.NO	FORMULATION CODE	DRYING TIME IN SECONDS
1	F1	120
2	F2	120
3	F3	120

SMOOTHNESS OF FLOW**F1****F2****F3**

SL.NO	FORMULATION	SMOOTHNESS OF FLOW
1	F1	Good
2	F2	Very good
3	F3	Good

WATER RESISTANCE TEST**F1****F2****F3**

SL.NO	FORMULATION	WATER RESISTANCE
1	F1	No colour change No blistering
2	F2	No colour change No blistering
3	F3	No colour change No blistering

ANTIFUNGAL ACTIVITY**F1****F2****F3**

SL.NO	PLATE	STANDARD	CONTROL	TEST
1	Plate 1	26mm	NIL	14mm
2	Plate 2	26mm	NIL	18mm
3	Plate 3	26mm	NIL	20mm

SUMMARY AND CONCLUSIONS

Nails are hard, keratin-based structures that grow from nail beds on the tips of fingers and toes. Onychomycosis is a fungal infection of nails that requires effective treatment.

Conventional antifungal therapies often come with side effects; therefore, the need for safer and more natural alternatives is increased. Nail lacquer commonly known as nail polish, is a cosmetic product applied to fingernails and toenails to enhance their appearance, protect the nail plate, and serve as a fashion statement.

The main aim of this project was to formulate and evaluate Anti-fungal nail lacquer with active ingredients such as Lawsonia from henna and Azadirachta extracts from neem were formulated into a nitrocellulose base to form the final product. Film-forming agent act as a drug depot for the controlled delivery of drugs at target sites. The evaluation tests, including physical appearance, drying time, smoothness of flow test, water resistance test, and antifungal activity, were conducted and indicate that the product is safe and faster effective for Onychomycosis. Based on the above said studies, F3 was selected as the best formula. F3 shows good zone of inhibition against *Aspergillus niger* which enhances the effectiveness of nail lacquer.

Both the herbal ingredients used provide individual as well as the combined benefits as they provide enhanced colour and shine, improved nail health, natural and sustainable product, moisturizing as well as nourishing properties for the nail lacquer that is prepared.

In conclusion, the herbal antifungal Nail lacquer offers potential nail benefits against fungal infections affecting the nail and provides natural, user-friendly solutions for Onychomycosis. This product aims to offer both aesthetic and health benefits while minimizing environmental impact and chemical exposure.

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