

Herbal Antifungal Cream to treat Onychomycosis

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ABSTRACT

Fungal skin infections are now incredibly common worldwide, affecting many people. It has been reported that 40 million individuals suffer from fungal infections. The World Health Organization (WHO) emphasizes the significant role of traditional medicine, including herbal remedies, in global primary health care. A substantial portion of the world's population, over 80% in more than 170 of the WHO's 194 member states currently relies on some form of traditional medicine for their primary healthcare needs.

Herbal antifungal creams play an important role in treating fungal infections by utilizing the natural antifungal properties of plants as their active ingredients. These creams are topical medications applied to the skin, scalp, or nails to treat fungal infections. They work by killing or inhibiting the growth of fungi. As a targeted drug delivery system, antifungal creams deliver the medication directly to the infected area. This approach reduces the amount of drug required and minimizes the risk of systemic side effects.

Herbal antifungal creams that include neem and tea tree oil harness the potent antifungal properties of these natural ingredients. Neem (*Azadirachta indica*) contains compounds such as Nimbidin and Azadirachtin, which disrupt fungal cell membrane integrity and replication, offering broad-spectrum antifungal, antibacterial, and anti-inflammatory benefits. Tea tree oil (*Melaleuca alternifolia*) is known for its strong antifungal activity, particularly against common skin fungi such as *Candida* species and Dermatophytes, by damaging their cell membranes. This study aims to be the first to assess the antifungal effectiveness of creams formulated with *Melaleuca alternifolia* (Tea tree oil) essential oil and *Azadirachta indica* (Neem).

Primary healthcare, antifungal, Antibacterial, Anti-inflammatory, *Melaleuca Alternifolia*, *Azadirachta indica*.

I. INTRODUCTION

For millennia, plants worldwide have served as a vital source of therapeutic agents, directly treating ailments and importantly providing

crucial building blocks or inspiration for the development of novel pharmaceuticals. This enduring relationship highlights the profound significance of the plant kingdom in the history and future of medicine. Over 50% of modern drugs have origins in natural products, highlighting their crucial role in the pharmaceutical industry. These natural compounds derived from various biological sources serve as inspiration and direct sources for drug development. The unique chemical diversity and biological relevance of these compounds ensure their ongoing importance in pharmaceutical research and development.^[1]

Dermatophyte infections, also known as tinea or ringworm, are among the earliest recognized fungal infections and remain remarkably common throughout the world. Dermatophytes, a group of fungi that thrive on keratin, are the primary culprits behind infections of the skin, hair, nails and in some cases subcutaneous tissues in both humans and animals. These infections are commonly known as ringworms or dermatomycoses.^[2]

Pharmacological treatment of fungal infections traditionally relies on a range of antifungal agents that target essential components and processes in fungal cells. These agents work by either killing the fungus (fungicidal) or inhibiting its growth (fungistatic). Recently, there has been increasing interest in the use of natural plant products as alternative or complementary approaches to inhibit causative fungal organisms. Research has identified numerous plants and their extracts or isolated compounds that possess antifungal properties. Since the late 19th century, scientific documentation has increasingly affirmed the long held traditional knowledge regarding the antimicrobial and antitoxin properties of numerous plants, herbs and their components. This growing body of evidence highlights their potential as safer alternatives to some chemically produced antifungal and antimicrobial compounds, offering a readily available resource for rural populations often most vulnerable to infections. For rural communities where access to conventional healthcare and expensive pharmaceutical drugs

may be limited, traditional plant-based medicines remain a crucial and accessible option for treating common infections. Their availability, often at little to no cost, makes them an integral part of primary healthcare in many parts of the world. The continued scientific investigation into these natural remedies not only validates traditional practices but also opens avenues for the development of new, effective and sustainable antimicrobial therapies.^[3,4]

Essential oil derived from the leaves of the Australian native plant *Melaleuca alternifolia*, commonly known as tea tree oil, is widely utilized in Australia as a topical therapeutic agent. Its medicinal benefits are primarily attributed to its potent anti-inflammatory and antimicrobial properties. The antimicrobial properties of tea tree oil are broad spectrum, demonstrating activity against bacteria, fungi and viruses. Due to this combination of anti-inflammatory and antimicrobial activities, tea tree oil is commonly used for topical applications such as treating acne, athlete's foot, nail fungus, insect bites and minor cuts and abrasions.^[5]

Neem is used for its antifungal and anti-inflammatory properties. It is also utilized to improve skin conditions by reducing scars, pigmentation, redness and itching.^[6,7] Neem leaf and seed extracts have demonstrated effectiveness against certain dermatophytes such as *Microsporum nanum* and *Epidermophyton floccosum*, as well as against *Candida albicans*, when tested using the tube dilution technique.^[8,9]

What is fungus?

1. Fungi are a group of eukaryotic organisms that possess a distinct mode of obtaining nutrients. Unlike animals that ingest food and digest it internally, fungi exhibit external digestion. A key characteristic of fungal cells is the presence of a rigid cell wall, primarily composed of chitin.
2. For a long time, fungi were mistakenly grouped with plants and considered primitive members of the plant kingdom, only slightly more advanced than bacteria.
3. Fungi primarily reproduce through spores and their main body, known as the thallus, consists of a network of microscopic, tube-like structures called hyphae.



Fig 1: Fungus

What is fungal infection?

Fungal infections, also known as mycoses, are skin diseases caused by various species of fungi. These fungi are widespread, living in soil, on plants, household surfaces, and even on our skin.

What is skin fungus?

Skin fungus, also called mycoses, are infections of the skin, hair, and nails caused by various fungi. These infections can show up in different ways, such as rashes, itching, or changes in how the skin looks. Fungal infections typically affect the skin and nails but can also occur in various internal areas like the mouth, throat, lungs, and urinary tract.

Symptoms of fungal infection

A fungal infection might cause:

1. Irritation
2. Scaly skin
3. Redness
4. Itching
5. Swelling
6. Blisters

Types of fungal skin infection:

1. Athlete's foot
2. Jock itch
3. Ringworm
4. Yeast infection
5. Onychomycosis



Fig 2: Types of fungal infection

Fungal skin infection risk factors:

1. Come into contact with an infected person or animal.
2. Borrow shoes or clothes that contain harmful fungus.
3. Take long- term or high dose of antibiotics.
4. Try new skin products.
5. Sweat a lot.
6. Wear tight clothing or footwear that doesn't breathe well.

Fungal skin infection treatment:

1. Topics antifungals –

- i. Creams
- ii. Lotions
- iii. Shampoo

Antifungal tablets –

- i. Griseofulvin
- ii. Itraconazole
- iii. Terbinafine^[10]

II. ANATOMY OF NAIL



Fig 3: Nails

Essentially, nails have protective coverings on the fingertips and toes of all primates. They're the primate equivalent of claws found in other animals. Both fingernails and toenails are composed of a strong protein called alpha-keratin, which is also the main component of claws, hooves, and horns in other vertebrates.^[11]

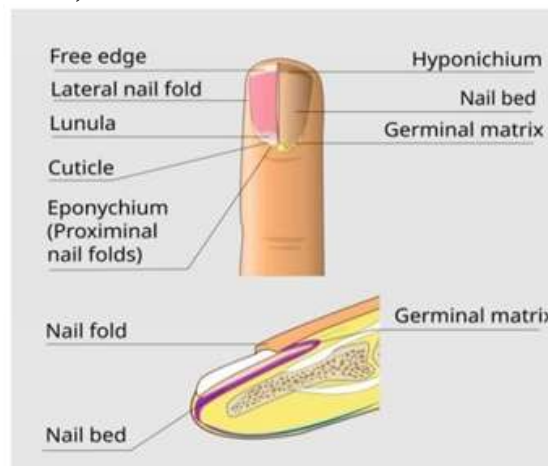


Fig 4: Parts of Nail

Structure of Nail:

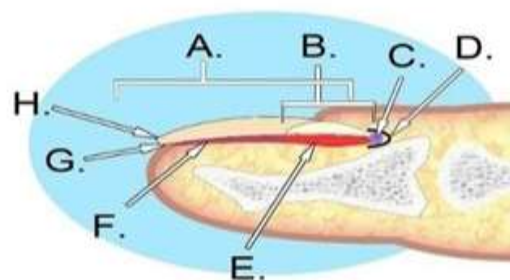


Fig 5: Structure of nail

A. Nail plate; B. Lunula; C. Root; D. Sinus; E. Matrix; F. Nail bed; G. Hyponichium; H. free margin.

The nail plate is a hard, visible part of the nail. The nail matrix is hidden beneath the skin at the base of the nail and is where new nail cells are produced. The nail bed is the skin underneath the nail plate. Grooves surround the nail plate.^[12]

Parts of the nail:

The nail matrix, also called the matrix unguis, keratogenous membrane, or onychostroma, is the living tissue at the base of the nail that creates new nail cells.^[13] These cells harden and move outward to form the nail plate.^[14] Located beneath the nail and containing nerves, lymph, and blood vessels, the matrix's size and shape influence

the nail plate's width, thickness, and overall form (flat, arched, or hooked, depending on the fingertip bone). If the matrix is healthy and nourished, it continuously produces new cells, pushing older ones forward.^[15] This compression makes the older cells flat and translucent, allowing the pink color of the capillaries in the nail bed underneath to show through.

The lunula is that whitish, crescent-shaped part you see at the base of your fingernail, especially noticeable on your thumb. It looks white because of how light reflects where the nail matrix and nail bed come together. You might not even see it on your little finger.

The nail bed is the skin under the nail plate that the nail rests on. It contains nerves and blood vessels that nourish the entire nail unit. Like other skins, it's made of the dermis and epidermis, which are connected by grooves called matrix crests. These grooves can become more visible as the nail plate thins with age. The nail bed is very sensitive due to its high nerve density, making nail plate removal extremely painful.

The different parts of the nail you've described:

Nail Sinus: This is where the nail root, or the base of the nail, is tucked underneath the skin. It's connected to the matrix, which is the active tissue that makes the nail grow.

Nail Plate: This is the hard, visible part of the nail, from the root to the free edge. It's made of layers of dead, packed keratin cells, making it strong but flexible. Its shape follows the bone underneath. Often, when people say "nail," they're just talking about this part. It's firmly attached to the nail bed and has no nerves or blood vessels.

Free Margin: This is the front edge of the nail plate – the part you file or cut.

Hyponychium: This is the skin just under the free edge of the nail, where the nail meets the fingertip skin. It creates a seal to protect the nail bed. Some people informally call this the "quick."^[16]

Onychodermal Band: This is the seal right under the free edge, where the nail plate meets the hyponychium and the nail bed ends. In people with fair skin, it can sometimes be seen as a glassy, greyish line. It's not visible to everyone.

Eponychium: The eponychium is a little band of living skin that sits right at the bottom of your nail, where the nail grows out. It's like a protective collar. This living eponychium produces the cuticle, which is that thin, almost invisible layer of dead skin cells that you see attached to the back of your nail.

Even though they're connected, and some people even use the terms interchangeably, it's important to know the difference. During a manicure, it's the dead cuticle that's carefully removed, but the living eponychium should be left alone to avoid infections.^[17] The perionych is specifically the part of the eponychium that slightly overlaps the little half-moon shape at the base of your nail, called the lunula.

Then you have the nail wall, which is the skin that folds over the sides and the base of your nail, kind of like a frame. The edges of your nail slide underneath these folds, and these little channels where the sides of your nail sit are called nail grooves.

Paronychium: The paronychium is essentially the soft skin that frames the sides of your nail.^[18] It's also sometimes called the paronychia edge. This area is prone to issues like hangnails and ingrown nails. Importantly, an infection of this surrounding skin is called paronychia. So, paronychium is an anatomical area, and paronychia is what happens when it gets infected.

Hyponychium: Hyponychium is the layer of skin located just underneath the free edge of your nail, that part that extends beyond your finger. It's a slightly thicker area of skin and is sometimes referred to as the "quick," which is what you might hear when someone talks about cutting their nails too short and hitting that sensitive spot.

III. DISORDER

What is Onychomycosis?

Onychomycosis is a fungal infection affecting the nails, and it's notably more common in toenails compared to fingernails.^[19] Onychomycosis, or *tinea unguium*, is a prevalent fungal nail infection. It accounts for a significant portion of nail problems, making up 15% of all nail disturbances. Furthermore, fungal nail infections represent 10% of all cases involving dermatophytes in patients.^[20] In 2012, Hay and Baran's estimations highlighted that onychomycosis, or fungal nail infection, might be less prevalent than commonly thought, accounting for only 18% to 40% of all observed nail disorders. Furthermore, they indicated that nail involvement was also a relatively less frequent occurrence in dermatomycoses, representing approximately 30% of all fungal skin infections.^[21, 22]



Fig 6: Types of fungal nail infections

Etiology:

Onychomycosis, a fungal nail infection, is mostly caused by *Trichophyton rubrum*. However, other dermatophytes like *Trichophyton mentagrophytes* and *Epidermophyton floccosum* can also be responsible. Dermatophytes are the identified cause in a significant majority of toenail (90%) and fingernail (50%) onychomycosis cases.^[23] *Candida albicans* is a culprit in about 2% of onychomycosis (nail fungus), and it tends to show up more in fingernails. Non-dermatophyte moulds are more common in toenail infections. Some of the usual suspects in these mould infections are *Fusarium*, *Aspergillus*, *Acremonium*, *Scytalidium*, and *Scopulariopsis brevicaulis*.^[24] Overall, these non-dermatophyte moulds are responsible for roughly 8% of all nail infections.^[25]

Epidemiology:

Onychomycosis, a fungal nail infection, is becoming more common. Initially, in the US, *Trichophyton rubrum* was mistaken for a contaminant in lab cultures. However, with increased international travel to Asia, this fungus has become the primary cause of onychomycosis in the United States.^[26] Mycotic infections are the cause in at least half of all abnormal toenails. The prevalence of these infections ranges from 1% to 8% and is on the rise. Genetic predisposition, following an autosomal dominant inheritance pattern, makes some individuals more susceptible to dermatophyte infections. Several factors increase the risk of developing onychomycosis, including older age, diabetes, athlete's foot, psoriasis,

weakened immune systems, and living with someone who has the condition.^[27, 28]

Pathophysiology:

Fungal organisms thrive in places like hotel carpets, public showers, and pool decks. An early, often unnoticed, dry and thickened skin infection on the feet (tinea pedis) can lead to nail fungus (onychomycosis). The environment inside shoes – dark, warm, and moist, combined with minor injuries to the nail – makes it easier for the fungus to get under the nail. Similarly, frequent water exposure can weaken fingernails. Remember, these fungi only feed on the dead keratin found in skin, nails, and hair.^[29]

Dermatophyte infections in the foot typically start between the smaller toes due to the fungi producing keratinases. From there, the infection spreads to the thickened skin on the sole of the foot. It can then extend to the area under the toenails at the tips of toes that have experienced small injuries. The dermatophytes can penetrate and infect the nail bed by entering through this area under the nail (hyponychium) and then spread backward, causing the nail to detach (onycholysis) and the area under the nail to thicken (subungual hyperkeratosis).^[30]

Initially, it causes mild inflammation, but over time, it can become a long-term infection leading to the complete destruction of the nail. Interestingly, the microscopic features of this early infection look like what's seen in psoriasis, with changes like swelling between skin cells (spongiosis), thickening of the outer skin layer (acanthosis), finger-like projections (papillomatosis) with fluid buildup (oedema), and increased keratin production (hyperkeratosis).^[31] This onychomycosis also ends up infecting the living part of the nail that produces new nail tissue (the nail matrix). As the nail bed thickens to get rid of the fungus, it damages the nail matrix. The fungus also spreads into the nail itself, causing it to detach and become deformed. Eventually, the nail plate lifts and becomes distorted, leading to the chronic, severe stage of onychomycosis known as Total Dystrophic Onychomycosis (TDO).^[32] This chronic stage of infection shows significant thickening of the skin layers (compact hyperkeratosis, hypergranulosis, acanthosis) and finger-like projections (papillomatosis), along with a minimal inflammatory response around blood vessels. Fungal infections of the skin (dermatophytosis) and fluid collections under the nail (subungual seromas) can also happen.

Research indicates elevated levels of interleukin-6 and interleukin-10 in both the nail bed and bloodstream in onychomycosis (nail fungus). Furthermore, many fibers containing human beta defensin-2 are present within the infected nail bed and plate.

Histopathology:

In the early stages of a fungal nail infection (onychomycosis), you typically see:

- Spongiosis (fluid buildup in the skin)
- Acanthosis (thickening of the skin layer)
- Papillomatosis with oedema (finger-like projections with swelling)
- Hyperkeratosis (thickening of the outer skin layer)

As the infection becomes chronic, the changes include:

- Large amounts of compact hyperkeratosis (very dense thickening of the outer skin layer)
- Hypergranulosis (increased granular layer in the skin)
- Acanthosis (thickening of the skin layer)
- Papillomatosis with sparse perivascular infiltrate (finger-like projections with a few inflammatory cells around blood vessels)

Additionally, keep in mind that:

- Other fungal skin infections (dermatophytosis) can occur alongside it.
- Fluid-filled pockets under the nail (subungual seromas) might also develop.^[33]

Treatment/Management:

The "complete cure rate" in onychomycosis (nail fungus) studies is defined by both a negative fungal test Treatment/Management The most powerful treatments are oral antifungal medications. Doctors might especially recommend these for more serious infections or if someone has diabetes because there's a higher chance of skin problems under the nail. Using a combination of oral medication with topical treatments, regular nail trimming, or even removing the nail with chemicals can work better than just taking pills by themselves. However, for milder to moderate cases, the newer topical antifungal drugs are getting better at curing the infection, which is good news for people who'd rather avoid oral medications. Research shows that terbinafine pills are about 76% effective at getting rid of the fungus, itraconazole given in pulses works about 63% of the time, and fluconazole is effective around 48% of the time. On

the other hand, topical treatments have lower cure rates, with efinaconazole at 55% and tavaborole or ciclopirox around 36%.^[34,35] (negative mycology) and a completely clear nail (100% clear nail) is a very strict measure. This is because even after successful treatment, the nail might still show some lingering signs of damage from the long-term infection, preventing it from being perfectly clear. Conversely, a nail might look completely normal, but the fungus could still be present. The complete cure rate for systemic terbinafine, using this strict definition, is around 38%.^[36]

So, while oral medications are highly effective for severe nail fungus, they aren't suitable for everyone, and some people prefer not to take them. Topical treatments would be ideal if they worked better. Although there are many options available, suggesting a lack of truly effective ones, recent data shows improvement in prescription topical treatments. Complete cure rates have gone up from 8.5% with ciclopirox lacquer to 18% with efinaconazole solution.^[37] Tavaborole solution shows a 9.1% complete cure rate for mild to moderate distal subungual onychomycosis.^[38]

Before starting continuous therapy, it's important to check a patient's history for alcohol use disorder and hepatitis. Ordering alanine aminotransferase (ALT) and Aspartate Aminotransferase (AST) tests sets a baseline for liver function. If the patient has lived in a region where hepatitis is common, a hepatitis screening panel should also be done. Follow-up liver function tests after 5 weeks can help catch rare reactions. If these follow-up tests show a significant increase in liver enzyme levels, the medication can be stopped, and the tests repeated.^[39]

Oral antifungals might not be an option if a patient is taking other medications. While terbinafine can interact with some drugs and requires monitoring, the concern about drug interactions with statins and systemic antifungal therapy primarily involves the azole class of antifungals, not terbinafine.^[40] It might be best to avoid systemic antifungal therapy for patients taking psychotropic medications. Specifically, terbinafine should not be used with phenothiazines and pimozide due to an increased risk of a heart condition called QT prolongation.^[41] Terbinafine can generally be used safely in children and older adults. However, clinicians should be careful when treating patients by taking multiple medications. In such cases, especially for a condition that isn't life-threatening, it might be best to use a different treatment or simply remove the affected nail

regularly to manage symptoms and avoid potential drug interactions.

IV. NEED FOR INVESTIGATION

- The current study focuses on herbal antifungal creams, which are very popular right now with the goal of minimizing negative effects caused by the available synthetic components.
- The current is on the formulation and evaluation of herbal antifungal cream by using herbal ingredients.
- The herbal ingredients do not have any side effects.
- Antifungal creams are topical treatments for fungal infections of the skin, hair, and nails.
- They work by targeting the infection directly, reducing symptoms like itching and redness, and preventing the spread or recurrence of the infection, all while minimizing systemic side effects.
- Herbal antifungal creams are gaining traction as a natural alternative to synthetic options due to their minimal side effects and potential effectiveness in treating fungal infections. They offer a promising approach for those seeking gentler treatment.

V. AIM & OBJECTIVE

Aim:

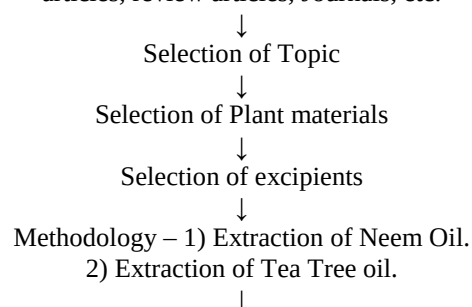
Formulation and evaluation of herbal antifungal cream containing *Melaleuca alternifolia* (Tea tree oil) and *Azadirachta indica* (Neem).

Objective:

1. This research aimed to develop an antifungal cream that is safe and effective, specifically focusing on formulations that do not cause side effects or adverse reactions.
2. The study also sought to evaluate the efficacy and safety of the prepared cream.

VI. PLAN OF WORK

Collection of information from the research articles, review articles, Journals, etc.



Experimental Work- 1) Formulation of herbal antifungal cream.

2) Evaluation of herbal antifungal cream.

- I. Physical properties
 - II. Determination of pH
 - III. Spreadability Test
 - IV. Washability Test
 - V. Irritancy Test
 - VI. Homogeneity
- ↓
Result
↓
Conclusion

VII. DRUG PROFILE

i. Neem:



Fig 7: Neem Oil

Biological Source:

Neem consists of almost all the parts of the plant which are used as the drug *Azadirachta indica*. It belongs to the family *Meliaceae*. It is also known as *Margosa*, *Indian lilac* and *Azadirachta indica*.

Geographical source:

India is native of *Azadirachta*. It is also cultivated in Nepal, Pakistan, Bangladesh, and Sri Lanka. Neem is a fast-growing tree that can reach a height of 15-20 m, rarely to 35-40m. It is evergreen.

Chemical constituents:

1. Azadirachtin:

Azadirachtin, a natural insecticide from neem seeds, acts as a feeding deterrent in two ways. Primary antifeedancy occurs through chemoreception, where it blocks the insect's taste receptors that are normally stimulated by food. Secondary antifeedancy involves a reduction in food intake after azadirachtin application, even if the mouth receptors are bypassed, likely due to toxic effects once consumed.^[42]

2. Nimbin:

Nimbin, a triterpenoid compound found in neem (*Azadirachta indica*), exhibits a wide range of biological activities, including anti-inflammatory, antimicrobial, antifungal, and antimalarial effects. It is also reported to have antipyretic, antihistamine, and antiseptic properties. Nimbin is believed to be a key component of neem oil that contributes to its various medicinal uses.

3. Nimbidin:

Nimbidin, a bitter extract from neem seed kernel oil, exhibits various biological activities. Several tetranortriterpenes, including nimbin, nimbinin, nimbidinin, nimbolide, and nimbidic acid, have been isolated from this extract.^[43] Nimbidin, a bitter principle from neem seed kernel oil, has demonstrated antifungal activity by inhibiting the growth of *Tinea rubrum*.^[44]

4. Nimbidol:

Nimbidol compound from the neem tree, possesses several biological activities. These include antipyretic (fever-reducing), anti-inflammatory, and antimicrobial effects. Research also suggests its potential in treating diabetes, cancer, and malaria.

5. Sodium nimbidinate:

Sodium nimbidinate, a phytochemical derived from the neem plant, has demonstrated diuretic activity when administered to dogs. This suggests that sodium nimbidinate could potentially be used as a diuretic agent in veterinary medicine.^[45]

6. Gedunin:

Gedunin, a limonoid isolated from neem seed oil, has demonstrated both antifungal and antimalarial properties. This suggests its potential utility in treating fungal infections and as an antimalarial agent.^[45]

7. Quercetin:

Quercetin, a flavonoid present in many plants, has shown antifungal activity, specifically against *Candida albicans*. Its mechanisms include inhibiting biofilm formation, adhesion, and invasion by *C. albicans*. Additionally, quercetin may improve the efficacy of other antifungal medications.

Uses of Neem:

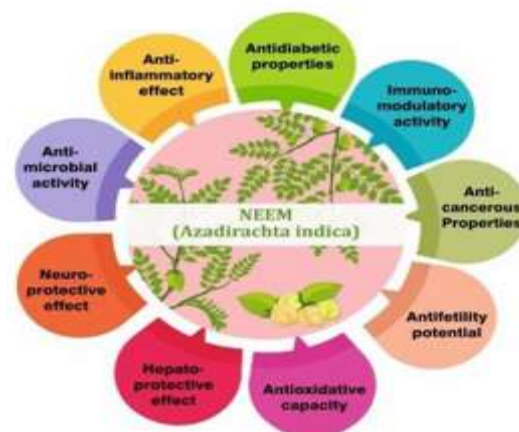


Fig 8: Uses of *Azadirachta indica*

1. Antifungal activity – Neem oil is a natural antifungal treatment for toenail fungus also; they have antibacterial and anti-inflammatory properties.
2. Biopesticides – Neem oil is a widely used natural biopesticide in organic farming, valued for its efficacy against a broad spectrum of insect pests.
3. Insecticides – Neem extracts and oil serve as natural insect repellents.
4. Oral health – Neem-based oral care products, such as mouthwash and toothpaste, promote better oral hygiene by fighting tooth decay and freshening breath.
5. Skin care – Neem offers therapeutic benefits for several skin issues, including acne, eczema, and psoriasis. It also helps calm skin that is inflamed and irritated.

ii. Tea Tree Oil:



Fig 9: Tea Tree Oil

Biological source:

Tea tree oil comes from the leaves of *Melaleuca alternifolia*, a small tree native to Australia also called the tea tree. The oil is obtained by steaming the leaves. The *Melaleuca* genus, part of the Myrtaceae family, includes about

230 species, with nearly all of them being native to Australia.

Geographical source:

Tea tree oil mainly comes from *Melaleuca alternifolia*, a plant native to the northeast coast of New South Wales and Southeast Queensland in Australia. Although some production occurs in China and Africa, Australia is the primary source.

Chemical constituents:

Tea tree oil (TTO) is a complex mixture of terpene hydrocarbons, primarily monoterpenes and sesquiterpenes, along with their corresponding alcohols. Terpenes, which give TTO its characteristic aroma, are essentially polymers of isoprene. Initial studies identified a relatively small number of components in TTO, with reports mentioning 12^[46], 21^[47], 48^[48] constituents. However, a comprehensive study by Brophy and coworkers analyzed over 800 TTO samples using advanced techniques like gas chromatography and gas chromatography-mass spectrometry. This in-depth analysis revealed a much more intricate composition, identifying approximately 100 different components and their respective concentration ranges within the oil.

Composition of Tea Tree oil:

Components	Composition (%)	
	Typical Composition ^[49]	ISO 4730 Range ^[50]
Terpinen-4-ol	40.1	>30 ^C
γ - Terpinene	23.0	10-28
α - Terpinene	10.4	5-13
1,8-Cineole	5.1	<15 ^d
Terpinolene	3.1	1.5-5
ρ -Cymene	2.9	0.5-12
α -Pinene	2.6	1-6
α -Terpineol	2.4	1.5-8
Aromadendrene	1.5	Trace-7
δ -Cadinene	1.3	Trace-8
Limonene	1.0	0.5-4
Sabinene	0.2	Trace-3.5
Globulol	0.2	Trace-3
Viridiflorol	0.1	Trace-1,5

Table 1: Composition of Tea Tree Oil

TTO is a nonpolar substance with a relative density between 0.885 and 0.906^[50], meaning it is less dense than water and will float. It

exhibits limited solubility in water but readily mixes with nonpolar solvents.

Uses:



Fig 10: Uses of *Melaleuca alternifolia*

1. Skin condition – Tea tree oil (TTO) can be beneficial for treating skin conditions like acne, eczema, and psoriasis due to its antimicrobial and anti-inflammatory properties.
2. Fungal infection – Tea tree oil is effective in treating fungal nail infections and athlete's foot.
3. Hair and scalp – Tea tree oil may alleviate dandruff and lice and potentially stimulate hair growth.
4. Wound care – Tea tree oil's antiseptic qualities can aid in the healing of minor cuts, burns, and insect bites.
5. Natural cleanser – Tea tree oil can serve as a natural disinfectant when added to cleaning solutions.

VIII. EXCIPIENT PROFILE

EXCIPIENTS	Role
1. Bees wax	Emulsifying agent
2. Borax	Buffering agent
3. Liquid Paraffin	Emollient
4. Methyl Paraben	Preservative
5. Rose oil	Perfume
6. Distilled Water	Solvent

Table 2: Excipients & their roles

i. **Bees wax:**



Fig 11: Bees Wax

Biological source:

Honeybees, from the genus *Apis*, are the biological source of beeswax. These bees belong to the family *Apidae*, which is part of the order *Hymenoptera*.

Beeswax, or *cera alba*, is a natural wax created by honeybees. Worker bees produce it in their abdominal glands and use it within the hive to build cells for storing honey and protecting larvae and pupae. Its primary chemical components are esters of fatty acids and long-chain alcohols.

Geographical source:

East and West Africa are significant regions for both beeswax production and export. Countries like Ethiopia, Angola, and Kenya are notable producers in Africa. India is a significant global producer of beeswax, contributing a substantial portion of the world's total output. In 2022, India was ranked as the top beeswax producer globally, accounting for over 37% of the total production. Major producing states include Uttar Pradesh, Punjab, and Maharashtra. China is a leading global producer and exporter of beeswax.

China is a major producer and exporter of beeswax; it's worth noting that some reports also indicate India as a significant producer in Asia. In terms of exports, China held the largest share in 2023 for "Beeswax, other insect waxes and spermaceti" and was a leading exporter of "Vegetable waxes and beeswax".

Chemical constituents:

Beeswax is primarily composed of the wax ester triacontanyl palmitate^[51], which has an approximate chemical formula^[52]. This major component, along with palmitoleate and oleate esters of long-chain (30-32 carbons) aliphatic alcohols, forms the bulk of beeswax.

Specifically, the ratio of triacontanyl palmitate ($\text{CH}_3(\text{CH}_2)_{29}\text{O}-\text{CO}-(\text{CH}_2)_{14}\text{CH}_3$) to cerotic acid ($\text{CH}_3(\text{CH}_2)_{24}\text{COOH}$), its two main constituents, is 6:1.

Beeswax can be categorized into European and Oriental types, distinguished by their saponification values: European beeswax has a lower value (3-5), while Oriental types have a higher value (8-9). High-temperature gas chromatography is a method used for its analytical^[53].

Uses:



Fig 12: Uses Of Bees Wax

1. **Candle making** – Beeswax is a favored material for crafting candles because of its inherent qualities, including a clean burn and a naturally enduring scent.
2. **Cosmetics and skin care** – Beeswax is frequently used in cosmetics, lotions, and lip balms due to its moisturizing and protective benefits for the skin.
3. **Food processing** – Beeswax serves as a glaze and coating for various food items, such as fruits and vegetables, to improve their visual appeal and extend their freshness.
4. **Industrial applications** – Beeswax finds application in diverse industrial settings, such as metal casting, textile manufacturing, and the production of varnishes.

ii. Borax:



Fig 13: Borax

Formula-

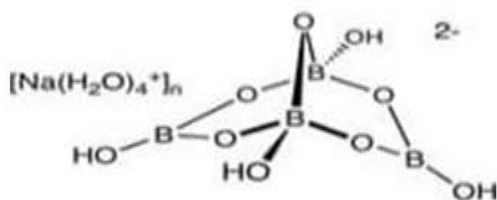


Fig 14: Structural Formula of Borax

The IUPAC name:

"disodium;3,7-dioxido-2,4,6,8,9-pentaoxa-1,3,5,7-tetraborabicyclo[3.3.1]nonane;decahydrate", refers to Borax, specifically Borax decahydrate.

It is also commonly known as sodium borate decahydrate or sodium tetraborate decahydrate. Borax, also known as sodium borate, tincal, or tincar, is an ionic compound (salt) that is a hydrated or anhydrous form of sodium borate, with the chemical formula ^[54].

Borax is a colorless, crystalline solid that forms a basic solution when dissolved in water. It is widely used in powder or granular form for various industrial and household applications. These include its use as a pesticide, a flux for metal soldering, a component in glass, enamel, and pottery glazes; for tanning; artificially ageing wood; preserving against wood fungus; and as a pharmaceutical alkalizer. In chemical labs, it acts as a buffering agent. ^[54, 55] The terms "tincal" and "tincar" refer to naturally occurring borax, historically sourced from dry lake beds in Asia. ^[56]

Chemistry:

Chemically, borax contains the complex ion $[B_4O_5(OH)_4]^{2-}$, featuring both four-coordinate and three-coordinate boron atoms. It exhibits proton conductivity above 21 °C, with maximum conductivity along the b-axis ^[57]. Borax readily converts to boric acid and other borates, having diverse applications. For instance, it reacts with hydrochloric acid to yield boric acid: $Na_2B_4O_7 \cdot 10H_2O + 2 HCl \rightarrow 4H_3BO_3 + 2 NaCl + 5 H_2O$. Its stability makes it suitable as a primary standard in acid-base titrations. ^[58] Molten borax dissolves various metal oxides to form glasses, a property utilized in metallurgy and the borax bead test. While soluble in many solvents, it is notably insoluble in ethanol.

Uses:

1. Disinfectant: It is effective for sanitizing surfaces due to its disinfecting properties.
2. Fungicide/Herbicide: Borax can be applied as fungicide and herbicide in specific contexts.
3. Water Softener: It aids in softening hard water, which is advantageous for laundry and cleaning.
4. Cosmetics: It's an ingredient in certain cosmetic formulations, particularly for skincare.
5. Ceramics and Glass: Borax is a key component in creating glazes for ceramics and glass.
6. Food Additive: While permitted as a preservative in some countries, its use in food products is prohibited in the United States.

iii. Liquid Paraffin:



Fig 15: Liquid Paraffin

Structure:

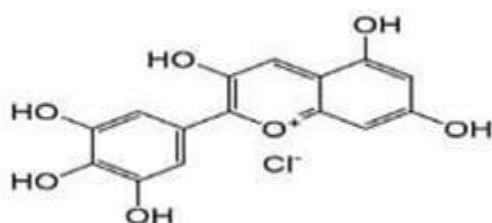


Fig 16: Structural Formula of Liquid Paraffin

Chemistry:

Liquid paraffin, also referred to as paraffinum liquidum, paraffin oil, or Russian mineral oil, is a highly refined mineral oil used in cosmetic and medicinal applications. It is important not to confuse this with kerosene, which is also known as paraffin but is used as fuel.

Due to the general chemical term "paraffin" referring to alkanes, the meanings of "paraffin" and "paraffin oil" vary regionally. Chemically, liquid paraffin is a transparent, colorless, nearly odorless, and oily liquid made up of saturated hydrocarbons sourced from petroleum.^[59]

iv. Methyl Paraben:



Fig 17: Methyl Paraben

IUPAC name and other name Methylparaben, also known by its preferred IUPAC name Methyl 4-hydroxybenzoate, is a common preservative. Other names for it include Methyl p-hydroxybenzoate, Methyl Para hydroxybenzoate, Nipagin M, Tegosept, and Mycocten. It is also identified by E number E218.

Chemistry:

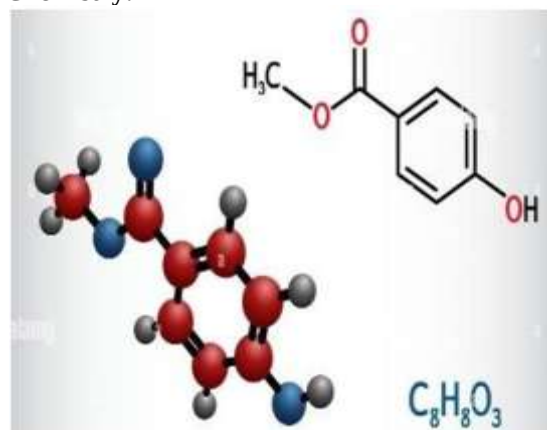


Fig 18: Structural formula & Chemical formula of Methyl Paraben

Methylparaben ($\text{CH}_3\text{O}_2\text{CC}_6\text{H}_4\text{OH}$) is a widely used preservative, belonging to the paraben family, which includes similar esters like ethyl-, propyl-, and butylparaben. These parabens are frequently employed in cosmetics and foods due to their advantageous properties: they are inexpensive, colorless, stable, odorless, and easily biodegradable.^[60]

v. Rose Oil:



Fig 19: Rose Oil

Rose oil, also known as rose otto or attar of roses, is an essential oil derived from rose petals. It's primarily produced via steam distillation (rose ottos) or solvent extraction (rose absolutes), with absolutes being more common in perfumery. Originating in Greater Iran, rose oil remains a highly prized and widely used essential oil in perfumery, despite its high cost and the availability of synthetic alternatives.

Rose oil is highly valued for its diverse properties and uses. In aromatherapy, it is prized

for its calming and balancing effects, helping to alleviate stress, anxiety, and emotional tension. For skincare, it's known for its ability to moisture, soothe, and reduce redness, improving overall skin health. Its captivating fragrance makes it a popular and enduring component in various perfumes and fragrances. Additionally, traditional and herbal medicine systems utilize rose oil for potential medicinal benefits, including aiding digestive health, promoting healing, and managing certain skin conditions.

IX. METHODOLOGY & EQUIPMENT

Soxhlet Apparatus

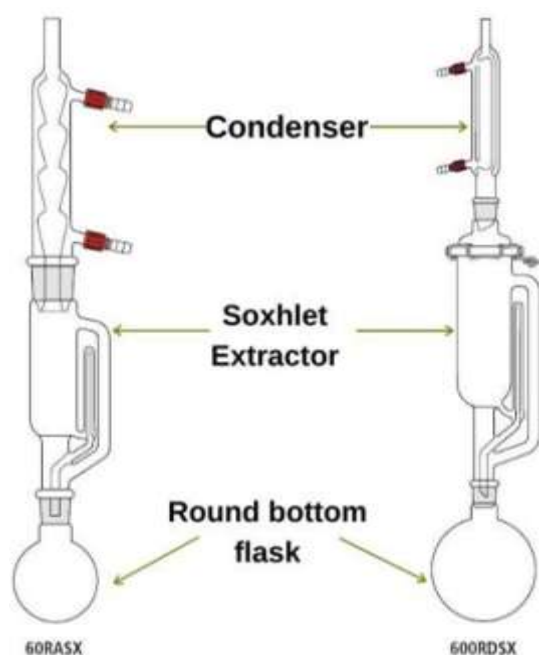


Fig 20: Diagram of Soxhlet Apparatus

The Soxhlet extraction method is a cost-effective and efficient technique for extracting soluble compounds from a solid material using a small amount of solvent. It operates on the principles of solvent reflux and syphoning.

Procedure:

1. **Sample Preparation:** The solid sample is placed inside a thimble-shaped filter paper, which is then positioned within a glass cylinder (the Soxhlet extractor).
2. **Assembly:** This cylinder, equipped with a syphon tube and an inlet tube, is connected at the top to a water condenser. The entire

assembly is then fitted onto a round-bottom flask containing the extraction solvent.

3. **Heating and Vaporization:** The solvent in the flask is heated (typically using a water bath or sand bath), causing it to vaporize.
4. **Condensation and Extraction:** The solvent vapours travel up through the inlet tube into the glass cylinder and condense as they pass into the condenser. The condensed, pure solvent then drips down onto the solid sample in the thimble, dissolving the soluble components.
5. **Syphoning:** As the solvent level in the cylinder rises and exceeds the maximum height of the syphon tube, the solvent containing the extracted compounds is syphoned back into the solvent flask.
6. **Continuous Cycle:** This process repeats continuously: fresh, pure solvent evaporates, condenses, extracts the sample, and then the extract-rich solvent syphons back into the flask. This ensures that the solid material is constantly exposed to pure solvent for efficient extraction, and the extracted material becomes concentrated in the flask.
7. **Product Recovery:** Once the extraction is complete, heating is stopped. The solvent in the flask is then distilled off, leaving the concentrated extracted organic compound behind.

I. Neem Oil Extraction:

1. 100 g of neem powder is placed in a thimble within a Soxhlet extractor.
2. 500 ml of a chosen solvent is added to a round-bottom flask, and the Soxhlet apparatus is assembled.
3. Distillation is initiated to begin the extraction process.
4. After extraction, the solvent is evaporated using a water bath, leaving the extracted neem oil.^[61]

II. Tea tree oil extraction:

The process involved:

1. **Soxhlet Extraction:** 50g of tea tree powder was extracted for approximately 6 hours (6 cycles) using 300mL of each solvent heated at its boiling point.
2. **Concrete Formation:** The crude extract was then purified using a rotary evaporator at 40°C under vacuum to remove the solvent, yielding a light yellow, waxy "tea tree concrete."
3. **Wax Removal and Absolute Formation:** This concrete was dissolved in 6mL of methanol,

heated at 40°C for 30 minutes, and then refrigerated at -15°C for 24 hours to precipitate natural waxes and heavy albuminous compounds.^[62]

4. Filtration and Final Purification: The mixture was vacuum filtered to separate the essential oils and methanol from the waxes. The filtrate was again purified via rotary evaporation to remove excess methanol, resulting in "tea tree absolutes" (essential oils with trace waxes).^[63]
5. Component Analysis: The obtained tea tree absolutes were then analyzed using GC-MS to identify and quantify key components (terpinen-4-ol, 1,8-cineole, γ -terpinene, and α -terpineol) and compare their compositions with standard tea tree oil and the international Melaleuca oil standard (ISO 4730).

X. FORMULATION OF HERBAL ANTIFUNGAL CREAM

Formulation Table:

Sr.no	Ingredients	F1	F2	F3
1.	Neem oil	1.5ml	3ml	4.5ml
2.	Tea tree oil	0.5ml	1ml	1.5ml
3.	Bees wax	3gm	3gm	3gm
4.	Borax	2gm	2gm	2gm
5.	Liquid Paraffin	10ml	10ml	10ml
6.	Methyl Paraben	0.02gm	0.02gm	0.02gm
7.	Rose oil	q.s	q.s	q.s
8.	Distilled water	q.s	q.s	q.s

Table 3: Formulation Table of Herbal Antifungal Cream

List of Glassware:

Sr.no	Glassware
1.	Glass Rod
2.	Beaker
3.	Measuring Cylinder
4.	Water bath
5.	Mortar Pestle

Table 4: List of Glassware

Procedure:

1. Weigh all Ingredients accurately.

2. Prepare oil phase and aq. phase
3. For the aqueous phase, add Borax and Methyl Paraben into Distilled water. In oil phase add Bees wax into Liquid Paraffin.
4. Heat both phases till their temperature is the same as 60-75°C.
5. Then add an aqueous phase into the oil phase and mix continuously in the mortar pestle.
6. Add remaining ingredients and mix continuously till thick cream is formed.
7. Store the cream in a well closed container.



Fig 21: Preparation of Herbal Antifungal Cream



Fig 22: Formulation of Herbal Antifungal Cream

XI. EVALUATION TESTS

1. Physical properties:

Physical properties encompass the measurable attributes of a substance without altering its chemical composition. These characteristics include color, odor, density, melting

point, and solubility, offering insights into how a substance behaves under specific conditions. Essential for material identification, physical properties help determine appearance, structure, and behavior. They enable scientists and researchers to comprehend the nature of substances, aiding in classifications and applications across various fields, from chemistry to materials science. Understanding physical properties is fundamental for assessing the suitability and performance of substances in diverse contexts, contributing to scientific analysis and practical applications.^[64]

Table of physical properties:

Sr.no	Properties	Observations
1.	Color	Slightly yellow
2.	Odour	Pleasant
3.	Appearance	Smooth & Creamy

Table 5: Physical Properties & their Observations

2. Determination of pH:

Smooth and creamy the pH of various formulation was determined by using digital pH Meter. The 0.5g of the weighed formulation was dispersed in 50ml of distilled water.

Table of pH:

Sr.no	Test	Observation
1.	pH at 27° C + 2° C	9 gm.cm/sec

Table 6: pH Observation

3. Spreadability test:

Glass slides are used to test the spreadability of a cream recipe. Spreadability is measured by the interval of separation of the two slides when a specific load is applied.

The time that is taken up by two slides for the separation indicates the ability to spread by the cream. A shorter time suggests better spreadability, meaning the cream can spread up easily to cover a superior area.^[65]



Fig 23: Spreadability test

Procedure:

1. Place a small amount of antifungal cream between two glass plates.
2. Apply a specific load onto the upper glass plate.
3. Start a stopwatch and gently separate the glass plates.
4. Record the time it takes for the plates to separate.
5. Repeat the test with different loads if necessary.
6. Calculate the spreadability by analyzing the time taken for separation under each load.
7. Shorter separation times indicate better spreadability of the cream.

$$\text{Spreadability} = M \times L / T$$

Where,

M = Mean weight of cream applied (in grams)

L = Length of path spread by cream (in centimeters) T = Time taken for the plates to separate (in seconds)

4. Washability Test:

0.5 gm of prepared formulation was applied on the skin. And it was washed with lukewarm water. The time taken for removal of preparation was noted.

5. Irritancy test:

Mark an area of 1sq cm on the left hand dorsal surface. The cream was applied to the specified area and time was noted. Irritancy erythema, edema was checked if any for regular intervals upto 24 hrs and reported.

6. Homogeneity:

The formulation was tested for homogeneity by visual appearance and by touch.

XII. RESULT

Sr.No	Properties	Observation F1	Observation F2	Observation F3
1.	Color	Whitish yellow	Yellowish	Yellow
2.	Odour	Pleasant	Pleasant	Pleasant
3.	Appearance	Pleasant	Pleasant	Pleasant
4.	pH	6.0	6.0	6.5
5.	Spreadability	9 g.cm/sec	9 g.cm/sec	12 g.cm/sec
6.	Irritancy	No	No	No

Table 7: Properties & their Observations F1, F2 and F3

In Above results, we are observed F3 formulations is safe and effective formulation as compared to the F1 & F2 Formulations.

XIII. CONCLUSION

This work explores the potential of herbal extracts for cosmetic applications, given the rising demand for personal care products. Bioactive ingredients from these extracts can positively impact skin health by influencing biological functions and providing essential nutrients. The cosmetic formulations developed demonstrated good spreadability, no phase separation, and consistent texture throughout the study. Furthermore, stability tests — including visual appearance, consistency, and fragrance — showed no significant changes over the study period.

Significant research is underway to evaluate the medicinal potential of plant extracts, primarily because a large portion of the global population, particularly in underdeveloped countries, still relies on herbal treatments for their healthcare needs. This study aims to investigate and confirm the promise of these plant extracts for therapeutic applications.

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