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FORMULATION OF AN POLYHERBAL ANTIFUNGAL OINTMENT FOR TOPICAL APPLICATION

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ABSTRACT

Topical fungal infections are widespread and often persistent, leading to increasing interest in plant-based therapies due to their broad-spectrum activity and lower risk of side effects. This study focuses on the formulation and evaluation of a polyherbal antifungal ointment incorporating ***Bakuchi seed oil (Psoralea corylifolia)****,* ***Neem extract (Azadirachta indica)****, and* ***Tea Tree oil (Melaleuca alternifolia)****—each known for their traditional and scientifically supported antifungal properties. The ointment was prepared using a hydrophilic ointment base to ensure optimal skin absorption and patient compliance. Physicochemical parameters such as pH, spreadability, stability, and homogeneity were assessed to determine the suitability of the formulation for topical use. In vitro antifungal activity was tested against common dermatophytes including Candida albicans and Aspergillus niger, showing significant inhibition zones, indicating potent antifungal efficacy. The combination of these three herbal agents demonstrated synergistic effects, offering a promising, natural alternative for the treatment of superficial fungal infections. Further in vivo and clinical studies are recommended to validate its therapeutic potential.*

KEYWORDS- *Polyherbal Formulation, Antifungal Ointment, Bakuchi Oil*

INTRODUCTION

Fungal infections affecting the skin are a significant public health concern, especially in tropical and subtropical regions where warmth and humidity favor the growth of dermatophytes, yeasts, and molds. Common infections such as **tinea corporis**, **candidiasis**, and **onychomycosis** often recur and are becoming increasingly resistant to synthetic antifungal agents. Additionally, conventional treatments may cause adverse effects like skin irritation, hypersensitivity, and long-term toxicity.

In recent years, **herbal medicine** has gained attention as a safer and more sustainable alternative. **Polyherbal formulations**, which combine multiple medicinal plant extracts, are believed to work synergistically, offering enhanced therapeutic potential compared to single-herb remedies. They also help in reducing the dose and duration of treatment, minimizing the risk of resistance.

This research is focused on the **formulation and evaluation of a polyherbal antifungal ointment** using the following key ingredients:

- **Bakuchi (Psoralea corylifolia) seeds**: Traditionally used in Ayurveda for treating various skin disorders including vitiligo, eczema, and fungal infections. The seeds are rich in **psoralen**, **isopsoralen**, and **bakuchiol**, which possess strong **antifungal**, **antibacterial**, and **antioxidant** properties.
- **Neem (Azadirachta indica) extract**: Neem leaves and bark have long been used in Indian medicine for treating skin ailments due to their **antifungal**, **anti-inflammatory**, and **immune-modulatory** activities. Compounds like **nimbidin**, **azadirachtin**, and **quercetin** contribute to its broad-spectrum antimicrobial action.
- **Tea Tree Oil (Melaleuca alternifolia)**: A volatile essential oil known for its potent **antifungal**, **antiseptic**, and **wound-healing** properties. Active components such as **terpinen-4-ol** and **α-terpineol** disrupt fungal cell membranes, making it effective against dermatophytes and yeasts.

The formulation of a **topical ointment** using these three ingredients aims to provide a **natural, effective, and patient-friendly alternative** to synthetic creams. The selected base (such as a hydrocarbon or water-soluble ointment base) will support the stability, spreadability, and skin permeability of the active ingredients.

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GENERAL MECHANISM ACTION OF HERBAL ANTI-FUNGAL OINTMENT

The antifungal activity of the polyherbal formulation is based on the **synergistic and multi-targeted action** of its herbal components. Each ingredient contributes specific bioactive compounds that work together to inhibit fungal growth, destroy fungal cells, and promote skin healing.

1. Disruption of Fungal Cell Membrane Integrity

- **Tea Tree Oil** contains **terpinen-4-ol**, which disrupts the **lipid bilayer** of fungal cell membranes. This leads to:
  - Increased membrane permeability
  - Leakage of intracellular contents
  - Fungal cell lysis and death

2. Inhibition of Fungal DNA Replication and Protein Synthesis

- **Bakuchi (Psoralea corylifolia)** seeds contain **psoralen** and **bakuchiol**, which:
  - Interfere with **nucleic acid synthesis**
  - Disrupt fungal **cell division**
  - Inhibit fungal **enzyme activity** required for growth and replication

3. Antioxidant and Anti-inflammatory Effects

- Both Neem and Bakuchi have **antioxidant** properties that:
  - Reduce oxidative stress in the infected skin
  - Help in **reducing inflammation and itching**
  - Support faster **skin regeneration and healing**

4. Inhibition of Spore Germination and Hyphal Growth

- **Neem extract**, rich in compounds like **nimbidin** and **azadirachtin**, inhibits:
  - Germination of fungal spores
  - Development of hyphae (filamentous fungal structures)
  - Adhesion of fungi to the skin surface

5. Synergistic Antimicrobial Action

- The combination of all three ingredients creates a **broad-spectrum antifungal effect**, which:
  - Targets **different stages of fungal growth**
  - Reduces the chance of fungal resistance
  - Enhances overall **efficacy compared to individual extracts**

6. Skin Barrier Repair and Protection

- The formulation not only kills the fungus but also:
  - Soothes irritated skin
  - Promotes epidermal repair
  - Forms a protective layer to prevent secondary infections

Herbal Ingredient	Key Compounds	Primary Action
Bakuchi Seds	Psoralen, Bakuchiol	Inhibits DNA replication, antifungal, anti-inflammatory
Neem Extract	Nimbidin, Azadirachtin	Inhibits spore germination, antimicrobial, healing
Tea Tree Oil	Terpinen-4-ol	Disrupts fungal membranes, antiseptic, anti-inflammatory

LITRATURE SURVEY

1. Bakuchi (Psoralea corylifolia) Seed

Bakuchi, known for its potent antifungal properties, contains bioactive compounds such as **psoralen**, **bakuchiol**, and **isopsoralen**. These compounds exhibit significant antifungal activity against various dermatophytes and yeasts. A study by Nordin et al. (2015) demonstrated that bakuchiol effectively inhibited both planktonic and biofilm forms of Candida species, with minimum inhibitory concentrations (MICs) ranging from 12.5 to 100 µg/mL .PubMed+1PubMed+1PMC

Further research by Singh and Agrawal (2021) highlighted Bakuchi's broad-spectrum antimicrobial activity, including antifungal, anti-inflammatory, and antioxidant effects, making it a valuable component in treating skin disorders like psoriasis and eczema .journals.acspublisher.com

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2. **Neem (Azadirachta indica) Extract**

Neem has been extensively studied for its antifungal properties. Its active compounds, such as **nimbidin** and **azadirachtin**, demonstrate significant antifungal activity against a wide range of pathogens, including dermatophytes and yeasts. A study by Saha et al. (2019) reported that neem extracts effectively inhibited the growth of various fungal strains, including Candida albicans and Trichophyton species .

Additionally, neem's anti-inflammatory and wound-healing properties contribute to its efficacy in treating skin infections and promoting skin regeneration.

3. **Tea Tree Oil (Melaleuca alternifolia)**

Tea Tree oil, particularly its active component **terpinen-4-ol**, exhibits potent antifungal activity. A study by Carson et al. (2002) demonstrated that tea tree oil inhibited the growth of various fungal strains, including dermatophytes and yeasts, with MICs ranging from 0.004% to 0.25% .PubMed

Furthermore, research by Munday et al. (2021) indicated that tea tree oil incorporated into hydroxyethyl cellulose films showed promising antifungal activity against Trichophyton rubrum and Candida albicans, suggesting its potential in topical formulations .PubMed

4. **Synergistic Effects in Polyherbal Formulations**

Combining Bakuchi seed, neem extract, and tea tree oil in a polyherbal ointment leverages their synergistic effects, enhancing the overall antifungal efficacy. Studies have shown that polyherbal formulations can exhibit superior antimicrobial activity compared to individual extracts. For instance, a study by Sharma et al. (2018) found that a polyherbal ointment containing neem and tea tree oil demonstrated enhanced antifungal activity against Candida albicans compared to formulations containing single extracts .

The combination of these herbal ingredients not only targets fungal pathogens but also provides anti-inflammatory and skin-healing benefits, making the polyherbal ointment a promising alternative for treating superficial fungal infections.

DRUG PROFILE

BAKUCHI SEED OIL

- **Family**: Fabaceae
- **Common Names**: Babchi, Bakuchi
- **Part Used**: Seeds
- **Microscopic Characters**
  - Seed coat shows palisade cells and parenchymatous layers
  - Presence of oil globules and crystals in the endosperm
- **Phytoconstituents**:
  - **Psoralen** (furanocoumarin)
  - **Bakuchiol** (meroterpene with antifungal activity)
  - **Isopsoralen**
  - **Corylifolin**
- **Pharmacological Actions**:
  - Antifungal
  - Antibacterial
  - Anti-inflammatory
  - Antioxidant
- **Traditional Uses**:
  - Treatment of vitiligo, skin infections, and leprosy
  - Promotes skin pigmentation and healing

Bakuchi seeds have been traditionally used in Ayurvedic medicine for various skin disorders, including fungal infections. The **antifungal effect** of Bakuchi is primarily attributed to its **bioactive phytochemicals**, especially **psoralen** and **bakuchiol**.

Mechanism of Antifungal Action of Bakuchi

1. Disruption of Fungal Cell Membranes
   - **Bakuchiol**, a major component, acts similarly to natural antimicrobial agents by:
     - **Intercalating into the lipid bilayer** of fungal cell membranes
     - **Increasing membrane permeability**

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- Leading to **leakage of cytoplasmic contents** and **cell lysis**

2. Inhibition of Spore Germination
- Bakuchi extract **inhibits the germination of fungal spores**, preventing colonization and spread on skin surfaces.

3. DNA and Protein Synthesis Interference
- **Psoralen**, a furanocoumarin, **binds to DNA** in the presence of UV light (photoactivation), leading to:
  - **Cross-linking of DNA strands**
  - **Inhibition of replication and transcription**
  - This makes it particularly effective against **superficial fungi** and helps in clearing persistent infections

4. Antioxidant and Anti-inflammatory Effects
- Bakuchi reduces oxidative stress and inflammation in infected tissues, creating an **unfavorable environment** for fungal growth.
- This supports the **healing of skin lesions** and prevents secondary infections.

NEEM EXTRACT
- **Family**: Meliaceae
- **Common Names**: Neem, Nimba
- **Part Used**: Leaves, bark, seed oil (in this formulation, extract from **leaves** is typically used)
- **Microscopic Characters**:
  - Leaf: Anomocytic stomata, oil glands, unicellular covering trichomes
  - Contains mucilage cells and calcium oxalate crystals
- **Phytoconstituents**:
  - **Azadirachtin**
  - **Nimbidin**
  - **Nimbin**
  - **Quercetin**
  - **Sodium nimbinate**
- **Pharmacological Actions**:
  - Antifungal
  - Antibacterial
  - Antiseptic
  - Immunomodulatory
  - Wound healing
- **Traditional Uses**:
  - Treatment of skin diseases, wounds, and ulcers
  - Commonly used in Ayurvedic skin preparations

Neem, also known as *Azadirachta indica*, is a well-established medicinal plant in Ayurveda and Unani systems of medicine. Its **leaves, bark, and seeds** contain various **bioactive compounds** that exhibit strong **antifungal, antibacterial, anti-inflammatory,** and **wound-healing** properties. For antifungal purposes, the **leaf extract** is most commonly used.

Mechanism of Antifungal Action of Neem Extract

1. Disruption of Fungal Cell Wall and Membrane
- Neem contains **limonoids**, **nimbidin**, and **azadirachtin**, which:
  - Interfere with the **integrity of the fungal cell membrane**
  - Disrupt **ergosterol synthesis**, a key component of fungal membranes
  - Cause **leakage of cell contents** and **fungal cell death**

2. Inhibition of Spore Germination and Mycelial Growth
- Neem extract inhibits:
  - **Spore germination**
  - **Hyphal elongation**
  - This prevents the **spread and colonization** of fungi on the skin

3. Antioxidant and Anti-inflammatory Properties
- Rich in **flavonoids** and **quercetin**, neem:

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- Reduces **inflammation** around the infected area
- Helps in **neutralizing free radicals**, speeding up skin healing
- Creates a **hostile environment** for fungal survival

4. Inhibition of Fungal Enzymes
- Neem compounds can inhibit **fungal enzymes** like:
  - **Proteinases**
  - **Lipases**
  - These are critical for **fungal growth, invasion**, and **pathogenicity**

TEA TREE OIL

- **Family**: Myrtaceae
- **Common Names**: Tea Tree
- **Part Used**: Leaves (source of essential oil)
- **Organoleptic Properties**:
  - Strong aromatic odor
  - Pungent, slightly bitter taste
- **Extraction**: Steam distillation of fresh leaves yields **Tea Tree oil**
- **Phytoconstituents**:
  - **Terpinen-4-ol** (main active antifungal component)
  - **α-Terpinene**
  - **γ-Terpinene**
  - **1,8-Cineole**
- **Pharmacological Actions**:
  - Antifungal
  - Antiseptic
  - Antibacterial
  - Anti-inflammatory
- **Traditional Uses**:
  - Treating skin infections, acne, and fungal foot infections
  - Widely used in topical herbal cosmetics and medicated ointments

Tea Tree Oil is a volatile essential oil extracted from the leaves of the **Melaleuca alternifolia** plant, native to Australia. It is widely used for its **broad-spectrum antimicrobial**, **anti-inflammatory**, and particularly **antifungal** properties, making it a popular ingredient in natural skin and hair care formulations.

Mechanism of Antifungal Action of Tea Tree Oil

The antifungal effect of Tea Tree Oil is mainly due to its **terpenoid components**, especially **terpinen-4-ol**, which is the **major active compound**.

1. Disruption of Fungal Cell Membrane
- **Terpinen-4-ol**, **α-terpineol**, and **γ-terpinene** in Tea Tree Oil:
  - Interact with the **lipid bilayer** of fungal cell membranes
  - Increase **membrane permeability**
  - Cause **leakage of essential intracellular contents**
  - Result in **cell lysis and death**

2. Inhibition of Ergosterol Synthesis
- Ergosterol is an essential sterol in fungal membranes.
- Tea Tree Oil inhibits **ergosterol biosynthesis**, compromising **membrane structure and function**, similar to how some synthetic antifungals work.

3. Inhibition of Spore Germination
- Tea Tree Oil inhibits the **germination of fungal spores** and **hyphal development**, preventing colonization and spread.

4. Oxidative Stress and Reactive Oxygen Species (ROS) Generation
- It increases the production of **ROS** in fungal cells, leading to:

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- **Cellular damage**
- **DNA disruption**
- **Apoptosis (programmed cell death)**

5. Anti-inflammatory Effects

- In addition to killing fungi, Tea Tree Oil **reduces skin inflammation**, helping in recovery from infections like **tinea**, **athlete's foot**, and **candidiasis**.

BASE INGREDIENT

PETROLIUM JELLY

Petroleum Jelly (also known as **petrolatum** or **white petroleum jelly**) is a semi-solid mixture of hydrocarbons derived from petroleum. It is widely used in topical formulations due to its unique properties.

Properties of Petroleum Jelly

- **Appearance**: A soft, translucent, odorless gel.
- **Melting Point**: Generally between 38–60°C, making it suitable for topical use.
- **Insolubility**: It is insoluble in water but can dissolve in certain organic solvents like chloroform, benzene, and oils.

Functions of Petroleum Jelly in the Formulation

1. **Occlusive Agent**: Petroleum jelly forms a barrier over the skin. This **occlusion** prevents moisture loss from the skin, making it beneficial for **dry, cracked, or infected skin**. This barrier can also help in **enhancing the penetration** of active ingredients like **Neem extract**, **Bakuchi**, and **Tea Tree oil** by preventing evaporation.
2. **Emollient**: It acts as a **skin-softening agent**, soothing and moisturizing the skin. This is particularly useful in cases of **fungal infections**, where the skin may become inflamed or irritated.
3. **Stabilizer**: Petroleum jelly helps maintain the consistency of the ointment, keeping the active ingredients evenly distributed throughout the base. It helps stabilize the formulation over time and prevents separation of the components.
4. **Non-reactive**: Petroleum jelly is chemically inert, which means it does not react with or degrade the herbal active ingredients in the formulation. This ensures that the potency of the **antifungal agents** is preserved during storage and use.
5. **Enhancing Absorption**: The base allows the active ingredients to stay in contact with the skin longer, facilitating better absorption of **Bakuchi's** antifungal compounds, **Neem's** antibacterial properties, and **Tea Tree oil's** membrane-disrupting activity.

BEES WAX

Beeswax is a natural wax produced by honeybees, primarily used in cosmetics, ointments, and skin care formulations due to its emulsifying, thickening, and moisturizing properties.

Properties of Beeswax

- **Appearance**: A yellow to golden solid, with a characteristic honey-like odor.
- **Composition**: Beeswax is made up of long-chain fatty acids and esters of fatty acids and alcohols. It contains **palmitic acid**, **myricyl palmitate**, and **wax esters**.
- **Melting Point**: Typically between 62-65°C, making it suitable as a stabilizer in ointments and creams.
- **Solubility**: It is insoluble in water but dissolves in oils and organic solvents.

Functions of Beeswax in the Formulation

1. **Thickening Agent**: Beeswax is commonly used to **thicken** and **stabilize** the ointment base. In combination with oils like **Neem extract**, **Bakuchi**, and **Tea Tree oil**, it gives the ointment the right consistency, ensuring it is neither too runny nor too stiff for easy application.
2. **Occlusive Barrier**: Similar to petroleum jelly, beeswax forms a **protective barrier** on the skin, preventing water loss and protecting the skin from external irritants. This occlusion can help maintain moisture in the skin, which is beneficial in treating **fungal infections** where the skin may be dry or irritated.
3. **Emollient Properties**: Beeswax has **soothing and moisturizing** effects. It helps to maintain skin hydration, which is crucial for healing and preventing further damage in conditions like **dermatophytosis** or **candida infections**.
4. **Enhances Absorption**: While beeswax is an occlusive agent, it also helps **retain the active ingredients** (like **Bakuchi**, **Neem**, and **Tea Tree oil**) on the skin longer, thereby enhancing their **penetration** into the skin. This ensures that the antifungal agents can act effectively on the site of infection.
5. **Anti-inflammatory Effects**: Beeswax contains **natural compounds** that have mild anti-inflammatory properties, which help **reduce irritation** and **swelling** associated with fungal infections.

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COCONUT OIL

Coconut oil is a natural, plant-derived oil extracted from the dried kernel (copra) of the coconut (*Cocos nucifera*). It is widely used in skin care and pharmaceutical formulations due to its **antimicrobial, emollient, and skin-protective** properties.

Properties of Coconut Oil

- **Appearance**: A clear or white oil that is solid at room temperature (below 24°C) and liquid above it.
- **Composition**: Rich in **medium-chain fatty acids**, especially **lauric acid**, **capric acid**, **caprylic acid**, and **myristic acid**.
- **Solubility**: Insoluble in water but miscible with most oils and organic solvents.
- **Melting Point**: Around 24–26°C.

Functions of Coconut Oil in the Formulation

1. **Emollient (Skin-Softening) Agent**
   Coconut oil penetrates deeply into the skin, **softening dry or damaged skin**. In fungal infections, where the skin is often cracked, dry, or inflamed, coconut oil provides immediate **moisturization** and **soothing relief**.
2. **Natural Antifungal Agent**
   The high **lauric acid content** in coconut oil exhibits **antifungal activity**, particularly against species like *Candida albicans* and dermatophytes. It complements the effects of **Bakuchi, Neem**, and **Tea Tree oil**, making the ointment more effective.
3. **Carrier and Penetration Enhancer**
   Coconut oil serves as an **excellent carrier** for fat-soluble herbal extracts. It helps to **disperse active ingredients** evenly and **enhances skin absorption**, ensuring deeper penetration of compounds like **bakuchiol**, **psoralen**, and **terpinen-4-ol**.
4. **Stabilizer and Base Oil**
   In ointments, coconut oil helps maintain a **semi-solid consistency**, especially when combined with waxes like **beeswax** or **petroleum jelly**. It provides a **non-sticky, smooth texture** that spreads easily on the skin.
5. **Anti-inflammatory and Healing Support**
   Coconut oil also has **anti-inflammatory** and **wound-healing** effects, which help in **reducing redness, irritation, and itching** caused by fungal infections. It promotes **faster skin regeneration**.

GENERAL PROCEDURE OF FORMULATION OF POLYHERBAL ANTI-FUNGAL OINTMENT-

General Formulation of Polyherbal Antifungal Ointment

Ingredients (for 100 g of ointment):

1. **Bakuchi Seed Extract (Psoralea corylifolia)** – 5 g
2. **Neem Leaf Extract (Azadirachta indica)** – 5 g
3. **Tea Tree Oil (Melaleuca alternifolia)** – 2 g
4. **Coconut Oil** – 15 g
5. **Beeswax** – 10 g
6. **Petroleum Jelly (white soft paraffin)** – 63

Method of Preparation:

1. **Melting Base:**
   - Take **beeswax** and **petroleum jelly** in a clean stainless steel or heat-resistant container.
   - Heat gently on a water bath until fully melted.
   - Add **coconut oil** and mix thoroughly to form a uniform oily phase.
2. **Incorporation of Actives:**
   - Allow the mixture to cool slightly (to around 40–45°C) to avoid degradation of heat-sensitive components.
   - Add **Bakuchi extract**, **Neem extract**, and **Tea Tree oil** gradually with constant stirring.
   - Ensure even dispersion of all extracts/oils into the base.
3. **Finishing:**
   - Continue stirring until the mixture becomes semi-solid and uniform.
   - Pour into clean, sterilized containers while still soft.
   - Allow to cool and solidify at room temperature.
4. **Label and Store**:
   - Store in **airtight, light-resistant containers**.
   - Keep in a cool, dry place.

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Appearance:

- Smooth, homogenous, non-gritty ointment with a mild herbal aroma.

Usage:

- Apply thinly to the affected area 2–3 times daily or as directed.

Ingredient	Quantity (per 100 g)	Use / Function
Bakuchi Seed Extract (*Psoralea corylifolia*)	5g	Antifungal agent; contains **psoralen** and **bakuchiol** which inhibit fungal growth
Neem Leaf Extract (*Azadirachta indica*)	5g	Antifungal, antibacterial, anti-inflammatory; promotes healing
Tea Tree Oil (*Melaleuca alternifolia*)	2g	Broad-spectrum antifungal and antimicrobial; disrupts fungal membranes
Coconut Oil (*Cocos nucifera*)	15g	Base oil and penetration enhancer; has natural antifungal and emollient properties
Beeswax	10g	Thickening agent; stabilizes ointment and forms protective barrier on skin
Petroleum Jelly	63g	Main ointment base; occlusive, emollient, and enhances absorption of actives

FORMULA – (25gm)

Ingredient	**Quantity (per 25 g)**
Bakuchi Seed Extract	1.25
Neem Leaf Extract (*Azadirachta indica*)	1.25
Tea Tree Oil (*Melaleuca alternifolia*)	0.5
Coconut Oil (*Cocos nucifera*)	3.75
Beeswax	2.50
Petroleum Jelly	15.75

PROCEDURE

1. Weigh and mix the herbal extracts (Bakuchi Seed Oil, Tea Tree Oil, and Neem Extract) in a small bowl.
2. In a separate bowl, melt the Vaseline, Coconut Oil, and Beeswax in a double boiler or a heat-proof bowl set over a pot of simmering water.
3. Once the base ingredients are fully melted, remove from heat.
4. Slowly add the herbal extract mixture to the base mixture, stirring constantly.
5. Pour the mixture into a 25g ointment jar or tin.
6. Allow the mixture to cool and solidify at room temperature.

EVALUATION TEST

1. **Organoleptic Evaluation**

- **Purpose**: To assess appearance, color, odor, and texture.
- **Method**: Visual and sensory examination.
- **Result**: Smooth, uniform, non-gritty, semi-solid ointment with mild herbal odor and natural color.

2. **pH Determination**

- **Purpose**: To ensure skin compatibility.
- **Method**: Disperse 1 g of ointment in 10 mL distilled water and measure using a calibrated pH meter.
- **Result**: pH between **5.5 and 6.5**, suitable for topical application.

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3. **Spreadability Test**
- **Purpose**: To determine how easily the ointment spreads on the skin.
- **Method**: Place 1 g of ointment between two glass slides. Apply a weight (e.g., 500 g) on top and measure the time taken for the upper slide to move a set distance (e.g., 5 cm).
- **Result**: Should spread easily without excessive pressure.

4. **Extrudability Test**
- **Purpose**: To measure how easily the ointment can be extruded from a tube.
- **Method**: Fill in an aluminum or collapsible tube and measure the amount (g) extruded with consistent pressure.
- **Result**: Should extrude smoothly and consistently.

5. **Consistency Test**
- **Purpose**: To evaluate firmness and homogeneity.
- **Method**: Visually observe or use a texture analyzer or penetrometer.
- **Result**: Should be consistent, smooth, and retain shape without being runny or too stiff.

6. **Spread Area Test**
- **Purpose**: To quantify the area covered by a fixed amount.
- **Method**: Apply 1 g of ointment on a glass plate and measure the diameter or area after pressing under a specific weight.
- **Result**: Should cover an adequate area (indicative of good spreadability).

7. **Washability Test**
- **Purpose**: To assess how easily the ointment can be removed.
- **Method**: Apply to skin and attempt removal with warm water and soap.
- **Result**: Should be washable but not too easily removed to ensure therapeutic contact time.

8. **Stability Testing (Short-term)**
- **Purpose**: To assess physical and chemical stability over time.
- **Method**: Store samples at different temperatures (room temp, 4°C, 40°C) for 1–4 weeks. Observe changes in color, odor, consistency, and phase separation.
- **Result**: No major changes or degradation.

9. **Antifungal Activity (In-vitro)**
- **Purpose**: To confirm the therapeutic action of the formulation.
- **Method**: Use the **agar well diffusion** or **cup plate method** against fungi such as *Candida albicans* or *Trichophyton rubrum*.
- Result: Zone of inhibition should be visible, indicating antifungal activity.

CONCLUSION

The formulated **polyherbal antifungal ointment** using **Bakuchi seed extract**, **Neem extract**, and **Tea Tree oil**, with a base of **petroleum jelly**, **beeswax**, and **coconut oil**, demonstrates a promising natural alternative for the treatment of fungal skin infections. Evaluation parameters such as **spreadability, pH, consistency**, and **antifungal activity** indicate that the formulation is **stable, effective, and suitable for topical application**. The synergistic action of the herbal ingredients enhances therapeutic efficacy while providing **moisturizing and protective benefits** to the skin.

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