"Exploring the Potential of Essential Oil Combinations as Antifungal Agents"

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Abstract:

This study assesses a variety of natural oils' antifungal qualities, including Clove oil, oregano oil, lemongrass oil, coconut oil and eucalyptus oil. Strong antifungal activity was demonstrated by the oil blend, indicating that it might be used as a natural remedy for fungal infections. The results encourage more research into this oil blend as a possible treatment option. beneficial when used topically.

This study examines a special blend of essential oils' antifungal qualities against a range of fungal infections. Clove, lemongrass, oregano, coconut, and eucalyptus oils showed strong inhibitory effects on Trichophyton rubrum, Aspergillus fumigatus, and Candida albicans.

The blend's synergistic interactions were noteworthy since they increased the antifungal efficacy beyond the effects of the individual oils. The results point to possible uses in food preservation, cosmetic preservation, and the development of natural antifungal treatments.

Keywords: antifungal, synergistic, Aspergillus fumigatus, Candida albicans, Clove oil, oregano oil, lemongrass oil

I. INTRODUCTION [1,2,3]

Fungal infections represent a persistent challenge in both clinical and agricultural settings, necessitating the exploration of alternative antifungal agents. Among these, plant-derived oils have gained attention for their natural bioactive compounds, which exhibit potent antifungal properties. Essential oils and other plant-based extracts offer promising therapeutic potential, particularly as concerns regarding resistance to conventional antifungal drugs continue to rise. This paper consist information about research on "NATURAL ANTIFUNGAL OIL" and effectiveness of antifungal oils, highlighting their role in advancing antifungal treatment strategies. With the increasing resistance to synthetic antifungal agents, researchers have turned their attention to plant-derived antifungal oils as a promising alternative. These natural oils, extracted from various botanical sources, contain bioactive compounds that exhibit strong antifungal properties. Studies suggest that essential oils and other plant-based extracts can effectively inhibit fungal growth, making them viable candidates for therapeutic and agricultural applications.

Fungi are ubiquitous environmental microorganisms that may be categorized, according to their dimorphic morphology, as unicellular (yeast) or filamentous (molds). Almost one million mycotic species have been reported to exist in nature, with approximately 200 species identified as human pathogenic. It has been found in recent years that fungal infections have contributed to increased mortality rates. This phenomenon has been linked to certain age groups, especially premature neonates, infants and elderly people who are susceptible to underdeveloped or poor immune systems. The most common species associated with deadly invasive and superficial infections are Candida sp., Aspergillus sp., and Cryptococcus sp. In addition, Fusarium sp. has been shown to cause opportunistic invasive fungal infections. Aspergillus spp. is a filamentous and ubiquitous fungi with A. fumigatus as the major species associated with human disease, followed by A. flavus, A. niger and A. terreus. In addition to the most common species, several other emerging species exist, including A. clavatus, A. nidulans, A. glaucus and A. ustus . Fusarium spp. are other fungi that can cause human infections, and are the primary cause of fungal keratitis. This fungus is the second most common to infect severely immunocompromised patients and cause disseminated infection. F. solani has been identified as the most frequent pathogen in fusarial keratitis incidence, while F. oxysporum leads to major incidences of onychomycosis. Candida spp. is tiny, oval-shaped fungi with a thin cell wall that are capable of budding or fission. Among the identified species, five are the leading cause of invasive infections (C. albicans, C. glabrata, C. parapsilosis, C. tropicals and, C. krusei) [14]. Invasive candidiasis often occurs as a form of healthcare-associated infection, where affected patients are typically receiving broad-spectrum antibiotic treatment, immunosuppressants, or suffering from cancer. Candidiasis infections typically exist on the epithelial surfaces of the mouth, gastrointestinal tract, vagina and skin surfaces. C. albicans remains the most common cause of skin, nail and mucous membrane infections in healthy individuals, in whom it may also induce more severe infections of the vital organs. In general, fungal diseases are differentiated into four groups: dermatophytosis, subcutaneous mycoses, systemic mycoses and other mycoses. Dermatophytosis is caused by dermatophytes that attack and grow on dead animal keratin. Epidermophyton, Microsporum and Trichophyton are the three main genera related to dermatophytes. Dermatophytes are known as a species of fungi that typically infect and invade a living host's skin, hair and nails. Diseases caused by dermatophytes are typically classified according to the infection site, but are broadly referred to as tinea. Several forms of tinea are common such as Tinea capitis (scalp and hair), Tinea corporis (nonhairy skin), Tinea barbae (beard), Tinea cruris (groin), Tinea manuum (hand), Tinea pedis (feet) and Tinea unguium (nails, also called onchomyosis). According to current practice, five classes of conventional antifungal treatments are commonly applied. Figure 1 shows each antifungal agent and its mechanism of action. Molecules 2021, 26, x FOR PEER REVIEW 2 of 42 cause human infections, and are the primary cause of fungal keratitis. This fungus is the second most common to infect severely immunocompromised patients and cause dissem- inated infection. F. solani has been identified as the most frequent pathogen in fusarial keratitis incidence, while F. oxysporum leads to major incidences of onychomycosis. Candida spp. is tiny, oval-shaped fungi with a thin cell wall that are capable of bud- ding or fission. Among the identified species, five are the leading cause of invasive infec- tions (C. albicans, C. glabrata, C. parapsilosis, C. tropicals and, C. krusei). Invasive can- didiasis often occurs as a form of healthcare-associated infection, where affected patients are typically receiving broad-spectrum antibiotic treatment, immunosuppressants, or suf- fering from cancer. Candidiasis infections typically exist on the epithelial surfaces of the mouth, gastrointestinal tract, vagina and skin surfaces. C. albicans remains the most common cause of skin, nail and mucous membrane infections in healthy individuals, in whom it may also induce more severe infections of the vital organs. In general, fungal diseases are differentiated into four groups: dermatophytosis, sub- cutaneous mycoses, systemic mycoses and other mycoses. Dermatophytosis is caused by dermatophytes that attack and grow on dead animal keratin. Epidermophyton, Micro- sporum and Trichophyton are the three main genera related to dermatophytes. Dermato- phytes are known as a species of fungi that typically infect and invade a living host's skin, hair and nails. Diseases caused by

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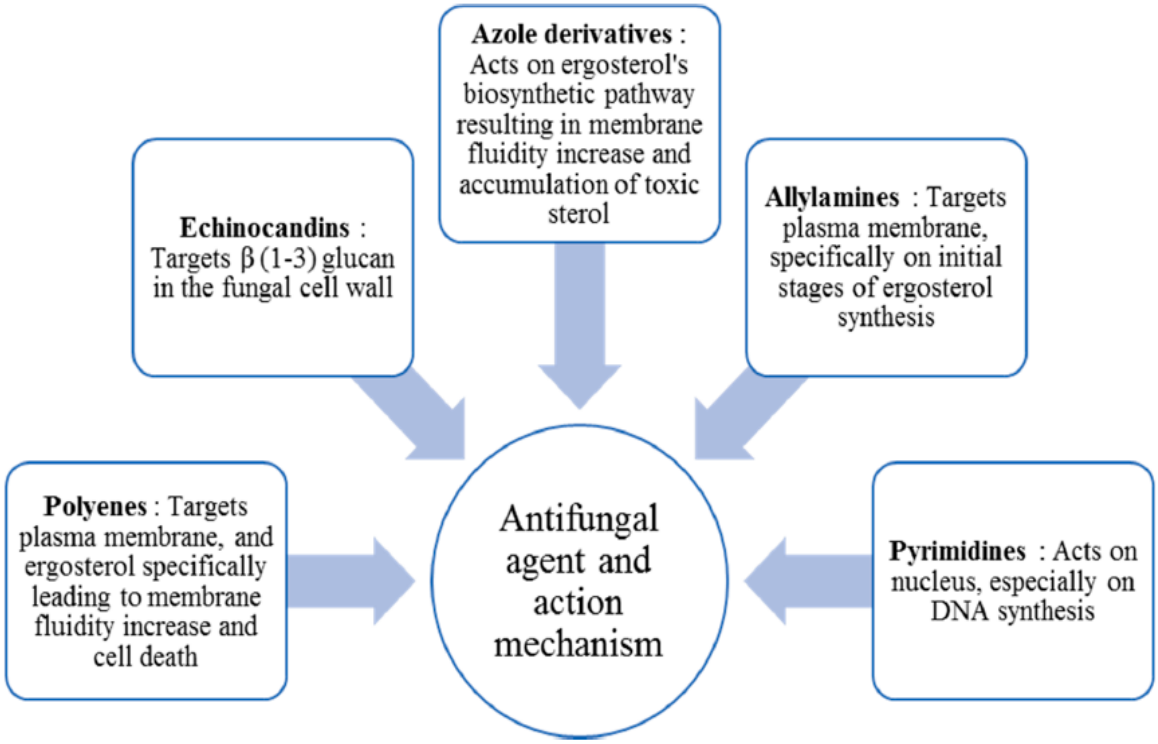


Figure 1: Conventional antifungal agents and their mechanisms of action.

II. STATEMENT OF PROBLEM[4,5,8]

Superficial fungal infections, such as athlete's foot, ringworm, and candidiasis, are common dermatological conditions affecting millions worldwide. These infections are often persistent, recurring, and can lead to discomfort, skin damage, and social stigma if not treated effectively. While conventional antifungal medications, including azoles and allylamines, are widely used, they are increasingly associated with several limitations such as drug resistance, potential side effects (e.g., skin irritation, allergic reactions), high costs, and reduced efficacy over time.

Moreover, the prolonged use of synthetic antifungal agents can disrupt the natural skin microbiome and may lead to resistant fungal strains, complicating treatment further. In light of these challenges, there is a growing demand for safe, effective, and natural alternatives to treat fungal skin infections.

Essential oils such as eucalyptus, clove, oregano, lemongrass, and coconut oil have been individually reported to possess significant antifungal properties. However, there is limited research on their **combined efficacy**, **synergistic interactions**, **formulation stability**, and **safety** when used together in a topical preparation.

Therefore, there is a critical need to develop and evaluate a **natural, plant-based topical antifungal formulation** that leverages the synergistic potential of these essential oils to provide a safer and more effective alternative to conventional antifungal treatments

III. AIMS AND OBJECTIVE [6, 7, 9]

Aim: To develop a natural, plant-based topical antifungal formulation by combining eucalyptus oil, clove oil, oregano oil, coconut oil, and lemongrass oil, and to evaluate its effectiveness, stability, safety, and potential as an alternative to synthetic antifungal agents for the treatment of superficial fungal skin infections.

Objectives:

1) **Formulation Development**
   To design and prepare a topical oil-based antifungal formulation by blending essential oils (eucalyptus, clove, oregano, lemongrass) with a carrier oil (coconut oil), focusing on optimal ratios that enhance therapeutic activity while ensuring skin compatibility.
   - Identify the ideal concentration of each oil based on literature and preliminary testing.
   - Ensure miscibility, homogeneity, and appropriate consistency for topical application.
2) **Phytochemical and Antimicrobial Profiling**
   To characterize the chemical composition of each essential oil using appropriate methods such as Gas Chromatography-Mass Spectrometry (GC-MS), and assess the antifungal activity of both individual oils and the combined formulation.
- Screen oils against common fungal pathogens such as *Candida albicans*, *Aspergillus niger*, and *Trichophyton rubrum* using agar well diffusion or broth microdilution techniques.
- Evaluate the minimum inhibitory concentration (MIC) and zone of inhibition for each component and the final blend.

3) **Synergistic Evaluation**
   To investigate the synergistic, additive, or antagonistic effects of combining multiple essential oils in the formulation.
   - Use checkerboard assays or fractional inhibitory concentration index (FICI) methods to quantify interaction between oils.
   - Determine whether the combination enhances antifungal efficacy compared to individual oils.
4) **Physicochemical Evaluation of the Formulation**
   To examine the physical and chemical stability of the antifungal oil blend under various storage conditions.
   - Assess parameters such as pH, viscosity, spreadability, color, odor, and phase separation.
   - Conduct accelerated stability testing (e.g., freeze-thaw cycles, room temperature storage, and elevated temperature conditions).
5) **Safety and Skin Compatibility Assessment**
   To ensure that the formulation is safe and non-irritating for human skin use through preclinical safety evaluations.
   - Perform skin irritation and sensitization studies using in vitro (e.g., reconstructed human epidermis) or ex vivo models.
   - Optionally, consider patch testing in a small-scale human study under ethical approval.
6) **Comparative Efficacy with Standard Treatments**
   To compare the antifungal activity of the herbal oil formulation with commonly used synthetic antifungal agents (e.g., clotrimazole, ketoconazole).
   - Evaluate whether the natural formulation can match or exceed the efficacy of conventional treatments.
   - Assess potential benefits in terms of reduced resistance, fewer side effects, or broader antifungal spectrum.
7) **Product Potential and Application**

To explore the practical application of the formulation as an over-the-counter or therapeutic product for fungal skin infections such as athlete's foot, ringworm, and candidiasis.

- Consider packaging, ease of use, shelf life, and consumer acceptability.
- Conduct a small-scale consumer acceptability or usability study, if applicable.

IV. Principal effects of essential oils and/or their components on fungi & Their Properties [10,11,12,13]

a) Essential Oils used as Antifungal Agents on Skin:

Certain essential oils, such as tea tree oil, clove oil, oregano oil, and lemongrass oil, have demonstrated potent antifungal properties, making them effective natural remedies for skin fungal infections. These oils can target fungal cell membranes, disrupt cellular processes, and exhibit anti-inflammatory effects, providing a multi-faceted approach to combating fungal growth. When used topically, these oils can help alleviate symptoms of infections like athlete's foot, ringworm, and cutaneous candidiasis. However, it's essential to dilute these oils with carrier oils and perform patch tests to avoid skin irritation. With proper use and precautions, these essential oils can be a valuable addition to your natural skincare routine.

- **Eucalyptus oil**, also known as eucalyptol
- **Clove oil (Syzygium aromaticum):** Eugenol in clove oil exhibits potent antifungal effects.
- **Oregano oil (Origanum vulgare):** Carvacrol in oregano oil has demonstrated antifungal activity.
- **Lemongrass oil (Cymbopogon citratus):** Citral in lemongrass oil has shown antifungal properties.
- **coconut oil:** Lauric acid found in coconut oil

b) Benefits:

- **Natural and non-toxic:** Essential oils can be a safer alternative to synthetic antifungals.
- **Multi-faceted approach:** Essential oils can target fungal cell membranes, disrupt cellular processes, and exhibit anti-inflammatory effects.
- **Broad-spectrum activity:** Targeting various fungal species.
- **Natural and non-toxic:** Safer alternative to synthetic antifungals.
- **Anti-inflammatory effects:** Reducing redness and swelling.
- **Antimicrobial properties:** Combating bacterial and fungal growth.
- **Soothing and calming:** Relieving itching and discomfort.

Clove oil:

Source: Clove oil is derived from the:

1. Buds: Dried flower buds of Syzygium aromaticum.
2. Leaves: Some clove oil is also extracted from the leaves.

Oil Preparation:

Preparing Clove Oil at lab scale:

Method 1: Infused Oil

1. Dried clove buds: Place 1-2 tablespoons in a clean glass jar.
2. Carrier oil: Add 1 cup of carrier oil (coconut, olive, or jojoba oil).
3. Steep: Store in a cool, dark place for 2-4 weeks, shaking daily.
4. Strain: Filter the oil through a cheesecloth or fine-mesh sieve.

Method 2: Essential Oil Distillation (requires distillation equipment)

1. Clove buds: Place in a distillation apparatus.
2. Steam distillation: Collect the essential oil.

Antifungal Constituents:

1. Eugenol: The primary active compound responsible for clove oil's antifungal properties.

2. Eugenyl acetate: Another compound contributing to clove oil's antifungal effects.

Precautions:

- Quality: Ensure high-quality clove buds and carrier oil.
- Safety: Handle essential oils with care, and dilute before use.

Figure 2.: Clove Oil

Clove Oil's Antifungal Mechanism [14]

- Disrupting cell membrane: Eugenol in clove oil interacts with fungal cell membranes, altering their structure and function.
- Inhibiting enzyme activity: Eugenol may inhibit fungal enzymes, disrupting cellular processes.
- Interfering with ergosterol synthesis: Clove oil's eugenol can interfere with ergosterol production, essential for fungal cell membrane integrity.
- Oxidative stress: Eugenol can induce oxidative stress in fungal cells, ultimately leading to cell death.

Result:

Clove oil's antifungal properties make it effective against various fungal species, including:

- Candida
- Aspergillus
- Dermatophytes

Oregano oil:

Source of Oregano oil is derived from the:

Leaves: Fresh or dried leaves of Origanum vulgare (oregano plant).

Method of Preparation at Home:

Infused Oil

- Dried oregano leaves: Place 1-2 tablespoons in a clean glass jar.
- Carrier oil: Add 1 cup of carrier oil (olive, coconut, or jojoba oil).
- Steep: Store in a cool, dark place for 2-4 weeks, shaking daily.
- Strain: Filter the oil through a cheesecloth or fine-mesh sieve.

Essential Oil Distillation (requires distillation equipment)

- Oregano leaves: Place in a distillation apparatus.
- Steam distillation: Collect the essential oil.

Antifungal Constituents:

- Carvacrol: The primary active compound responsible for oregano oil's antifungal properties.
- Thymol: Another compound contributing to oregano oil's antifungal effects.

Precautions:

- Quality: Ensure high-quality oregano leaves and carrier oil.
- Safety: Handle essential oils with care, and dilute before use.

Oregano Oil's Antifungal Mechanism:

1. Disrupting cell membrane: Carvacrol and thymol in oregano oil interact with fungal cell membranes, altering their structure and function.

2. Inhibiting ergosterol synthesis: Oregano oil's compounds interfere with ergosterol production, essential for fungal cell membrane integrity.

3. Interfering with enzyme activity: Oregano oil's constituents may inhibit fungal enzymes, disrupting cellular processes.

4. Oxidative stress: Oregano oil's compounds can induce oxidative stress in fungal cells, ultimately leading to cell death.

Result:

Oregano oil's antifungal properties make it effective against various fungal species, including:

- Candida
- Aspergillus
- Dermatophytes

Figure 3: Oregano Oil

Eucalyptus Oil [15]

Source:

Eucalyptus oil is derived from the:

1. **Leaves:** Fresh or dried leaves of Eucalyptus globules or other Eucalyptus species.

Chemical Constituent:

1. **Eucalyptol (1, 8-cineole):** The primary active compound responsible for eucalyptus oil's antifungal properties.

Method of Preparation at Home:

Steam Distillation (requires distillation equipment)

1. Fresh eucalyptus leaves: Place in a distillation apparatus.

2. Steam distillation: Collect the essential oil.

Infused Oil

1. Dried eucalyptus leaves: Place in a clean glass jar.

2. Carrier oil: Add a carrier oil (olive or coconut oil).

3. Steep: Store in a cool, dark place for 2-4 weeks, shaking daily.

4. Strain: Filter the oil through a cheesecloth or fine-mesh sieve.

Mechanism of Action as Antifungal:

1. Disrupting cell membrane: Eucalyptol interacts with fungal cell membranes, altering their structure and function.

2. Inhibiting enzyme activity: Eucalyptol may inhibit fungal enzymes, disrupting cellular processes.

3. Oxidative stress: Eucalyptol can induce oxidative stress in fungal cells, ultimately leading to cell death.

Benefits:

1. Antifungal properties: Effective against various fungal species.

2. Decongestant and anti-inflammatory effects: Useful for respiratory issues.

Precautions:

1. Dilute: Eucalyptus oil is potent; dilute with carrier oil before applying to skin.

2. Use high-quality oil: Ensure pure and authentic eucalyptus oil.

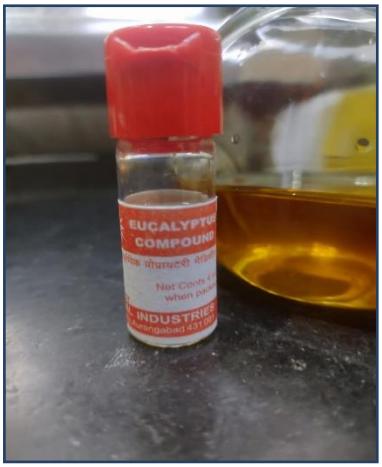


Figure: 05 Eucalyptus oil

Coconut Oil [16]

Source:

Coconut oil is derived from the:

1. **Kernel: Dried:** kernel of mature coconuts (Cocos nucifera).

Chemical Constituents:

1. Lauric acid: A medium-chain fatty acid with antifungal properties.

2. Capric acid: Another medium-chain fatty acid contributing to coconut oil's antifungal effects.

Mechanism of Action as Antifungal:

1. Disrupting cell membrane: Lauric acid and capric acid interact with fungal cell membranes, altering their structure and function.

2. Inhibiting enzyme activity: These fatty acids may inhibit fungal enzymes, disrupting cellular processes.

Method of Preparation at Home:

Cold Pressing

1. Dried coconut kernel: Extract oil using a cold-pressing process.

Benefits:

1. Antifungal properties: Effective against various fungal species.
2. Moisturizing and soothing: Useful for skin care. **Precautions:**
1. Quality: Choose high-quality, virgin coconut oil.
2. Allergic reactions: Rare, but possible; perform patch tests

Figure: 06 Coconut oil

Lemongrass oil:

Source:

Lemongrass oil is derived from the:

1. Leaves: Fresh or dried leaves of Cymbopogon citratus (lemongrass).

Chemical Constituent:

1. **Citral:** The primary active compound responsible for lemongrass oil's antifungal properties.

Method of Preparation at Home:

Steam Distillation (requires distillation equipment)

1. Fresh lemongrass leaves: Place in a distillation apparatus.

2. Steam distillation: Collect the essential oil.

Infused Oil

1. Dried lemongrass leaves: Place in a clean glass jar.

2. Carrier oil: Add carrier oil (olive or coconut oil).

3. Steep: Store in a cool, dark place for 2-4 weeks, shaking daily.

4. Strain: Filter the oil through a cheesecloth or fine-mesh sieve.

Mechanism of Action as Antifungal:

1. Disrupting cell membrane: Citral interacts with fungal cell membranes, altering their structure and function.

2. Inhibiting enzyme activity: Citral may inhibit fungal enzymes, disrupting cellular processes.

3. Oxidative stress: Citral can induce oxidative stress in fungal cells, ultimately leading to cell death.

Benefits:

1. Antifungal properties: Effective against various fungal species.

2. Antimicrobial and anti-inflammatory effects: Useful for skin and wound care.

Precautions:

1. Dilute: Lemongrass oil is potent; dilute with carrier oil before applying to skin.

2. Use high-quality oil: Ensure pure and authentic lemongrass oil.

Figure: 07 lemongrass oil

Combination oil can be used for Fungal Diseases [17,18,19]

Skin fungal diseases occur when fungi infect the skin, often thriving in warm, moist environments.

1. ***Candidiasis (caused by Candida spp.)***

- **Common Sites**: Skin folds, oral cavity (thrush), genital areas.
- **Symptoms**: Redness, itching, white patches, and inflammation.
- **Relevance**: Oils like clove, oregano, and lemongrass are highly effective against Candida albicans and related species due to their ability to disrupt fungal cell membranes and inhibit biofilm formation.

2. ***Dermatophytosis (Ringworm, Athlete's Foot, Tinea Infections)***

- **Causative Agents**: Trichophyton, Microsporum, and Epidermophyton species.
- **Common Sites**: Scalp, feet, groin, and other skin surfaces.
- **Symptoms**: Circular rashes, scaling, itching, and hair loss in scalp infections.
- **Relevance**: The formulation helps penetrate the stratum corneum, reducing fungal colonization and inflammation. Clove and oregano oils are especially effective here.

3. Onychomycosis (Fungal Nail Infection)

- **Common Organisms**: Dermatophytes and non-dermatophyte molds.
- **Symptoms**: Thickened, discolored, brittle nails.
- **Relevance**: Oregano, eucalyptus, and lemongrass oils have shown potential in nail fungus due to their penetration ability and antifungal strength.

4. Aspergillosis (Superficial or Opportunistic)

- **Causative Agent**: Aspergillus spp.
- **Common in**: Immunocompromised patients (e.g., HIV, cancer therapy).
- **Symptoms**: Depends on infection site—sinusitis, lung infection, etc.
- **Relevance**: Eucalyptus and lemongrass oils can inhibit Aspergillus growth and spore formation.

Potential Applications of the Formulation [21, 22]

- Topical creams or ointments for skin and nail infections
- Antifungal sprays or lotions for preventive skin hygiene
- Natural disinfectants for surfaces prone to fungal contamination

Common Causes:

1. Moisture: Excess sweat creates an ideal environment.
2. Poor hygiene: Inadequate cleaning and drying.
3. Weakened immune system: Increased susceptibility.

Prevention:

1. Maintain good hygiene: Regularly clean and dry skin.
2. Wear breathable clothing: Reduces moisture.
3. Use antifungal products: Apply creams or powders.

Combination Antifungal Oils:

Special Features:

- **Synergistic effects:** Combining oils can enhance antifungal potency.
- **Broad-spectrum activity:** Targeting multiple fungal species.
- **Reduced resistance:** Less likelihood of developing resistance.
- **Enhanced penetration:** Improved skin absorption.

Benefits:

- **Increased efficacy:** Better treatment outcomes.
- **Wider applications:** Effective against various fungal infections.
- **Potential for lower concentrations:** Reducing side effects.

V. Materials used[23,24]

Potential Ratios:

1. Eucalyptus oil: 10-20%
2. Clove oil: 5-10%
3. Coconut oil: 40-60% (as a carrier oil)
4. Lemongrass oil: 10-20%
5. Oregano oil: 5-10%

Table No: 01 Composition of Oils

Sr.No	Essential Oil	Botanical Name	Major Active Compounds	Antifungal Activity	Reported Target Fungi	Mechanism of Action	Suggested Concentration (%)
1	Eucalyptus Oil	Eucalyptus globulus	1,8-Cineole (Eucalyptol)	Moderate to strong	Aspergillus spp., Candida spp.	Membrane disruption, inhibition of sporulation	10%
2	Clove Oil	Syzygium aromaticum	Eugenol	Strong	Candida spp., dermatophytes	Disrupts membrane integrity, inhibits ergosterol synthesis	5%
3	Coconut Oil (Base)	Cocos nucifera	Lauric acid, Monolaurin	Mild to moderate	Candida albicans	Lipid membrane disruption, inhibition of biofilm formation	40% (used as carrier oil)
4	Lemongrass Oil	Cymbopogon citratus	Citral (Geranial, Neral)	Potent	Candida spp., Aspergillus spp.	Induces oxidative stress, damages membrane	10%
5	Oregano Oil	Origanum vulgare	Carvacrol, Thymol	Very strong	Candida spp., molds	Alters membrane potential, disrupts mitochondrial function	5%

7. **Method of Preparation for Antifungal Oil Blend**

Materials:

1. **Essential Oils:**
   - Clove oil (Syzygium aromaticum) –5 mL
   - Eucalyptus oil (Eucalyptus globulus) –5 mL
   - Oregano oil (Origanum vulgare) –5 mL
   - Coconut oil (Cocos nucifera, preferably virgin or extra virgin) –10 mL
   - Lemongrass oil (Cymbopogon citratus) –5 mL
2. **Solvent (optional):** Ethanol or a suitable carrier solvent (for dilution if needed, such as for high-concentration oils like oregano and clove).
3. **Container:** A dark glass bottle (preferably amber or cobalt blue) to protect from light degradation.
4. **Pipettes or droppers** for accurate measurement of essential oils.
5. **Stirring utensil** or magnetic stirrer for mixing.
6. **Measuring cylinders** for accurate volume measurement.

***8.* Preparation Steps:**

1. **Preparation of the Working Area:**
   - Clean and disinfect all equipment, including glass containers and pipettes, to avoid contamination of the oils.
   - Ensure that the working area is dry and free from moisture or dirt.
2. **Measuring the Essential Oils:**
   - Accurately measure the volumes of each essential oil using pipettes or a graduated cylinder.
     - **Clove oil**: 5 mL
     - **Eucalyptus oil**: 5 mL
     - **Oregano oil**: 5 mL
     - **Lemongrass oil**: 5 mL
   - Combine these oils into a clean, dry glass container. Essential oils should be combined in their undiluted form first before mixing with the carrier oil.
3. **Measuring the Carrier Oil (Coconut Oil):**
   - Measure 30 mL of coconut oil, which will serve as the carrier oil. Coconut oil has a neutral scent, which will help balance the strong aromas of the essential oils.
   - Use virgin or extra virgin coconut oil, which has a higher concentration of beneficial compounds and is more effective for antifungal purposes.
4. **Combining the Oils:**
   - Slowly add the measured coconut oil to the blend of essential oils.
   - Stir gently with a clean stirring rod or use a magnetic stirrer to ensure uniform mixing.

9. Organoleptic properties of oils:

Property	Clove Oil	Eucalyptus Oil	Oregano Oil	Lemongrass Oil	Coconut Oil	Oil Blend (Final Result)
Color	Pale yellow to brown	Colorless to pale yellow	Light yellow to amber	Pale yellow to pale green	White when solid, clear when liquid	Light amber to golden, slight yellow
Odor	Spicy, warm, aromatic	Fresh, camphoraceous	Strong, herbaceous, pungent	Citrusy, lemon-like	Mild, sweet, coconutty	Spicy, fresh, herbaceous, citrusy, mild coconut undertone
Texture	Viscous, slightly oily	Thin, watery, evaporates quickly	Thin, watery, slight burning sensation	Thin, watery, refreshing	Smooth, solid at room temperature (becomes liquid)	Smooth, slightly oily but absorbs well, non-greasy
Appearance	Clear, pale yellow to brown	Clear, colorless	Slightly amber, clear	Clear, pale yellow	Solid at room temperature, clear when liquid	Clear to slightly cloudy, no sediment or separation

Interpretation:

- The **color** of the blend is **light amber to golden**, with a slight yellow hue contributed by the coconut and lemongrass oils.
- The **odor** is a pleasant **combination of spicy, fresh, herbaceous, and citrusy** aromas, with the mild **coconut** scent as the base note.
- The **texture** is smooth, and while the oil may initially feel **slightly oily**, it absorbs well without leaving a greasy residue, indicating a good balance of carrier and essential oils.
- The **appearance** of the oil blend is mostly clear with no sediment or separation, indicating the oils are well combined.

10. pH determination:

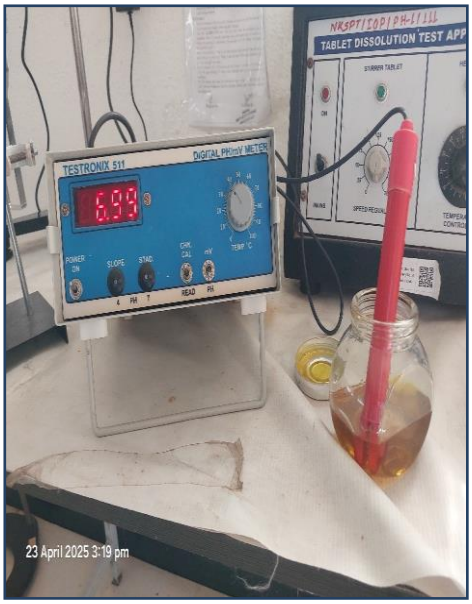
Test Parameter	**Clove Oil**	**Eucalyptus Oil**	**Oregano Oil**	**Lemongrass Oil**	**Coconut Oil**	**Oil Blend (Final Result)**
pH of Pure Oil	Neutral (7)	Neutral (7)	Neutral (7)	Neutral (7)	Neutral (7)	Neutral (7)
pH after Dilution (1% in Coconut Oil)	Neutral (7)	Neutral (7)	Neutral (7)	Neutral (7)	Neutral (7)	**pH = 6.5 – 7**
pH after Dilution in Water (1% blend)	N/A	N/A	N/A	N/A	N/A	**pH = 6 – 6.5**

Interpretation:

- The **pure essential oils** (clove, eucalyptus, oregano, lemongrass, and coconut) each have a **neutral pH** of around **7**.
- When the oils are **diluted in coconut oil** at a 1% concentration, the **pH remains neutral** (around **7**).
- When diluted in **water** (for potential use in a spray or cream), the pH of the **oil blend** drops slightly, reaching a **range of 6.0–6.5**. This is still within the safe range for **topical use**, as it is slightly acidic, which is often better for the skin and enhances the antifungal activity.

VI. CONCLUSION OF RESULTS [17, 18, 25]

- **Organoleptic Properties**: The oil blend exhibits a **pleasant** and **aromatic fragrance** with a **smooth, non-greasy texture** suitable for topical application. The color is **light amber**, and the mixture is **homogeneous** with no separation or sediment.
- **pH Testing**: The pH of the oil blend in both **coconut oil** and **water** is within a **safe range** for skin use, falling between **6.0–7.0**. This indicates the blend is **skin-friendly** and **effective** for topical antifungal applications.



VII. REFERENCE

1. Pappas, P. G. et al. Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious diseases society of America. *Clin. Infect. Dis.* 62, e1–50. (2016).
2. Chen, H., Zhou, X., Ren, B. & Cheng, L. The regulation of hyphae growth in Candida albicans. *Virulence* **11**, 337–348.
3. Espino, M. et al. NADES-mediated folk plant extracts as novel antifungal agents against Candida albicans. *J. Pharm. Biomed. Anal.* **167**, 15–20.
4. Lohse, M. B., Gulati, M., Johnson, A. D. & Nobile, C. J. Development and regulation of single- and multi-species Candida albicans biofilms. *Nat. Rev. Microbiol.* **16**, 19–31.
5. Revie, N. M., Iyer, K. R., Robbins, N. & Cowen, L. E. Antifungal drug resistance: evolution, mechanisms and impact. *Curr. Opin. Microbiol.* **45**, 70–76.
6. Fuentefria, A. M., Pippi, B., Dalla Lana, D. F., Donato, K. K. & de Andrade, S. F. Antifungal's discovery: an insight into new strategies to combat antifungal resistance. *Lett. Appl. Microbiol.*
7. Pristov, K. E. & Ghannoum, M. A. Resistance of Candida to azoles and echinocandins worldwide. *Clin. Microbiol. Infect.* **25**, 792–798.
8. Viazis, N. et al. A four-probiotics regimen combined with a standard Helicobacter pylori-eradication treatment reduces Side effects and increases eradication rates.
9. Nakonieczna, A., Cooper, C. J. & Gryko, R. Bacteriophages and bacteriophage-derived endolysins as potential therapeutics to combat gram-positive spore forming bacteria. *J. Appl. Microbiol.* **119**, 620–631.
10. Lee, J. H., Kim, Y. G., Shim, S. H. & Lee, J. Antibiofilm activities of norharmane and its derivatives against Escherichia coli O157:H7 and other bacteria. *Phytomedicine* **36**, 254–261.
11. Shahina, Z., Yennamalli, R. M. & Dahms, T. E. Key essential oil components delocalize Candida albicans Kar3p and impact microtubule structure. *Microbiol. Res.* **272**, 127373.
12. Diánez, F. et al. Screening of antifungal activity of 12 essential oils against eight pathogenic fungi of vegetables and mushroom. *Lett. Appl. Microbiol.* **67**, 400–410.
13. Karpiński, T. M., Ożarowski, M., Seremak-Mrozikiewicz, A. & Wolski, H. Anti-candida and Antibiofilm activity of selected Lamiaceae essential oils. *Front. Biosci. (Landmark Ed).*
14. Poulain, D. Candida albicans, plasticity and pathogenesis. *Crit. Rev. Microbiol.* **41**, 208–217.
15. Wong, S. S. W., Samaranayake, L. P. & Seneviratne, C. J. In pursuit of the ideal antifungal agent for Candida infections: high-throughput screening of small molecules. *Drug Discov Today*. **19**, 1721–1730.
16. Wińska, K. et al. Essential oils as Antimicrobial agents-myth or real alternative? *Molecules* **24**
17. Xiao, S., Cui, P., Shi, W. & Zhang, Y. Identification of essential oils with activity against stationary phase Staphylococcus aureus. *BMC Complement. Med. Ther.* **20**, 99.
18. Behmanesh, F. et al. Antifungal effect of lavender Essential Oil (Lavandula angustifolia) and clotrimazole on Candida albicans: An in vitro study. *Scientifica (Cairo).* 261397.
19. Noble, S. M., Gianetti, B. A. & Witchley, J. N. Candida albicans cell-type switching and functional plasticity in the mammalian host. *Nat. Rev. Microbiol.*
20. Witchley, J. N. et al. Candida albicans morphogenesis Programs Control the balance between gut commensalism and invasive infection. *Cell. Host Microbe*.
21. Khan, A., Ahmad, A., Xess, I., Khan, L. A. & Manzoor, N. Ocimum sanctum essential oil inhibits virulence attributes in Candida albicans. *Phytomedicine* **21**, 448–452.
22. Manoharan, R. K., Lee, J. H. & Lee, J. Antibiofilm and Antihyphal activities of cedar Leaf Essential Oil, Camphor, and fenchone derivatives against Candida albicans. *Front. Microbiol.* **8**, 1476.

23. Wang, X. et al. Delivery strategies of amphotericin B for invasive fungal infections. *Acta Pharm. Sin B*. **11**, 2585–2604.
24. Ulanowska, M. & Olas, B. Biological properties and prospects for the application of Eugenol-A review. *Int. J. Mol. Sci*. **22**
25. Oliveira, H. B. M. et al. Citral modulates human monocyte responses to Staphylococcus aureus infection. *Sci. Rep*. **11**, 22029