



FACULTY OF SCIENCE AND EDUCATION

DEPARTMENT OF CHEMISTRY

**FORMULATION AND EVALUATION OF A TOPICAL ANTIFUNGAL
CREAM FROM TRICHODERMA EXTRACTS**

By

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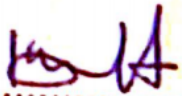
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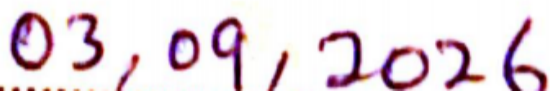
A RESEARCH PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF
CHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE
AWARD OF THE DEGREE OF BACHELOR OF SCIENCE AND EDUCATION OF
BUSITEMA UNIVERSITY

AUGUST, 2026

DECLARATION

I Kwures Hosea, declare that this project report is my original work and has not been submitted to any other institution of higher learning for any academic award.

Signature: 

Date : 

APPROVAL

This research project has been submitted with the approval of the supervisor

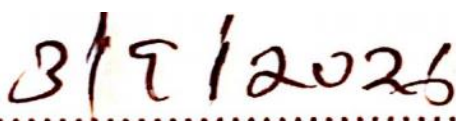
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DEDICATION

This work is dedicated to my beloved parents, Mr. Kwures Patrick and Mrs. Chelangat Jackline, my sister Cherotich Doreen, my brother Kwures Timothy, Sande Aron, Kwures Elly, Mr. Yesho Tom my primary teacher Forest View primary school, Mr. Chemutai Emmanuel teacher Kabei SSS and Mr. Jimmy teacher St Lawrence SS Sonde , for their unwavering support, sacrifices, and encouragement throughout my academic journey. I pray that the good Lord always grant your heart's desires and may He increase your faith, love and hope in Him in your old age

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the Almighty God for granting me good health, wisdom, and strength throughout the course of this research. I extend my deepest appreciation to my supervisor, Dr. Andima Moses, for his invaluable guidance, support, and constructive advice during the entire research process. I am also grateful to my family members for their spiritual, moral, and financial support throughout my studies.

Special thanks go to my friends and colleagues for their encouragement, cooperation, and the useful ideas they contributed, which greatly supported the completion of this project.

ABSTRACT

Fungal skin infections are a major public health problem in Uganda, affecting many people and increasingly showing resistance to conventional antifungal drugs. Local communities in Nagongera traditionally use *Trichoderma*, a naturally occurring soil fungus, to treat skin fungal infections because of its antifungal properties. *Trichoderma* produces enzymes and secondary metabolites that inhibit and destroy pathogenic fungi.

This study aimed to investigate the antifungal activities of *Trichoderma* compounds and formulate an antifungal cream for treating skin fungal infections. Laboratory experiments were conducted to evaluate the effectiveness of the extract and assess the cream's properties including pH, spreadability, viscosity, stability, washability, texture, skin irritation, and retention time on the skin.

The formulated cream showed good physicochemical properties, stability, and minimal skin irritation. The *Trichoderma* extract demonstrated effective antifungal activity against common skin fungal pathogens.

The study concluded that *Trichoderma* compounds have significant potential as a natural and effective treatment for fungal skin infections and can be successfully formulated into a topical antifungal cream.

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CHAPTER ONE; INTRODUCTION

1.1 Background

With a population of about 45 million people, the yearly burden of serious fungal diseases affects about 9% of Ugandans totaling around 4,099,357 individuals .we estimate the burden of candidiasis as follows ; recurrent candidiasis vaginitis (656340 cases) in HIV infected people ,5553 cases of cryptococcal meningitis , 4604 cases of pneumocystis pneumonia in adults and 2010 cases in children for aspergillosis syndromes, invasive aspergillosis annual incidence (3607 cases), 26765 cases of chronic pulmonary aspergillosis and 635745-year-period prevalent cases,75931 cases of allergic bronchopulmonary aspergillosis ,severe asthma with fungal sensation of 100228 cases. Tinea capitis is common with 3047989 prevalent cases, for other mycoses, we estimate annual incidence of histoplasmosis to be 646 case and mucormycosis at 9 cases. Serious fungal diseases affect nearly 9% of Ugandans every year(Felix Bongomin 2024).

Fungi that cause infections in the skin are called dermatophytes, and infections caused by dermatophytes are also known as ringworm or tinea. One example of a very common fungal infection is athlete's foot, also known as tinea pedis. Another common fungal infection is jock itch, an infection affecting the groin area and known as tinea cruris.

In ancient times, plant extracts were utilized to treat skin and nail fungal infections, contributing to the development of antifungal agents as drugs for combating fungal infections. The earliest available pharmacologic agents for the treatment of fungal infections included undecylenic acid, phenolic dye and weak acid. The modern era of antifungal therapy began in the mid-20th century, following the discovery of griseofulvin in 1939, the first synthetic antifungal drug, which was approved for clinical use in early 1960 and became available for oral use that year. Griseofulvin belongs to the antifungal class of polyenes, was isolated from the fungus *penicillium griseofulvum* and remains effective against dermatophyte infections. Besides being available for oral use, antifungal medications are available as topical and intravenous agents as well, and are used to treat different types of fungal infections; however, all

these routes of administration have their respective limitations and side effects. Numerous antifungals have since been developed after the discovery of the first antifungal agent in the 20th century. These are grouped into classes including azoles, echinocandins, polyenes, and flucytosine, with each class exhibiting different mechanisms of action. Therefore, appropriate combination therapy may ultimately yield a positive outcome, even for patients who have drug-resistant fungal infections(Pranal R . Gagbhiye 2023).

Despite the treatment of fungal infections there is an increasing drug resistance by the pathogen which has led to the widespread selection of resistant strains particularly in trichophyton species. systemic antifungals often carry risks of hepatotoxicity and drug – drug interaction .Poor penetration, topical formulations often struggle to penetrate the thick keratinized layers of the skin

Due to the increased persistence of ringworm and candidiasis infections to the drugs, the people of Nagongera are treating tinea locally using the extracts of a naturally occurring organism called Trichoderma.

Trichoderma produces secondary metabolites such as antibiotics that help inhibit other microorganisms. Enzyme production, Trichoderma produces enzymes like chitinases, guanase's and protease. Chitinases and glucanases hydrolyses the cell walls of the target fungus and causing it to burst.

The known antifungal metabolites include; Trichodermin which inhibits protein synthesis in target fungi stopping their growth, peptaiboles which form pores on the membrane of target cells leading to leakage and cell death, gliotoxin, harzianic acid, viridian, koniginins.

Trichoderma derived metabolites are often formulated in to topical creams. creams are preferred because creams allows direct application of the active metabolite to the side of infection ensuring a high concentration of the antifungal agent where it is needed most. Bioavailability and penetration, the semi- solid nature of creams helps in maintaining contact with the target surface, creams are user friendly since they are easy to apply on the skin evenly reducing the risk of wastage and ensuring consistent coverage creams also sensitive secondary metabolites from environmental degradation

Despite the potential of Trichoderma metabolites several gaps remain and they include; standardization, where it becomes difficult to scale up the production of specific secondary metabolites. Safety profiles, limited clinical data on the long-term topical safety and potential skin sensitization of Trichoderma-derived compounds. Mechanism of action, while bio-control mechanisms are well studied the specific molecular pathways by which these metabolites inhibit human dermatophytes are not fully elucidated.

The rise of multi-drug-resistant fungi necessitate the discovery of novel, potent and safe antifungal agents. Trichoderma species represent an untapped reservoir of bioactive compounds. by investigating these metabolites and integrating in to advanced topical delivery systems, we can develop a new antifungal therapy that are not only effective against resistant strains but also minimize the side effects associated with conventional synthetic drugs

In conclusion therefore Trichoderma is a naturally occurring soil fungus, characterized by sweet odour and a powdery texture .it possesses anti-fungal activities which effectively kills most of the fungal skin infections by production of antibiotics and enzymes that degrade the cell walls of the pathogen(Felix Bongomin 2024).

1.2 statement of the problem

The increasing global burden of dermatophyte infections particularly those caused by trichophyton species, represents a critical public health challenge that is increasingly difficult to manage due to the wide spread emergency of drug-resistant fungal strains, which renders synthetic antifungal – such as azoles-frequently ineffective and prone to systemic side effects. This situation is particularly agent in the Nagongera community, where the failure of treatment necessities the exploration of the alternatives, sustainable therapies, in this context, Trichoderma species have emerged as a promising solution due to their unique, multi - model bioactive metabolites that effectively inhibits pathogens without inducing resistance. However, the lack of scientific standardization regarding these compounds prevents their formal clinical adaptation. Therefore, this study aims to isolate and quantify these potent Trichoderma metabolites and incorporate them into a specialized topical cream formulation, which is essential to ensure targeted high concentration delivery to the

sites of infection, improve skin penetration and provide a scientifically validated, patient- compliant, alternative to failing conventional treatment.

1.3 Objectives of the study

1.3.1 General objectives

To develop and evaluate topical antifungal cream formulated from Trichoderma extracts.

1.3.2 Specific objectives

To extract antifungal metabolites from Trichoderma

To evaluate antifungal activity of Trichoderma extracts against selected fungal pathogens.

To formulate a topical cream containing Trichoderma extract.

1.4 Significance of the study

Because of the increased costs in medical facilities, this research will provide knowledge and skills on how to treat fungal infections at a low-cost favoring the poor in terms of finances.

Basing on the studies, it provides wide knowledge on various compounds of Trichoderma spp against fungal infections.

1.5 Scope

This research will be experimental on studying of Trichoderma spp antifungal compounds, and it will be carried out within Nagongera.

CHAPTER TWO; LITERATURE REVIEW

2.0 Abstract

Dermatophyte infections represent a significant global health burden, with increasing antifungal resistance necessitating exploration of novel therapeutic agents.

Trichoderma species, long recognized for their biocontrol properties in agriculture, produce a diverse array of secondary metabolites with potent antifungal activity. This literature review examines the current evidence regarding Trichoderma-derived compounds, particularly trichodermin, for potential application in topical antifungal creams targeting skin infections. The review synthesizes findings from in vitro studies, mechanistic investigations, and in vivo animal models to evaluate both the therapeutic potential and safety considerations of Trichoderma-based antifungals. Key findings demonstrate that trichodermin exhibits broad-spectrum activity against clinically relevant dermatophytes including Trichophyton mentagrophytes, Candida species, and Aspergillus fumigatus, with mechanisms involving mitochondrial disruption and metabolic interference. In vivo rabbit models confirm efficacy in promoting lesion healing, though dose-dependent toxicity necessitates careful formulation optimization. The review identifies significant research gaps, including the absence of standardized clinical trials and the need for advanced formulation strategies to balance efficacy with dermal safety (patriya chanyachailert 2023).

2.1 Introduction

Fungal infections of the skin, hair, and nails affect approximately 20-25% of the global population, with dermatophytes from the genera Trichophyton, Microsporum, and Epidermophyton representing the primary etiological agents. Superficial fungal infections, while rarely life-threatening, significantly impair quality of life and present substantial treatment challenges due to recurrence, chronicity, and emerging antifungal resistance. Current therapeutic options include topical formulations of azoles, allylamines, and polyenes, though treatment failure rates remain considerable, particularly in immuno-compromised populations.

The search for novel antifungal compounds from natural sources has gained momentum, with fungal metabolites representing an underexplored reservoir of

bioactive molecules. *Trichoderma* species, filamentous fungi widely distributed in soil and plant ecosystems, have been extensively studied for their biocontrol mechanisms against phytopathogenic fungi. These organisms produce an array of secondary metabolites, including non-ribosomal peptides, terpenoids, and polyketides, many of which demonstrate potent antimicrobial properties (Shentu 2015).

Among these metabolites, trichodermin, a simple trichothecene-type sesquiterpene, has emerged as a particularly promising candidate for dermatological applications. While trichothecenes have historically been studied primarily in the context of mycotoxin contamination in agriculture, recent investigations have refocused attention on their therapeutic potential when appropriately formulated and dosed. This review critically evaluates the current evidence base for *Trichoderma*-derived antifungal creams, examining the molecular mechanisms, preclinical efficacy data, safety considerations, and future directions for clinical translation.

2.1.1 Antifungal Metabolites from *Trichoderma*

Biosynthesis and Spectrum of Activity

2.1.2 Trichodermin: The Principal Bioactive Compound

Trichoderma brevicompactum produces trichodermin, a simple trichothecene-type toxin that shares initial biosynthetic steps with other sesquiterpene compounds from *Fusarium* species. The biosynthesis of trichodermin begins with the cyclisation of farnesyl pyrophosphate to trichodiene, a reaction catalyzed by the enzyme trichodiene synthase, encoded by the *tri5* gene. Through homologous overexpression of *tri5* in *T. brevicompactum*, Tijerino and colleagues demonstrated that increased expression of this gene leads to enhanced trichodermin production and correspondingly greater antifungal activity compared to wild-type strains.

The broad-spectrum antifungal activity of trichodermin has been well documented through agar diffusion assays. Purified trichodermin demonstrates significant activity against a range of clinically relevant fungi, including *Saccharomyces cerevisiae*, *Kluyveromyces Marxianus*, *Candida albicans*, *Candida glabrata*, *Candida topicals*, and *Aspergillus fumigatus*. Notably, in most cases, trichodermin exhibited antifungal activity superior to that of amphotericin B and hygromycin, two established antifungal agents. This finding is particularly significant given that amphotericin B remains a

gold-standard treatment for severe systemic fungal infections, albeit with considerable toxicity concerns.

2.2 Activity Against Dermatophytes

The specific activity of *Trichoderma* metabolites against dermatophytes, the primary causative agents of skin fungal infections has been investigated by Xiao and colleagues. Using trichodermin derived from the plant endophytic fungus *Trichoderma taxi*, these researchers examined its effect on *Trichophyton mentagrophytes*, an important zoonotic dermatophyte responsible for significant skin pathology in humans and animals.

The results demonstrated dose-dependent suppression of *T. mentagrophytes* growth, with complete inhibition observed at a concentration of 1000 µg/mL trichodermin. Furthermore, spore production and germination critical factors in fungal transmission and infection establishment were markedly reduced following trichodermin exposure. These findings establish that *Trichoderma* metabolites possess direct antifungal activity against dermatophytes, supporting their potential utility in treating conditions such as *tinea pedis*, *tinea corporis*, and *tinea cruris*.

2.3 Other Antifungal Compounds

While trichodermin represents the most extensively characterized antifungal metabolite from *Trichoderma*, the genus produces numerous other bioactive compounds with potential dermatological applications. These include peptaibols (linear peptides containing the non-proteinogenic amino acid α -aminoisobutyric acid), diketopiperazines (including gliotoxin and gliovirin), and various volatile terpene. The diversity of chemical structures produced by *Trichoderma* suggests that multiple compounds may contribute to antifungal activity, potentially offering synergistic effects that could be exploited in formulation development.

2.4 Mechanisms of Antifungal Action

2.4.1 Mitochondrial Disruption and Metabolic Interference

Understanding the mechanisms by which *Trichoderma* metabolites exert antifungal effects is essential for optimizing therapeutic applications and anticipating potential resistance mechanisms. Xiao and colleagues conducted transcriptomic analysis of *T.*

mentagrophytes following trichodermin exposure, revealing significant alterations in cellular processes.

Exposure to trichodermin resulted in a marked increase in mitochondrial numbers, accompanied by downregulation of several mitochondria-related genes a pattern suggestive of mitochondrial damage and compensatory cellular responses. This observation aligns with the known mechanism of trichothecene toxins, which inhibit protein synthesis by binding to the peptidyl transferase center of ribosomes. The disruption of protein synthesis would be expected to impair mitochondrial function and trigger stress responses, consistent with the observed transcriptional changes(srivastava 2015).

Gene Ontology Enrica hymen analysis identified several biological processes significantly affected by trichodermin treatment, including carbohydrate metabolic processes, membrane integrity, and carbohydrate binding. Kyoto Encyclopedia of Genes and Genome pathway analysis further revealed enrichment in biosynthesis of secondary metabolites, glycolysis/gluconeogenesis, and carbon metabolism pathways. These findings indicate that trichodermin exerts pleiotropic effects on fungal physiology, disrupting core metabolic processes essential for survival and proliferation.

2.4.2 Membrane-Related Effects

The enrichment of membrane-related terms in transcriptomic analyses suggests that trichodermin may also affect fungal membrane integrity or function. This is consistent with observations that trichothecenes can intercalate into membranes and alter their physical properties. Additionally, the presence of membrane-associated transporters in the sensitivity mechanism further implicates membrane function in trichodermin activity.

2.4.3 Fungal Resistance Mechanisms

Xiao and colleagues explored potential resistance mechanisms by comparing wild-type *T. mentagrophytes* with a gene deletion strain lacking CDR1, an ABC transporter encoding gene. The CDR1 deletion strain exhibited altered sensitivity to trichodermin, indicating that this efflux pump plays a role in fungal susceptibility to this compound. ABC Transporters are well-established mediators of multidrug resistance in fungi, and

their involvement in trichodermin sensitivity suggests potential pathways for clinical resistance that would need monitoring in therapeutic applications.

2.4.4 Preclinical Evidence for Topical Application

2.4.5 In Vivo Efficacy in Animal Models

The translational potential of *Trichoderma* metabolites for treating skin fungal infections is supported by in vivo evidence from animal models. Xiao and colleagues evaluated trichodermin efficacy in a rabbit model of dermatophytosis, wherein skin lesions were experimentally induced with *T. mentagrophytes* and subsequently treated with topical trichodermin formulations.

Treatment of lesions with 1 mg/ml trichodermin significantly promoted the healing process compared to untreated controls. This concentration proved effective in reducing fungal burden and accelerating clinical resolution of dermatophyte lesions. The study design, employing direct application to infected skin, closely mimics the intended clinical use of a topical antifungal cream and provides critical proof-of-concept for *Trichoderma*-based dermatological preparations.

2.5 Dose-Response and Safety Considerations

A critical finding from the rabbit model concerns the narrow therapeutic window of trichodermin. While 1 mg/ml demonstrated efficacy without observable adverse effects, application of 20 mg/ml trichodermin was associated with probable skin toxicity. This dose-dependent toxicity profile highlights the importance of careful formulation optimization to achieve concentrations that are fungicidal yet below the threshold for dermal irritation or damage.

The toxic potential of trichodermin at higher concentrations is consistent with its classification as a trichothecene mycotoxin. Trichothecenes are known to inhibit eukaryotic protein synthesis and can cause dermal irritation, inflammation, and necrosis at sufficiently high concentrations. However, the existence of an efficacious and apparently safe concentration (1 mg/mL) suggests that a therapeutic window exists for topical application, provided formulations are carefully designed to deliver fungicidal doses without exceeding local toxicity thresholds(vibhavari m. chatur 2025).

2.6 Comparison with Conventional Antifungals

Direct comparative studies between trichodermin and established topical antifungals in dermatophyte infection models are limited. However, the in vitro data demonstrating superior activity of trichodermin compared to amphotericin B against multiple *Candida* species and *A. fumigatus* provides a benchmark for potency. Amphotericin B, while highly effective, is associated with significant toxicity and is rarely used topically; the comparison primarily serves to establish the relative potency of trichodermin against clinically relevant fungi.

For context, contemporary research on topical antifungal formulations continues to explore both novel compounds and reformulations of established agents. For example, a recent clinical study demonstrated that clioquinol 3% cream effectively treated interdigital tinea pedis, with efficacy associated not only with reduced abundance of *Trichophyton* but also with restoration of normal skin microbial diversity. This emphasis on microbiome restoration as a therapeutic outcome represents an evolving paradigm in dermatological therapy and may offer insights for evaluating Trichoderma-based formulations in future studies.

2.7 Formulation Challenges and Opportunities

2.7.1 Optimization of Trichodermin Concentration

The preclinical data indicating both efficacy at 1 mg/mL and toxicity at 20 mg/mL underscore the necessity for precise formulation development. Several factors require consideration in optimizing trichodermin concentration for topical creams:

1. Minimum inhibitory concentration (MIC) determination: Systematic MIC testing against a panel of clinical dermatophyte isolates would establish the concentration range required for fungicidal activity.
2. Skin penetration studies: The ability of trichodermin to penetrate stratum corneum and reach fungal elements in different skin layers requires investigation using ex-vivo human skin models.
3. Safety margin calculation: The ratio between efficacious concentrations causing dermal irritation must be established through dose-ranging studies in appropriate animal models or human skin equivalents.

2.7.2 Delivery Systems and Excipients

The choice of cream formulation and excipients can significantly influence both efficacy and tolerability. Several formulation strategies merit consideration:

- Liposomal or microemulsion systems: Encapsulation of trichodermin in lipid-based delivery systems could enhance skin penetration while potentially reducing local irritation through controlled release.
- Combination with penetration enhancers: Safe and well-characterized penetration enhancers might allow reduction of the total trichodermin concentration while maintaining efficacy.
- Synergistic combinations: Incorporation of additional antifungal compounds, whether other *Trichoderma* metabolites or established agents, could permit dose reduction of trichodermin while achieving equivalent or enhanced efficacy.

2.7.3 Stability Considerations

Trichodermin, like other trichothecenes, possesses chemical stability under appropriate storage conditions, but formulation components could potentially affect its stability. Accelerated stability studies under varying temperature and humidity conditions would be necessary prior to clinical development. Additionally, compatibility with common cream preservatives and antioxidants requires assessment to prevent degradation or loss of activity.

2.8 Clinical Development Status and Research Gaps

2.8.1 Current Clinical Trial Landscape

A systematic search of clinical trial registries reveals no completed or ongoing clinical trials specifically evaluating *Trichoderma*-derived antifungal creams for skin infections. The sole registered trial identified in this review investigated Silver Nova Skin Cream, a silver-based formulation, as an adjuvant to conventional antifungal, rather than a *Trichoderma*-derived product. This absence of clinical trial data represents the most significant gap in the evidence base for *Trichoderma*-based dermatological(Felix Bongomin 2024).

2.8.2 Critical Research Needs

The translation of preclinical findings to clinical applications requires addressing several key research priorities:

1. Standardized Good Manufacturing Practice production: Development of reproducible fermentation and purification processes for trichodermin and other *Trichoderma* metabolites, with appropriate quality control measures.
2. Regulatory toxicology studies: Comprehensive dermal toxicity assessment, including skin irritation and sensitization potential, following regulatory guidelines to support Investigational New Drug applications.
3. Dose-ranging clinical trials: Phase I/2 studies in healthy volunteers and patients with dermatophytosis to establish optimal dosing, safety, and preliminary efficacy signals.
4. Comparative effectiveness studies: Head-to-head comparisons with established topical antifungal to determine relative efficacy and identify patient populations most likely to benefit.
5. Resistance surveillance: Baseline characterization of dermatophyte susceptibility to trichodermin and monitoring for emergence of resistance during treatment.

2.8.3 Microbiome Considerations

The recent emphasis on skin microbiome preservation in antifungal therapy raises important questions about the effects of *Trichoderma* metabolites on commensal skin microorganisms. Future studies should characterize the spectrum of activity of trichodermin against non-pathogenic skin fungi and bacteria to determine whether, like clioquinol, it selectively targets pathogens while allowing restoration of normal microbial diversity (Abdelmotaal 2016).

2.9 Safety and Toxicity Profile

2.9.1 Mycotoxin Concerns

The classification of trichodermin as a trichothecene mycotoxin necessitates careful consideration of safety in any therapeutic application. Trichothecenes are associated with a range of toxic effects in humans and animals, including gastrointestinal toxicity, immunosuppression, and dermal irritation at sufficient exposure level.

However, it is essential to distinguish between the effects of systemic exposure to dietary mycotoxin and the localized, controlled application of purified compounds in topical formulations.

The dose-dependent toxicity observed in the rabbit model demonstrates that while high concentrations may be toxic, lower concentrations can be both effective and apparently safe. This finding supports the feasibility of developing topical formulations that remain below the threshold for local toxicity while achieving therapeutic concentrations.

2.9.2 Systemic Absorption Considerations

For topical antifungal creams intended for application to intact or disrupted skin, the potential for systemic absorption requires investigation. Trichodermin, with its relatively low molecular weight, could potentially penetrate skin and enter the systemic circulation, particularly if applied to large surface areas or damaged skin. Future toxicokinetic studies should quantify absorption following topical application and assess the margin of safety relative to known systemic toxicity thresholds (Pranal R . Gagbhiye 2023).

2.9.3 Comparative Safety Assessment

A comprehensive safety assessment should compare trichodermin-based formulations with existing topical antifungal agents. Established agents such as terbinafine, clotrimazole, and ciclopirox have well-characterized safety profiles with low rates of serious adverse events. Any novel therapy must demonstrate at least comparable safety while offering advantages in efficacy, resistance profiles, or treatment duration to justify clinical development and regulatory (vibhavari m. chatur 2025).

CHAPTER THREE; METHODOLOGY

3.1 Cultivation

3.1.1 Materials

Three Duran bottles, water, measuring cylinder, Trichoderma, spatula, analytical balance, source of power, incubator, sterilizer, potato dextrose broth beakers.

3.1.2 Procedure

Measure 333.3 milliliters of water using a measuring cylinder, pour into one of the cleaned Duran bottles.

Weigh 8 grams of potato dextrose broth using a beam balance, put into the bottle containing water and shake vigorously until it dissolves.

Repeat the above procedure for the other two Duran bottles.

Cover the bottles tightly

Place the bottles containing potato dextrose broth into the sterilizer at 15 lbs pressure and a temperature of 121°C for 15 minutes.

Stop the sterilizer and allow the solution to cool

Add a spatula of Trichoderma into each of the bottles and shake gently.

Close the bottles loosely and place into the incubator for 18 days

Shake at least once in a day to ensure all food is consumed by Trichoderma

3.2 Extraction

3.2.1 Materials

Ethyl acetate, clamp and retort stand, burette, conical flasks, beakers

3.2.2 Procedure

Mix 450 milliliters of the solution with equal volume of ethyl acetate.

Shake for 15 minutes and pour into the burette.

Allow it to form two layers, Trichoderma compounds migrate to the organic layer.

Titrate the aqueous layer into a conical flask and titrate the organic layer into a different conical flask.

Repeat the above procedure to aqueous layer two times

Evaporate off the ethyl acetate using a rotatory evaporator set at a temperature of 70°C.

If the extract contains water, evaporate water off using a water bath set at 70°C

Keep the extract in to a dry clean covered container

3.3 Formation of the cream base

3.3.1 Materials

Water bath, thermometer, two stirring Rodes, distilled water, eight 500ml glass beakers, Ceto-stearyl alcohol, stearic acid, cetyl alcohol, shea butter, glycerin, coconut oil, two 50 ml and 100ml measuring cylinder.

3.3.2 Procedure

Measure 61ml of distilled water into a beaker and mixed with 3.9ml of glycerin and stir using the stirring rode to form aqueous phase

weigh 6.0 g of Ceto-stearyl alcohol, 8.0g of stearic acid, 3.0g of cetyl alcohol, 5.0g of shea butter, 10.9 ml of coconut oil, all into one beaker, stir continuously on the water bath regulated at 75°C until it all melts to form a uniform mixture of the oil phase.

Maintain the aqueous phase and the oil phase at 70°C and slowly pour the oil phase into the aqueous phase while stirring vigorously on the water bath for 8 minutes.

Remove the mixture from the from the water bath while stirring continuously until it cools to form the cream base.

3.4 Formulation of cream containing Trichoderma extracts as an antifungal agent

3.4.1Procedure

Weigh 0.153g of Trichoderma extract.

Dissolve the weight Trichoderma extract in 3 ml warm glycerin

Slowly transfer the dissolved Trichoderma extract into the cream base while stirring

Add 2 drops of lavender tree oil and two drops of tea tree oil.

Stire gently for uniform distribution

CHAPTER FOUR; RESULTS AND DISCUSSION

4.1 Introduction

Skin fungal infections are among the most common dermatological conditions affecting humans. These infections are mainly caused by fungi such as *Candida* species, *Trichophyton*, and *Microsporum*, which thrive in warm and moist environments. Antifungal creams are commonly used to manage these infections because they allow direct application of the active ingredient onto the affected skin area. Recently, natural bioactive compounds obtained from fungi such as *Trichoderma* species have gained attention due to their antimicrobial and anti-fungal properties.

A skin antifungal cream formulated using *Trichoderma* extract must possess acceptable physicochemical and dermatological properties to ensure effectiveness, safety, and patient acceptability. Parameters such as pH, texture, washability, spreadability, stability, viscosity, skin irritation, and retention time on the skin are important in determining the quality and performance of the cream formulation. Therefore, characterization of the formulated cream is essential in evaluating whether the product is suitable for topical application.

The extraction yield of *Trichoderma* extract was 0.153g.

4.2 Presentation of Results

PARAMETER	OBSERVATION
pH	6.38
Texture	Smooth
Homogeneity	Homogeneous and free from lumps
Washability	Easily washable with water
Stability	Stable below 37°C
Spreadability	Easily spreads on the skin
Skin irritation	No redness or itching
Time stays on the skin	12 hours without rapid removal

4.3 Analysis of Results

4.3.1 pH

The formulated Trichoderma antifungal cream showed a slightly acidic pH that is close to the natural pH of human skin. Skin normally has a pH between 4.5 and 6.5 therefore, the cream was considered compatible with the skin surface. A suitable pH helps to avoid skin irritation and supports the maintenance of the skin barrier.

4.3.2 Texture

The cream exhibited a smooth and uniform texture with no visible particles or grittiness. This indicates proper mixing of ingredients and good emulsion formation. A smooth texture enhances user comfort during application and improves consumer acceptability.

4.3.3 Washability

The cream was easily removed with water, indicating good washability characteristics. This suggests that the formulation contained a balanced proportion of water-soluble components, making it convenient for users to clean the skin after use when necessary.

4.3.4 Spreadability

Good spreadability was observed as the cream spread evenly on the skin surface with little effort. This characteristic is important because it ensures uniform distribution of the active antifungal agent over the infected area, improving therapeutic effectiveness.

4.3.5 Stability

The formulation remained physically stable during storage and showed no signs of phase separation, color change, or unpleasant odor. Stability indicates compatibility among formulation ingredients and suggests that the cream can maintain its effectiveness during storage of below 37°C.

4.3.6 Skin Irritation

No irritation effects such as redness, swelling, or itching were observed after application. This indicates that the formulation was safe for topical use and that the Trichoderma extract and excipients used were non-irritant at the tested concentration.

4.3.7 Time Stayed on the Skin

The cream remained on the skin for a duration of 12 hours before being removed naturally or washed away. Good retention time improves prolonged contact between the active ingredient and the infected area, which may enhance antifungal activity.

4.4 Discussion of Results

The characterization results demonstrate that the formulated Trichoderma skin antifungal cream possessed desirable pharmaceutical and dermatological properties suitable for topical administration. The slightly acidic pH obtained is important because creams with highly acidic or alkaline pH values may damage the skin barrier and cause irritation. Therefore, the observed pH suggests that the formulation is compatible with normal skin physiology.

The smooth texture and homogeneity indicate effective emulsification and proper dispersion of ingredients such as stearic acid, cetyl alcohol, glycerin, and Trichoderma extract within the cream base. These properties improve patient acceptability and ensure consistent delivery of the active ingredient.

Good spreadability and moderate viscosity are closely related characteristics. A cream with proper viscosity spreads easily while remaining adhered to the skin surface. This balance is important because highly viscous creams may be difficult to apply, whereas creams with low viscosity may flow away from the application site too quickly. The observed results suggest that the formulation achieved an acceptable consistency for topical use.

The washability result indicates that the cream can easily be removed from the skin without excessive scrubbing, improving user convenience and hygiene. Additionally, the absence of irritation demonstrates that the ingredients used in the formulation were safe and suitable for skin application. This is particularly important for antifungal creams because they are often applied repeatedly over long treatment periods.

The stability of the cream suggests that the formulation maintained its physical integrity during storage of below 37°C. Lack of phase separation or changes in appearance indicates good compatibility between the oil and aqueous phases. Stable formulations are essential for maintaining product quality, efficacy, and self-life.

Finally, the prolonged retention time of the cream on the skin is beneficial because it allows extended contact between the antifungal compounds from Trichoderma and the infected skin area. This may improve antifungal effectiveness and reduce the frequency of application.

Overall, the results indicate that the formulated Trichoderma skin antifungal cream possesses acceptable characteristics for topical antifungal application and has potential for further pharmaceutical and clinical evaluation

CHAPTER FIVE; RECOMMENDATION AND CONCLUSION

5.1 Recommendations

Based on the results obtained from the testing of the Trichoderma skin antifungal cream, several recommendations can be made:

Analysis of all Trichoderma compounds with their antifungal activities

Further microbiological and clinical studies should be conducted to confirm the antifungal effectiveness of the cream against different fungal skin infections such as ringworm, athlete's foot, and candidiasis.

Long-term stability studies are recommended to determine how storage conditions such as temperature, humidity, and light exposure affect the quality and shelf life of the cream over time.

Optimization of the formulation may be carried out to further improve properties such as spreadability, viscosity, and skin retention time for enhanced patient acceptability and therapeutic action.

Additional skin safety tests involving a larger number of participants should be performed to fully confirm the non-irritant nature and safety of the cream for prolonged use.

Comparative studies between the Trichoderma cream and commercially available antifungal creams are recommended to evaluate its effectiveness, affordability, and consumer acceptability.

More research should be conducted on the extraction and purification of Trichoderma metabolites to improve the concentration and activity of the bioactive antifungal compounds in the formulation.

Proper packaging and storage conditions should be recommended to maintain the stability and effectiveness of the cream during transportation and use.

The cream should be produced under hygienic and standardized manufacturing conditions to ensure consistent quality, safety, and effectiveness.

5.2 Conclusion

The results obtained from the evaluation of the Trichoderma skin antifungal cream showed that the formulated cream possessed acceptable physical and functional characteristics for topical application. The pH of the cream was within the suitable range for human skin, indicating that the formulation is less likely to disrupt the natural skin barrier or cause irritation during application. The cream also demonstrated good smoothness and texture, which are important for user comfort and easy application on the affected skin area.

The spreadability results indicated that the cream could easily spread over the skin surface with minimal effort, ensuring uniform distribution of the active Trichoderma extract. Good washability was observed, showing that the cream can be removed easily from the skin without leaving excessive residue. In addition, the formulation exhibited acceptable viscosity and stability, suggesting that the ingredients were well incorporated and that the cream could maintain its consistency and effectiveness during storage.

The irritability test showed little or no irritation on the skin, indicating that the formulation is relatively safe for topical use. The retention time of the cream on the skin was also satisfactory, meaning the cream remained on the skin long enough to allow prolonged contact between the antifungal agent and the infected area, which may improve therapeutic effectiveness.

Overall, the findings suggest that Trichoderma skin antifungal cream has promising potential as a topical antifungal preparation. The formulation demonstrated desirable physicochemical properties and acceptable skin compatibility, indicating that Trichoderma extract can successfully be incorporated into a stable and user-friendly antifungal cream.

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