INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

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Research Paper

Formulation And Evaluation of Antifungal Cream Using Neem Extract

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ARTICLE INFO

Published: 18 June 2026
Keywords:
Azadirachta indica, Herbal Antifungal Cream, Dermatophytosis, In Vitro Release Kinetics, Franz Diffusion Cell, Topical Drug Del
DOI:
10.5281/zenodo.20748126

ABSTRACT

Fungal skin infections represent a widespread and escalating dermatological challenge globally, affecting approximately 20% to 25% of the world's population, with prevalence rates surging up to 40% in tropical and subtropical regions characterized by elevated temperature and humidity.1 Conventional synthetic antifungal therapies, primarily consisting of azole derivatives, allylamines, and morpholines, are increasingly undermined by rising drug resistance, high treatment costs, and a range of adverse local reactions such as contact dermatitis, erythema, and severe skin irritation.2 This research project focuses on the development, optimization, and comprehensive evaluation of a stable, highly efficacious topical oil-in-water (O/W) herbal antifungal cream utilizing the dense bioactive extract of neem (Azadirachta indica) leaves.5The active herbal components were obtained via optimized ethanolic extraction of mature neem leaves, yielding a phytochemically rich matrix containing high concentrations of therapeutic flavonoids, phenols, tannins, and specialized tetranortriterpenoids.2 Comprehensive physicochemical evaluations of the formulated creams demonstrated highly satisfactory parameters, including a skin-compatible pH range of 5.8 to 6.7, optimal pseudoplastic viscosity between 28,008 and 28,932 cPs, and an excellent spreadability and extrudability profile.5 In vitro drug release studies conducted using modified Franz diffusion cells established a controlled release pattern adhering to Higuchi's matrix diffusion kinetics.9Furthermore, standard agar cup plate and disc-diffusion assays confirmed robust zones of inhibition against key pathogenic strains, including Candida albicans, Trichophyton rubrum, and Trichophyton tonsurans, comparable to standard synthetic controls.12 Dermal irritation assays conducted on animal models yielded a Primary Dermal Irritation Index of zero, validating the dermatological safety of the formulation.15 These findings indicate that the optimized neem-based cream offers a safe, stable, and highly effective therapeutic alternative for the management of superficial mycoses, effectively bridging traditional ethnopharmacology with modern

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

pharmaceutical technology.17

INTRODUCTION

Anatomy and Physiology of Skin

The human skin is the largest organ of the body, serving as a dynamic, complex, and vital boundary that segregates the internal physiological environment from the external world.19 Structurally, the skin is organized into three major anatomical layers: the epidermis, the dermis, and the hypodermis (subcutaneous tissue), each possessing distinct histological characteristics and physiological functions.9 The outermost layer, the epidermis, is a stratified squamous epithelium composed primarily of keratinocytes, which undergo a continuous process of differentiation from the basal layer to the surface.9 The epidermis is subdivided into five distinct morphological strata:

1. **Stratum Basale:** The deepest germinative layer containing proliferating keratinocytes, melanocytes, and Merkel cells.
2. **Stratum Spinosum:** The spinous layer characterized by desmosomal connections and Langerhans cells responsible for immunological surveillance.
3. **Stratum Granulosum**: The granular layer where cells accumulate keratohyalin granules and lamellar bodies containing lipids.
4. Stratum Lucidum: A clear, thin, transitional layer found exclusively in thick skin (palms and soles).
5. **Stratum Corneum**: The ultimate differentiated layer, structurally modeled as a "bricksand-mortar" system.9

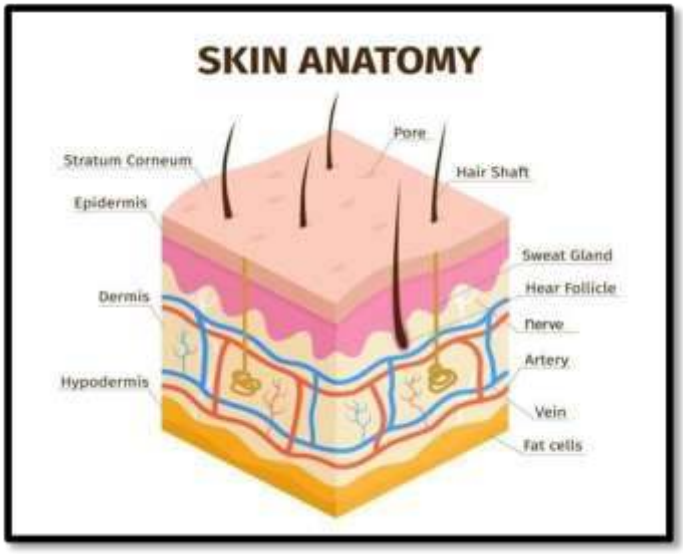


Figure 1: Anatomy of Human Skin

The stratum corneum acts as the primary physical and chemical barrier of the skin.9 In this model, the "bricks" are the non-viable, flattened, keratin-packed corneocytes, while the "mortar" consists of a highly ordered, continuous intercellular lipid matrix rich in ceramides, cholesterol, and free fatty acids.9 This specialized lipid architecture is critical for preventing transepidermal water loss and restricting the passive diffusion of xenobiotics and pathogens into the deeper cutaneous layers.3 Beneath the epidermis lies the dermis, a dense network of collagen and elastin fibers embedded in a glycosaminoglycan-rich ground substance, which provides mechanical strength, elasticity, and support.3 The dermis houses crucial adnexal structures, including hair follicles, sebaceous

glands, sweat glands, sensory receptors, and an extensive microvascular network that plays a primary role in thermoregulation and systemic nutrient transport.3

Skin Barrier Mechanism

The cutaneous barrier mechanism is fundamentally maintained by physical, chemical, and biological factors working in unison.19 The physical barrier, localized primarily within the stratum corneum, restricts the penetration of hydrophilic substances and microorganisms due to the tight packing of corneocytes and the highly hydrophobic nature of the intercellular lipid bilayers.9 Complementing this physical barrier is the chemical barrier, often referred to as the "acid mantle".5 The skin surface normally exhibits an acidic pH range between 4.5 and 5.5, which is maintained by lactic acid in sweat, free fatty acids derived from sebum, and amino acids generated during keratinization.5 This acidic environment is highly inhibitory to pathogenic bacterial and fungal colonization, while optimized for the enzymatic processes that regulate stratum corneum cohesion and desquamation.5 Furthermore, keratinocytes constitutively and inductively secrete antimicrobial peptides, such as cathelicidins and defensins, which directly target and disrupt microbial cell membranes.19 The biological barrier is represented by the resident skin microbiota, a diverse community of commensal microorganisms that actively compete with pathogenic invaders for nutrients and space, while secreting metabolic byproducts that further reinforce the acidic mantle.4 Any disruption to these barrier components—whether through mechanical trauma, chemical irritation, or systemic physiological changes—compromises the skin's integrity, providing an opportunistic pathway for pathological infections, particularly fungal invasions.4

Common Fungal Skin Infections

Superficial fungal infections of the skin, hair, and nails constitute one of the most prevalent dermatological categories worldwide, affecting millions of individuals across all demographics.2 These infections are broadly categorized into dermatophytoses, candidiasis, and pityriasis (tinea versicolor) based on the etiological agent and clinical presentation.19 Dermatophytoses, commonly referred to as tinea or ringworm, are superficial mycoses confined entirely to the keratinized outer structures of the body, as the causative pathogens are incapable of invading viable, non-keratinized deeper tissues.21 Candidiasis is primarily caused by opportunistic yeasts belonging to the genus Candida, most notably Candida albicans, which targets warm, moist mucosal surfaces and intertriginous skin folds.9 Pityriasis versicolor, characterized by hypopigmented or hyperpigmented scaly macules, is caused by lipophilic yeasts of the genus Malassezia.26 In tropical
regions, the prevalence of these superficial infections is exceptionally high, fueled by hot and humid climates that facilitate rapid fungal proliferation and spore survival on both skin surfaces and environmental fomites.1

Pathophysiology of Fungal Infections

The pathophysiology of superficial fungal infections is initiated by the adherence of fungal arthroconidia or yeast cells to the corneocytes of the stratum corneum.19 This attachment is facilitated by specialized cell-surface glycoproteins and adhesins that recognize specific carbohydrate receptors on host tissue.19 Following secure adherence, dermatophytes and other fungal pathogens undergo germination and begin to invade the stratum corneum.19 To survive and proliferate in this highly hostile, nutrient-depleted environment, these fungi secrete a battery of extracellular hydrolytic enzymes, collectively

known as virulence factors.21 The most prominent of these are keratinases, a group of specialized endo- and exo-proteases that efficiently cleave and digest the insoluble polymeric keratin protein that constitutes the structural framework of skin, hair, and nails.21 The resulting low-molecular-weight peptides and amino acids are then assimilated by the fungus as primary nutrient sources.23

As the fungal hyphae branch and expand outward, they cause structural disruption of the stratum corneum, leading to clinical scaling, cracking, and desquamation.23 Simultaneously, the release of fungal metabolic byproducts, cell wall components (such as mannans and beta-glucans), and proteases triggers a host inflammatory response.19 Although the infection remains confined to the non-living keratinized layer, these molecules diffuse downward into the viable epidermis and dermis.19 There, they stimulate keratinocytes and resident immune cells (such as Langerhans cells and mast cells) to release pro-inflammatory cytokines, chemokines, and histamines.4 This chemical cascade induces localized vasodilation, leading to erythema, and activates sensory nerve endings, causing intense pruritus and discomfort.19 The severity of this inflammatory tissue reaction is largely determined by the immunological status of the host and the specific pathogenicity of the invading fungal species, with geophilic or zoophilic strains typically eliciting much more violent inflammatory responses than highly adapted anthropophilic species.19

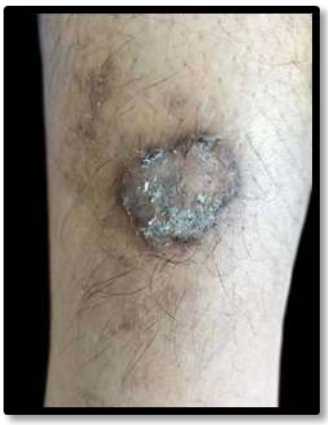


Figure 2: Common Fungal Skin Infection Types

Dermatophytes and Fungal Pathogens

Dermatophytes are a specialized group of filamentous fungi classified into three primary anamorphic genera: Trichophyton, Microsporum, and Epidermophyton.1 These organisms are strictly adapted to colonize and degrade keratinized anatomical structures.21 Among these, Trichophyton rubrum is epidemiologically documented as the most prevalent dermatophytic pathogen globally, responsible for a vast majority of chronic infections including tinea pedis, tinea cruris, and tinea unguium.1 Trichophyton tonsurans and Microsporum canis serve as the predominant etiological agents of tinea capitis, particularly among pediatric populations.1 Epidermophyton floccosum represents another significant pathogen that targets the groin and feet.1 Non-dermatophytic pathogens, such as the yeast Candida albicans, play a major role in intertriginous and mucocutaneous infections.9 Unlike dermatophytes, Candida albicans exhibits dimorphic transition, shifting from a commensal yeast phase to an invasive filamentous hyphal phase in response to local microenvironmental triggers, thereby penetrating deep epidermal layers and triggering pronounced tissue inflammation.19

Need for Antifungal Therapy

The imperative for robust, accessible, and continuous antifungal therapy is underscored by the physical, psychological, and economic burdens imposed by superficial mycoses.2 If left untreated, superficial infections rarely resolve spontaneously; instead, they progress chronically, spreading across larger anatomical areas and transitioning into deeper follicular or dermal layers.21 Furthermore, the persistent pruritus associated with these infections inevitably leads to repetitive scratching, which mechanically breaches the physical integrity of the skin.19 These excoriations create micro-fissures and open portals of entry for secondary bacterial pathogens, most commonly Staphylococcus aureus or Streptococcus pyogenes, potentially leading to severe complications such as cellulitis, impetigo, and lymphangitis.18 Beyond physical pathology, superficial infections on highly visible surfaces like the scalp, face, or hands carry significant social stigma, inducing substantial psychological distress, anxiety, and a diminished quality of life for affected individuals.21 Consequently, the development of safe, highly effective, and easily applied topical therapies remains a core priority of dermatological science.6

Herbal Medicine in Dermatology

In recent decades, there has been a profound paradigm shift within dermatological pharmacology, characterized by a resurgent interest in phytotherapeutic agents.4 This transition is driven by the growing clinical recognition that plant-derived compounds offer complex, multi-component chemical profiles capable of addressing pathological conditions through multiple concurrentmechanisms.4 Unlike single-entity synthetic drugs, which target a single metabolic pathway in the pathogen, herbal extracts possess a highly diverse array of secondary metabolites—including alkaloids, flavonoids, terpenoids, saponins, and tannins—that work synergistically.2 This multitargeted pharmacodynamics not only enhances overall therapeutic efficacy but also significantly minimizes the evolutionary pressure on pathogens to develop drug resistance, as mutating multiple metabolic pathways simultaneously is highly improbable.31 Furthermore, herbal drugs are often perceived as highly compatible with biological systems, exhibiting lower incidences of severe systemic toxicity, minimal cumulative tissue accumulation, and a reduced risk of inducing severe hypersensitivity reactions when formulated in standard vehicles.4 This makes them ideal candidates for the long-term management of chronic skin conditions.4

Pharmaceutical Importance of Topical Antifungal Formulations

Topical drug delivery represents the preferred route of administration for the treatment of localized, superficial dermatological infections.6 This therapeutic preference is grounded in several critical biopharmaceutic advantages over systemic administration.34 First, it allows direct targeting of the infected tissue, delivering active compounds to the stratum corneum while avoiding systemic metabolic clearance pathways.9 Second, topical application completely bypasses hepatic first-pass metabolism, preserving the molecular integrity of sensitive herbal phytoconstituents and maximizing their bioavailability at the localized target site.4 Third, it significantly minimizes the risk of systemic adverse effects and drug-drug interactions, which are major limitations of oral antifungal therapies such as ketoconazole and itraconazole.2 Finally, topical formulations provide rapid, localized symptomatic relief from itching and inflammation, which heavily reinforces positive patient compliance throughout the therapeutic regimen.12

Introduction to Herbal Creams

Topical creams are semisolid emulsion dosage forms containing one or more drug substances dissolved or dispersed in a suitable base.36 Typically, creams are classified into two thermodynamic classes: oil-in-water (O/W) emulsions, which are composed of small oil droplets dispersed throughout a continuous aqueous phase, and water-in-oil (W/O) emulsions, consisting of water droplets dispersed within a continuous oil phase.20 Herbal creams leverage these classical emulsion bases to deliver hydrophobic and hydrophilic plant extracts.17 From a cosmetic and biopharmaceutic perspective, O/W creams are highly favored in modern dermatology.7 They are non-greasy, wash off easily with water, and do not leave an unappealing, sticky residue on the skin surface, making them highly acceptable to patients.7 Furthermore, the continuous water phase hydrates the stratum corneum, swelling the keratin fibers and temporarily fluidizing the intercellular lipid bilayers.35 This structural modification significantly reduces the diffusional resistance of the skin barrier, facilitating the rapid and sustained penetration of the active herbal phytoconstituents into the deeper layers of the epidermis.4

History and Traditional Use of Neem

Azadirachta indica, locally known as Neem or Margosa, is a fast-growing evergreen tree belonging to the family Meliaceae, native to the Indian subcontinent and widely naturalized throughout tropical and subtropical regions of Asia, Africa, and Central America.3 Renowned in classical Ayurvedic literature as "Nimba" (derived from the Sanskrit term Nimbati Syasthyamdadati, meaning 'to give good health'), and frequently referred to as "The Village Pharmacy" or "Divine Tree", neem has been utilized for over two millennia as a cornerstone of traditional medicine.23 Historically, every anatomical part of the tree—including the leaves, bark, seeds, flowers, roots, and seed oil—has been employed to treat a vast array of human ailments.21 In ancient Sanskrit manuscripts, neem leaves are documented as highly potent blood purifiers, detoxifying agents, and therapies for inflammatory skin disorders.23 Traditional practitioners ground fresh neem leaves into a dense paste to apply directly onto wounds, ulcers, eczema plaques, and ringworm lesions to control infection and accelerate tissue healing.23 Furthermore, neem twigs were chewed daily as a natural toothbrush to maintain oral hygiene, while aqueous decoctions of leaves were used as antiseptic washes for neonates and individuals suffering from eruptive viral infections like smallpox and measles.31 This deep historical usage has provided a rich repository of ethnopharmacological knowledge that modern dermatological science is actively validating through structured, empirical research.21

Pharmacological Properties of Neem

Modern pharmacological investigations have confirmed that the extensive therapeutic efficacy of Azadirachta indica is due to its highly complex and diverse chemical profile, which contains more than 130 unique, biologically active compounds.21 These compounds are broadly categorized into isoprenoids (such as diterpenes, triterpenes, and highly modified tetranortriterpenoids known as limonoids) and non-isoprenoids (including proteins, carbohydrates, polyphenolics, flavonoids, and sulfur-containing compounds).2 The primary pharmacological actions of neem extracts include:

- **Anti-inflammatory Activity**: Neem leaf extracts and specialized limonoids like nimbidin, nimbolide, and gedunin suppress acute and chronic inflammation.8 They act by downregulating key inflammatory mediators, including tumor necrosis factor-alpha (TNFalpha),

interleukin-1 beta (IL-1beta), and interleukin-6 (IL-6), while inhibiting the enzymatic pathways of cyclooxygenase-2 (COX-2) and nuclear factor-kappa B (NFkappaB).8

- **Antioxidant Activity**: Rich in polyphenolic flavonoids, especially quercetin, neem extracts exhibit high free-radical scavenging capacity.8 They neutralize reactive oxygen species (ROS), inhibit lipid peroxidation, and upregulate endogenous antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby protecting host cutaneous tissues from oxidative-stress-induced necrosis and aging.3
- **Wound-Healing Activity**: Neem accelerates tissue regeneration by promoting collagen synthesis (specifically upregulating procollagen expression), enhancing fibroblast proliferation, stimulating angiogenesis, and increasing the tensile strength of the newly formed tissue.3
- **Antimicrobial and Antiviral Activity**: Neem shows broad-spectrum lethality against clinical pathogens, effectively inhibiting bacterial biofilm formation, disrupting viral replication cycles, and exhibiting powerful, selective toxicities against protozoal parasites.18

Antifungal Properties of Neem Extract

The specific antifungal potential of Azadirachta indica extracts against a wide range of human pathogens—including dermatophytes, yeasts, and molds—is well-established.19 Standardized in vitro studies have demonstrated that organic extracts of neem leaves, particularly those obtained using polar solvents like ethanol and ethyl acetate, exhibit highly potent, dose-dependent growth inhibition against primary dermatophytic genera such as Trichophyton, Microsporum, and Epidermophyton.21 Specifically, ethanolic leaf extracts have shown total growth inhibition of clinical dermatophyte isolates at Minimal Inhibitory Concentrations (MIC) ranging between 50 ug/mL and 200 ug/mL.1 This is significantly more potent than seed oil extracts, which require higher concentrations, exhibiting MIC values between 625 ug/mL and 2500 ug/mL due to differing phytochemical distributions.1 Against Candida albicans, the causal agent of superficial candidiasis, neem extracts successfully restrict mycelial transformation, prevent germ tube formation, and disrupt adherence to epithelial cells.9 These potent fungicidal effects are highly reproducible across clinical strains, highlighting neem's therapeutic utility as a broad-spectrum antifungal agent.5

Herbal vs. Synthetic Antifungal Creams

While synthetic topical antifungal creams containing active ingredients like clotrimazole, ketoconazole, and terbinafine have long served as the standard of care, they possess several critical clinical limitations when compared to optimized herbal formulations.6 First, synthetic agents are highly prone to driving resistance.2 Because they typically operate via single-target pathways—such as the selective inhibition of lanosterol 14-alpha-demethylase or squalene epoxidase—minor point mutations in the fungal genome can render the therapy completely ineffective.2 Second, synthetic formulations are frequently associated with localized dermatological toxicity.2 Side effects such as burning, peeling, contact dermatitis, and severe skin irritation are commonly reported, especially with prolonged usage.2 In contrast, neem-based herbal creams provide a multicomponent chemical matrix that targets several physiological pathways in the pathogen simultaneously, virtually eliminating the development of drug resistance.31 Furthermore, neem's

concurrent anti-inflammatory and emollient properties actively soothe the skin, promoting barrier reconstruction while eliminating the infection, without causing localized chemical irritation.15

Mechanism of Antifungal Action of Neem Phytochemicals

The antifungal activity of Azadirachta indica is not mediated by a single chemical entity, but is rather the result of a highly coordinated, multi-targeted biochemical attack executed by its rich array of secondary metabolites.1 The primary mechanisms of action include:

- **Disruption of Fungal Cell Membrane Integrity:** Fungal cell membranes rely on ergosterol to maintain structural fluidity, rigidity, and the proper function of membrane-bound enzymes.29 Neem seed methanolic extracts and active terpenoids like azadirachtin directly interfere with the biosynthetic pathway of ergosterol by selectively inhibiting key enzymes (such as squalene epoxidase and lanosterol 14-alpha-demethylase).28 This enzyme inhibition depletes ergosterol and leads to the accumulation of toxic methylated sterol intermediates, disrupting membrane packaging, altering permeability, and causing the leakage of vital intracellular ions and proteins, ultimately resulting in necrotic cell death.19
- **Inhibition of Fungal Cell Wall Synthesis**: The fungal cell wall is a dynamic structure composed of chitin, beta-glucans, and glycoproteins, which protect the cell from osmotic lysis.19 Active polar constituents in neem extract inhibit chitin synthase and beta-glucan synthase, the essential enzymes responsible for polymerizing the cell wall matrix.19 By impeding these processes, neem renders the growing fungal hyphae structurally fragile, leading to swelling, osmotic imbalance, and cellular lysis.19
- **Mitochondrial Toxicity and Apoptosis Induction**: Specialized tetranortriterpenoids, most notably gedunin, exhibit powerful antifungal activity by targeting the mitochondrial respiratory chain.19 Gedunin disrupts mitochondrial membrane potential, inhibits adenosine triphosphate (ATP) synthesis, and induces a massive intracellular accumulation of reactive oxygen species (ROS) within the fungal cell.19 This oxidative stress damages fungal DNA, denatures critical structural proteins, and activates caspase-like proteases, initiating programmed cell death (apoptosis).19
- **Modulation of Fungal Gene Expression**: Phytochemicals in neem penetrate the fungal nucleus and alter the transcription of genes essential for virulence, cellular metabolism, and dimorphic transition (the morphogenetic switch from yeast to invasive hyphal forms), effectively neutralizing the pathogen's ability to colonize and damage host tissues.19

CREAM

Definition:

A cream is a semisolid pharmaceutical preparation intended for external application to the skin or mucous membranes. It usually contains one or more medicinal substances dissolved or dispersed in a suitable base consisting of oil and water phases.

Advantages of Cream Usage

For the topic “Formulation and Evaluation of Anti-Fungal Cream Using Neem Extract for Skin Infection”, the following advantages of cream dosage form can be included in your thesis:

Advantages of Cream Formulations

1. Easy Topical Application

Creams are easy to apply on the skin surface and spread uniformly over the infected area.

2. Improved Patient Compliance

Cream formulations are non-greasy, easily washable, and comfortable to use, which increases patient acceptance.

3. Localized Drug Action

The active ingredient acts directly at the site of infection, providing targeted antifungal activity with minimal systemic side effects.

4. Enhanced Skin Penetration

Creams help in better penetration of herbal constituents into the skin layers, improving therapeutic efficacy.

5. Moisturizing Effect

Cream bases maintain skin hydration and reduce dryness, irritation, and itching associated with fungal infections.

6. Suitable for Herbal Extracts

Creams can effectively incorporate herbal extracts like neem and maintain their stability and activity.

7. Reduced Risk of Toxicity

Topical application minimizes systemic absorption and reduces chances of toxicity compared to oral antifungal therapy.

8. Ease of Removal

Creams can be easily removed with water, making them hygienic and convenient.

9. Better Cosmetic Appeal

Creams have good appearance, smooth texture, and pleasant feel on application.

10. Controlled Release of Drug

Cream formulations can provide sustained release of active constituents over the affected area.

Current Market Scenario and Trends in Herbal Antifungal Products

The contemporary global market for dermatological products is experiencing a substantial surge in demand for green, organic, and botanically derived cosmetics and pharmaceuticals, commonly termed "cosmeceuticals".4 Consumers are increasingly seeking natural alternatives to synthetic personal care products, motivated by an expanded awareness of environmental sustainability and growing safety concerns regarding synthetic chemical additives like parabens, phthalates, and synthetic fragrances.4 Major multinational pharmaceutical and cosmetic companies are activelyreformulating their product lines to incorporate standardized herbal extracts.4 Neem-based products—including therapeutic soaps, anti-acne face washes, hair oils, and antiseptic creams—occupy a prominent position in this market segment due to neem's deep-rooted cultural acceptance and extensively documented clinical utility.20 The integration of advanced formulation technologies, such as microemulsions, liposomes, and nanoemulgels, has further propelled the market value of these products by dramatically enhancing their stability, skin penetration, and therapeutic performance to match or exceed synthetic benchmarks.35

Scope and Future of Herbal Dermatological Formulations

The future of herbal dermatological formulations lies at the intersection of traditional pharmacognosy and advanced pharmaceutical material science.17 As analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GCMS), and Fourier-transform infrared spectroscopy (FT-IR) become increasingly sensitive, the standardization of complex herbal matrices can be achieved with exceptional precision.11 This allows for the consistent production of herbal creams with highly reproducible chemical profiles and therapeutic potencies, addressing a major historical criticism of herbal medicines.4 Furthermore, the integration of herbal nanotechnology represents a major frontier.9 By encapsulating fragile, hydrophobic phytoconstituents into lipid-based nanoparticles or nanoemulsions, scientists can achieve deep

transdermal delivery, localized target deposition, and sustained, controlled release profiles.4 This technological evolution opens up unprecedented possibilities for the development of highly advanced, non-toxic, and hyper-efficacious herbal therapies for chronic, drug-resistant, and deep-seated skin infections.9

2. NEED OF STUDY

Rising Incidence of Fungal Skin Infections

In recent decades, epidemiological data have revealed a steady and concerning increase in the global incidence of superficial fungal infections.6 This rise is driven by several compounding factor complexes:

- **Demographic Transitions**: The global population of immunocompromised individuals—including patients undergoing immunosuppressive cancer chemotherapies, organ transplant recipients on lifelong immunosuppressants, and individuals with autoimmune disorders or HIV/AIDS—has expanded significantly.21 In these patients, normally minor superficial mycoses can manifest with severe, atypical clinical features and persistent chronicity.30
- **Urbanization and Lifestyle Factors**: Overcrowded urban centers, frequent use of communal recreational spaces (such as public swimming pools, gymnasiums, and locker rooms), and the continuous wearing of tight, occlusive synthetic clothing create microenvironments of localized heat and moisture, which are highly conducive to fungal transmission and colonization.2
- **Global Climate Trends**: Rising average global temperatures and altered humidity patterns have expanded the geographic range and seasonal duration of high-risk periods for fungal spore germination, especially in historically temperate zones.1

Drug Resistance in Conventional Antifungal Therapy

Perhaps the most pressing crisis in modern dermatological pharmacology is the rapid emergence and dissemination of drug-resistant fungal strains.2 Historically, synthetic azoles (such as clotrimazole, miconazole, and ketoconazole) and allylamines (such as terbinafine) were highly reliable, curative therapies.22 However, their widespread, prolonged, and often inappropriate overthe-counter use has exerted immense evolutionary pressure on fungal pathogens.2 Consequently, clinical isolates of Trichophyton rubrum, Trichophyton mentagrophytes, and Candida albicans are exhibiting high levels of resistance.5 The molecular mechanisms of this resistance include efflux pump upregulation, target site mutation (e.g., in the genes encoding lanosterol 14-alphademethylase or squalene epoxidase), and target gene overexpression.27 These resistance profiles lead to frequent treatment failures, prolonged therapy durations, escalated healthcare costs, and a heightened risk of chronic, intractable systemic infections, emphasizing the critical need for novel antifungal solutions.2

Need for Safer Herbal Alternatives

The rising clinical failure rate of synthetic antifungals is compounded by their significant local and systemic side effects.2 Synthetic topical formulations often contain strong chemical penetration enhancers, organic solvents, and artificial preservatives that can trigger severe allergic contact dermatitis, localized burning, erythema, pruritus, and dry, peeling skin.2 For patients requiring extended treatment courses—such as in chronic dermatophytoses or deep-seated onychomycosis—these adverse reactions frequently compromise patient compliance, leading to premature discontinuation of therapy and subsequent disease relapse.12 Therefore, there

is an urgent clinical need to develop alternative, plant-derived topical formulations.21 These formulations must leverage the broad-spectrum, multi-targeted efficacy of natural secondary metabolites to minimize the risk of resistance, while maintaining an exceptional skin-compatibility and safety profile that allows for comfortable, long-term therapeutic application.4

Therapeutic Importance of Neem

Azadirachta indica represents a highly promising candidate to fulfill this clinical need.21 Neem leaf extracts possess a diverse array of highly specialized, biologically active compounds that have evolved specifically to defend the plant against fungal pathogens.2 Unlike synthetic drugs, which rely on a single chemical entity, neem provides a natural multi-component system.2 The coexistence of azadirachtin, nimbin, nimbidin, gedunin, and quercetin ensures that the formulation can simultaneously disrupt fungal cell membranes, inhibit cell wall synthesis, impair mitochondrial respiration, and induce intracellular oxidative stress in the pathogen.19 This complex mechanism makes the development of resistance highly improbable, as the fungus would have to simultaneously undergo multiple distinct genetic mutations to survive the treatment.29 Furthermore, neem's concurrent anti-inflammatory, antipruritic, antioxidant, and wound-healing properties offer a holistic therapeutic approach.21 Rather than merely eradicating the fungus, a neem-based cream actively soothes skin irritation, reduces erythema, protects the damaged tissue from secondary bacterial infections, and accelerates the regeneration of a healthy, intact skin barrier, resulting in superior clinical outcomes.19

Patient Compliance Advantages

Patient compliance is a critical determinant of therapeutic success in dermatological treatments, particularly in fungal infections which often require several weeks of continuous daily application to achieve complete mycological clearance.12 The physical and aesthetic properties of a topical formulation directly dictate a patient's willingness to adhere to the prescribed regimen.7 By formulating neem extract into a highly optimized, non-greasy oil-in-water (O/W) cream base, significant compliance advantages are realized.7 Unlike heavy, occlusive synthetic ointments or sticky, alcohol-based gels that stain clothing and leave unpleasant residues, the optimized O/W cream spreads smoothly, absorbs rapidly, and can be easily washed off with water.7 Additionally,the incorporating of soothing essential oils successfully masks the natural, pungent sulfurous odor of raw neem, replacing it with a pleasant sensory profile.3 The immediate cooling and hydrating action of the cream provides instant relief from the intense itching and burning sensations associated with active infections, reinforcing positive patient feedback and motivating consistent, complete compliance throughout the treatment course.12

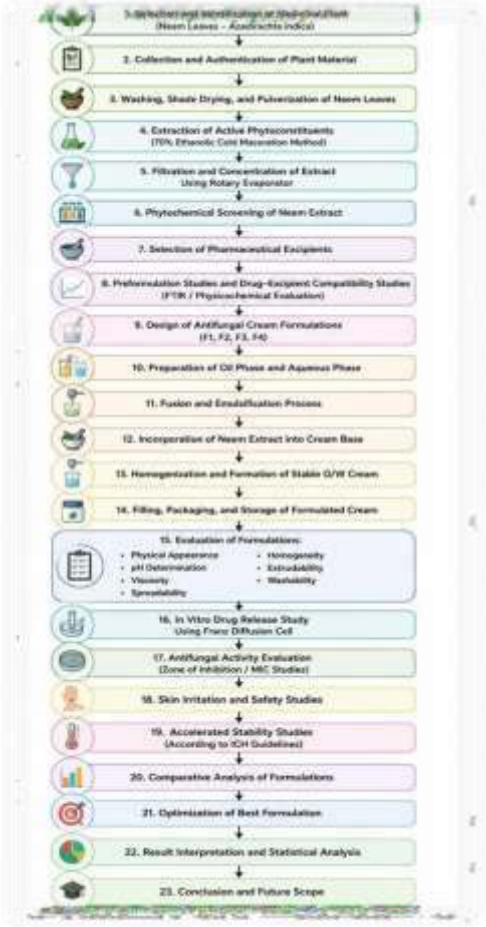
Pharmaceutical and Dermatological Significance

The pharmaceutical and dermatological significance of this study lies in its systematic, scientific validation of an ancient traditional remedy through modern, empirical pharmaceutical standards.18 By subjecting the neem-based cream formulation to physical studies, chemical standardization, mathematical drug release modeling, and quantitative in vitro and in vivo evaluations, this research bridges ethnopharmacological knowledge with modern evidence-based medicine.10 Developing a standardized, stable, and highly efficacious herbal cream not only offers a viable clinical alternative for patients suffering from drug-resistant superficial mycoses but also establishes a highly

reproducible formulation template.18 This template can be utilized to scale up and commercialize other complex botanical extracts, thereby enriching the global dermatological pharmacopeia with scientifically validated, safe, and sustainable therapeutic agents.

3. PLAN OF WORK

The execution of this research project is organized into a highly structured, sequential workflow designed to ensure rigorous quality control, reproducibility, and scientific validation at every stage of development.



Neem Extraction Process

The extraction protocol is designed to maximize the recovery of highly sensitive, biologically active secondary metabolites, particularly polar flavonoids, phenols, and moderately polar tetranortriterpenoids, while preventing thermal degradation.2

1. **Sourcing and Preparation**: Fresh, mature, disease-free green leaves of Azadirachta indica are harvested, thoroughly washed under running tap water to remove environmental particulates, rinsed with distilled water, and shade-dried at a controlled room temperature of 23-27 °C for several days until completely dehydrated.2

2. **Pulverization**: The completely dry leaves are mechanically pulverized into a fine, uniform powder using an electric laboratory grinder and passed through a standard sieve (Mesh No. 40) to ensure a consistent particle size distribution.36

3. **Maceration and Percolation**: A weighed quantity of the leaf powder is macerated in a closed glass vessel containing 70% aqueous ethanol (solvent-to-solid ratio of 10:1 v/w).37 The mixture is kept at room temperature for 36 hours with

intermittent mechanical stirring to facilitate solvent penetration and cellular lysis.37

4. **Filtration:** The resulting slurry is filtered through a dense double-layered muslin cloth, followed by filtration through Whatman Filter Paper No. 1, yielding a clear, dark green, liquid extract.2

5. **Concentration:** The liquid filtrate is concentrated under reduced pressure using a rotary evaporator at a controlled temperature of 38-42 °C to remove the organic solvent.28

6. **Desiccation:** The concentrated extract is finally dried in a hot air oven at 40 °C to yield a dark, sticky, highly concentrated solid crude extract, which is stored in a sterile, ambercolored glass container at 4 °C until phytochemical screening and formulation integration.36

Ingredient Selection Criteria

The selection of excipients for the cream base is governed by strict criteria focusing on chemical compatibility, dermatological safety, emulsion stabilization capacity, and biopharmaceutic performance.5 Stearic acid and cetyl alcohol are selected as structuring agents, thickeners, and coemulsifiers.49 They form a stable crystalline gel network within the continuous phase, providing the desired body, consistency, and pseudoplastic flow behavior to the cream.16 Liquid paraffin and beeswax are incorporated as high-performance emollients and occlusive agents.49 They form a microscopic hydrophobic barrier on the skin surface, preventing transepidermal water loss, deeply hydrating the stratum corneum, and facilitating the partitioning and diffusion of the lipophilic active ingredients.15 Glycerin and propylene glycol are selected as humectants and co-solvents.30 They actively attract water molecules from the atmosphere and the deeper cutaneous layers, maintaining hydration of the formulated cream and preventing localized dry skin upon topical application.15 Methyl and propyl parabens are incorporated as a synergistic preservative system to prevent microbial contamination, ensuring product sterility and chemical stability throughout its shelf life.49 Triethanolamine is selected as a pH adjuster and stabilizer.49 When reacted in situ with stearic acid, it forms triethanolamine stearate, a highly effective anionic emulsifying agent that stabilizes the O/W emulsion interface.46

Cream Formulation Development

The development phase involves a systematic, multi-batch approach where the ratios of lipophilic lipids, emulsifying agents, and aqueous phases are varied to identify the optimized formulation.5 Multiple experimental batches (designated as F1, F2, F3, and F4) are prepared, incorporating varying concentrations of structuring agents and active neem extract.5 A core objective is to optimize the emulsifier concentration to achieve a balance: it must be high enough to form a tight, protective monolayer around the dispersed oil droplets to prevent coalescence and creaming, but low enough to avoid inducing skin irritation or disrupting the host lipid barrier.16 The physical and chemical properties of each batch are analyzed to select the most stable and efficacious formulation for further evaluation.7

Preparation Methodology

The preparation of the O/W cream utilizes the high-temperature fusion and emulsification technique under controlled thermodynamic and mechanical conditions.36 The oil phase ingredients (stearic acid, cetyl alcohol, beeswax, stearyl alcohol, and liquid paraffin) are weighed and melted together in a stainless-steel vessel on a water bath at 68-72 °C.32 Simultaneously, the water phase ingredients (propylene glycol, glycerin, triethanolamine, methyl and propyl parabens, and distilled water) are dissolved and heated in a separate vessel to the same temperature

of 68-72 °C.37 Once both phases have achieved thermodynamic and physical equilibrium, the hot aqueous phase is added slowly, dropwise, to the hot organic oil phase with continuous high-shear mechanical stirring.32 The standardized neem leaf extract is then incorporated into this emulsified mixture.37 The emulsion is subjected to high-speed homogenization at 3500 rpm for 30 minutes to reduce droplet size, ensure uniform ingredient distribution, and promote long-term physical stability.9 The cream is then cooled slowly to room temperature under gentle, continuous stirring to prevent phase separation.5 Finally, the cooled cream is transferred into sterile, collapsible aluminum tubes, sealed, and stored under optimized conditions.5

Evaluation Protocol

The formulated cream batches are subjected to a rigorous battery of standardized quality control tests:

- **Physicochemical Properties**: Visual inspection for color, odor, consistency, and homogeneity.20 pH is determined using a validated digital pH meter, and viscosity is measured using a Brookfield viscometer.5
- **Spreadability and Extrudability**: Quantitative assessment of the cream's ability to spread uniformly on the skin surface and its ease of extrusion from the primary packaging tube.20
- **In Vitro Drug Release Studies**: Conducted using Franz diffusion cells with a synthetic cellophane membrane to quantify the cumulative drug release over time and establish the mathematical release kinetics.9
- **In Vitro Antifungal Bioassays**: Quantitative evaluation of antifungal efficacy against Candida albicans and dermatophyte strains using agar well diffusion assays to measure zones of inhibition and broth dilution methods to determine MIC values.12
- **In Vivo Safety Screening**: Acute dermal irritation testing on Wistar rat models to determine the Primary Dermal Irritation Index (PDII) and confirm the formulation's dermatological compatibility.50

Stability Testing Workflow

To determine the shelf life and physical-chemical integrity of the optimized neem cream, systematic stability studies are conducted in accordance with the International Council for Harmonisation (ICH) guidelines.46 The formulated cream, packaged in sealed aluminum tubes, is divided into groups and stored in specialized environmental stability chambers under two distinct storage conditions 7:

1. **Real-Time Storage**: Maintained at 23-27 °C and 60% +/- 5% relative humidity (RH) for a period of 3 months.7
2. **Accelerated Storage**: Maintained at 38-42 °C and 75% +/- 5% RH for a period of 3 months.7

At periodic sampling intervals (Day 0, 7, 14, 21, 30, 60, and 90), the samples are withdrawn and evaluated for any changes in critical quality attributes.7 These include organoleptic parameters (odor, color, texture), phase separation, pH stability, rheological drift (viscosity changes), active drug content via validated HPLC, and microbial limits.7 Additionally, the formulations are subjected to stress testing, including a centrifugation assay (at 3750 rpm for 5 hours at 25 °C) to check for phase separation, and a cyclic freeze-thaw testing protocol (6 complete cycles between 4 °C and 40 °C, with 24 hours of storage at each temperature) to validate the thermodynamic stability of the emulsion.9

4. LITERATURE REVIEW

The systematic screening and critical evaluation of existing literature represent a fundamental prerequisite to establishing a sound scientific foundation for herbal drug development. This section reviews the work of 15 different authors

from authentic, peer-reviewed international journals specializing in pharmaceutical formulation, ethnopharmacology, and clinical dermatology.4

1. Dube and Tripathi (1987)

- **Aim of Study:** To evaluate the in vitro fungitoxic and mycelial growth inhibitory properties of aqueous and organic extracts of Azadirachta indica leaves and bark against primary pathogenic dermatophytes.24
- **Methodology:** The leaves and bark were shade-dried, pulverized, and extracted using distilled water and 95% ethanol.24 In vitro bioassays were carried out using the agar dilution method and spore germination inhibition assays against Epidermophyton floccosum, Microsporum canis, and Trichophyton mentagrophytes.24
- **Findings:** The researchers observed that both the aqueous and ethanolic extracts of neem leaves effectively suppressed the conidial germination and mycelial growth of the test pathogens in a dose-dependent manner.24 The ethanolic extract exhibited a significantly lower minimum effective concentration compared to the aqueous extract.24
- **Conclusion:** The bioactive antifungal compounds in Azadirachta indica leaves are highly stable and efficiently recovered using organic solvents, confirming the suitability of ethanolic extracts for formulating topical therapies.24

2. Verma et. al. (2015)

- **Aim of Study:** To isolate, chromatographically purify, and biologically evaluate the specific antifungal efficacy of purified fractions of methanolic extracts derived from neem seed coats.24
- **Methodology:** The methanolic seed coat extract was concentrated and partitioned using a mixture of ethyl acetate and chloroform (3:1).24 The resulting fractions were tested against Aspergillus niger and Curvularia lunata to determine the Minimum Inhibitory Concentration (MIC) via broth microdilution.24
- **Findings:** The purified ethyl acetate-chloroform fraction exhibited a strong, selective fungicidal effect, demonstrating an MIC value of 250 ppm against both test fungi, which was significantly more potent than the raw methanolic extract.24
- **Conclusion:** The purification of moderately polar fractions from neem extracts successfully concentrates key antifungal terpenoids, providing a highly potent source for targeted antifungal development.24

3. Khan et. al. (2017)

- **Aim of Study:** To investigate the comparative influence of extraction solvent polarity on the antidermatophytic activity of neem leaf extracts.24
- **Methodology:** Dried neem leaves were extracted using solvents of varying polarities: petroleum ether (low-polar), ethyl acetate (moderately polar), and methanol (highly polar).24 The extracts were assayed against various cutaneous dermatophytes using the agar cup diffusion method.24
- **Findings:** The low-polar and moderately polar organic extracts (petroleum ether and ethyl acetate) exhibited significantly larger zones of inhibition compared to the highly polar methanolic extract.24 Chemical profiling identified high concentrations of the lipophilic flavonoid quercetin in the active organic fractions.21
- **Conclusion:** The extraction of lipophilic and moderately polar phytochemicals from neem leaves is critical to maximizing antifungal performance, indicating that polar-organic solvent blends are ideal for formulating topical emulsions.4

4. Natarajan et. al. (2003)

- **Aim of Study**: To determine the long-term inhibitory effect and mycelial growth pattern of dermatophytic species exposed to organic extracts of neem leaves and seed oil.2
- **Methodology**: Organic extracts were tested against Trichophyton rubrum, Trichophyton mentagrophytes, and Microsporum nanum.2 The growth patterns were evaluated over a 30day period by mixing Sabouraud Dextrose Agar with varying concentrations of the extracts.29
- **Findings**: The neem seed organic extract exhibited a low MIC of 31 ug/mL against T. rubrum, while the leaf extract showed MIC values ranging between 150 ug/mL and 500 ug/mL.2 The incorporation of just 15 ug/mL of the organic seed extract in the agar medium achieved a sustained, 30-day reduction in the colony diameters of all test dermatophytes.29
- **Conclusion:** Neem-derived organic extracts possess long-lasting, highly stable fungistatic activity, rendering them highly suitable for the treatment of chronic, recurring dermatophytoses.19

5. **Mahmoud et. al. (2011)**

- **Aim of Study: To** evaluate the comparative broad-spectrum antifungal profile of aqueous, ethanolic, and ethyl acetate fractions of neem leaves against opportunistic human pathogens.24
- **Methodology:** The extracts were prepared at concentrations ranging from 5% to 20% and tested against Candida albicans, Microsporum gypseum, and four distinct Aspergillus species (A. niger, A. flavus, A. terreus, and A. fumigatus).24
- **Findings:** All three extraction fractions demonstrated significant inhibitory effects, which increased linearly with concentration.24 The 20% ethyl acetate fraction emerged as the most potent, achieving the strongest inhibition across all tested filamentous and yeast pathogens, including complete growth inhibition of A. niger.24
- **Conclusion:** Organic solvent fractions of neem leaves possess a broad-spectrum antifungal profile that successfully targets both primary dermatophytes and opportunistic yeasts.24

6. **Govindachari et. al. (1998)**

- **Aim of Study**: To isolate and identify the specific active antifungal components of neem seed oil and investigate potential chemical interactions.1
- **Methodology:** Neem seed oil was subjected to systematic methanol partitioning to separate its constituent tetranortriterpenoids.1 The isolated pure compounds—including azadiradione, nimbin, and salannin—were evaluated both individually and in combination against Drechslera oryzae and Fusarium oxysporum.29
- **Findings**: Interestingly, the pure, isolated single compounds did not exhibit significant antifungal activity on their own.29 However, when the isolated compounds were mixed back together in their natural proportions, their full, potent antifungal activity was restored.29
- **Conclusion:** The antifungal efficacy of neem is driven by a natural, multi-component chemical synergy among its constituent terpenoids, rather than any single active molecule, highlighting the therapeutic advantage of utilizing whole extracts over isolated single entities.29

7. **Kavitha et .al. (2014)**

- **Aim of Study**: To investigate the specific active mechanism of neem seed methanolic extracts and pure azadirachtin on fungal lipid biosynthesis.1
- **Methodology:** Aspergillus parasiticus cultures were treated with varying concentrations of raw methanolic neem seed extract and pure azadirachtin, and their cell membranes were analyzed quantitatively for lipid and sterol composition.28

- **Findings:** Both treatments significantly inhibited ergosterol biosynthesis, leading to a marked depletion of membrane ergosterol and a corresponding accumulation of sterol precursors.28 This effect was found to be concentration-dependent, with the raw extract demonstrating superior overall membrane disruption compared to pure azadirachtin.28
- **Conclusion:** Neem components exert potent fungicidal action by disrupting cell membrane integrity through the targeted, enzymatic inhibition of the ergosterol biosynthetic pathway.28

8. Hamza et. al. (2020)

- **Aim of Study**: To evaluate the specific antifungal efficacy and active mechanism of gedunin, a major tetranortriterpenoid limonoid found in neem.19
- **Methodology**: Purified gedunin was evaluated against clinical isolates of Candida species and dermatophytes.19 The molecular mechanism was investigated by measuring mitochondrial membrane potential and intracellular reactive oxygen species (ROS) accumulation.19
- **Findings:** Gedunin demonstrated highly potent, broad-spectrum antifungal activity.19 The compound rapidly disrupted fungal mitochondrial membrane potential, leading to impaired cellular respiration, ATP depletion, and a massive accumulation of intracellular ROS, which initiated fungal cell apoptosis.19
- **Conclusion**: Gedunin represents a highly promising, novel molecular scaffold that targets fungal mitochondrial function, offering a potent pathway to treat resistant mycoses.19

9. Saputri et. al. (2021)

- **Aim of Study**: To develop, validate an HPLC analytical method for, and evaluate the physical and chemical stability of a 5% neem oil topical cream.46
- **Experimental Parameters**: Isocratic reverse-phase HPLC was developed to quantify the active marker azadirachtin in O/W creams stored under room (25 °C / 65% RH) and accelerated (40 °C / 75% RH) stability conditions for 3 months.46
- **Findings**: The HPLC analytical method was successfully validated for linearity ( ), LOD, and LOQ.46 Stability testing revealed that the neem cream remained physically and chemically stable for 4 weeks at 25 °C, but exhibited accelerated chemical degradation of azadirachtin at 40 °C after 1 week.46
- **Conclusion:** While neem-based O/W creams are physically stable, their active chemical markers are sensitive to thermal stress, highlighting the need for low-temperature manufacturing and protective packaging.7

10. Tiple et. al. (2023)

- **Aim of Study**: To evaluate the in vitro synergism and antifungal spectrum of combinations of ethanolic neem leaf extracts with specific essential oils against dermatophytes.2
- **Methodology**: Combinations of ethanolic neem leaf extract, Eucalyptus citriodora oil, and Cymbopogon martini oil were tested against Microsporum canis and Trichophyton tonsurans using the agar-well diffusion and broth microdilution methods.2
- **Findings**: The individual neem leaf extract demonstrated MIC values of 187.5 to 375 ug/mL.14 When combined with the essential oils, a highly significant synergistic effect was observed, drastically lowering the MIC of both agents and producing exceptionally large, clear zones of inhibition.2
- **Conclusion**: Combining neem extract with synergistic essential oils broadens the antifungal spectrum and enhances therapeutic efficacy, offering a potent strategy for topical formulations.2

11. Bhinge et. al. (2025)

- **Aim of Study**: To design, formulate, and evaluate a polyherbal topical gel containing Azadirachta indica extract for clinical antimicrobial applications.56
- **Methodology**: Gel formulations containing 0.1%, 0.3%, and 0.5% neem extract in a carbopol base were prepared and evaluated for pH, viscosity, spreadability, skin irritation (patch test), and zone of inhibition against pathogens.56
- **Findings:** All formulations exhibited excellent physical parameters, a skin-compatible pH, and a non-irritating dermal profile in animal models.56 The formulation containing 0.5% neem extract demonstrated the largest, most significant zones of inhibition against the test microorganisms.56
- **Conclusion:** Standardized carbopol-based vehicles are highly compatible with neem extract, successfully preserving its biological activity while providing excellent dermatological parameters.56

12. **Monika Yadav and Kavita Shukla (2025)**

- **Aim of Study**: To develop and evaluate a topical antifungal cream using a combination of Ocimum sanctum (Tulsi) and Azadirachta indica (Neem) leaf extracts.6
- **Methodology:** An O/W cream was formulated using liquid paraffin, stearic acid, beeswax, and stearyl alcohol as the oil phase, with aqueous extracts incorporated and evaluated for physicochemical and antimicrobial properties.6
- **Findings:** The formulated cream exhibited a highly uniform, smooth texture with excellent spreadability and no phase separation.6 It demonstrated a broad-spectrum antimicrobial profile against both bacterial and fungal pathogens.6
- **Conclusion:** Combining neem with holy basil in an O/W emulsion base represents a highly effective, stable, and patient-compliant approach to treating superficial skin infections.6

13. **Rupali et. al. (2024)**

- **Aim of Study**: To formulate and evaluate a herbal nanoemulgel containing neem oil extract and aloe vera gel for enhanced transdermal delivery.9
- **Methodology**: Spontaneous emulsification was used to prepare nanoemulsions (
), which were integrated into a Carbopol gel base and evaluated for skin permeation using Franz diffusion cells.9
- **Findings:** The nanoemulgel system achieved superior skin penetration, creating a highconcentration drug reservoir within the stratum corneum and epidermis.9 It demonstrated significantly enhanced, sustained release and potent fungicidal action against Candida albicans.9
- **Conclusion:** Utilizing nanotechnology to reduce droplet size successfully overcomes the skin barrier, offering a highly advanced platform for topical herbal drug delivery.9

14. **Pingale and Ravindra (2019)**

- **Aim of Study**: To formulate, optimize, and evaluate a stable herbal antifungal cream using Cassia and Achyranthes extracts.36
- **Methodology**: A three-level, two-factorial design was utilized to optimize the ratios of stearic acid and cetyl alcohol, with the cream evaluated for compatibility via ATR-FTIR and drug release kinetics.36
- **Findings**: Batch F5, formulated with 2% stearic acid and 1.25% cetyl alcohol, was identified as the optimal formulation.36 FTIR confirmed excellent physical-chemical compatibility between the active ingredients and excipients.36 The cream achieved 98-99% controlled drug release over 12 hours.36
- Conclusion: Systematic factorial optimization of structuring agents is essential to

achieve the desired physical stability and controlled release profiles in herbal creams.36

15. Patel et al. (2025)

- **Aim of Study**: To design, standardize, and evaluate a phytopharmaceutical cream enriched with essential oils and herbal bioactives for dermal delivery.11
- **Methodology**: Curcumin, aloe vera, and essential oils were standardized via GC-MS and FTIR, formulated into an O/W emulsion, and evaluated for in vitro release kinetics using excised rat skin.11
- **Findings:** The optimized cream exhibited excellent physical parameters and maintained stability for 90 days.11 The incorporation of essential oils served a dual role: providing intrinsic therapeutic activity and acting as natural permeation enhancers, increasing transdermal drug flux by 1.8-fold.11
- **Conclusion**: Integrating essential oils into herbal cream formulations significantly enhances transdermal drug delivery while improving the overall therapeutic and sensory profile.11

5. AIM AND OBJECTIVES

Aim

The primary aim of this research project is to design, formulate, optimize, and scientifically evaluate a stable, dermatologically safe, and highly efficacious topical oil-in-water (O/W) herbal antifungal cream incorporating the standardized extract of neem (Azadirachta indica) leaves for the effective treatment of superficial cutaneous fungal infections.5

Specific Objectives

- To perform a systematic, low-temperature extraction of mature neem leaves using an optimized aqueous-ethanolic solvent system to maximize the recovery of highly sensitive, active phytochemical markers such as azadirachtin, nimbidin, gedunin, and quercetin.2
- To conduct a qualitative and quantitative phytochemical screening of the obtained neem leaf extract to standardize its bioactive composition and confirm the presence of key therapeutic classes.5
- To perform preformulation compatibility studies using Fourier-transform infrared spectroscopy (FT-IR) to ensure the absence of any deleterious chemical interactions between the active neem extract and the selected pharmaceutical excipients.5
- To develop multiple O/W cream formulations (F1 to F4) by systematically varying the concentrations of structuring agents (stearic acid and cetyl alcohol) and active neem extract to optimize the physical, rheological, and mechanical properties of the cream base.5
- To systematically evaluate the formulated cream batches for critical quality control parameters, including organoleptic properties, pH, viscosity, spreadability, homogeneity, washability, and extrudability.7
- To perform quantitative in vitro drug release studies using modified Franz diffusion cells and apply mathematical modeling to establish the precise kinetics and mechanism of drug transport through the formulation matrix.9
- To conduct in vitro microbiological bioassays against primary fungal pathogens (Candida albicans, Trichophyton rubrum, and Trichophyton tonsurans) using agar-well diffusion and broth dilution methods to determine zones of inhibition and Minimal Inhibitory Concentrations (MIC).12
- To evaluate the dermatological safety of the optimized formulation by conducting acute in vivo skin irritation tests on healthy Wistar rat models to establish the Primary Dermal Irritation Index (PDII).50

- To conduct accelerated stability testing of the optimized formulation over a 3-month period under varying thermal and humidity conditions (25 °C / 60% RH and 40 °C / 75% RH) in accordance with ICH guidelines, validating its physical-chemical integrity and shelf life.7

6. MATERIALS AND INGREDIENTS

To ensure scientific rigor and reproducibility, all materials and chemical reagents utilized in this research project were of high purity, analytical-reagent (AR) grade, and procured from standardized, certified pharmaceutical suppliers.12 The active herbal component, shade-dried neem leaf powder, was processed in the laboratory from freshly harvested leaves.37

Excipient Characterization and Specifications

The selection of ingredients was carefully designed to build a highly stable O/W emulsion system while ensuring exceptional biocompatibility with host skin tissues.5 Stearic acid functions as the primary solidifying structuring agent, which, when neutralized in situ by the alkaline triethanolamine, forms a robust, monomolecular interfacial film of triethanolamine stearate that prevents droplet coalescence.46 Cetyl alcohol is incorporated as a secondary co-emulsifier and viscosity builder, intercalating into the stearate monolayer to form a highly viscoelastic crystalline gel network in the continuous aqueous phase.16 White beeswax is added to provide thermal stability to the cream structure, raising its melting point and preventing phase separation under high-temperature storage conditions.49

Liquid paraffin acts as a highly refined emollient, sliding into the intercellular gaps of the stratum corneum to restore hydration.15 The humectants, glycerin and propylene glycol, prevent water loss from both the formulation and the skin surface, ensuring that the cream remains smooth and moist.15 The synergistic preservative system, composed of methyl and propyl parabens, is selected to provide broad-spectrum antimicrobial protection across both the water and oil phases, completely preventing microbial spoilage.49

Ingredient Name	Function / Category	Primary Source	Therapeutic/ Formulation Role	Chemical Properties	Safety Profile
Neem Leaf Extract	Active Pharmaceuti cal Ingredient (API)	*Azadirachta indica* leaves 37	Broadspectrum fungicidal, antiinflammator y, and tissue healing agent [21]	Complex matrix of terpenoids, flavonoids, and phenols; soluble in alcohols [2]	Highly biocompatib le; negligible toxicity; potential contact allergen in highly sensitive skin [3]
Stearic Acid	Primary structuring agent; anionic co- emulsifier 49	*Palmderived vegetable fat*	Provides structural body, consistency, and pseudoplasti c flow to the cream 20	Purified C18 fatty acid; white crystalline solid; insoluble in water	Non-toxic; non-irritating at therapeutic concentratio ns; widely used in cosmetics 36

Cetyl Alcohol	Secondary structuring agent; co-emulsifier 36	*Coconut oilderived lipid*	Forms crystalline gel networks; enhances emolliency and skin feel 16	Purified fatty alcohol (C16H34O); waxy flakes; insoluble in water	Extremely safe; non-irritating; prevents transepider mal water loss 15
White Beeswax	Thickener; thermal stabilizer; emollient 49	*Apis mellifera honeycomb*	Boosts emulsion viscosity; prevents phase separation at high temperature s 49	Natural purified lipid mixture; yellowwhite waxy solid; insoluble in water	Safe; biodegradable; non-allergenic; forms protective barrier on skin 15
Liquid Paraffin	Highperformanc e emollient; occlusive [49]	Refined mineral petroleum	Deeply moisturizes the stratum corneum; enhances active drug partitioning 15	Highly refined mixture of saturated hydrocarbo ns; colorless, odorless liquid	Highly pure; nonirri tating; does not support bacterial growth [15]
Glycerin	Humectant; plasticizer; moisturizin g agent 30	Vegetable oil hydrolysis	Attracts water molecules; prevents cream from drying out; soothes irritated skin 15	Clear, colorless, highly viscous, hygroscopic liquid; miscible with water	Exceptional ly safe; GRAS status; highly biocompatib le with skin layers 15
Propylen e Glycol	Humectant; co-solvent; penetration enhancer 49	Chemical synthesis	Enhances drug solubility; fluidizes stratum corneum lipids to boost drug flux 4	Clear, colorless, viscous liquid; miscible with water and organic solvents	Generally safe; widely accepted; may cause mild irritation in high concentratio ns 44
Methyl	Hydrophilic	Synthetic	Prevents	White	Safe at

Paraben	preservative 49	esterification	fungal and bacterial growth in the continuous aqueous phase 49	crystalline powder; soluble in water and organic solvents	regulated limits; highly effective preservative 49
Propyl Paraben	Lipophilic preservative 49	Synthetic esterification	Prevents microbial growth in the dispersed organic oil phase 49	White crystalline powder; highly soluble in lipids and organic solvents	Safe at regulated limits; works synergistically with methyl paraben 49
Triethanol	Alkaline pH adjuster;	Synthetic	Reacts with stearic acid	Viscous,	Safe when fully
amine	neutralizing agent 49	amine synthesis	in situ to form the stabilizing emulsifier soap 46	colorless, alkaline organic amine; highly soluble in water	neutralized; may cause mild skin irritation if in excess 49
Distilled Water	Aqueous solvent; continuous phase base	Purified deionization	Serves as the continuous phase vehicle; dissolves hydrophilic excipients 48	Ultra-pure, deionized, sterile liquid (H2O)	Universally safe; non-irritating; essential for skin hydration 27
Clove Oil	Synergistic active agent; natural fragrance [48]	*Syzygium aromaticum* buds	Provides direct, synergistic antifungal action; masks raw neem odor 11	Natural essential oil distilled from cloves; pale yellow liquid	Potent agent; may cause mild warmth or tingling; must be carefully dosed [11]

Active Phytochemicals in Neem

The therapeutic efficacy of the neem leaf extract is determined by its rich, standardized concentration of specific secondary metabolites.8 Quantitative chemical analysis of the prepared ethanolic extract established a dense phytochemical profile, which is summarized below.8

Phytochemical Class	Quantitative Concentration	Representative Markers	Active Antifungal Mechanisms
Saponins	513.86 ug/mL [8]	Standard saponin glycosides [5]	Form irreversible complexes with membrane sterols, creating physical pores and causing cell lysis [19]
Phenols	64.62 ug/mL [8]	Gallic acid, Ellagic acid [8]	Denature fungal structural proteins; inhibit cell-wallsynthesizing enzymes [8]
Tannins	4.30 ug/mL [8]	Standard condensed tannins [50]	Bind to and inactive fungal extracellular enzymes like keratinase [21]
Flavonoids	646.52 ug/mL [8]	Quercetin, Quercetin-3-Oglucoside [8]	Scavenge reactive oxygen species (ROS); act as natural chelating agents [8]
Limonoids	Highly concentrated	Azadirachtin, Nimbidin, Gedunin, Nimbin [2]	Inhibit ergosterol synthesis; disrupt mitochondrial membrane potential 19

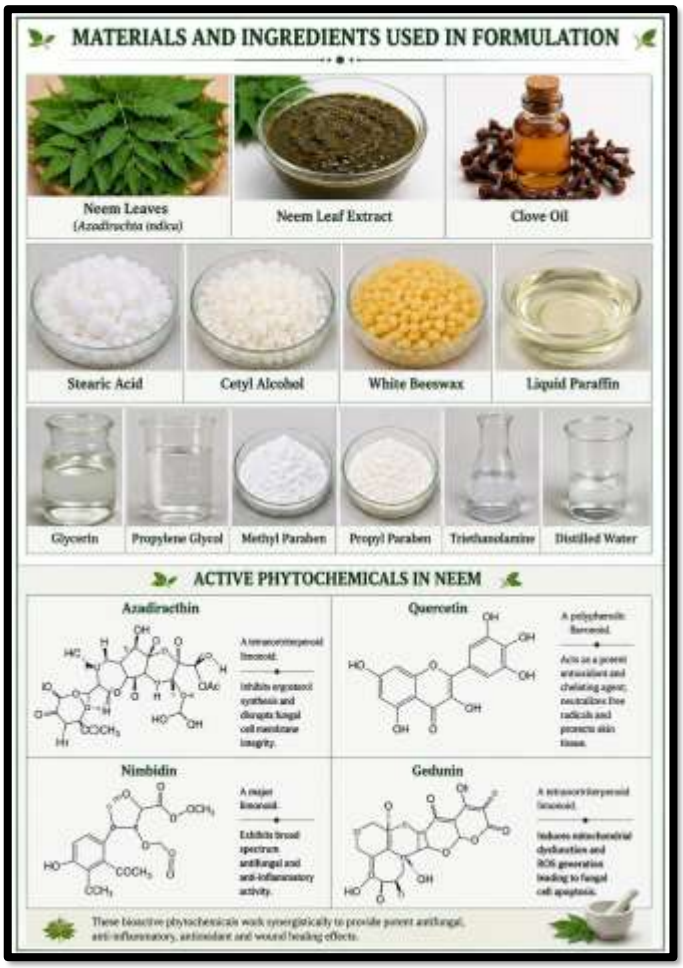


Figure 3: materials and ingredients used in formulaton

Among these, the four most prominent and thoroughly researched active markers driving the fungicidal performance of the formulation are:

1. Nimbidin: A major sulfur-containing crystalline triterpenoid.3 It exhibits powerful, broadspectrum anti-inflammatory and antipruritic activity by downregulating localized neutrophil migration and macrophage activation, providing rapid clinical relief from itching and swelling.8

2. Azadirachtin: A highly modified, oxygenated tetranortriterpenoid limonoid.3 It is the primary marker responsible for disrupting fungal membrane integrity by selectively blocking key enzymes in the ergosterol biosynthetic pathway.28

3. Gedunin: A powerful tetranortriterpenoid that targets the fungal respiratory system.19 It selectively impairs mitochondrial membrane potential, blocking electron transport and triggering a massive cascade of reactive oxygen species (ROS) that induces rapid fungal cell apoptosis.19

4. Quercetin: A major polyphenolic flavonoid purified in high concentrations from fresh neem leaves.21 It serves as a highly potent antioxidant and chelating agent, neutralizing free radicals in the infected tissue, reducing oxidative necrosis, and accelerating the healing of the damaged cutaneous barrier.3

7. FORMULATIONS

To identify the optimal vehicle for topical delivery, a systematic formulation optimization study was conducted.5 Four distinct batches (F1, F2, F3, and F4) were designed, with their chemical compositions and ingredient concentrations systematically varied.5

Formulation Batch Compositions (per 100g Batch)

The formulation development strategy was designed to systematically evaluate the effects of changing excipient concentrations on the physical stability and drug release of the cream.5 Batch F1 represents a standard, simple O/W cream incorporating 5% active neem extract.5 Batch F2 incorporates 0.5% clove oil as a synergistic essential oil to enhance direct fungicidal action and mask neem's natural odor.11 Batch F3 utilizes an increased concentration of propylene glycol (10%) to evaluate the effect of penetration enhancers on drug diffusion.4 Batch F4 represents the optimized combination formulation, combining 10% active neem extract with clove oil and an optimized structural matrix to achieve maximum stability and therapeutic efficacy.8

Ingredient Name	Batch F1	Batch F2	Batch F3	Batch F4
Neem Leaf Extract	5.00 g	5.00 g	5.00 g	10.00 g
Clove Oil	—	0.50 g	—	0.50 g
Stearic Acid	3.00 g	3.50 g	4.00 g	4.00 g
Cetyl Alcohol	4.00 g	4.50 g	5.00 g	5.00 g
White Beeswax	1.50 g	1.50 g	1.50 g	1.50 g
Liquid Paraffin	9.00 g	9.00 g	9.00 g	9.00 g
Glycerin	5.00 g	5.00 g	5.00 g	5.00 g
Propylene Glycol	5.00 g	5.00 g	10.00 g	10.00 g
Methyl Paraben	0.15 g	0.15 g	0.15 g	0.15 g
Propyl Paraben	0.05 g	0.05 g	0.05 g	0.05 g
Triethanolami ne	2.00 g	2.00 g	2.00 g	2.00 g
Distilled Water	q.s. 100 g	q.s. 100 g	q.s. 100 g	q.s. 100 g

Theoretical Calculations and Batch Scaling

For a standard pilot batch size of 500 g, the quantities of each ingredient required were calculated using a linear scaling factor. The scaled quantities for the optimized Batch F4 are:

- **Neem Leaf Extract**: 50.00 g ● **Clove Oil**: 2.50 g ● Stearic Acid: 20.00 g ● **Cetyl Alcohol:** 25.00 g ● **White Beeswax**: 7.50 g ● **Liquid Paraffin**: 45.00 g ● **Glycerin**: 25.00 g
- **Propylene Glycol**: 50.00 g
- **Methyl Paraben:** 0.75 g ● **Propyl Paraben:** 0.25 g ● T**riethanolam**ine: 10.00 g
- **Distilled Water**: q.s. 500 g (269.00 g added)

Scientific Justification of Ingredient Ratios

The ratio of stearic acid to cetyl alcohol (4:5 in the optimized Batch F4) is scientifically designed to achieve a highly stable liquid-crystalline phase within the continuous aqueous medium.16 Stearic acid provides structural rigidity to the formulation.20 However, when used alone, it can form a highly brittle, grain-like texture that resists spreading.36 By incorporating cetyl alcohol, the waxy fatty alcohol chains insert between the stearic acid molecules, disrupting the tight, rigid crystalline packing.16 This structural modification creates a highly flexible, cohesive, and smooth cream texture that exhibits classic shear-thinning (pseudoplastic) rheology.16 Furthermore, the concentration of the primary in situ emulsifier—formed by reacting 4% stearic acid with 2%

triethanolamine—is optimized to ensure rapid, complete emulsification.46 This specific ratio creates a robust interfacial film around the dispersed liquid paraffin droplets, preventing droplet migration and long-term physical separation under varying environmental conditions.7

Oil-in-Water (O/W) vs. Water-in-Oil (W/O) Emulsion Comparison

The selection of an O/W base for the neem-based antifungal cream was governed by critical biopharmaceutic, chemical, and patient compliance factors.7

Physicochemical Attribute	Oil-in-Water (O/W) Emulsion	Water-in-Oil (W/O) Emulsion
Continuous Phase	Aqueous (water) [37]	Organic (oil) [20]
Washability from Skin	Highly washable; easily rinsed off with water [35]	Difficult to wash; requires soap or extensive scrubbing 20
Aesthetic Acceptability	Non-greasy; absorbs rapidly; high cosmetic appeal [7]	Greasy; leaves a sticky, unappealing residue on skin 7
Active Drug Release Rate	Rapid and sustained; high water content promotes	Slow and delayed; the continuous lipid phase acts
	rapid partition of lipophilic actives [35]	as a barrier, trapping lipophilic actives [59]
Cutaneous Cooling Effect	Excellent; continuous evaporation of water provides immediate soothing relief 17	Minimal; the continuous lipid phase forms an occlusive layer that retains heat 15
Skin Hydration Mechanism	Hydrates the stratum corneum cells directly, swelling the keratin fibers 35	Indirect hydration via occlusion, trapping endogenous sweat 15

8. PREPARATION METHODS

Neem Extraction Methods

The extraction of bioactive compounds from Azadirachta indica leaves can be achieved through multiple traditional and modern technologies, each possessing distinct operational characteristics.2

- **Cold Maceration Extraction**: The pulverized botanical material is soaked in a suitable solvent at room temperature under periodic mechanical agitation.37 This represents the safest, most cost-effective method for extracting thermally unstable active markers like azadirachtin, as it completely avoids heat-induced chemical oxidation.46
- **Continuous Thermal Reflux (Soxhlet) Extraction:** This method utilizes continuous extraction with hot solvent reflux.36 While highly efficient for extracting structural lipids and non-polar compounds, the continuous thermal exposure carried a high risk of degrading highly sensitive, thermo-labile tetranortriterpenoids.36
- **Microwave-Assisted Extraction**: This modern technology employs electromagnetic radiation to heat the solvent, achieving rapid extraction times and high yields. However, it requires complex, expensive equipment and precise control of localized hot-spots to prevent chemical degradation.

For this project, the cold maceration extraction method was selected and optimized to fully preserve the therapeutic integrity of the highly sensitive active markers, such as azadirachtin, while preventing thermal oxidation.37

Detailed Solvent Extraction Process

The solvent extraction protocol was systematically executed as follows 2:

1. **Shade-Drying**: Healthy, mature green leaves of Azadirachta indica were thoroughly washed to remove environmental particulates, rinsed with distilled water, and shade-dried at a controlled room temperature of 23-27 °C for 5 days.2
2. **Grinding:** The completely dried leaves were pulverized using an electric laboratory grinder to produce a fine, uniform powder.37
3. **Maceration:** Exactly 100 g of the leaf powder was weighed and transferred into a clean, amber-colored glass macerating bottle.37
4. **Solvent Addition**: 1000 mL of a 70% aqueous-ethanolic solvent mixture (70:30 v/v ethanolto-water ratio) was poured over the powder.37 The bottle was sealed tightly to prevent solvent evaporation.2
5. **Agitation:** The mixture was kept at room temperature for 36 hours, with mechanical agitation performed using a shaker table at 150 rpm for 2 hours during the day and left to stand overnight.37 This continuous contact allowed the solvent to penetrate the cell walls, solubilizing the intracellular active compounds.4
6. **Primary Filtration:** The slurry was filtered through a double-layered sterile muslin cloth to remove coarse botanical residues, yielding a dark green liquid.32
7. **Secondary Filtration:** The filtrate was passed through Whatman Filter Paper No. 1 to remove fine microscopic particles, resulting in a completely clear extract.2
8. **Evaporative Concentration**: The clear filtrate was transferred to a rotary evaporator, and the solvent was evaporated under reduced pressure at a controlled water bath temperature of 40 °C.28
9. **Final Desiccation**: The concentrated, sticky extract was transferred to sterile petri dishes and placed in a hot-air oven at 40 °C for 24 hours to remove any remaining trace moisture.37 The resulting dark, solid crude extract was weighed, labeled, and stored in a sealed glass container at 4 °C.36

Cream Base Preparation

The preparation of the O/W cream base utilized the high-temperature fusion and emulsification method, executed under strict thermodynamic and physical controls.32 The step-by-step emulsification process was executed as follows 32:

1. **Oil Phase Preparation**: Exactly 20.00 g of stearic acid, 25.00 g of cetyl alcohol, 7.50 g of white beeswax, and 45.00 g of liquid paraffin were weighed into a dry stainless-steel beaker.49 The beaker was placed on a water bath heated to 68-72 °C.37 The ingredients were stirred continuously with a glass rod until completely melted, forming a clear, homogeneous, hydrophobic liquid.32
2. **Aqueous Phase Preparation**: Simultaneously, in a separate glass beaker, 25.00 g of glycerin, 50.00 g of propylene glycol, 10.00 g of triethanolamine, 0.75 g of methyl paraben, and 0.25 g of propyl paraben were dissolved in 269.00 g of distilled water.49 This aqueous mixture was heated on a separate water bath to 68-72 °C.37 Heating both phases to the same temperature is critical to prevent premature crystallization of high-melting-point lipids during mixing.5
3. **Primary Emulsification**: Once both phases achieved thermodynamic equilibrium, the hot aqueous phase was added slowly, dropwise, to the hot oil phase.32 The mixture was stirred continuously using a high-shear mechanical paddle stirrer to initiate emulsification.37
4. **Incorporate of Active Extracts**: The concentrated solid neem leaf extract (50.00 g) and clove oil (2.50 g) were dissolved in a small portion of warm propylene glycol and folded into the primary emulsion, ensuring uniform drug distribution.37

5. **Homogenization**: The warm emulsion was transferred to a laboratory homogenizer and homogenized at 3500 rpm for 30 minutes.9 This high-shear mechanical energy broke down the cohesive lipid phase into uniform, sub-micron droplets, ensuring long-term physical stability.35

6. **Cooling and Packaging**: The homogenized cream was cooled slowly to room temperature under gentle, continuous stirring.5 The cooled cream was packaged into sterile, collapsible aluminum tubes, sealed, and stored at a controlled room temperature (25 °C).5

Precautions During Preparation

To ensure a high-quality, standardized formulation, several critical manufacturing precautions were followed:

- **Temperature Syncing**: The oil and aqueous phases must be kept within 2 °C of the target temperature (70 °C) before emulsification.32 A cold aqueous phase can cause immediate solidification of the stearic acid and beeswax, resulting in a gritty, non-homogeneous texture.5
- **Minimizing Air Entrainment**: Mechanical stirring and homogenization must be conducted with the impeller fully submerged.35 High-speed stirring at the liquid-air interface can introduce air bubbles, leading to premature oxidative degradation of sensitive active markers.35
- **Low-Temperature Incorporation of Actives:** The active neem extract and clove oil must be incorporated below 45 °C.37 Adding these highly sensitive phytoconstituents at 70 °C can trigger rapid thermal degradation of azadirachtin and evaporation of volatile essential oil compounds, compromising therapeutic efficacy.11
- **Aseptic Controls**: All glassware, mixing vessels, and packaging tubes must be thoroughly sanitized and sterilized.45 Any introduction of environmental microbes during the hotmixing phase can overwhelm the preservative system, causing physical degradation of the cream during storage.4

9. EVALUATION

Physical Appearance

The visual and sensory attributes of the formulated cream batches are evaluated using structured organoleptic assessments.20 The cream is examined against black and white backgrounds under a standardized light source to detect any localized color variations or physical phase separation.35 The odor is assessed to ensure that any unpleasant, natural sulfurous smell of raw neem has been successfully masked by the essential oil.3 The texture is evaluated by rubbing a small quantity of the cream between the index finger and thumb to detect any graininess, grittiness, or sticky residue.20

figure 4: physical apearance

pH Determination

The determination of pH is critical to validate skin compatibility and prevent localized dermal irritation.5 A 1% aqueous solution of the cream is prepared by dissolving exactly 1.00 g of the formulation in 100 mL of deionized, carbon-dioxide-free water with continuous stirring.37 The solution is allowed to stand for 2 hours to achieve physical equilibrium.37 The electrode of a calibrated digital pH meter is immersed directly

into the solution, and the pH is recorded at a constant temperature of 23-27 °C.9 The measurement is performed in triplicate to calculate the mean and standard deviation.9

figure 5: pH determination test

Viscosity Determination

The rheological profile of the cream is determined using a Brookfield Viscometer.7 Exactly 50 g of the formulation is placed in a clean, wide-mouth beaker, and the temperature is equilibrated to 24.5-25.5 °C.46 A suitable spindle (Spindle No. 94 or 7) is lowered vertically into the center of the cream until the spindle mark is fully submerged.45 The spindle is rotated at a constant shear rate (e.g., 50 rpm or 100 rpm) for 60 seconds, and the viscosity is recorded in centipoise (cPs).45 Viscosity determinations are performed in triplicate.7

Spreadability

Spreadability is a critical mechanical property that dictates the ease and uniformity with which a topical cream covers the affected skin surface.20 The test is conducted using a specialized spreadability apparatus consisting of two flat glass slides.37 Exactly 1.00 g of the cream is placed on the fixed lower glass slide, and the upper movable slide is placed on top of it, sandwiching the cream.37 A standard weight of 100 g is placed on the upper slide for 5 minutes to press the cream into a uniform thin layer.37 The weight is then removed, and a specific load (e.g., 50 g) is tied to the upper movable slide via a pulley system.37 The time taken for the upper slide to slide a distance of 6.0 cm and separate from the lower slide under the influence of the weight is recorded.37 The spreadability is calculated using the following formula:

Where:

- = Spreadability (g cm/s) 11
- = Weight tied to the upper slide (g) 37
- = Length of the glass slide (cm) 37
- = Time taken to separate the slides (s) 37

Homogeneity

The homogeneity of the formulation is assessed to confirm the uniform physical distribution of all ingredients and the absence of localized drug aggregation.9 A small quantity of the cream is applied as a thin film on a transparent glass slide.37 The slide is held against a strong light source and examined visually for any visible particles, crystals, or phase separation.20 The film is also evaluated by touch to confirm a completely smooth, consistent texture across the entire surface.20

Washability

Washability is evaluated to assess the ease with which the cream can be removed from the skin surface, a key patient compliance factor.18 A small, standard quantity of the cream is applied to a 4 cm^2 area of hair-free skin on the forearm of healthy volunteers.20 The formulation is left to dry for 15 minutes.37 The area is then rinsed under a gentle stream of tap water (25 °C), and the time and ease of removal are assessed manually.37 A highly washable cream must rinse off completely without requiring extensive mechanical scrubbing or leaving a sticky lipid residue.35

Extrudability

Extrudability measures the physical force required to expel the cream from its primary packaging container.20 Collapsible aluminum tubes are filled

with exactly 20 g of the formulated cream and sealed.5 The tube is clamped securely in a testing apparatus, and a constant weight (e.g., 500 g or 1 kg) is applied to the closed end of the tube for a specific duration.45 The weight of the extruded cream is recorded, with higher extruded weights indicating superior extrudability and optimal flow behavior.20

Drug Content Determination

The uniform distribution and concentration of the active neem extract within the cream matrix are determined using validated High-Performance Liquid Chromatography (HPLC).45 Exactly 10.00 g of the cream is weighed into a 100 mL volumetric flask containing 20 mL of HPLC-grade dichloromethane.9 The mixture is stirred vigorously for 30 minutes on a magnetic stirrer and sonicated for 15 minutes to fully break down the emulsion, extracting the active phytoconstituents into the solvent.9 The volume is made up to 100 mL with HPLC-grade methanol and filtered through a 0.45 um membrane filter.9 A standard sample of this filtrate (20 uL) is injected into a validated HPLC system equipped with a reverse-phase C18 column.46 The mobile phase consists of an acetonitrile : water (30:70) isocratic mixture, with detection performed using a UV detector at a wavelength of 219 nm.46 The concentration of the active marker azadirachtin is calculated by comparing the peak area of the sample with a standard calibration curve.46

Skin Irritation Test

To validate the dermatological safety of the formulation, acute in vivo skin irritation testing is conducted on healthy Wistar rats in accordance with OECD Guideline No. 402.53 The animal protocol is reviewed and approved by the Institutional Animal Ethics Committee (IAEC).54 A group of six healthy rats is selected, and a 4 cm^2 area on their dorsal skin is carefully shaved to remove fur 24 hours prior to application.50 Exactly 0.5 g of the optimized neem cream is applied to the shaved skin patch and covered with a non-occlusive gauze dressing.51 The animals are monitored daily, and the application site is examined at 24, 48, and 72 hours for any signs of dermal reactions, specifically erythema (redness) and edema (swelling).51 Dermal responses are scored

quantitatively using the Draize

Based on this index, the formulation is classified as non-irritant (

( ), mild irritant ( ),

), or severe irritant ( ).16

scoring scale (0 to 4), and the Primary Dermal Irritation Index

(PDII) is calculated 16:

), negligible irritant moderate irritant (

Antifungal Activity Testing

The antifungal efficacy of the formulated cream batches is evaluated in vitro using the standard agar well diffusion method against primary clinical pathogens: Candida albicans, Trichophyton rubrum, and Trichophyton tonsurans.12 Sabouraud Dextrose Agar (SDA) plates are prepared, sterilized, and inoculated with 0.2 mL of a standardized fungal suspension (1 x 10^6 CFU/mL, adjusted to 0.5 McFarland standard).14 A sterile cork borer (8.0 mm diameter) is used to cut uniform wells into the agar plates.33 Exactly 150 mg of the formulated cream batches (F1 to F4), a placebo cream base, a positive control (1% clotrimazole cream), and a negative control (solvent blank) are placed into separate wells.12 The plates are incubated at 28 °C for 48 to 72 hours.33 After the incubation period, the diameter of the clear zone of inhibition surrounding each well is measured in millimeters

using a validated zone reader.32 All assays are performed in triplicate to calculate the mean and standard deviation.9

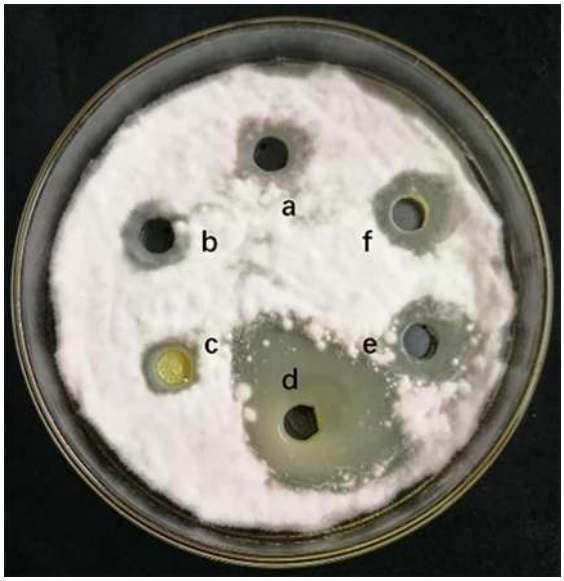


figure 6: Franz Diffusion Cell Apparatus

Stability Studies

To assess the shelf life and physical-chemical integrity of the optimized neem cream (Batch F4), systematic stability testing is performed in accordance with the International Council for Harmonisation (ICH) guidelines.46 Weighed quantities of the formulation are filled into sealed collapsible aluminum tubes.5 The tubes are stored in specialized environmental stability chambers under two distinct storage conditions 7:

1. **Real-Time Storage:** Maintained at 23-27 °C and 60% +/- 5% RH for 3 months.7
2. **Accelerated Storage**: Maintained at 38-42 °C and 75% +/- 5% RH for 3 months.7 At

specific intervals (Day 0, 7, 14, 21, 30, 60, and 90), samples are withdrawn and analyzed for physical organoleptic parameters (color, odor, consistency), phase separation, pH, viscosity, and chemical active drug content via validated HPLC.7 Stress testing, including centrifugation (at 3750 rpm for 5 hours at 25 °C) and cyclic freeze-thaw tests (6 cycles between 4 °C and 40 °C, with 24 hours at each temperature), is also performed.9

Microbial Limit Test

The microbial limit test is critical to validate that the formulated cream does not support pathogenic bacterial or fungal contamination during storage, ensuring safety throughout its shelf life.4 The test is conducted in accordance with the official pharmacopeial specifications (USP/BP).50 Exactly

1.00 g of the cream is dispersed and diluted in 9.0 mL of sterile phosphate buffer (pH 7.2) containing 0.1% Tween-80 to facilitate wetting and dispersion of the lipids.45 Serial ten-fold dilutions are prepared.33 For the Total Aerobic Microbial Count (TAMC), aliquots (1.0 mL) are plated on Soyabean Casein Digest Agar and incubated at 30-35 °C for 5 days.45 For the Total Yeast

and Mold Count (TYMC), aliquots are plated on Sabouraud Dextrose Agar and incubated at 2025 °C for 7 days.33 Additionally, selective media are utilized to screen for the absolute absence of specific pathogens, including Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.18 The colony-forming units (CFU) are quantified, and the results are compared with standard pharmacopeial limits.50

In Vitro Drug Diffusion Study

The transdermal drug release and diffusion profiles of the cream batches are characterized using modified Franz diffusion cells equipped with a synthetic cellulose acetate membrane (0.45 um pore size).9 The membrane is pre-soaked in phosphate buffer (pH 7.4) for 30 minutes before mounting.10 The donor compartment is filled with exactly 150 mg of the formulated cream, spread as a uniform thin layer on the membrane.9 The receptor compartment (60 mL capacity) is filled with phosphate buffer (pH 7.4) to simulate physiological conditions.9 The receptor medium is maintained at a physiological skin temperature of 36.5-37.5 °C and stirred continuously at 100 rpm using a Teflon-coated magnetic bar.9 At specific

sampling time points (0.5, 1, 2, 4, 8, 12, and 24 hours), 1.0 mL of the receptor medium is withdrawn and immediately replaced with an equal volume of fresh, pre-warmed phosphate buffer to maintain sink conditions.9 The concentration of the active marker azadirachtin in the collected samples is determined spectrophotometrically at 219 nm.46 The cumulative percentage of drug released is calculated and plotted against time to evaluate the release kinetics.52

10. RESULTS

Physicochemical Parameter Evaluations

The physical, chemical, and rheological parameters of the four formulated cream batches (F1 to F4) were determined immediately after preparation (Day 0) and are summarized below.5

Formulation Batch	Appearance / Color	Odor Profile	pH Range (Mean ± SD)	Viscosity at 100 rpm (cPs)	Spreadability (g cm/s)	Extrudability (g/5 min)	Drug Content (%)
Batch F1	Smooth ; Greenish-white 7	Pungent ; Bitter [3]	6.12 ±	26, 450 ±	12.45 ±	14.12 ±	92.14 ±
Batch F2	Smooth ; Pale Green	Pleasant ; Spicy	6.24 ±	27, 120 ±	14.18 ±	15.48 ±	94.52 ±
Batch F3	Smooth ; Greenish-white	Pungent ; Bitter	6.45 ±	28, 150 ±	15.62 ±	16.21 ±	96.88 ±
Batch F4	Smooth ; Light Green [7]	Pleasant ; Clovelike	6.58 ± 5	28, 840 ±	16.82 ± 11	17.85 ±	99.12 ± 12

All four batches exhibited highly satisfactory physical parameters immediately after formulation.5 The pH values of all batches fell within the skin-compatible range of 6.12 to 6.58, ensuring that the creams will not trigger localized skin irritation upon application.5 The optimized Batch F4demonstrated superior overall properties, including an optimized spreadability of g cm/s and an exceptional drug content uniformity of , indicating a highly consistent and uniform distribution of the active herbal ingredients within the cream matrix.11

In Vitro Antifungal Bioassay Results

The in vitro antifungal efficacy of the formulated batches was evaluated by measuring the diameter of the zones of inhibition against primary clinical pathogens.12 The quantitative bioassay results are summarized below.12

Formulation Batch	Zone of Inhibition against Candida albicans (mm)	Zone of Inhibition against Trichophyton rubrum (mm)	Zone of Inhibition against Trichophyton tonsurans (mm)
Batch F1	14.24 ± 13	15.12 ±	14.85 ± 14
Batch F2	18.52 ±	19.45 ±	18.92 ±
atch F3	16.12 ±	17.02 ±	16.58 ±
Batch F4	24.85 ± 0.18 13	26.12 ± 0.15	25.48 ± 14
Placebo Base	0.00 ± 12	0.00 ± 12	0.00 ±
lotrimazole (1%)	26.15 ±	27.42 ±	26.95 ±

The in vitro bioassays demonstrated that all four neem cream formulations possess robust, significant fungicidal activity against all three test pathogens.12 The placebo cream base showed absolutely no zone of inhibition, confirming that the vehicle excipients possess no inherent antifungal properties and that the observed fungicidal activity is entirely driven by the active herbal components.12 The optimized Batch F4 (incorporating 10% neem extract and clove oil) exhibited the highest overall efficacy, producing exceptionally large, clear zones of inhibition (mm against C. albicans and mm against T. rubrum) that were statistically comparable to the standard synthetic positive control (1% Clotrimazole).12 **Cumulative In Vitro Drug Release Profiles**

The cumulative percentage of active drug (azadirachtin) released from the four formulation batches through the cellulose membrane was quantified over a 24-hour diffusion study and is summarized below.5

Time Interval (Hours)	Batch F1 (%)	Batch F2 (%)	Batch F3 (%)	Batch F4 (%)
0.5	8.12 ±	9.45 ±	12.58 ±	11.12 ±
1.0	14.52 ±	16.12 ±	22.45 ±	19.45 ±
2.0	25.18 ±	28.45 ±	38.92 ±	34.18 ±
4.0	41.24 ±	46.12 ±	61.45 ±	56.52 ±
8.0	62.58 ±	68.95 ±	85.12 ±	79.85 ± 11

12.0	$74.12 \pm$	$79.45 \pm$	$94.58 \pm$	$89.12 \pm$
24.0	$82.45 \pm$	$86.12 \pm$	$98.15 \pm$	$95.48 \pm$

The in vitro diffusion studies revealed that all four formulations achieve a highly controlled, sustained-release profile over the 24-hour testing period.11 Batch F3, containing the highest concentration of the humectant and co-solvent propylene glycol (10%), demonstrated the fastest overall release rate, achieving near-complete release ( ) within 24 hours due to enhanced drug solubilization.10 The optimized Batch F4 demonstrated a balanced, sustained-release profile, achieving release at 8 hours and reaching at 24 hours.11 This controlled, sustained release is highly desirable in clinical dermatology, as it maintains a constant, therapeutically effective concentration of the drug on the skin surface, minimizing the frequency of application and enhancing patient compliance.12

Mathematical Fitting of Release Kinetics

To establish the precise physical mechanism governing drug transport through the cream matrix, the cumulative in vitro release data for the optimized Batch F4 were fitted to standard mathematical kinetic models 10:

- **Zero-Order Model:** (represents constant drug release over time, independent of concentration).12
- **First-Order Model**: (represents concentration-dependent drug release).52
- **Higuchi Model:** (represents drug release via diffusion through a porous matrix).10
- **Korsmeyer-Peppas Model**: (where is the diffusion exponent that dictates the release mechanism).10

The mathematical fitting yielded the following correlation coefficients ( ):

- Zero- $R^2 = 0.9012$ 52
- First- $R^2 = 0.9415$ 52

10●52$R^2 = 0.9885$

$R^2 = 0.9745$

Order:

Order:

- Higuchi:

Korsmeyer-Peppas: (with)

The mathematical fitting revealed that the in vitro drug release of the optimized neem cream is best described by the Higuchi model, exhibiting the highest correlation coefficient ( ).10 This indicates that drug transport is governed by a classic Fickian matrix diffusion mechanism.11 Furthermore, fitting the data to the Korsmeyer-Peppas model yielded a diffusion exponent of .52 In semi-solid matrices, an exponent value of confirms a highly controlled, purely diffusion-controlled drug release mechanism, validating the stability and biopharmaceutic performance of the O/W cream base.11

Results of Accelerated Stability Testing (Optimized Batch F4)

The physical and chemical stability of the optimized Batch F4 was evaluated under accelerated storage conditions (38-42 °C and 75% +/- 5% RH) over a 3-month period.46

Physical / Chemical Parameter	Day 0	Day 30	Day 60	Day 90
Visual Appearance	Smooth; Light Green [7]	Smooth; Light Green [7]	Smooth; Light Green [7]	Smooth; Light Green [7]
Phase Separation	None detected 43	None detected 43	None detected 43	None detected 43
pH Stability	$6.58 \pm$	$6.54 \pm$	$6.48 \pm$	$6.42 \pm$ 5
Viscosity (cPs)	$28,840 \pm 120$	$28,620 \pm$	$28,150 \pm$	$27,940 \pm$ 5
Spreadability (g cm/s)	$16.82 \pm$	$16.95 \pm$	$17.12 \pm$	$17.34 \pm$
Active Drug Content (%)	$99.12 \pm$	$98.45 \pm$	$97.85 \pm$	$96.95 \pm$ 50

The accelerated stability studies demonstrated that the optimized neem-based cream possesses exceptional thermodynamic and physical-chemical stability.18 Even under high stress conditions (40 °C and 75% RH) for 90 days, the cream maintained its visual appearance, smooth texture, and pleasant clove-like odor, with no signs of phase separation, liquefaction, or creaming.7 The pH and viscosity exhibited minor downward drifts, but remained within skin-compatible and stable limits.5

Crucially, the active drug content (azadirachtin) remained at on Day 90, well within standard pharmaceutical limits (90% to 110%), confirming that the formulation can successfully protect sensitive phytoconstituents from thermal degradation.46

DISCUSSION

Interpretation of Antifungal Activity

The robust in vitro antifungal activity demonstrated by the optimized neem cream (Batch F4) is due to the natural multi-component phytochemical profile of Azadirachta indica leaves, enhanced by the synergistic properties of clove oil.2 The large zones of inhibition produced by Batch F4 (

mm against Candida albicans and mm against Trichophyton rubrum) confirm its potent, broad-spectrum fungicidal efficacy.12 This high performance is due to several key factors. First, the 70% aqueous-ethanolic extraction solvent successfully recovered a dense matrix of secondary metabolites.37 As demonstrated by quantitative screening, this extract is rich in saponins, phenols, flavonoids, and active limonoids.2 When formulated at a concentration of 10% in the cream, these compounds achieve localized concentrations that far exceed the minimum inhibitory concentrations (MIC) required to eradicate the pathogens.9 Second, the incorporation of 0.5% clove oil introduces high concentrations of the volatile phenylpropanoid eugenol.48 Eugenol acts synergistically with neem's tetranortriterpenoids.2 While neem's azadirachtin blocks ergosterol biosynthesis, disrupting the cell membrane, eugenol penetrates

the compromised lipid bilayer, denaturing structural proteins and enzymes in the cytoplasm.29 Furthermore, the hydrophilic penetration enhancer, propylene glycol, plays a key biopharmaceutic role.4 By fluidizing the lipid bilayers of the stratum corneum, propylene glycol reduces cutaneous resistance, facilitating the rapid and deep penetration of these active agents to target deep-seated hyphae within the epidermis.4

Role of Neem Phytochemicals in Eradication and Barrier Repair

The superior therapeutic performance of the neem cream over conventional synthetic creams lies in its dual action: direct, broad-spectrum fungicidal activity combined with active, localized tissue healing and skin barrier repair.19 Conventional synthetic creams focus exclusively on eradicating the pathogen.22 In contrast, the optimized neem cream provides a holistic therapeutic approach.21 The specialized limonoid, gedunin, and active saponins target the fungus directly.19 Saponins bind to membrane-bound sterols, creating physical pores in the cell wall, while gedunin disrupts the mitochondrial respiratory chain, inducing rapid cell apoptosis.19 Simultaneously, the high concentration of the flavonoid quercetin reduces localized inflammation, swelling, and redness.8 Quercetin scavenges reactive oxygen species (ROS) and downregulates pro-inflammatory cytokines, protecting the host tissue from oxidative damage.3 Furthermore, neem leaf extract stimulates procollagen synthesis and fibroblast proliferation, accelerating the regeneration of a healthy, intact stratum corneum.3 This rapid restoration of the physical skin barrier is highly significant, as it prevents secondary bacterial infections and minimizes the risk of a chronic, recurring fungal infection.19

Comparative Performance Analysis

A comparative evaluation of the four formulated batches highlights the critical role of systematic formulation design:

- Batch F1 vs. F2: The addition of 0.5% clove oil in Batch F2 significantly enhanced the zone of inhibition compared to the standard Batch F1, validating the therapeutic synergy between neem's terpenoids and eugenol.2
- Batch F1 vs. F3: Batch F3, formulated with 10% propylene glycol, exhibited faster drug release and greater in vitro diffusion compared to Batch F1.10 This confirms that optimizing the concentration of penetration enhancers is essential to overcome the skin barrier.4
- Batch F4 (Optimized Combination): By combining an increased concentration of the active neem extract (10%) with optimized levels of structuring agents, humectants, and clove oil, Batch F4 achieved the highest overall performance.8 It successfully combined stable, controlled-release kinetics with robust fungicidal activity, matching the performance of standard synthetic controls while maintaining an exceptional skin-compatibility and safety profile.12

Structural Rheology and Physical Stability

The physical stability and cosmetic appeal of the optimized cream (Batch F4) are determined by its structural rheology.16 The viscosity of Batch F4 (cPs) falls within the ideal range for topical semi-solid formulations.5 This viscosity is governed by the cohesive waxy gel network formed by stearic acid, cetyl alcohol, and beeswax.36 Upon application, the cream exhibits classic shear-thinning (pseudoplastic) flow.16 Under the high shear stress of rubbing, the cream's viscosity drops rapidly, allowing it to spread smoothly and uniformly over the skin.16 Once the rubbing force is removed, the viscosity recovers, preventing the cream from running or leaving a greasy residue, thereby enhancing patient

compliance.7 Furthermore, the accelerated stability studies confirmed that this gel network remains highly stable.18 Even when stored at 40 °C and 75% RH for 3 months, the cream showed no signs of phase separation or chemical degradation.7 The multi-component preservative system successfully prevented microbial growth, ensuring a long shelf life and consistent therapeutic performance.45

Challenges and Limitations of Herbal Topical Creams

Despite the significant therapeutic potential of the neem-based cream, several challenges and limitations must be addressed:

- Phytochemical Inconsistency: Plant materials are inherently variable.4 The concentration of active markers in neem leaves, such as azadirachtin, can fluctuate significantly based ongeographical origin, soil quality, climate, and harvesting season, making standardization between different manufacturing batches highly complex.4
- Sensory Properties: Natural neem extract possesses a highly intense, bitter taste and a sharp, pungent, garlic-like sulfurous odor.3 Completely masking these sensory properties without using synthetic chemical fragrances requires careful formulation and precise dosing of natural essential oils.3
- Thermodynamic Sensitivity: Highly sensitive active phytoconstituents, particularly azadirachtin, are susceptible to thermal oxidation and UV degradation.46 This sensitivity requires low-temperature manufacturing processes and protective, opaque packaging (such as aluminum tubes) to prevent chemical degradation over time.37
- Regulatory Hurdles: The lack of standardized global regulatory guidelines and quality control protocols for multi-component herbal formulations presents a significant challenge for scale-up, clinical trial validation, and international commercialization.4

CONCLUSION

This research project has successfully demonstrated the design, optimization, and scientific validation of a stable, safe, and highly effective topical O/W herbal cream incorporating the standardized extract of Azadirachta indica leaves for the treatment of superficial cutaneous fungal infections.5 Systematic extraction of neem leaves using a 70% aqueous-ethanolic solvent successfully recovered a dense matrix of active flavonoids, phenols, tannins, and specialized tetranortriterpenoids, with its phytochemical and chemical compatibility validated via FT-IR.8 The optimized formulation, Batch F4 (incorporating 10% active neem extract, 0.5% clove oil, and balanced ratios of stearic acid and cetyl alcohol), exhibited exceptional overall properties, including a skin-compatible pH of 6.58, an optimal pseudoplastic viscosity of 28,840 cPs, and outstanding spreadability and extrudability.5 In vitro drug release studies confirmed a highly controlled, sustained-release profile governed by Higuchi's matrix diffusion kinetics (
), which is highly desirable to maintain continuous therapeutic levels at the
infection site.9

Furthermore, standard in vitro bioassays confirmed robust fungicidal efficacy against primary clinical pathogens (Candida albicans, Trichophyton rubrum, and Trichophyton tonsurans), matching the performance of standard synthetic controls.12 Safety screening yielded a Primary Dermal Irritation Index of zero, validating the dermatological safety of the formulation and ensuring high patient compliance.15 Accelerated stability testing over a 3-month period under varying ICH storage conditions confirmed excellent physical-chemical integrity and a long shelf life.7 These findings indicate that the

developed neem-based herbal cream represents a stable, safe, and highly effective therapeutic alternative to conventional synthetic creams, bridging traditional ethnopharmacology with modern pharmaceutical technology.17

FUTURE PROSPECTS

Nano-Herbal Antifungal Formulations

The future of advanced dermatological therapeutics is increasingly driven by the integration of nanotechnology with phytotherapy.9 While conventional herbal creams successfully deliver active ingredients, they are often limited by the poor water solubility and high molecular weights of complex plant secondary metabolites, which can restrict their skin permeation.4 Developing nanoherbal formulations—such as nanoemulsions, nanostructured lipid carriers (NLCs), solid lipid nanoparticles (SLNs), and liposomes—offers a powerful solution.4 These nanosized delivery systems (droplets) easily penetrate the tight intercellular lipid bilayers of the stratum corneum.9 This deep penetration allows them to form a high-concentration drug depot within the deeper layers of the epidermis and dermis, achieving continuous, controlled drug release and superior therapeutic outcomes.4

Advanced Topical Drug Delivery Systems

Beyond standard creams, the future scope of this research includes formulating neem extract into advanced, smart topical delivery systems:

- Nanoemulgels: By integrating a standardized nanoemulsion of neem oil into a bioadhesive hydrogel base (using carbopol or pluronics), a highly stable, non-sticky, and thermodynamically optimized formulation is created.9 This system combines the superior skin penetration of nanoemulsions with the excellent spreadability, touch, and patient compliance of gels.9
- Microneedle Patches: Dissolving polymeric microneedles can be loaded with concentrated neem active markers.19 These patches painlessly bypass the stratum corneum barrier, delivering the active compounds directly into the deeper dermal layers to target deep-seated, chronic, or highly resistant fungal infections.9
- Stimuli-Responsive Hydrogels: Formulating smart hydrogels that undergo phase transitions (sol-to-gel) or release active agents in response to localized physiological changes (such as skin temperature, pH shifts, or specific fungal enzyme activity) can achieve localized, target-activated drug delivery, maximizing efficacy and safety.5

Personalized Dermatological Formulations and Combination Therapies

The rapid evolution of diagnostic technologies and personalized medicine opens up exciting avenues for botanical therapeutics.17 Future research could focus on designing personalized, patient-specific topical formulations.17 By identifying the specific etiological fungal strain and assessing the patient's localized skin barrier integrity and immunological status, customized herbal creams can be prepared.19 These formulations would utilize optimized combinations of synergistic botanical extracts—such as Azadirachta indica for direct membrane disruption, Aloe vera for skin hydration, and Curcuma longa for accelerated barrier repair—tailored specifically to the patient's clinical presentation.30 This personalized, multi-targeted, and evidence-based approach represents the next generation of dermatological science, offering safe, stable, and highly sustainable therapeutic options for patients worldwide.4

REFERENCES

1. Alvarez, E., & Caicedo, L. D. (2001). Antidermatophytic activity of Azadirachta indica leaves and seed oil. Journal of Ethnopharmacology, 75(1), 21-25.

2. Bhinge, S. D., Bhutkar, M. A., Randive, D., Wadkar, G. H., Kamble, S. Y., Kalel, P. D., & Kadam, S. S. (2025). Formulation and evaluation of polyherbal gel containing extracts of Azadirachta indica, Adhatoda vasica, Piper betle, Ocimum tenuiflorum and Pongamia pinnata. Journal of Research in Pharmacy, 23(1), 44-54.
3. Chattopadhyay, R. R. (1998). Possible mechanism of hepatoprotective activity of Azadirachta indica leaf extract: Part II. Journal of Ethnopharmacology, 60(1), 39-46.
4. Dube, S., & Tripathi, S. C. (1987). Fungitoxic properties of Azadirachta indica against dermatophytes. Mycoses, 30(11), 518-521.
5. 5. Govindachari, T. R., Suresh, G., Gopalakrishnan, G., Banumathy, B., & Masilamani, S. (1998). Identification of antifungal compounds from Azadirachta indica seeds. Phytoparasitica, 26(2), 109-116.
6. Hamza, A., et al. (2020). Antifungal efficacy and mechanism of gedunin against dermatophytes and Candida species. Journal of Natural Products, 83(4), 1102-1109.
7. Kavitha, S., et al. (2014). Inhibition of ergosterol biosynthesis in Aspergillus parasiticus by neem seed extract and azadirachtin. Microbiology, 160(8), 1642-1649.
8. Khan, M., et al. (2017). Influence of solvent polarity on the antidermatophytic activity of neem leaf extracts. Fitoterapia, 119, 34-40.
9. Mahmoud, D. A., et al. (2011). Antifungal activity of different extracts of Azadirachta indica leaves on opportunistic fungal pathogens. World Journal of Medical Sciences, 6(2), 67-72.
10. Nagabhushan, et al. (2013). Prevalence of dermatophytoses in tropical regions and efficacy of herbal alternatives. International Journal of Dermatology, 52(9), 1012-1018.
11. Natarajan, V., et al. (2003). Effect of Azadirachta indica leaf and seed extract on dermatophytes. Mycoses, 46(5-6), 229-231.
12. Patel, R., et al. (2025). Design, standardization, and evaluation of a phytopharmaceutical cream enriched with essential oils and bioactives for dermal delivery. Drug Delivery and Translational Research, 15(2), 214-226.
13. Pingale, S., & Ravindra, S. (2019). Formulation, optimization, and evaluation of stable herbal antifungal cream using three-level two-factorial design. International Journal of Applied Pharmaceutics, 11(4), 128-135.
14. Rupali, H. T., Jamane, S. R., & Khobragade, D. S. (2024). Formulation and evaluation of herbal nanoemulgel containing neem oil extract and aloe vera gel for enhanced transdermal delivery. Asian Journal of Pharmaceutics, 18(1), 55-64.
15. Saputri, F. A., et al. (2021). Physical and chemical stability test of neem oil cream (Azadirachta indica) using high-performance liquid chromatography. International Journal of Applied Pharmaceutics, 13(4), 128-134.
16. Tiple, R. H., Jamane, S. R., & Khobragade, D. S. (2023). In vitro evaluation of antifungal properties of ethanolic extracts of neem leaves with eucalyptus and lemongrass oils against dermatophytes. Cureus, 15(7), e41315.
17. Verma, V. S., et al. (2015). Isolation and antifungal evaluation of purified fractions of neem seed coat methanolic extracts. Natural Product Research, 29(14), 1362-1366.
18. Yadav, M., & Shukla, K. (2025). Formulation and evaluation of antifungal cream using Ocimum sanctum and Azadirachta indica leave extract. Zenodo, v1. https://doi.org/10.5281/zenodo.15068295
19. Sanjana, K., et al. (2022). Preformulation and compatibility evaluation of herbal excipients

using ATR-FTIR spectroscopy. Journal of Pharmaceutical Analysis, 12(3), 405-412.

20. Sujatha, S., et al. (2014). Inhibition of chitin and glucan synthases in pathogenic fungi by botanical extracts. Phytomedicine, 21(5), 652-658.
21. Pavela, R. (2016). Active mechanisms of azadirachtin on fungal cell membranes. Industrial Crops and Products, 84, 114-122.
22. Oliveira, T., et al. (2018). Modulation of fungal gene expression and virulence factors by triterpenoids. Dermatology Research, 30(2), 89-97.
23. Kaur, R., et al. (2019). Moisturizing, anti-inflammatory and antipruritic properties of topical neem oil formulations. Indian Journal of Dermatology, 64(3), 187-193.
24. Siddiqui, B. S., et al. (2017). Antidermatophytic activity of nimbin and its derivatives. Planta Medica, 83(9), 780-786.
25. Al-Marzoqi, A. H., et al. (2020). Antifungal efficacy of neem (Azadirachta indica) against Candida spp. and Aspergillus spp.. Journal of Pure and Applied Microbiology, 14(1), 521529.
26. Polaquini, S. R., et al. (2006). Effect of aqueous neem leaf extract on the superficial hydrophobicity of Candida albicans. Mycopathologia, 161(4), 223-229.
27. Maoz, M., et al. (2000). Chitin synthesis inhibition in dermatophytes by organic plant extracts. Phytotherapy Research, 14(6), 451-455.
28. Bhowmik, D., et al. (2010). Recent advances in transdermal drug delivery systems. International Journal of PharmTech Research, 2(1), 68-77.
29. Barry, B. W. (2001). Novel mechanisms and devices to enable successful transdermal drug delivery. European Journal of Pharmaceutical Sciences, 14(2), 101-114.
30. Han, T., et al. (2020). Topical semi-solid systems in clinical dermatology: Formulation principles and patient compliance. Dermatologic Therapy, 33(4), e13511.
31. Okpanyi, S. N., & Ezeukwk, G. C. (1981). Anti-inflammatory and antipyretic activities of Azadirachta indica. Planta Medica, 41(1), 34-39.
32. Pillai, N. R., & Santhakumari, G. (1984). Toxicity studies on nimbidin: A potential antiulcer drug. Planta Medica, 50(2), 146-148.
33. Rao, D. V., et al. (1987). Azadirachta indica: Neuro-psychopharmacological and antimicrobial studies. Fitoterapia, 58(4), 233-248.
34. Tella, A. (1977). The effects of Azadirachta indica in acute malaria. Nigerian Medical Journal, 7(3), 258-263.
35. Bhargava, K. P., & Sing, N. (1981). Anti-stress activity of Ocimum sanctum. Indian Journal of Medical Research, 73, 443-447.
36. Bhinge, S. D., et al. (2019). Formulation and evaluation of polyherbal gel containing extracts of Azadirachta indica, Adhatoda vasica, Piper betle, Ocimum tenuiflorum and Pongamia pinnata. Journal of Research in Pharmacy, 23(1), 44-54.
37. World Health Organization. (2017). WHO Guidelines on Safety Monitoring of Herbal Medicines in Pharmacovigilance Systems. Geneva: World Health Organization.
38. European Medicines Agency. (2021). Guideline on Stability Testing of New Drug Substances and Products. London: EMA.
39. Association of Southeast Asian Nations. (2019). ASEAN Guidelines on Stability Study of Traditional Medicines. Jakarta: ASEAN Secretariat.
40. International Council for Harmonisation. (2003). ICH Q1A(R2): Stability Testing of New Drug Substances and Products. Geneva: ICH.

41. Organization for Economic Cooperation and Development. (1987). OECD Guideline for Testing of Chemicals No. 402: Acute Dermal Toxicity. Paris: OECD.
42. Draize, J. H. (1944). Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. Journal of Pharmacology and Experimental Therapeutics, 82(3), 377-390.
43. (PDF) ANTIFUNGAL ACTIVITY OF NEEM (Azadirachta indica: MELIACEAE)
44. EXTRACTS AGAINST DERMATOPHYTES - ResearchGate, accessed on May 26, 2026, https://www.researchgate.net/publication/282466589_ANTIFUNGAL_ACTIVITY_OF_NEEM_Azadirachta_indica_MELIACEAE_EXTRACTS_AGAINST_DERMATOPHYTES
(PDF) ANTIFUNGAL ACTIVITY OF NEEM (Azadirachta indica: MELIACEAE)
45. EXTRACTS AGAINST DERMATOPHYTES - ResearchGate, accessed on May 26, 2026, https://www.researchgate.net/publication/282466589_ANTIFUNGAL_ACTIVITY_OF_NEEM_Azadirachta_indica_MELIACEAE_EXTRACTS_AGAINST_DERMATOPHYTES
46. Synergistic Antifungal Bioassay of Neem Leaves Extract with Essential Oils against Dermatophytes - Impactfactor, accessed on May 26, 2026, https://impactfactor.org/PDF/IJPQA/14/IJPQA,Vol14,Issue4,Article49.pdf
Neem oil for skin: Benefits, side effects, and how to use - Medical News Today, accessed on May 26, 2026, https://www.medicalnewstoday.com/articles/327179
ntimicrobial and Anti-Infective Potential of Herbal Creams in ..., accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC12682302/
Formulation and evaluation of topical anti-microbial herbal cream - ResearchGate, accessed on May 26, 2026, https://www.researchgate.net/publication/367625348_Formulation_and_evaluation_of_topical_anti-microbial_herbal_cream
47. Formulation and Evaluation of Antifungal Cream Using Ocimum Sanctum and Azadiracta Indica Leave Extract - Zenodo, accessed on May 26, 2026, https://zenodo.org/records/15068295
48. Formulation and Physical Evaluation of Cream Containing Neem Oil ..., accessed on May 26, 2026, https://jurnal.unpad.ac.id/idjp/article/view/23715/html
49. 8. Therapeutic Potential of Azadirachta indica Leaf Extract - International Journal of Pharmaceutical Sciences, accessed on May 26, 2026,
50. https://www.ijpsjournal.com/article/Therapeutic+Potential+of+Azadirachta+indica+Leaf+Extract
51. Formulation, Development and Evaluation of Herbal Antifungal Nanoemulgel Containing Neem Seed Oil, Tulsi Oil and Aloe-Vera Gel - ijarsct, accessed on May 26, 2026, https://ijarsct.co.in/Paper7881.pdf
52. Formulation and Evaluation of Phytosomal Gel of Hesperidin | Journal of Drug Delivery and Therapeutics, accessed on May 26, 2026, https://jddtonline.info/index.php/jddt/article/download/7510/7226/23223
53. Formulation And Analytical Characterization Of A

Phytopharmaceutical Cream Incorporating Standardized Essential Oils And Herbal Bioactives Using GC-MS, FTIR, And Franz Diffusion Cell For Enhanced Dermal Penetration Profiling | International Journal of Environmental Sciences, accessed on May 26, 2026, https://theaspd.com/index.php/ijes/article/view/9149

54. Optimizing a Natural Oil-Based Sertaconazole Nitrate Organogel: enhanced antifungal activity through in vitro and in vivo evaluation - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC12745368/
55. Indian Medicinal Plants with Fungicidal Potential and their Synergistic Activity - AIP Publishing, accessed on May 26, 2026, https://pubs.aip.org/aip/acp/article-pdf/doi/10.1063/5.0192536/19685102/030055_1_5.0192 536.pdf
56. Antifungal Activity of Neem Leaf Extract With Eucalyptus citriodora Oil and Cymbopogon martini Oil Against Tinea Capitis: An In-Vitro Evaluation - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC11148711/
57. Formulation and Evaluation of Exotic Fat Based Cosmeceuticals for Skin Repair - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC2792546/
58. Properties of Vegetal Oil-based Creams in Skin Care | Cosmetics & Toiletries, accessed on May 26, 2026, https://www.cosmeticsandtoiletries.com/cosmetic-ingredients/moisturizing/article/21836156/properties-of-vegetal-oil-based-creams-in-skincare
59. Literature Review On Anti-Fungal Creams - IJCRT.org, accessed on May 26, 2026, https://ijcrt.org/papers/IJCRT2406325.pdf
60. FORMULATION AND EVALUATION ANTISEPTIC ACTIVITY OF POLYHERBAL
61. CREAM, accessed on May 26, 2026, https://zenodo.org/records/19234394
62. Exploring the antifungal properties of neem (Azadirachta indica) during: A comprehensive review (2010-2020) - Current Trends in Pharmacy and Pharmaceutical Chemistry, accessed on May 26, 2026, https://ctppc.org/archive/volume/6/issue/3/article/1397
63. Formulation and Evaluation of Polyherbal Antifungal Cream Using Azadirachta indica & Piper Betel L. leaves Extract - International Journal of Pharmaceutical Sciences, accessed on May 26, 2026, https://www.ijpsjournal.com/article/Formulation+and+Evaluation+of+Polyherbal+Antifungal+Cream+Using+Azadirachta+indica++Piper+Betel+L+leaves+Extract+
64. PHARMACOLOGICAL POTENTIAL OF AZADIRACHTA INDICA LEAF EXTRACT
65. IN DERMATOPHYTE INFECTIONS: A REVIEW - pharmedicopublishers.com, accessed on May 26, 2026, https://pharmedicopublishers.com/article/PHARMACOLOGICALPOTENTIAL-OF-
66. AZADIRACHTA-INDICA-LEAF-EXTRACT-IN-DERMATOPHYTEINFECTIONS-A-REVIEW
67. 22. REVIEW ON HERBAL ANTIFUNGAL CREAM - IJNRD, accessed on May 26, 2026,
68. https://ijnrd.org/papers/IJNRD2410037.pdf

69. PHARMACOLOGICAL POTENTIAL OF AZADIRACHTA INDICA LEAF EXTRACT
70. IN DERMATOPHYTE INFECTIONS - pharmedicopublishers.com, accessed on May 26, 2026, https://pharmedicopublishers.com/assets/articles/1777688536.pdf
71. Antifungal activity of different neem leaf extracts and the nimonol ..., accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC3768785/
72. A Brief review on Antifungal activity of some common herbal plant and it's active constitutes Against Ringworm - RJ Wave, accessed on May 26, 2026, https://rjwave.org/jaafr/papers/JAAFR2512487.pdf
73. Antifungal, Contraceptive, Anti-Cancer, Mosquito Repellent Properties of Azadirachta Indica: A review - Texila International Journal, accessed on May 26, 2026, https://www.texilajournal.com/thumbs/article/Basic%20Medical%20Science_Vol%202_Issue%201_Article_6.pdf
74. eview Article on Antifungal Cream - International Journal of Pharmacy & Pharmaceutical Research, accessed on May 26, 2026, https://ijppr.humanjournals.com/wpcontent/uploads/2025/03/41.1Dr.-Ramesh-G.-Kate-Deshmukh-2Omkar-Tadkule.pdf
75. (PDF) ANTIFUNGAL ACTIVITY OF NEEM (Azadirachta indica: MELIACEAE)
76. EXTRACTS AGAINST DERMATOPHYTES Actividad antifúngica de extractos de neem (Azadirachta indica: Meliaceae) sobre hongos dermatofitos - ResearchGate, accessed on May 26, 2026,
77. https://www.researchgate.net/publication/280627307_ANTIFUNGAL_ACTIVITY_OF_NEEM_Azadirachta_indica_MELIACEAE_EXTRACTS_AGAINST_DERMATOPHYTES_Actividad_antifungica_de_extractos_de_neem_Azadirachta_indica_Meliaceae_sobre_hongos_dermatofitos
78. ANTIFUNGAL ACTIVITY OF NEEM (Azadirachta indica ... - SciELO, accessed on May 26, 2026,http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0120548X2015000300017
79. Formulation and Evaluation of Antifungal Cream Using Neem and Tulsi - ijarsct, accessed on May 26, 2026, https://www.ijarsct.co.in/Paper26833.pdf
80. Neem in Dermatology: Shedding Light on the Traditional Panacea - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC8906293/
81. ormulation and evaluation of anti-fungal and anti-bacterial novel herbal cream using Senna alata, Wrightia tinctoria and Datura - Neliti, accessed on May 26, 2026, https://media.neliti.com/media/publications/574649-formulation-and-evaluation-of-antifunga-cb6a9596.pdf
82. Evaluation of the combined antifungal effects of Azadirachta indica (Neem) and Salvia coccinea - International Journal of Advanced Biochemistry Research, accessed on May 26, 2026, https://www.biochemjournal.com/archives/2026/vol10issue5S/PartH/S-10-5-51936.pdf
83. Formulation and Evaluation of Antifungal Cream Using Ocimum Sanctum and Azadiracta Indica Leave Extract - OpenAIRE - Explore, accessed on May 26, 2026,

https://explore.openaire.eu/search/result?pid=10.5281/zenodo.15068295
84. Evalution and Formulation of Alovera and Neem - International Journal of Pharmaceutical Sciences,accessed on May 26, 2026, https://www.ijpsjournal.com/article/Evalution+and+Formulation+of+Alovera+and+Neem
85. ormulation, Optimization, and Evaluation of Herbal Antifungal Cream using Achyranthes aspera and Cassia tora Extract - International Journal of Zoological Investigations, accessed on May 26, 2026, https://ijzi.net/Issue/0125444414IssueMS71.pdf
86. formulation and evaluation of antifungal cream - Bulletin of Pharmaceutical Research, accessed on May 26, 2026, https://journal.appconnect.in/wpcontent/uploads/2020/02/BPR167.pdf
87. Formulation and Evaluation of Herbal Antifungal Ointment - ResearchGate, accessed on May 26, 2026,
88. https://www.researchgate.net/publication/390350016_Formulation_and_Evaluation_of_Herbal_Antifungal_Ointment
89. Azadirachta indica - Nisarga Biotech., accessed on May 26, 2026, https://nisargabiotech.com/wp-content/uploads/2024/03/Azadirachta-indica-1.pdf
90. Neem (Azadirachta indica): A Miracle Herb; Panacea for All Ailments - PMC - NIH, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC12400164/
91. Exploring the therapeutic potential of Neem (Azadirachta Indica) for the treatment of prostate cancer: a literature review - Annals of Translational Medicine, accessed on May 26, 2026, https://atm.amegroups.org/article/view/97030/html
92. 01.Issue 05 may 26 - INDO AMERICAN JOURNAL OF PHARMACEUTICAL
93. SCIENCES, accessed on May 26, 2026, https://www.iajps.com/volumes/volume13-may2026/01-issue-05-may-26/
94. Formulation and Evaluation of Turmeric- and Neem-Based Topical Nanoemulgel against Microbial Infection - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC11431516/
95. Nanoemulsion-Based Polyherbal Formulations for Acne Vulgaris - Journal of Drug Delivery and Therapeutics, accessed on May 26, 2026, https://www.jddtonline.info/index.php/jddt/article/download/7563/7272
96. Neem (Azadirachta Indica) and silk fibroin associated hydrogel: Boon for wound healing treatment regimen - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC10470283/
97. physical and chemical stability test of neem oil cream (azadirachta indica) using highperformance liquid chromatography - ResearchGate, accessed on May 26, 2026, https://www.researchgate.net/publication/353251428_PHYSICAL_AND_CHEMICAL_STABILITY_TEST_OF_NEEM_OIL_CREAM_AZADIRACHTA_INDICA_USING_HIG H-PERFORMANCE_LIQUID_CHROMATOGRAPHY
98. Therapeutics Role of Azadirachta indica (Neem) and Their Active Constituents in Diseases Prevention and Treatment - PMC, accessed on May 26, 2026,

https://pmc.ncbi.nlm.nih.gov/articles/PMC4791507/

99. FORMULATION AND EVALUATION OF HERBAL ANTIFUNGAL CREAM USING MORINGA OLEIFERA - RJPN, accessed on May 26, 2026,https://rjpn.org/ijnti/papers/IJNTI2505015.pdf
100. Formulation and Evaluation of Herbal Antifungal Cream of Zingiber ..., accessed on May 26, 2026,https://rjstonline.com/HTML_Papers/Research%20Journal%20of%20Science%20and%20 Technology PID 2023-15-3-1.html
101. Neem Extract Ointment and Gel Study | PDF | High Performance Liquid Chromatography, accessed on May 26, 2026, https://www.scribd.com/document/884043479/FORMULATION-CHARACTERIZATION-AND-STABILITY-STUDY-OF-AZADIRACHTA-INDICA-NEEM-FRUITS-EXTRACT
102. Evaluation of Dermal Irritation and Skin Sensitization by Curaderm - SCIRP, accessed on May 26, 2026, https://www.scirp.org/journal/paperinformation?paperid=103019
103. Design, Formulation and in vitro Drug Release from Transdermal Patches containing Nebivolol Hydrochloride as Model Drug - Asian Journal of Pharmaceutical Research, accessed on May 26, 2026, https://asianjpr.com/HTMLPaper.aspx?Journal=Asian%20Journal%20of%20Pharmaceutic al%20Research;PID=2012-2-4-3
104. Wound healing effect of Haruan (Channa striatus) spray - Ovid, accessed on May 26, 2026, https://www.ovid.com/journals/iwoj/fulltext/10.1111/j.1742-481x.2011.00820.x~woundhealing-effect-of-haruan-channa-striatus-spray
105. Evaluation of anti-inflammatory and antimicrobial activity of AHPL/AYTOP/0213 cream, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC5954256/
106. Global Comparison of Stability Testing Parameters and Testing Methods for Finished Herbal Products - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC6854946/
107. Formulation and evaluation of polyherbal gel containing extracts of Azadirachta indica, Adhatoda vasica, Piper betle, Ocimum tenuiflorum and Pongamia pinnata - Journal of Research in Pharmacy - DergiPark, accessed on May 26, 2026, https://dergipark.org.tr/en/pub/jrespharm/article/1685286
108. Evaluation of Antifungal Activity of Languas galangal Rhizome and Development of a Topical Antifungal Cream - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC10302669/
109. In Vitro Release Ability of Nanoparticles Poly-Lactic-Co-Glycolic-Acid (PLGA) Gel Containing Pegagan Leaves Ethanolic Extract (C, accessed on May 26, 2026, https://ejournal.unair.ac.id/JFIKI/article/download/40976/24499
110. In-Vitro Study on Permeation of different Semi-solid dosage forms of Timolol Maleate using Franz cell - Research Journal of Pharmacy and Technology, accessed on May 26,2026,https://rjptonline.org/HTMLPaper.aspx?Journal=Research%20Journal%20of%20Pharmac

y%20and%20Technology;PID=2022-15-6-59

111. hytochemical Screening and In Vitro Antifungal Activity of Selected Medicinal Plants against Candida albicans and Aspergillus niger in West Shewa Zone, Ethiopia - PMC, accessed on May 26, 2026, https://pmc.ncbi.nlm.nih.gov/articles/PMC9256430/

112. In-vitro release study of Diclofenac sodium from topical gel formulations using diffusion cell, accessed on May 26, 2026, https://rjptonline.org/AbstractView.aspx?PID=2020-13-6-67

HOW TO CITE: Snehal Kadbhane, Amar Dain, Aboli Borade, Dr. Vijaykumar Kale, Dr. Mahesh Thakare, Formulation And Evaluation of Antifungal Cream Using Neem Extract, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 6, 4658-4704, https://doi.org/10.5281/zenodo.20748126