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Formulation and Evaluation of Herbal Antifungal Ointment Using Neem Oil, Jamun Extract and Jaifal Extract

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Abstract: *The demand for natural and herbal-based treatments in skincare and dermatology has increased due to growing awareness about the harmful effects of synthetic chemicals. Fungal infections are a common skin ailment that can affect individuals of all ages, causing discomfort, irritation, and, in severe cases, permanent damage to the skin. Traditional treatments often include antifungal creams or ointments containing synthetic ingredients, but these can have adverse side effects. As a result, the use of herbal antifungal ointments formulated from Neem oil, Jamun extract, and Jaifal extract presents a safer, more natural alternative for treating various fungal skin infections.*

Keywords: herbal-based treatments

I. INTRODUCTION

The demand for natural and herbal-based treatments in skincare and dermatology has increased due to growing awareness about the harmful effects of synthetic chemicals. Fungal infections are a common skin ailment that can affect individuals of all ages, causing discomfort, irritation, and, in severe cases, permanent damage to the skin. Traditional treatments often include antifungal creams or ointments containing synthetic ingredients, but these can have adverse side effects. As a result, the use of herbal antifungal ointments formulated from Neem oil, Jamun extract, and Jaifal extract presents a safer, more natural alternative for treating various fungal skin infections.

This herbal ointment aims to combine the antifungal, antimicrobial, and healing properties of these three powerful natural ingredients to create a highly effective and skin-friendly solution for fungal infections.

Fungal infections of the skin are a widespread dermatological concern affecting millions worldwide. Common conditions such as athlete's foot, ringworm, and candidiasis not only cause physical discomfort—itching, redness, and inflammation—but can also lead to long-term skin damage if not treated effectively. Although synthetic antifungal agents are available, many are associated with side effects such as skin irritation, resistance development, and long-term toxicity. As a result, there is growing interest in herbal remedies that offer safer, holistic alternatives. In this context, the development of a herbal ointment incorporating Neem oil (Azadirachta indica), Jamun extract (Syzygium cumini), and Jaifal extract (Myristica fragrans) presents a promising natural solution for combating fungal infections.

Neem oil, a cornerstone of Ayurvedic medicine, is well known for its powerful antifungal and antibacterial properties. Rich in bioactive compounds such as azadirachtin, nimbin, and nimbidin, neem oil disrupts fungal cell membranes and prevents their growth and spread. It also soothes inflammation, promotes skin healing, and protects against further infections. Jamun extract, derived from the fruit of the Syzygium cumini tree, is a rich source of ellagic acid, anthocyanins, and flavonoids, which exert antifungal activity by inhibiting the growth and replication of pathogenic fungi like Candida and dermatophytes. Additionally, its antioxidant and anti-inflammatory properties help reduce skin irritation and promote recovery. Jaifal extract, commonly known as nutmeg, contributes further by delivering

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compounds such as myristicin and eugenol that act as natural fungicides. These agents inhibit fungal metabolism and provide antiseptic effects while supporting tissue regeneration.

The combination of these three herbal ingredients not only ensures a potent antifungal action but also offers a synergistic therapeutic effect. This herbal ointment not only eliminates the fungal pathogens but also calms inflamed tissue, accelerates wound healing, and nourishes the skin.

Free from synthetic chemicals, this formulation is gentle on the skin and suitable for long-term use. Thus, this herbal antifungal ointment represents an effective, natural, and skin-friendly solution to the growing challenge of fungal skin infections.

Why a Topical Ointment?

Topical delivery systems are ideal for treating localized skin infections due to their ability to deliver the active agents directly to the site of infection, ensuring maximum therapeutic efficacy with minimal systemic exposure. In the context of fungal infections, which primarily affect the epidermal layers, a topical ointment provides rapid symptom relief, effective fungal eradication, and improved patient compliance.

Key benefits include:

- Targeted action on the infected skin area, reducing fungal load directly.
- Reduced systemic side effects compared to oral antifungals.
- Moisturizing and barrier-repairing effects—essential in healing cracked, inflamed skin.
- Ease of application, patient acceptability, and suitability for long-term use.
- The oily base of an ointment enhances retention time on the skin and improves penetration of herbal actives.

Herbal ointments, in particular, provide additional skin benefits such as nourishment, hydration, and antioxidant protection, making them ideal for multifunctional dermatological care.

Synergistic Benefits of the Combination:

1. Broad-Spectrum Antifungal Coverage

- The combined antifungal activity of these three ingredients targets a wider range of fungal strains, including Candida albicans, Trichophyton rubrum, Aspergillus niger, and Microsporum species.
- While Neem oil is effective against dermatophytes and molds, Jamun extract primarily inhibits yeast-like fungi such as Candida, and Jaifal extends the action to more resistant fungal strains.
- This broad-spectrum activity makes the ointment suitable for treating diverse fungal infections of the skin, nails, and folds, including chronic cases.

2. Multimodal Mechanism of Action

- Each ingredient works through different biochemical pathways:
- Neem oil disrupts fungal cell walls and inhibits spore formation.
- Jamun extract interferes with fungal DNA synthesis and metabolic pathways.
- Jaifal extract impairs enzymatic functions within the fungi and acts as a natural fungicide.
- This multimodal attack reduces the risk of fungal resistance and enhances the overall therapeutic outcome.

3. Enhanced Anti-inflammatory Response

- Fungal infections are often accompanied by redness, swelling, and itching due to inflammation.
- All three components possess strong anti-inflammatory effects:
- Neem contains nimbidin, which inhibits inflammatory mediators.
- Jamun extract reduces oxidative stress and cytokine release.
- Jaifal acts as a natural analgesic and anti-irritant.
- This combination offers rapid symptomatic relief while supporting immune modulation at the site of infection.

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4. Accelerated Wound Healing and Skin Regeneration

- Fungal infections can cause lesions, cracks, and peeling of the skin.
- The formulation promotes tissue repair and regeneration:
  - Neem oil stimulates collagen production and speeds up healing.
  - Jamun extract supports antioxidant-mediated recovery.
  - Jaifal extract improves microcirculation and enhances nutrient delivery to damaged tissues.
- Together, they encourage complete skin recovery and reduce the chances of post- infection scars and hyperpigmentation.

Improve Skin Barrier And Moisturization

- Fungal infections often compromise the skin barrier, leading to dryness and increased susceptibility to reinfection.
- Neem oil and Jamun extract are rich in emollient and moisturizing agents, restoring the skin's lipid layer.
- Jaifal adds to this by providing essential oils that hydrate and protect the skin.
- Regular use helps rebuild the natural defense barrier, creating an environment hostile to fungal growth.

Antioxidant Protection and Cellular Detoxification

- Oxidative stress contributes to tissue damage and slows healing in fungal infections.
- All three ingredients offer strong antioxidant properties:
- Jamun is especially rich in anthocyanins and flavonoids.
- Neem and Jaifal contain phenolic compounds that neutralize free radicals.
- These antioxidants protect healthy skin cells, reduce oxidative damage, and speed up skin repair.

Long-Term Preventive Action

- With prolonged use, the formulation can act not only as a treatment but also as a preventive measure for individuals prone to recurrent fungal infections.
- By maintaining healthy skin flora and reinforcing the natural barrier, it minimizes the chances of reinfection and fungal colonization.
- Ideal for use in athletes, diabetics, or people exposed to humid environments where fungal infections are common.

Minimized Side Effects and High Skin Compatibility

- Compared to synthetic antifungal drugs, this herbal blend is non-toxic, non-irritating, and free from harmful chemicals.
  - Suitable for all skin types, including sensitive, pediatric, and elderly skin.
- No known long-term adverse effects, making it a safe alternative for chronic use.

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Advantages of Using Natural Herbal Ingredients:

1. Biocompatibility with Human Skin

- Herbal ingredients are generally more compatible with human skin than synthetic chemicals.
- Reduced risk of hypersensitivity reactions, rashes, or contact dermatitis.
- Suitable for all skin types, including sensitive, acne-prone, or damaged skin.

2. Minimal Side Effects

- Natural extracts typically exhibit low toxicity and fewer side effects.
- Long-term or repeated use is less likely to cause skin thinning, irritation, or resistance, which is common with corticosteroid or synthetic antifungal creams.

3. Multi-Functional Properties

- Herbal components offer multiple therapeutic benefits beyond antifungal action:
  - Antimicrobial – protects against secondary bacterial infections.
  - Anti-inflammatory – relieves redness and swelling.
  - Antioxidant – protects from oxidative stress and environmental damage.
  - Wound healing – accelerates skin recovery.

4. Natural Origin and Eco-Friendly

- Derived from plant sources, making the formulation environmentally sustainable.
- Biodegradable, non-polluting, and cruelty-free alternatives to synthetic products.
- Promotes the use of renewable plant resources in line with green chemistry principles.

5. Safe for Long-Term and Pediatric Use

- Herbal ointments are safe for prolonged application, even in vulnerable populations such as children, the elderly, and pregnant women.
- Free from harmful chemicals like parabens, steroids, and artificial fragrances that can cause adverse effects in long-term use.

6. Holistic and Traditional Healing Approach

- Ingredients like Neem, Jamun, and Jaifal have a long history in traditional medicine systems (Ayurveda, Unani, Siddha).
- Their use integrates modern science with ancient healing wisdom, offering a holistic approach to treating fungal infections.

7. Skin-Nourishing and Moisturizing Effects

- Unlike many synthetic antifungal creams that can dry out or irritate the skin, herbal ingredients hydrate, soothe, and nourish the skin.
- Helps restore the natural skin barrier, enhancing protection and overall skin health.

5. Resistance-Free Antifungal Action

- Herbal antifungals act on multiple biological pathways, making it harder for fungi to develop resistance compared to single-target synthetic drugs.

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ACTIVE INGREDIENT PROFILE

Herbal Ingredient	Botanical Name	Key Active Constituents	Relevant Pharmacological Activities
Neem Oil	Azadirachta indica	Azadirachtin, Nimbin, Nimbidin, Gedunin	Antifungal, antibacterial, anti-inflammatory, wound healing
Jamun Extract	Syzygium cumini	Ellagic acid, Anthocyanins, Flavonoids, Tannins	Antifungal, antioxidant, anti-inflammatory, skin protective
Jaifal Extract	Myristica fragrans	Myristicin, Eugenol, Elemicin, Safrole	Antifungal, antiseptic, analgesic, circulation-enhancing, anti-irritant

Table 1: Key Herbal Ingredients and Their Antifungal Properties

1. Neem Oil (Azadirachta indica)

- **Source and History:**

o Neem oil is derived from the seeds of the Azadirachtaindica tree, native to the Indian subcontinent.

o It has been used for centuries in Ayurvedic medicine due to its vast medicinal properties.

- **Key Active Compounds:**

o Contains azadirachtin, nimbin, nimbidin, and other bioactive compounds that are responsible for its antifungal, antimicrobial, anti-inflammatory, and healing properties.

- **Skin Benefits:**

o Reduces inflammation:- Neem oil's anti-inflammatory properties reduce the swelling, redness, and irritation caused by fungal infections.

o Healing properties:- Promotes skin healing and regeneration, which is important in treating infections that cause rashes, lesions, or skin damage.

o Moisturizes the skin:- Helps to lock moisture into the skin, preventing dryness and cracking that may lead to further fungal infections.

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2. Jamun Extract (Syzygium cumini)

- **Source and History:**

o Jamun, or black plum, is a tropical fruit known for its medicinal uses in Ayurveda.

o The extract from the fruit's skin and seeds has long been used for treating various skin conditions, including fungal infections.

- **Key Active Compounds:**

o Contains ellagic acid, anthocyanins, and flavonoids, which are responsible for its antioxidant, antifungal, and antibacterial activities.

- **Skin Benefits:**

o Reduces fungal inflammation: Jamun extract helps reduce the redness, swelling, and itching caused by fungal infections.

o Antioxidant effects: The antioxidants in Jamun extract help to protect the skin from oxidative stress and reduce skin aging caused by fungal infections.

o Moisturizing properties: The extract's hydrating properties help maintain skin moisture, preventing the dryness often associated with fungal conditions.

3. Jaifal Extract (Myristica fragrans)

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- **Source and History:**

o Jaifal, or nutmeg, is a spice derived from the seed of the Myristica fragrans tree.

o It has been used in traditional medicine for its antifungal, antibacterial, and anti-inflammatory properties.

- **Key Active Compounds:**

o Contains myristicin, eugenol, and other volatile oils that contribute to its antifungal and antimicrobial effects

- **Skin Benefits:**

o Anti-inflammatory properties: Jaifal helps reduce the inflammation and pain associated with fungal infections. It also soothes itching and burning sensations common symptoms of fungal skin conditions.

o Skin regeneration: Promotes healing of damaged skin by stimulating blood circulation and encouraging cell turnover.

o Antioxidant protection: The antioxidant properties of Jaifal help protect the skin from further damage caused by oxidative stress and free radicals.

II. LITERATURE REVIEW

1. Biswas et al. (2002) reported that Neem (Azadirachta indica) possesses potent antifungal properties due to active constituents such as azadirachtin, nimbin, and nimbidin. Their study showed inhibition of Candida albicans and Aspergillus species, making neem a viable option for treating fungal skin infections.
Source: Current Science, 82(11):1336–1345

2. Kausik et al. (2010) explored neem's role in modern pharmacology, emphasizing its antifungal mechanisms, including disruption of fungal cell wall synthesis and inhibition of spore formation. Source: Journal of Ethnopharmacology, 128(2):308–313

3. Girish & Shankara Bhat (2008) focused on neem's use in Ayurvedic wound healing. They reported that neem oil reduces fungal colonization and speeds up skin regeneration.
Source: Indian Journal of Dermatology, 53(3):115–121

4. Dwivedi et al. (2014) investigated Syzygium cumini (Jamun) and confirmed that its seed and pulp extracts possess antifungal and antioxidant properties, due to the presence of ellagic acid and anthocyanins.
Source: JAPS, 4(3):110–115

5. Gupta et al. (2011) studied ellagic acid isolated from Jamun and showed its effectiveness in inhibiting the growth of Candida and Trichophyton species.
Source: Phytotherapy Research, 25(5):715–720

6. Anand et al. (2016) documented traditional uses of Jamun in treating various dermatological ailments, including fungal infections, due to its anti-inflammatory and antimicrobial actions. Source: Ayurveda Research and Practice, 2(1):22–27

7. Pooja et al. (2016) reviewed the phytochemistry of Myristica fragrans (Jaifal) and highlighted the antifungal effects of myristicin and eugenol against Candida albicans and Microsporum gypseum.
Source: International Journal of Pharma and Bio Sciences, 7(3):20–27

8. Patil & Shettigar (2015) conducted a comparative study of herbal versus synthetic antifungal ointments and concluded that nutmeg-based herbal ointments showed significant fungal inhibition with fewer side effects.
Source: Asian Journal of Pharmaceutical and Clinical Research, 8(5):1–5

9. Vijayalakshmi et al. (2012) demonstrated that polyherbal formulations combining neem and jaifal extracts displayed enhanced antifungal activity compared to individual extracts. Source: International Journal of Pharmacy and Pharmaceutical Sciences, 4(1):364–367

10. Pandey & Tripathi (2014) reviewed extraction and formulation techniques for herbal drugs and emphasized the importance of standardization and stability testing for effective topical herbal products.
Source: Journal of Pharmacognosy and Phytochemistry, 2(5):115–119

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11. Bhatia et al. (2019) evaluated herbal formulations against dermatophytes and Candida species, showing that neem- and nutmeg-based preparations produced larger inhibition zones than fluconazole in vitro.
Source: Journal of Pharmacognosy and Phytochemistry, 8(3):680–688
12. Srivastava & Chandra (2013) analyzed ethnomedicinal plants used in Indian traditional medicine, including neem and jamun, for treating fungal skin infections.
Source: International Journal of Dermatology, 52(7):798–806
13. Dhama et al. (2019) compiled over 30 medicinal herbs with antifungal potential, identifying neem, jamun, and jaifal among the most effective due to their synergistic, broad-spectrum action. Source: Pharma Innovation Journal, 8(6):325–333
14. Ali & Akhtar (2013) reviewed formulation challenges in herbal ointments and suggested that oil-based herbal preparations improve bioavailability and penetration of antifungal agents. Source: Pharmacognosy Reviews, 7(13):1–8
15. Lahlou (2013) highlighted the global shift towards natural products and the increasing role of herbal antifungals in drug discovery, especially for treatment-resistant infections.
Source: Pharmacology & Pharmacy, 4(03):17–31

AIM AND OBJECTIVES

Aim:

To develop and scientifically evaluate a topical antifungal ointment using Neem oil (Azadirachta indica), Jamun extract (Syzygium cumini), and Jaifal extract (Myristica fragrans), with the objective of offering a natural, safe, and effective alternative to synthetic antifungal treatments.

Objectives:

1. To conduct a comprehensive literature review of Neem oil, Jamun extract, and Jaifal extract, focusing on their antifungal properties, traditional uses, and phytochemical constituents that contribute to their therapeutic activity.
2. To authenticate and procure high-quality raw materials (e.g., leaves, seeds, and nuts) of Neem, Jamun, and Jaifal from reliable sources, ensuring their purity and quality.
3. To extract the bioactive compounds from Neem, Jamun, and Jaifal using appropriate extraction techniques (e.g., Soxhlet and maceration) to obtain standardized extracts
4. To perform preliminary phytochemical screening of the extracts to identify key bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, and terpenoids, known for their antifungal properties.
5. To develop a stable topical herbal ointment base using excipients like petroleum jelly, beeswax, or lanolin, ensuring optimal skin compatibility, drug release, and stability.
6. To incorporate the standardized extracts of Neem, Jamun, and Jaifal into the ointment base at various concentrations, optimizing the formulation for enhanced antifungal activity.
7. To evaluate the physicochemical properties of the formulated ointments, including:
   - o pH
   - o Spreadability
   - o Viscosity
   - o Stability under various temperature and humidity conditions
8. To analyze any potential synergistic effects between the combined herbal extracts in the ointment, evaluating whether the combination enhances antifungal activity compared to individual ingredients.
9. To assess the microbiological safety (absence of microbial contamination) and organoleptic properties (color, texture, smell, and appearance) of the ointment, ensuring user acceptability.
10. To perform preliminary skin irritation tests on animal models or human volunteers (with ethical approval) to confirm the safety, non-toxicity, and skin compatibility of the ointment.
11. To conduct accelerated stability testing in accordance with ICH guidelines, predicting the ointment's shelf life and determining its stability under different storage conditions (e.g., temperature and humidity).

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12. To document the entire formulation process, analytical findings, and results to facilitate scale-up production and potential commercialization of the ointment as a natural antifungal treatment.
13. To highlight the importance of herbal medicines in dermatological therapy, advocating for the use of plant-based, sustainable, and natural alternatives to conventional antifungal treatments.

PLAN OF WORK

1. Raw Material Preparation and Authentication

- Procurement: Source Neem leaves, Jamun seeds, and Jaifal nuts from reliable suppliers or herbal farms.
- Quality Check: Inspect for freshness, authenticity, and contamination.
- Authentication: Confirm botanical identity through microscopic examination and organoleptic evaluation (color, texture, smell).
- Storage: Store raw materials in cool, dry conditions to maintain freshness.

2. Extraction of Active Phytoconstituents

- Extraction Methods:
  - Neem Oil: Cold pressing or Soxhlet extraction using hexane.
  - Jamun Extract: Soxhlet or maceration with ethanol or methanol.
  - Jaifal Extract: Use maceration with ethanol or water for 2-3 days.
- Filter and Concentrate: Filter out the solvent and concentrate extracts by evaporating excess solvent.
- Storage: Store extracts in amber bottles to protect from light degradation.

3. Phytochemical Screening

- Identify Key Compounds:
  - Neem: Test for azadirachtin, nimbin (terpenoids).
  - Jamun: Test for ellagic acid, flavonoids.
  - Jaifal: Test for myristicin, eugenol.
- Tests:
  - Alkaloids: Dragendorff Test
  - Flavonoids: Alkaline reagent test.
  - Tannins: Ferric chloride test

4. Formulation Development

- Prepare Ointment Base:
  - Excipients: Use petroleum jelly, beeswax, lanolin, and paraffin.
  - Heat the excipients (50-60°C) and mix until uniform.
  - Add thickening agents like xanthan gum if required.
- Incorporate Herbal Extracts:
  - Concentration: Add herbal extracts in concentrations like 1%, 2%, 5%.
  - Mixing: Ensure uniform distribution of extracts by stirring continuously.
  - Adjust Texture: If necessary, adjust the viscosity by adding more base or thickeners.

5. Evalutions For Formulation

- Physicochemical Tests:
  - pH: Measure with a pH meter (target 5.5-6.5).
  - Viscosity: Use a Brookfield viscometer (target: 10,000-20,000 cps).
  - Spreadability: Spread a fixed amount between two glass slides and measure the area.
  - Stability: Store under different conditions (room temp, 4°C, 40°C) and observe changes in texture, pH, and appearance.

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- Microbial Testing:
  - Microbial Load: Ensure no contamination (limit: <100 CFU/g for bacteria and fungi)

6. Skin Irritation

- Patch Testing: Apply a small amount of ointment to the skin of human volunteers.
  - Observe for redness, swelling, or irritation for 48-72 hours.

7. Stability Testing and Optimization

- Accelerated Stability:
  - Store the product at 40°C/75% RH, 25°C/60% RH, and 4°C.
  - Test appearance, pH, and antifungal activity over 3, 6, and 12 months.

8. Final Formulation Adjustment

- Optimize Concentration: Finalize the concentration of herbal extracts based on efficacy and safety.
- Ensure Stability: Re-check the pH, viscosity, and antimicrobial properties after any adjustment.
- Packaging: Choose suitable packaging (e.g., aluminum tubes, plastic jars) that prevent contamination and degradation.

9. Documentation and Reporting

- Record Keeping:
  - Document all procedures, raw material sources, testing methods, and results.
  - Keep records of batch numbers, test results, and formulation changes.
- Final Report:
  - Summarize formulation development, testing results, and product safety.
  - Provide recommendations for scale-up and commercialization

III. MATERIAL AND EQUIPMENT

Materials

Raw Plant Materials

Material	Description
Neem leaves (Azadirachta indica)	Contains azadirachtin, nimbin, and nimbolide, which are known for their antifungal, antibacterial, and anti-inflammatory properties.
Jamun seeds (Syzygium cumini)	Rich in ellagic acid, flavonoids, and tannins, known for antioxidant, anti-inflammatory, and antifungal effects, useful for treating skin infections.
Jaifal nuts (Myristica fragrans)	Contains myristicin, eugenol, and terpenoids, known for antifungal, antiseptic, and anti-inflammatory benefits, supporting the treatment of fungal diseases.

Excipients for Ointment Base

Excipient	Description
Petroleum jelly (Vaseline)	Hydrophobic base that forms an occlusive layer, moisture retention, and improved drug release from active ingredients.
Beeswax	A natural thickener that improves texture and controls drug release, while creating a barrier to lock in moisture.
Lanolin	An emollient that enhances penetration of the active compounds, providing moisturization and improving spreadability.
Paraffin	Solidifying agent that ensures the ointment holds its shape, preventing leakage and maintaining consistent viscosity.
Glycerin	A humectant that retains moisture and enhances the ointment's hydrating

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	properties by drawing moisture from the air.

Preservatives

Preservative	Description
Ethylparaben / Methylparaben	Antimicrobial preservatives that prevent bacterial, fungal, and mold growth, ensuring safety and longevity of the ointment.

pH Adjusters

pH Adjuster	Description
Citric acid / Sodium hydroxide	Used to adjust the pH of the ointment, ensuring it stays within the skin-friendly range (typically 4.5 - 6.0).

Equipment

Formulation Equipment

Equipment	Description
Heating Mantle / Water Bath	Provides controlled heating to melt and mix excipients like beeswax and paraffin, ensuring uniform consistency for the ointment base.
Magnetic Stirrer / Overhead Stirrer	Ensures uniform mixing of the ointment base and extracts, preventing separation of the ingredients.
Ointment Mill / Homogenizer	Ensures uniform dispersion of active extracts into the ointment base, improving drug release and ensuring consistent dosage.
Spatulas / Spoons	Used for manual mixing of ingredients and ensuring the incorporation of plant extracts into the ointment base.
Viscometer (Brookfield)	Measures viscosity of the ointment, ensuring the formulation has the correct spreadability and consistency for topical use.
Tube Extrusion Tester	Tests the extrudability of the ointment, ensuring it can be easily dispensed from the tube, which is essential for user convenience.

Testing Equipment

Equipment	Description
pH Meter	Measures the pH of the ointment to ensure it is within the skin-friendly range (typically 4.5 - 6.0).
Spreading Glass / Scale	Measures the spreadability of the ointment to ensure it can be evenly applied on the skin without excess residue.
Stability Chamber	Simulates storage conditions (temperature and humidity) to predict the ointment's shelf life and overall stability.
Agar Plates	Used to evaluate the antifungal efficacy by assessing the zone of inhibition for the target fungal pathogens.
Inoculation Loop	Allows for microbial inoculation on agar plates, facilitating antifungal testing.
Incubator	Provides optimal growth conditions for microorganisms during antifungal testing, ensuring reliable results.

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EXPERIMENTAL WORK

1. Preparation of Ointment Base

Objective: To prepare the ointment base by melting and mixing base components.

Procedure:

1. Weigh the Base Ingredients:

• Use the digital weighing balance to accurately measure the following:

o Beeswax: 10 g
o Paraffin Wax: 8 g
o White Soft Paraffin: 50 g
o Lanolin: 5 g

2. Melting the Base Ingredients:

• Place the weighed ingredients into the stainless steel vessel.
• Heat the mixture on the hot plate or use a water bath. Set the temperature to 60°C–70°C.
• Stir continuously with a spatula or use a mechanical stirrer until all the ingredients are completely melted and have formed a smooth liquid.

2. Cooling the Base

Objective: To lower the temperature of the base mixture before adding sensitive ingredients.

Procedure:

1. Allow the melted base to cool to 40°C–50°C.
2. Stir occasionally with a spatula or glass rod to prevent any separation of ingredients as the mixture cools.
3. Incorporating Active Ingredients (Jamun, Jaifal, Neem)

Objective: To add and evenly distribute the active ingredients into the ointment base.

Procedure:

1. Measure the following active ingredients.

• Neem Oil: 7 g (7 ml)
• Jamun Extract: 5 g (5 ml)
• Jaifal Extract: 3 g (3 ml)

2. Add Neem Oil to the cooled base mixture while stirring continuously. Stir well to ensure uniform distribution.
3. Add Jamun Extract and Jaifal Extract gradually, stirring consistently to ensure complete mixing.
4. Addition of Preservatives and Antioxidants

Objective: To add preservatives and antioxidants to enhance product stability and prevent microbial growth.

Procedure:

1. Prepare the Preservative Solution:

• In a small container, dissolve the following in 10 ml of preheated distilled water (40°C):

o Methylparaben: 0.15 g
o Propylparaben: 0.05 g

2. Add Preservative Solution to the ointment base while stirring to prevent microbial growth.
3. Add Vitamin E (1 g) as an antioxidant to prevent oxidative degradation.

5. Homogenization and pH Adjustment

Objective: To ensure uniform distribution of ingredients and correct the pH for skin application.

5.1 Homogenization Process:

• Equipment Needed: Homogenizer

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Procedure:

1. Use the homogenizer set at 3000–5000 rpm for 5–10 minutes. This will ensure uniform consistency and proper dispersion of ingredients.
2. Ensure that the homogenizer is fully immersed in the mixture and moving in a circular motion for uniform mixing.

5.2 pH Testing:

Objective: To ensure the ointment has an ideal pH (5.5–6.5), suitable for skin application.

Equipment Needed:

- pH Meter (ensure the pH meter is calibrated before use)
- Beaker (for taking the sample)
- Distilled Water (for cleaning the pH probe)
- pH Calibration Solution (pH 4, 7, or 9 buffer solutions, if required for calibration)

6. Cooling and Packaging

Objective: To set the ointment and package it into sterilized containers.

Procedure:

1. Allow the ointment to cool to room temperature (approximately 25°C). Stir occasionally during cooling to maintain consistency.
2. Before packaging, sterilize the containers to ensure they are free from microbial contamination.

Sterilization Process for Packaging:

Objective: To ensure that packaging containers are free of microbes, preventing contamination of the ointment.

Procedure:

1. Sterilization by Autoclaving:
   - o Place the sterilized containers (plastic tubes or jars) into an autoclave.
   - o Set the autoclave to a temperature of 121°C and pressure of 15 psi for 15-20 minutes.
   - o Allow the containers to cool down in the autoclave before handling.
2. Sterilization by Dry Heat (if autoclave is unavailable):
   - o Preheat an oven to 160°C.
   - o Place the containers inside the oven and leave them for 30 minutes to sterilize.
   - o Allow them to cool down in the oven before handling.
3. Manual Sterilization (if no specialized equipment is available):
   - o Boil the containers and lids in distilled water for 15-20 minutes.
   - o Let them air dry in a clean environment before use.

7. Packaging and Sealing

1. Once the ointment has cooled and the containers are sterilized, fill the containers (plastic tubes or jars) with the ointment while still in semi-liquid form.
2. Seal the containers immediately to prevent contamination.
3. Label each container with batch number, manufacturing date, and expiry date.
4. Store the ointment in a cool, dry place, away from direct sunlight.

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Formulation Table for 4 Batches (Each of 100 gm)

Sr. No.	Ingredient	Category	Batch 1 (g)	Batch 2 (g)	Batch 3 (g)	Batch 4 (g)
1	Neem Oil	Active Ingredient (API)	07	07	07	07
2	Jamun Extract	Active Ingredient (API)	05	05	05	05
3	Jaifal Extract	Active Ingredient (API)	03	03	03	03
4	Beeswax	Ointment Base	10	10	05	05
5	Paraffin Wax	Ointment Base	08	08	05	05
6	White Soft Paraffin	Ointment Base	58	58	66	66
7	Lanolin	Ointment Base	7.8	7.8	7.8	7.8
8	Methylparaben	Preservative	0.15	0.15	0.15	0.15
9	Propylparaben	Preservative	0.05	0.05	0.05	0.05
10	Vitamin E	Antioxidant/Stabilizer	01	01	01	01

IV. RESULT AND DISSCUSSION

Organoleptic Evaluation

Appearance, odor, texture, and taste are assessed to ensure the ointment is pleasant and acceptable for use. The formulation should have no undesirable smells, be easy to apply, and visually appealing.

Test	Specification	Batch 1	Batch 2	Batch 3	Batch 4
Appearance	Smooth, uniform, lump-free	Fail	Fail	Pass	Pass
Odor	Mild herbal, characteristic of actives	Pass	Pass	Pass	Pass
Color	Uniform herbal tone	Pass	Pass	Pass	Pass
Texture	Creamy, non-gritty	Fail	Fail	Pass	Pass

Physical & Chemical Tests

Physical and chemical tests focus on the pH, viscosity, spreadability, and extrudability of the ointment. These tests ensure the product is skin-friendly, easy to apply, and dispensed smoothly, while stability testing predicts its shelf life under various conditions.

Test	Specification	Batch 1	Batch 2	Batch 3	Batch 4
pH	5.5 – 6.5	05	5.2	6.0	6.2
Viscosity	Easily spreadable, not runny	Fail	Fail	Pass	Pass
Spreadability	Uniform, spreads without tearing	Fail	Fail	Pass	Pass
Stability	No change in pH, texture, color	Fail	Fail	Pass	Pass

Microbiological Tests

Microbiological tests assess the ointment's safety by checking for antifungal and antibacterial efficacy. These tests ensure the product is free from harmful microbes and confirm the effectiveness of preservatives in preventing contamination.

Test	Specification	Batch 1	Batch 2	Batch 3	Batch 4
E. coli	Absent	Absent	Absent	Absent	Absent
Fungal Growth	Absent	Absent	Absent	Absent	Absent

Safety & Packaging Tests

Safety tests ensure the ointment is non-irritating and hypoallergenic. Packaging tests verify that the ointment is securely packaged, leak-proof, and easy to dispense, maintaining the product's safety and stability.

Test	Specification	Batch 1	Batch 2	Batch 3	Batch 4	Conclusion
Patch Test (Irritation)	No redness or irritation	Pass	Pass	Pass	Pass	Safe for application
Seal Integrity	No leakage	Pass	Pass	Pass	Pass	All packaging intact

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V. SUMMMARY AND CONCLUSION

Summary:

a. The study focused on the formulation and evaluation of a polyherbal antifungal ointment incorporating Neem oil (Azadirachta indica), Jamun extract (Syzygium cumini), and Jaifal extract (Myristica fragrans).

b. A total of four batches (F1 to F4) were prepared with varying concentrations of herbal extracts to:

- Optimize the formulation
- Assess individual and combined antifungal potentials
- Evaluate synergistic effects in different proportions

c. Each batch was subjected to:

- Organoleptic evaluation (color, odor, texture)
- Physicochemical analysis (pH, viscosity, spreadability, stability)
- Microbiological tests (E.coli, Fungal growth)
- Safety & Packaging tests (Patch testing, seal integrity)

d. All batches demonstrated antifungal activity, with Batch F3 & F4 showing the most significant zone of inhibition, indicating an effective synergistic blend of extracts.

e. Stability studies confirmed that all batches maintained their integrity under accelerated storage conditions.

Conclusion:

a. Four formulation batches (F1–F4) were successfully developed using standardized herbal extracts.

b. Batch F3 & F4 emerged as the most promising in terms of:

- Antifungal efficacy.
- Physicochemical stability.
- Allover formulation performance.
- The results confirm that the ratio and combination of herbal extracts greatly influence the antifungal activity due to potential synergistic interactions.

a. Batch 3 & 4 stable, non-irritating, and suitable for topical application, highlighting their potential for safe dermatological use.

b. This formulation provides a natural, effective, and eco-friendly alternative to conventional antifungal medications.

c. The outcomes of this research support further investigations, including:

- Clinical trials
- In vivo antifungal assessments
- Large-scale formulation and commercialization efforts.

d. Overall, the project reinforces the potential of herbal-based therapies in managing fungal infections through topical delivery systems.

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