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FORMULATION AND EVALUATION OF ETHANOLIC PLANT EXTRACT IN MADRAS PEA PUMPKIN ANTIFUNGAL CREAM

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ABSTRACT

The present study was undertaken to formulate and evaluate an antifungal cream containing ethanolic extract of Madras pea pumpkin plant. The plant material was collected, authenticated, shade-dried, powdered, and subjected to extraction using ethanol. The obtained ethanolic extract was evaluated for its preliminary phytochemical constituents and screened for potential antifungal activity. Based on the phytochemical screening, the extract was incorporated into a suitable cream base at different concentrations to develop an effective topical antifungal formulation. The prepared cream formulations were evaluated for various physicochemical parameters such as appearance, colour, odour, homogeneity, pH, spreadability, extrudability, washability, viscosity, and skin irritation. The antifungal activity of the extract and formulated cream was evaluated against selected fungal strains using a suitable microbiological method, such as agar well diffusion. The formulation showing satisfactory physicochemical properties and antifungal activity was considered as the optimized formulation. The study aims to explore the potential of Madras pea pumpkin ethanolic extract as a natural source for the development of a topical antifungal cream.

1. INTRODUCTION

1. Introduction to Fungal Infections

Fungal infections, also known as mycoses, are diseases caused by fungi that can affect various parts of the human body. These infections range from superficial infections of the skin, hair, and nails to life-threatening systemic infections involving internal organs. Fungi are eukaryotic organisms that include yeasts, molds, and dimorphic fungi. Unlike bacteria, fungi possess complex cellular structures, including a defined nucleus and organelles, making them more similar to human cells. This similarity presents a significant challenge in developing antifungal agents that selectively target fungal cells without harming human host cells.[1]

Fungal infections have become increasingly significant in recent decades due to a rise in immune compromised populations, including patients with HIV/AIDS, cancer patients undergoing chemotherapy, organ transplant recipients, and individuals on long-term corticosteroid or immunosuppressive therapy. Additionally, widespread use of broad-spectrum antibiotics has disrupted normal microbial flora, creating an environment conducive to fungal overgrowth.[2]

2. Historical Background of Antifungal Therapy

The development of antifungal therapy began in the mid-20th century. Before the discovery of antifungal drugs, treatment options were limited and often ineffective. The introduction of polyene antibiotics such as nystatin and amphotericin B marked a major milestone in antifungal chemotherapy. Amphotericin B, discovered in the 1950s, remains one of the most potent antifungal agents for systemic infections despite its toxicity.[3]

The subsequent development of azole antifungals in the 1970s and 1980s revolutionized antifungal therapy due to their improved safety profile and oral availability. Later, echinocandins and allylamines were introduced, expanding the arsenal of antifungal drugs. Continuous research has led to the development of newer agents with enhanced efficacy and reduced toxicity.[4]

3. Classification of Fungal Infections (Mycoses)

Fungal infections are broadly classified based on the depth and severity of infection.

3.1 Superficial Mycoses

These infections affect the outermost layers of the skin and hair. Examples: Tinea versicolor, Piedra.

3.2 Cutaneous Mycoses

These involve deeper layers of the skin, hair, and nails. Examples: Dermatophytosis (ringworm), athlete's foot, onychomycosis.

3.3 Subcutaneous Mycoses

These occur in deeper tissues following traumatic implantation of fungi. Examples: Sporotrichosis, chromoblastomycosis.

3.4 Systemic Mycoses

These are severe infections affecting internal organs. Examples: Histoplasmosis, blastomycosis, coccidioidomycosis.

3.5 Opportunistic Mycoses

These occur in immuno-compromised individuals. Examples: Candidiasis, aspergillosis, cryptococcosis.[5,6]

4. Need for Antifungal Agents

The increasing incidence of fungal infections has created a growing need for effective anti-fungal agents. Factors contributing to this need include.

- Increased immuno-compromised population
- Emergence of drug-resistant fungal strains
- Limited number of antifungal drug classes
- Toxicity associated with existing drugs

Antifungal therapy aims to either kill fungi (fungicidal) or inhibit their growth (fungistatic) while minimizing harm to host tissues.[7]

5. Ideal Properties of Antifungal Agents

An ideal antifungal agent should possess the following characteristics.

- Selective toxicity toward fungal cells
- Broad spectrum of activity
- Minimal side effects
- Good bioavailability and tissue penetration
- Low potential for resistance development
- Cost-effectiveness

However, due to similarities between fungal and human cells, achieving selective toxicity remains a major challenge.[8]

6. Structure of Fungal Cell

Understanding the fungal cell structure is essential for antifungal drug design. Key components include.

6.1 Cell Wall

The fungal cell wall is composed of chitin, glucans, and mannoproteins. It provides structural integrity and protection. This structure is absent in human cells, making it an important target for antifungal drugs.

6.2 Cell Membrane

The fungal cell membrane contains ergosterol instead of cholesterol (found in human cells). Many antifungal drugs target ergosterol synthesis or function.

6.3 Cytoplasm and Organelles

Fungal cells contain a nucleus, mitochondria, endoplasmic reticulum, and ribosomes, similar to human cells.[9,10]

7. Mechanism of Action of Antifungal Agents

Antifungal agents act by targeting specific components of fungal cells.

7.1 Inhibition of Cell Membrane Synthesis

Drugs like azoles and allylamines inhibit ergosterol synthesis, leading to disruption of membrane integrity.

7.2 Binding to Ergosterol

Polyenes bind directly to ergosterol, creating pores in the membrane and causing cell death.

7.3 Inhibition of Cell Wall Synthesis

Echinocandins inhibit the synthesis of β-glucan, an essential component of the fungal cell wall.

7.4 Inhibition of Nucleic Acid Synthesis

Flucytosine interferes with DNA and RNA synthesis.

7.5 Disruption of Mitotic Spindle

Griseofulvin disrupts microtubule function, inhibiting fungal cell division.[11,12]

8. Classification of Antifungal Agents

Antifungal drugs are classified based on their chemical structure and mechanism of action.

8.1 Polyenes

Examples: Amphotericin B, Nystatin.

8.2 Azoles

- Imidazole,x Ketoconazole, Clotrimazole
- Triazoles: Fluconazole, Itraconazole

8.3 Echinocandins

Examples: Caspofungin, Micafungin

8.4 Allylamines

Examples: Terbinafine, Naftifine

8.5 Antimetabolites

Example: Flucytosine

8.6 Miscellaneous Agents

Examples: Griseofulvin, Ciclopirox[13,14]

9. Routes of Administration

Antifungal agents can be administered via different

routes depending on the type of infection.

- Topical (creams, ointments, powders)
- Oral (tablets, capsules)
- Intravenous (for systemic infections)[15]

10. Resistance to Antifungal Drugs

Antifungal resistance is an emerging problem. Mechanisms include.

- Alteration of drug target
- Efflux pump overexpression
- Biofilm formation
- Reduced drug uptake[16]

11. Adverse Effects of Antifungal Agents

Common side effects include.

- Hepatotoxicity
- Nephrotoxicity
- Gastrointestinal disturbances
- Skin irritation (topical agents)[17]

12. Applications of Antifungal Agents

Antifungal agents are used in.

- Treatment of fungal infections
- Prophylaxis in immunocompromised patients
- Agriculture (fungicides)[18]

13. Recent Advances in Antifungal Therapy

Recent developments include

- Liposomal formulations of amphotericin B
- New triazoles (voriconazole, posaconazole)
- Combination therapy approaches[19]

ANTIFUNGAL CREAM

1. Introduction to Fungal Infections

Fungal infections, also known as mycoses, are among the most common infectious diseases affecting humans worldwide. These infections are caused by various types of fungi including dermatophytes, yeasts, and molds, which can infect the skin, hair, nails, and mucous membranes. While many fungal infections are superficial and limited to the outer layers of the skin, some can become invasive, especially in immunocompromised individuals.[20]

The increasing prevalence of fungal infections can be attributed to several factors such as poor hygiene, humid climatic conditions, increased use of immunosuppressive drugs, diabetes, and prolonged antibiotic therapy. Countries with tropical and subtropical climates, such as India, are particularly prone to fungal infections due to high temperature and humidity, which provide an ideal environment for fungal growth.

Fungal infections are not only a medical concern but also a significant public health issue, as they affect quality of life, cause discomfort, and may lead to complications if untreated.[21]

2. Classification of Fungal Infections

Fungal infections are broadly classified into the following categories.

2.1 Superficial Mycoses

These infections affect the outermost layers of the skin, hair, and nails. Examples include.

- Tinea corporis (ringworm of the body)
- Tinea pedis (athlete's foot)
- Tinea cruris (jock itch)

2.2 Cutaneous Mycoses

These involve deeper layers of the epidermis and include infections caused by dermatophytes.

2.3 Subcutaneous Mycoses

These infections occur in the dermis and subcutaneous tissues, usually following trauma.

2.4 Systemic Mycoses

These infections affect internal organs and are more severe, often requiring systemic antifungal therapy.

Topical antifungal creams are primarily used for superficial and cutaneous mycoses.[22,23]

3. Need for Antifungal Therapy

Fungal infections often persist if untreated due to the resilient nature of fungal organisms. The need for antifungal therapy arises due to:

- Chronic nature of infections
- High recurrence rates
- Discomfort such as itching, redness, and inflammation
- Risk of spreading to other parts of the body or individuals

Antifungal therapy can be administered through topical or systemic routes, depending on the severity and location of infection. Topical therapy is preferred for localized infections due to fewer side effects and targeted action.[24]

4. Topical Drug Delivery System

Topical drug delivery refers to the application of drugs directly to the skin to treat localized conditions. This route offers several advantages.

- Direct application at the site of infection
- Reduced systemic absorption
- Fewer adverse effects
- Improved patient compliance

Among various topical formulations such as ointments, gels, lotions, and powders, creams are one of the most widely used dosage forms due to their ease of application and cosmetic acceptability.[25]

5. Definition of Antifungal Cream

An antifungal cream is a semi-solid topical formulation containing one or more antifungal agents intended for application on the skin to treat fungal infections.

These creams are typically emulsions (oil-in-water or water-in-oil) that allow for effective delivery of the active pharmaceutical ingredient (API) into the affected area.[26]

6. Composition of Antifungal Creams

Antifungal creams consist of several components:

6.1 Active Pharmaceutical Ingredient (API)

The antifungal agent responsible for therapeutic action. Common drugs include.

- Clotrimazole
- Miconazole
- Ketoconazole
- Fluconazole
- Terbinafine

6.2 Base or Vehicle

The cream base facilitates drug delivery and affects absorption. Types include:

- Oil-in-water (O/W) creams (non-greasy, easily washable)
- Water-in-oil (W/O) creams (more moisturizing)

6.3 Emulsifying Agents

These help stabilize the cream formulation.

6.4 Preservatives

Prevent microbial contamination (e.g., parabens).

6.5 Stabilizers and Humectants

Improve shelf-life and maintain moisture content.[27,28]

7. Mechanism of Action of Antifungal Agents

Antifungal drugs act by targeting the fungal cell membrane or cell wall. Common mechanisms include.

- Inhibition of ergosterol synthesis (e.g., azoles like clotrimazole)
- Disruption of cell membrane integrity (e.g., polyenes)
- Inhibition of fungal enzyme systems

Ergosterol is an essential component of fungal cell membranes; its inhibition leads to increased membrane permeability and cell death.[29]

8. Advantages of Antifungal Creams

Antifungal creams offer several benefits:

- Localized treatment
- Minimal systemic side effects
- Easy application
- Cost-effective
- Suitable for mild to moderate infections They are especially useful in treating:
- Athlete's foot
- Ringworm
- Fungal diaper rash
- Cutaneous candidiasis[30,31]

9. Limitations of Antifungal Creams

Despite their advantages, antifungal creams have certain limitations:

- Limited penetration in severe infections
- Require frequent application
- Possibility of skin irritation or allergic reactions
- Not effective for systemic infections

In such cases, oral or intravenous antifungal therapy may be required.[32]

10. Factors Affecting Efficacy of Antifungal Creams

Several factors influence the effectiveness of antifungal creams.

- Severity and type of infection
- Patient compliance
- Skin condition (hydration, thickness)
- Formulation characteristics
- Duration of treatment

Proper application and adherence to prescribed duration are crucial for successful treatment.[33]

11. Evaluation Parameters of Antifungal Creams

To ensure quality and effectiveness, antifungal creams are evaluated based on.

- Physical appearance
- pH
- Viscosity
- Spreadability
- Drug content
- Stability
- In-vitro antifungal activity[34]

12. Recent Advances in Antifungal Creams

Modern research has led to the development of advanced antifungal formulations such as.

- Nanoemulsions
- Liposomes
- Solid lipid nanoparticles
- Hydrogels

These advanced systems improve drug penetration, stability, and therapeutic efficacy.[35]

Common antifungal creams

- Clotrimazole – broad-spectrum; used for ringworm, athlete's foot, candidiasis
- Miconazole – similar to clotrimazole; also used for yeast infections
- Terbinafine – very effective for dermatophyte infections (e.g., athlete's foot)
- Ketoconazole – used for dandruff, seborrheic dermatitis, and skin infections
- Econazole – used for a wide range of fungal infections

Conditions treated

Antifungal creams are commonly used for.

- Athlete's foot (Tinea pedis)
- Ringworm (Tinea corporis)
- Jock itch (Tinea cruris)
- Candidiasis (yeast infections)

- Skin rashes caused by fungi

How to use antifungal cream

1. Clean and dry the affected area
2. Apply a thin layer of cream
3. Gently rub it in
4. Use once or twice daily (depending on the product)
5. Continue treatment for 1–2 weeks after symptoms disappear to prevent recurrence

Advantages

- Easy to apply
- Acts directly at the site of infection
- Fewer side effects compared to oral drugs

Side effects

- Mild irritation or redness
- Burning or itching sensation (rare)
- Allergic reactions (very rare)

Important precautions

- Avoid contact with eyes, mouth, or open wounds.
- Do not stop treatment early.
- Keep the affected area clean and dry.
- Use separate towels/clothes to prevent spreading infection.

MECHANISMS OF ACTION AND RESISTANCE

Understanding the mechanism(s) of action of different antimicrobial agents is an important prerequisite to understanding mechanisms of resistance. In fact, in many cases an elucidation of resistance mechanisms has allowed or enhanced our understanding of specific mechanisms of action. We therefore combine our discussions of mechanisms of action and resistance to individual antimicrobial classes, although the bulk of our attention is focused on mechanisms of resistance. Readers interested in a more in-depth discussion of the mechanisms of action of different antifungal agents.[36]

The three major groups of antifungal agents in clinical use, azoles, polyenes, and allylamine/thiocarbamates, all owe their antifungal activities to inhibition of synthesis of or direct interaction with ergosterol. Ergosterol is the predominant com ponent of the fungal cell membrane.[37]

PREVENTION AND CONTROL OF ANTIFUNGAL RESISTANCE

Strategies to avoid and suppress the emergence of antifungal resistance have not been defined. However, approaches analogous to those recommended for antibacterials could be suggested. These measures include (i) prudent use of antifungals, (ii) appropriate dosing with special emphasis on avoiding treatment with low antifungal dosage, (iii) therapy with combinations of existing agents, (iv) treatment with the appropriate antifungal (in cases where the etiological agent is known), and (v) use of surveillance studies to determine the true frequency of antifungal resistance. It should be emphasized that data supporting the use of the suggested measures is largely lacking, and ongoing studies may provide some specific guidelines in the near future. Additionally, advances in rapid diagnosis of fungi may be helpful in reducing the use of inappropriate antifungals to treat organisms that are resistant to a particular agent. Unfortunately, progress in developing diagnostic methods specific to fungi has been slow. The recent approval of a reference method for the antifungal susceptibility testing of yeast is encouraging and provides a means for performing surveillance studies.[38,39]

The expression of resistance to antimicrobial agents is the logical and inevitable consequence of using these agents to treat human infections. The availability of molecular genetic tools has led to a rapid expansion in our understanding of the mechanisms by which antibacterial resistance emerges and spreads and promises to greatly inform efforts to develop novel and effective compounds for future use. With increased use and availability of different classes of antifungal agents, it is anticipated that we will see an increasing number and variety of fungal species resistant to these agents. Continued efforts to study the mechanisms of antifungal resistance and the development of experimental systems (like those available in bacteria) in which individual resistance mechanisms can be studied will be important components of a strategy to limit the emergence of resistance to these agents and to develop safer and more potent compounds for the future.

2. LITERATURE REVIEW

1. B.H. More, S.N. Sakharwade, S.V. Tembhurne, D.M. Sakarkar et.al., (2013)[40]

Evaluation of sunscreen activity is an important aspect in the cosmetic industry, as exposure to sunlight is recognized as a major factor in the etiology of the progressive unwanted changes in the skin appearance and physiology due to UV rays present in the sunlight. In the present study, sunscreen activity of formulated cream containing the leaves extract (0.5, 1 and 1.5%) of Butea monosperma respectively was determined by absorption spectroscopy and transmission spectroscopy methods. In absorption spectroscopy, the absorption spectrum of dilute test solution of formulation from 290-320nm was studied to determine SPF against UV-B, most risky UV rays while in the transmittance spectroscopy, UVA and UVB protection and average UVA protection factor were calculated by taking transmission of formulations from 290nm-400nm using PVC as substrate. The results of the present study shows that the formulated creams have potency to protect against UV-rays with good SPF against UVB (Absorption spectroscopy), as well the formulations produced by incorporating different concentration of extracts can be applicable for different type of Skin respectively as per SPF value. Result of transmission spectroscopy also revealed that formulations has satisfied protection against UV-A and B rays with good average UVA protection factor

(Transmission spectroscopy) indicating sunscreen activity. From the result of the present study, it concludes that cream of Butea monosperma can be used as sunscreen for different types of skin as per SPF obtained for formulations containing different concentration of extract.

2. Ahmed Dhahir latif Al-Hussainy et.al., (2015)[41]

The aim of the study was to evaluate the antimicrobial activity ethanolic extracts of Nigella sativa (N.sativa), Zingiber officinal (Ginger), and Trigonella foenum graecum (Fenugreek), in three concentration (50mg/ml, 100mg/ml, and 200mg/ml). The antimicrobial activities have been evaluated against two-gram positive bacteria: Staphylococcus aureus, Streptococcus sp., and two-gram negative bacteria: Escherichia coli, Proteus sp. And two pathogenic fungi: Candida albicans, Sacromysis sp. Ethanolic extract of Ginger showed the maximum antimicrobial against gram positive bacteria and Candida albicans fungi while Fenugreek extract give the potent effect against gram negative bacteria and Sacromysis fungi. On the other hand N. sativa extract showed the minimum antimicrobial effects. The results indicate the efficacy of the plants as a potent antimicrobial agent.

3. Aremu Olusola Isaac and Adekoya Christiana Tolulope et.al., (2016)[42]

The plant Acalypha wilkesiana (Euphorbiaceae) has been ethnomedicinally reported to have antifungal activities. There is the need to have convenient dosage formulations that would be therapeutically useful to patients. The present study seeks to screen for the presence of secondary metabolites present in the plant and then evaluate the extract and cream formulations against certain selected fungal organisms. The extract was obtained from pulverized dried leaves with ethanol 95%v/v at powdered leaves to solvent ratio 1:10. The plant extract was screened phytochemically using standard procedures. The extract was formulated into cream at concentrations 0%w/w, 2.5%w/w, 5.0%w/w, 7.5%w/w and 10.0%w/w respectively. The cream formulations were evaluated against Tricophyton tonsurans and Epidermophyton floccosum as test organisms using ketoconazole cream as reference. The extraction gave a yield of 10.68%w/w. The 0phytochemical screening indicated presence of saponin, tannins and alkaloids. The extract showed activity against Tryhophyton tonsurans only with mean inhibition zones of 10.00mm to 16.00mm for all the concentrations tested. Cream formulations gave favourable physical characteristics and zones of inhibition of 12.00mm and 15.00mm against Tricophyton tonsurans at 7.5%w/w and 10.0%w/w concentrations respectively. This compares favourably with ketoconazole cream with inhibition zone of 18.00mm. The results of this work show that Acalypha wilkesiana leaf extract cream has potential in treating fungal infection of the skin due to presence of Tricophyton tonsurans.

4. Puri Dinesh, Choudhary Deepak, Bhandari Anil, Gaur Kumar Praveen, Yasir Mohd et.al., (2018)[43]

Aim: The aim of present work was to develop and evaluate antifungal topical preparation of aqueous extract of herbal plant *FagoniaschweinfurthiiHadidi*. Materials and Method: An oil in water (O/W) emulsion-based cream was formulated by using extract and various ingredients like oil soluble components white petrolatum, cetyl alcohol and water soluble components sodium lauryl sulphate, methyl paraban and water. Prepared formulation was subjected to various physiochemical parameters like pH, spread ability viscosity and stability study. Antifungal activity of cream was determined by cup plate method against three fungal strains. Result and Discussion: pH, spreadability and viscosity was found to be 7.2±0.1, 20.55±0.47 gm.cm/sec. and 11820±5 cp respectively. The values of diameters of zones of inhibitions (in mm) by prepared cream and marketed formulation was found to be 36.00 ± 1 and 35.66± 1.52, 31.56± 1.52 and 37.33 ±1.15, 32.66± 0.57 and 35.66± 0.57 respectively against *Candida albicans, Aspergillus niger*and *Aspergillus fumigates.*Results indicated that developed formulation has potent antifungal activity. Conclusion: On the basis of data of stability study and other evaluation parameters, it can be concluded that stable formulation was developed which has potent anti fungal activity and can be used in treatment of skin diseases caused by fungal infections.

5. Rety Setyawaty, Feriadi, Dewanto et.al., (2019)[44]

Cream is a semi-solid emulsion dosage form of both water-in-oil (W/O) or oil in water (O/W) type containing one or more dissolved or dispersed ingredients in the corresponding base material (containing no less than 60% water). Cream is usually used as emollient or containing active pharmaceutical ingredients on the skin (Ansel, 2008). The advantage of cream are the application practicallity, water washeability, and the easiness to spread evenly. In this research, we formulated cream containing rhizome Galangal rhizome. According to Darmono (2008), Galangal rhizome has various properties such as antifungal and antibacterial activities. Galangal rhizome contains 1-asetoksikhavikol asetat (ACA). ACA is an antifungal. ACA has good solubility in 70% ethanol. We maserated Galangal rhizome (Alpinia galangal L.) to extract ACA from the simplicia. As for the cream base, we use hydrophilic base containing emulgators stearic acid and triethanolamine, with glycerin as humectant. During the optimization, we chose three formulas, formula 1 (10% stearic acid, 2% triethanolamin, 5% glycerin, and 0.01% vitamin E), formula 2 (15% stearic acid, 3% triethanolamin, 10% glycerin, and 0.05% vitamin E), and formula 3 (20% stearic acid, 4% triethanolamin, 7.5% glycerin, and 0.09% vitamin E). We used the bases to contain 10% of the extract. The results show that formula 1, formula 2, and formula 3 had typical smell of Galangal rhizome, brown color, and thick consistency. All formulas are homogenous. Formula 1 the best stability. We conclude that Galangal rhizome

(Alpinia galanga L.) can be formulated in cream form with our formula 1 had the best stability among others.

6. Kusum KAUSHIK, Ram Babu SHARMA, Abhıshek SHARMA, Shweta AGARWAL et.al., (2020)[45]

In the last few decades, there has been an exponential growth in the field of herbal medicine. It gets popularized in developed and developing countries owing to its natural origin and lesser side effects. In this experimental study, herbal gel containing Ipomoea carnea Jacq. methanolic leaf extract was formulated and evaluated for antifungal activity. The base was prepared by using carbopol 934, propylene glycol 400, methyl paraben, propyl paraben, triethanolamine and required amount of water in a quantity sufficient to prepare 100 g. Different gel formulations were prepared by varying the carbopol 934 concentration (0.8 and 1%), plant extract concentration (2, 4 and 6%) and lavender oil (0.5 and 1%). Eight gel formulations were prepared and evaluated for physicochemical tests that was colour, pH, viscosity, spreadibility, extrudability, homogeniety and screened for their antifungal activity by agar well diffusion technique for zone of inhibition. The formulation F8 showed zone of inhibition 32 ± 1, 17.67 ± 1.15, 18.67 ± 0.57, 26 ± 1 mm against Aspergillus niger, Candida albicans, Rhizopus species and Penicillium notatum respectively greater than marketed preparations. Skin irritation study (patch test) was carried out on wistar rats with F8 formulation which did not exhibit any clinical signs hence the gel was considered safe and nonirritant. The gel can be used in the treatment of vaginal candidiasis, candidiasis of the skin, cutaneous aspergillosis, facial skin manifestations by Penicillium, cutaneous mucormycosis and other skin infections.

7. Roger Ducos Youmsi Fokouo, Patrick Valere Tsouh Fokou, Cedric Derick Jiatsa Mbouna, Elisabeth Zeuko'o Menkem, Fabrice Fekam Boyom et.al., (2020)[46]

Background: The increasing incidence of dermatophytoses in the world and the side effects of the current therapies encouraged the search of alternative drugs. Hence the objective of this work was to determine antidermatophytes activity of Syzigium aromaticum formulate antidermatophytic cream. Materials and Methods: The extracts were prepared by maceration of plant materials into methanol. Three formulations of creams were made, and the best was chosen according to its physicochemical stability and appearance. The acute dermal toxicity and antidermatophytic efficacy of the cream was performed on guinea-pig. Results: The methanolic extract of S. aromaticum was incorporated in the final cream formulation. The formulation containing shea-butter 58.5%, acetylic alcohol 2.5%, stearic acid 1.5%, bee-wax 10%, borax 1.5%, polysorbate 60 2.5%, 2 drops of lactic acid and water was chosen because of its good appearance and stability. The cream with methanolic extract of S. aromaticum did not reveal any dermal toxic effect. The cream efficacy was dose-dependent. The treatment with cream at 5% methanolic extracts of S. aromaticum revealed the best potency after 14 days of treatment. Conclusion: These results show that the cream at 5% methanolic extract of S. aromaticum seed is promising in the treatment of dermatophytoses and could be used as an alternative in the development of a new therapy.

8. Clement Olusola Ogidi, Ayokunbi Elizabeth Ojo, Oluwatayo Benjamin Ajayi-Moses, Oluwatoyin Modupe Aladejana, Oluwakemi Abike Thonda and Bamidele Juliet Akinyele et.al., (2021)[47]

Background: The frequent incidence of fungal infection and wide spread of antibiotic resistance are emergent concerns in public health. Hence, there is a need to harness the potential of natural bioactive compounds from plant towards treatment of fungal infection. Combination effect of antibiotic creams with natural products from plants is prospective strategy to produce new antifungal agent. This study therefore, revealed antifungal effect of combined Antifungal Creams (AFCs) with Turmeric Essential Oil (TEO) or Aloe vera Gel (AVG). Methods: Phytochemicals and bioactive compounds in TEO and AVG were revealed using GC-MS. Bioactive compounds in plant extracts were compared to known compounds in database library of National Institute of Standards and Technology (U.S.). Antifungal activity and synergistic effect of AFCs with TEO or AVG were carried out using agar well diffusion method. Results: Phenol, flavonoids, saponins, alkaloids, steroids, terpenoids and cardiac glycosides were present in TEO and AVG. GCMS revealed thirty-six (36) and eighteen (18) bioactive compounds in TEO and AVG, respectively. AFCs displayed zones of inhibition with values ranged from 5.0 to 14.3 mm, TEO was 5.0 to 11.0 mm and AVG was 8.0 to 11.7 mm against tested fungi. Minimum Inhibitory Concentration (MIC) by AFCs, TEO and AVG ranged from 1.25 to 10.0 mg/ml. Combinatory effects of AFCs with TEO or AVG revealed synergistic and indifferent properties. Conclusion: Development of novel products using bioactive ingredients from plants with commercially available AFCs will serve as potential alternative therapy to cure dermatological infections with no side effects.

9. M. F. Lubis, V. E. Kaban, J. O. Aritonang, D. Satria1, A. A. Mulina and H. Febriani et.al., (2022)[48]

Murraya koenigii (MK) plants are a type of plant whose bioactive compounds have been widely used in various countries as traditional medicines with metabolites containing alkaloids, flavonoids, tannins, steroids/triterpenoids and anthraquinones. The purpose of this study was to determine of acute toxicity effect and to test the antifungal effects of ointment MK ethanol extract. Acute toxicity testing is carried out with the initial stage of testing at a dose of 0.05. The formulated ointment was subjected to an organoleptic evaluation test, pH, spreadability, and homogeneity. MK leaves ethanol extract can be formulated into ointment because it is safe

to use with an LD50 value >2000mg/kgBW. The concentrations of 12.5% and 25% were the best concentration to inhibit the growth of Candida albicans.

10. OKAFO SE, ANIE CO, OMOH JO et.al., (2022)[49]

This research was conducted to evaluate creams formulated using ethanolic extract of Carica papaya leaves. The leaves were collected; dried and extracted by maceration method using ethanol. The antimicrobial activity of extract was determined using the pour plate method for viable counts and the minimum inhibitory concentration (MIC) by agar diffusion method. Test organisms used were Staphylococcus aureus, Bacilus subtilis, Pseudomonas aeruginosa, Escherichia coli, Aspergillus niger and Candida albicans. Carica papaya leaves ethanolic extract creams were formulated and evaluated for antimicrobial activity and physicochemical properties. The extract had good antimicrobial activity and MIC of 3.125 mg/ml against all the test organisms except Aspergillus niger which was not sensitive even at 100 mg/ml extract concentration. All the formulated creams retained their antibacterial activity but only Formulations F5 to F7 retained their antifungal activity against Candida albicans.

11. Miriam G.U. Nwaneri Ugochukwu M. Okezie Uchenna C. Ogwaluonye Ijeoma N. Ebenebe Chidimma R. Chukwunwejim Chinelo K. Ezejiegu Charles O. Esimone et.al., (2023)[50]

Creams and Ointments containing extracts of either Mitracarpus scaber or Occimum gratissimum were formulated for possible topical application. In-vitro sensitivity study of the ethanol extract of Mitracarpus scaber, Occimum gratissimum oil and standard antifungal drugs (Nystatin, Ketoconazole and Fluconazole) were assessed against the clinical isolates of Candida albicans, Aspergillus niger, Trichophyton spp, Fusarium spp, Cladosporium spp and Penicillium spp using a modified cup-agar diffusion plate method. The antifungal properties of the creams and ointments were evaluated in vitro against isolated organisms using the well-agar diffusion technique. Standard creams and ointments containing clotrimazole and miconazole respectively were evaluated in parallel as controls. The activity of the antifungal extracts competes well with that of the standard antifungal drugs. Evaluation of the topical formulations showed that the ethanol extract of M. scaber Cream has a lesser activity against the clinical isolates than the standard (clotrimazole); the Ointment could however not diffuse into the agar. On the other hand, the O. gratissimum Oil Creams and Ointments have a great activity against the isolates compared to the standards (clotrimazole and miconazole). This study confirms the broad-spectrum antifungal effects of herbal creams and ointments containing O. gratissimum Oil.

12. Sujata P. Choudhari, Rahul M. Dhawale, Vikrant V. Wadkar, Arun A. Pawar et.al., (2023)[51]

The aim of the study was to formulate a cream containing a composition to treat fungal infections of the skin and improve skin properties. This preparation belongs to medicinal creams containing antifungal agents. It reveals a formula for treating skin fungal infections. For skin infections, a topical approach is the best option. Developing a local drug delivery system with systemic action may be advantageous for a variety of drugs, as it offers many advantages over traditional drug administration routes. Ginger extract is an active pharmaceutical ingredient (API) used to treat fungal skin infections. The cream base also contains two primary and secondary emulsifiers, a waxy substance, a co-solvent, two preservatives, a buffer, a humectant, and water. When the active ingredients combine, they exert a powerful antifungal effect. Several experiments were performed to evaluate the physico-chemical properties of formulated creams, including B. Visual inspection, pH measurement, homogeneity, removal test, skin irritation test, viscosity, spreadability test, saponification value, acid value, etc. The antifungal effect of the cream was further analyzed using nutrient agar. The medicated cream had good viscosity and color. However, the scent of ginger was characteristic.

13. Santiago Ojeda-Riascos, Guisella Rivera, Leydy Nathaly Castillo, Chabaco Armijos, Jorge Ramírez, et.al., (2024)[52]

The present study describes the process of a technological adaptation performed at the laboratory level to prepare a topic cream with antifungal activity using the alcoholic extract (70%) of the endemic Piper ecuad orense Sodiro (Matico) species. During processing, the behavior of semisolid formulations (creams) was evaluated in terms of organoleptic and physicochemical characteristics and in vitro antifungal activity of the cream at three different storage temperature conditions: (i) Environment, (ii) 30 °C ± 2, and (iii) 45 °C ± 2 for three months. Properties such as aspect, texture, density, pH, and extensibility were considered to determine each cream's stability. According to the dosage of the ethanolic extract to inhibit fungal growth, the cream made using 1% of the extract turned out to be the most effective formulation, so it gave a positive response against the two dermatophytes to which it was exposed, Trichophyton mentagrophytes ATCC® 28185 and Trichophyton rubrum ATCC® 28188 according to a dosage of 1000 µg/mL during one month of evaluation. Due to promising antifungal results, the developed formula could be used in further studies to evaluate its clinical efficiency in treating topical skin conditions.

14. Reena D. Kokate (Pawar), Varsha K. Wagh, Rutuja S. Deshmukh, Suraj. R. Shinde, Mayuri. S. Bhamare et.al., (2025)[53]

The present study aims to investigate the antifungal potential of Epipremnum aureum and to formulate a topical antifungal preparation derived from its extract. This formulation falls under the category of medicinal creams containing herbal antifungal agents. For treating

skin infections, topical applications are generally preferred as they deliver the active ingredient directly to the affected site. Epipremnum aureum, commonly known as the Money Plant or Golden Pothos, is a rapidly growing climber belonging to the family Araceae. Widely cultivated as an ornamental indoor plant, it is also known for its remarkable air-purifying ability, effectively removing pollutants such as formaldehyde, benzene, and xylene from the environment. Phytochemical investigations have revealed that E. aureum contains several bioactive constituents, including flavonoids, alkaloids, tannins, saponins, and terpenoids, which contribute to its broad spectrum of biological activities. These compounds are believed to be primarily responsible for its antifungal efficacy. In addition to its antifungal effects, different parts of the plant exhibit antioxidant, antibacterial, antimalarial, anticancer, and wound-healing properties. However, many of the therapeutic potentials of Epipremnum species remain underexplored and warrant further scientific investigation for their benefits to human health and environmental applications. In this study, a herbal antifungal cream was developed using E. aureum extract, and the formulation was evaluated for physicochemical parameters such as pH, appearance, viscosity, spreadability, and skin irritation potential to ensure its suitability for topical use.

15. Kiran Dudhat, Krushil Thummar, Yuvrajsinh Jadeja, Kiran Dekavadiya et.al., (2026)[54]

The present study focuses on the formulation and evaluation of a herbal cream intended for the treatment of ringworm, a common fungal infection. With growing global interest in plant-based remedies, particularly those with antimicrobial properties, this research utilizes natural ingredients known for their antifungal efficacy. The cream was developed using ethanolic extracts of Aloe vera gel, Azadirachta indica (Neem), and Butea monosperma (Palash) prepared via maceration. The cream base comprised beeswax, liquid paraffin, borax, methylparaben, rose oil, and distilled water, and the formulation was completed using the slab method to ensure homogeneity. Five trial batches (F1H to F5H) were prepared and evaluated for physical appearance, pH, viscosity, phase separation, and skin irritancy. All formulations exhibited favorable visual properties, appropriate pH and viscosity, stability at room temperature, and no signs of skin irritation or erythema during application. Antifungal screening against Trichophyton, Microsporum, and Epidermophyton revealed that the herbal ingredients demonstrated notable antifungal activity. The overall results confirmed that all five formulations were physically stable, non-irritant, and suitable for topical application, highlighting the potential of herbal-based creams as effective, safe, and natural alternatives for managing fungal skin infections.

3. AIM AND OBJECTIVES

Aim

The aim of research was to formulate and evaluate the Antifungal cream using plant extract of *Madras pea pumpkin* that may have the major antifungal parameters.

Objectives

Primary Objective

- To formulate and evaluate an antifungal cream using the ethanolic extract of *Madras pea pumpkin.*

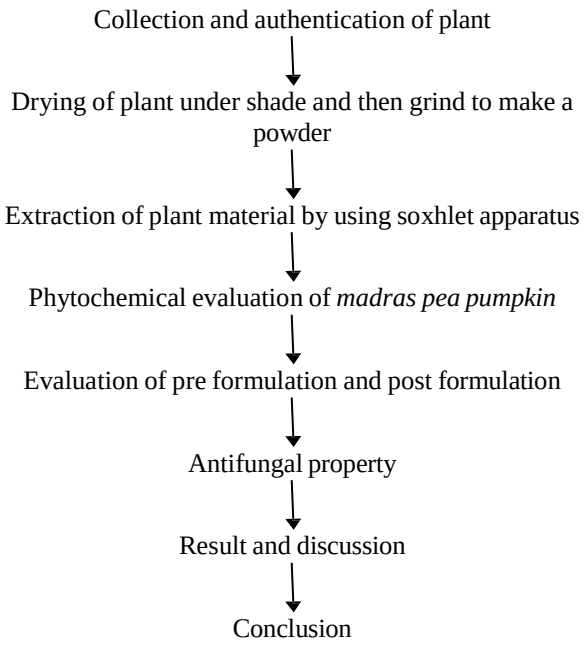
Secondary Objectives

- To collect, identify, and authenticate the *Madras pea pumpkin* plant material.
- To prepare the ethanolic extract of the plant using suitable extraction methods (e.g., Soxhlet or maceration).
- To perform preliminary phytochemical screening of the extract for active constituents such as alkaloids, flavonoids, tannins, etc.
- To formulate a topical cream incorporating the plant extract using appropriate excipients.
- To optimize the formulation for stability, consistency, and spreadability.

Evaluation Objectives

- To evaluate the prepared cream for physicochemical parameters such as:
  - pH
  - Viscosity
  - Spreadability
  - Appearance
- To assess the antifungal activity of the formulation against selected fungal strains.

4. PLAN OF WORK



5. PLANT PROFILE

Fig no.1: Madras Pea Pumpkin.

SYNONYMS

The term "*Madras pea pumpkin*" isn't a widely standardized common name in botany, so synonyms can vary depending on the plant it refers to locally. It is often associated with pumpkin/gourd-type plants (Cucurbita species) or regional varieties.

Here are possible synonyms / related names.

- Madras pea gourd
- Madras pumpkin
- Country pumpkin (South India)
- Local pea pumpkin variety
- Cucurbita spp. (botanical reference)
- Pumpkin (general common name)

TAXONOMICAL CLASSIFICATION

Taxonomical classification of *Madras pea pumpkin* plant such as.

Table; 1.

Kingdom	Plantae
Clade	Angiosperms
Clade	Tracheophytes
Clade	Eudicots
Clade	Rosids
Order	Cucurbitales
Family	Cucurbitaceae
Genes	Cucurbita
Species	*Cucurbita maxima* / *Cucurbita moschata*
Binomial Name	Cucurbita maxima

COMMON NAMES

Different vernacular names of *Madras pea pumpkin* have been reported in table Table; 2 Common names.

LANGUAGES	COMMON NAMES
English	Rough Bryony
Hindi	Bilari, Agumaki
Malayalam	Mukkapiriyan, Cenkummatti
Marathi	Chirati, Ghugri
Sanskrit	Gajachirbhata, Gopalakarkati
Tamil	Musumusukai, Appakovil
Telugu	Nugudosa
Kanada	Kaadapaavate

ORIGIN AND DISTRIBUTION

Origin

- The Madras pea pumpkin is believed to have originated in the tropical regions of India, particularly in South India (Tamil Nadu region).
- It has been traditionally used in indigenous systems of medicine such as Ayurveda and Siddha.
- The plant is adapted to warm and humid climatic conditions, which support its growth and phytochemical richness.

Distribution

- The plant is widely distributed in South India, especially in:
  - Tamil Nadu
  - Kerala
  - Karnataka
  - Andhra Pradesh
- It is also found in other tropical and subtropical regions of India.
- Outside India, similar species may be distributed in:
  - Southeast Asian countries
  - Parts of Africa (depending on species similarity)

- It commonly grows in.
  - Agricultural fields
  - Home gardens
  - Waste lands and roadsides (in some cases)

ECOLOGY

The *Madras pea pumpkin* grows well in tropical and subtropical climates with warm temperatures and moderate rainfall. It prefers well-drained, fertile soils such as loamy soil. The plant is commonly found in fields, gardens, and wastelands, growing as a creeper or climber. It requires adequate sunlight and moderate water for proper growth. It is usually propagated by seeds and may support soil fertility and pollinators.

PROPAGATION

Madras pea pumpkin is mainly propagated by seeds. Healthy and mature seeds are sown in well-prepared soil under warm conditions for better germination. The seeds usually germinate within 7–10 days with adequate moisture. The plant grows as a creeper or climber, so support may be provided for proper growth. Regular watering and sunlight help in successful establishment.

TRADITIONAL USES

Treatment of diabetes: Leaves and fruits are widely used in traditional medicine to help control blood sugar levels. Decoctions or juices are commonly consumed.
Digestive aid: The fruit is used to improve digestion and relieve constipation due to its mild laxative properties.
Skin diseases: Leaf paste or extracts are applied externally to treat skin infections, wounds, and itching because of their antimicrobial effects.
Fever and inflammation: Different parts of the plant are used in herbal remedies to reduce fever and inflammation.
Respiratory conditions: In some traditional systems, it is used to relieve cough and asthma symptoms.
Nutritional use: The young fruits are cooked as a vegetable, providing vitamins and minerals that support general health.

MORPHOLOGICAL CHARACTERS

1. Habit
- A perennial, fast-growing climbing or trailing herb (vine).
- Climbs using tendrils and spreads over fences, shrubs, or ground.

2. Root
- Tap root system with tuberous roots in some cases.
- Roots help in perennation and regeneration.

3. Stem
- Slender, green, herbaceous, and angular.
- May be slightly hairy (pubescent).
- Produces simple tendrils for climbing.

4. Leaves
- Simple, alternate leaves.
- Shape: cordate (heart-shaped) to palmately lobed (3–5 lobes).
- Margin: slightly toothed or smooth.
- Surface: soft and sometimes hairy.

5. Tendrils
- Simple, axillary tendrils used for climbing.
- Coiled structure helps the plant attach to support.

6. Flowers
- Unisexual (dioecious plant) – male and female flowers on separate plants.
- Colour: white or pale yellow.
- Male flowers: usually in clusters.
- Female flowers: generally solitary.
- Typical pentamerous structure (5 petals).

7. Fruit
- Berry type elongated or oval.
- Green with white stripes when immature, turns bright red when ripe.
- Soft and fleshy when mature.

8. Seeds
- Small, flat, oval seeds.
- Light brown or yellowish in colour.

6. MATERIALS AND METHODS

MATERIALS
- *Madras Pea Pumpkin*
- Liquid paraffin
- Stearic acid
- Bees wax
- Stearyl alcohol
- Glycerin
- Tween-80
- Methyl parabens
- Sorbitol solution
- Potassium hydroxide
- Distilled water

INSTRUMENT
- Soxhlet apparatus
- Digital pH
- Water bath
- Brookfield viscometer

PLANT MATERIAL

In this present study, *Madras pea pumpkin* was selected because it is considered for anti-fungal activity.

COLLECTION OF PLANT MATERIAL

The fresh *Madras pea pumpkin* whole plant was collected from Yelagiri, Tirupattur, Tamil Nadu.

PREPARATION OF EXTRACT

PREPARATION OF MADRAS PEA PUMPKIN PLANT EXTRACT

Cleaning and drying

Thoroughly clean the plant material collected to remove any dirt or debris. Allow it to air dry in a shaded area to prevent degradation of active compounds alternatively, you can use a food dehydrator or an oven set to a low temperature for drying.

Grinding or crushing

Once grind, grind the plant material into a coarse powder using a mortar and pestle or an electric grinder. Ensure that the powder is uniform in texture to facilitate extraction.

Extraction

There are various methods for extracting bioactive compounds from plant material.

Figure no. 2: Soxhlet Apparatus (plant extraction).

Common extraction method includes (Soxhlet apparatus) Ethanol extraction

The solid powdered plant material is placed in the sox thimble, the ethanolic solvent was used for this extraction. The 70% ethanolic solvent was placed in the round bottom flask, and the solvent is heated under the reflex by using heating mantle under the control temperature. Seal the container and let it soak for a specified period, with occasional shaking or stirring after extraction, filter the mixture to remove solid particles, and then evaporate the ethanol under reduced pressure using a rotary evaporator or by air-drying to obtain the crude extract.

Concentration and drying

Once the extraction is complete, concentrate on the extract using a rotary evaporator or by air-drying. The resulting concentrated extract was stored there in a cool, dry place for further use.

Quality Control

Performed appropriate quality control tests to assess the phytochemical composition, purity and potency of the extract. This includes assays for specific bioactive compounds, such as flavonoids, alkaloids, or phenolic compounds, as well as tests for contaminants.

Standardization

Standardizes the extract to ensure consistency in this composition and therapeutic effects. They involved adjusting the extraction parameters or adding standardized markers for quantification. It is important to note that the specific extraction method and conditions vary depending on the desired phytochemical and intended use of the extract. Additionally, I ensured compliance with ethical and legal guidelines regarding the collection and use of plant materials.

Yield and color determination

The color of the extract was observed visually. The yield of the extract was calculated using the following formula.

%yield= (weight of the extract\ weight of powder taken) x100

FORMULATION OF ANTI-FUNGAL CREAM

Procedure

The formulation trails were done as per formula. The formulation containing Madras pea pumpkin Was formulated by the following method; Different amount of ingredients were incorporated together in 2 phases i.e., oil phase and aqueous phase separately. The oil phase consists of liquid paraffin, bee wax, stearyl alcohol, tween-80 and stearic acid while the aqueous phase was composed of methyl paraben, sorbitol solution and potassium hydroxide. Both aqueous and oil phase were heated to 75c on a water bath separately. The aqueous phase was then added drop wise to the oil phase with continuous stirring and finally the herbal extracts of Madras pea pumpkin against the fungi *candida albican* were incorporated in the emulsion. Gradually temperature decreased with continuous stirring and emulsion was formed which was then stored in the airtight wide-mouth container.

Figure.no.3: F1, F2 and F3.

COMPOSITION OF MADRAS PEA PUMPKIN CREAM.

Ingredients	F1	F2	F3	USES
Madras pea pumpkin	1gm	1.5gm	2.5gm	A.P.I
Liquid paraffin	2.5ml	2.5ml	2.5ml	Softening agent
Stearic acid	1.5ml	1.5ml	1.5ml	Cleansing property
Bees wax	2.5gm	2.5gm	2.5gm	Hydrating agent
Stearyl alcohol	5gm	5gm	5gm	Emollient
Glycerin	10ml	10ml	10ml	Humectant
Tween-80	4ml	4ml	4ml	Solubilizer
Methyl parabens	0.6gm	0.6gm	0.6gm	Preservative
Sorbitol solution	3gm	3gm	3gm	Moisturizing
Potassium hydroxide	2.5gm	2.5gm	2.5gm	PH Adjuster

PREFORMULATION STUDY OF RAW MATERIALS

1) PHYTOCHEMICAL SCREENING OF CHEMICAL CONSTITUENTS

TEST	PROCEDURE	OBSERVATION
Saponins	**Foam test:** Place a small extract i a test tube with a small amount of water. Shake it vigorously. If foam remains for 10 minutes, it indicate the presence of saponin.	it indicates the presence of saponin.
Alkaloids	• **Mayer's test:** 2-3 ml of the filtrate, when mixed with a small amount of Mayer's reagent, • **Wagner's test:** When 2-3 ml o the filtrate is mixed with a small amount of Wagner's reagent,	forms a precipitate. It produces a reddish-brown color**.**
Tannins	**Ferric chloride test**: Add a few drops of neutral ferric chloride solution to the alcoholic extract. The development of a green color.	indicates the presence of tannins
Steroids	**Liebermann's reaction:** Mix 3 milliliters of the extract with an equal amount of acetic anhydride. Heat the mixture and allow it to cool. Introduce a small amount of concentrated sulfuric acid.	This process results in the appearance of a blue color.
Flavonoids	**Alkaline reagent test**: When the test solution reacts with sodium hydroxide, it exhibits a heightened yellow coloration	which reverses to colorlessness upon the addition of a small amou of dilute acid.
Terpenoids	**Salkowski reaction:** Mix 2 ml of the extract with 2 ml of chlorofor and 2 ml of oncentrated sulfuric acid. Shake the mixture thoroughl	The chloroform layer will turn red while the acid layer will exhibit a fluorescence that appears greenish yellow.
Reducing sugar	**Benedict's test:** Combine equal amounts of Benedict's reagent and the test extract in a test tube. Heat the mixture in a boiling water bath for 5 minutes.	The color of the solution changes green, yellow, or red, indicating th quantity of reducing sugars in the test solution.

2) DETERMINATION OF TOTAL ASH

Useful for detecting low grade products useful for detecting exhausted products, useful for detecting excess of sandy useful for detecting earthy matter with drug.

Weigh accurately about 2-3 gm of the powdered drug in a tard silicas crucible. Incinerate the powdered drug by gradually increasing the temperature $55^{O}C$ until free from carbon and cool keep it in desiccators. Weigh the ash and calculate the % of ash with reference to the air-dried sample.

FORMULA

$$\%\text{Total ash} = \frac{\text{Ash weight}}{\text{Weight of sample}}$$

3) Solubility Test

As adding solute for solubility analysis is small incremental amount to fixed the volume of solvents such as ethanol, acetone and chloroform. After undissolved particles will be examined**.**

Evaluation Parameters of Antifungal Cream

1. **Physical Evaluation**: Physical parameters such as color and appearance were evaluated.

2. Type of Emulsion

Mix the Dye: Take a small sample of the test emulsion on a clean glass slide or in a watch glass. Add a tiny amount of either a water-soluble dye (like Amaranth) and mix gently.

Incubate: Allow the mixture to stand for a minute to ensure uniform contact between the dye and the emulsion phases.

Microscopic Observation: Place a cover slip over the sample and observe it under a microscope.

Interpretation of Results: Using a Water-Soluble Dye (e.g., Amaranth): O/W Emulsion: The continuous (external) phase appears red, while the dispersed oil globules remain colorless.

W/O Emulsion: The continuous phase remains colorless, and the dispersed water globules appear red.

3. pH

The pH of different cream formulations was assessed using a digital pH meter. Specifically, 2.5 grams of each cream sample was precisely weighed and dispersed in 25 mL of distilled water, allowing it to sit for two hours. pH measurements for each formulation were conducted three times, and the average values were reported. The pH of the dispersions was determined using a pH meter.

4. Spreadability

The Spreadability of creams was assessed using a wooden block apparatus equipped with a pulley at one end. This method measured the slip and drag characteristics of creams. Approximately 2 grams of the cream under study were placed on a fixed ground slide. Another glass slide of the same dimensions as the ground slide, equipped with a hook, was placed on top to sandwich the cream. A weight of 1 kg was applied to the top slide for 5 minutes to remove air and ensure a uniform cream film between the slides. Excess cream was removed from the edges. Subsequently, a 50 g weight was pulled with a string attached to the hook, and the time (in seconds) taken for the top slide to travel a distance of 6.5 cm was recorded. A shorter time interval indicated better Spreadability. Spreadability was calculated using the following formula: $S = M \times L / T$ Where, S = Spreadability,

M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide and T = Time (in sec.) taken to separate the slide completely each other.

Figure: Spreadability Test.

5. **Viscosity**: The viscosity of the herbal cream was measured using a Brookfield rotational viscometer with spindle no. 64 at various speeds: 5, 10, 20, 30, and 50 rpm. Each measurement was taken after allowing the sample to reach equilibrium for two minutes. This procedure was repeated three times to ensure accuracy.[23-26]
6. **Irritancy**: An area ($1cm^2$) on the dorsal left-hand surface was marked. The cream was applied on this marked surface. Then irritancy, erythema, edema were checked for regular time interval up to 24 hrs and the time was noted and reported.
7. **Microbial test**: Nutrient agar, nutrient broth media, was used in microbial growth study. In this method the blank and sample Petri plates were used, and cream sample were aseptically transferred on to the sample plates in a cross pattern, the microbial growth was observed. Antimicrobial activity was assessed against staphylococcus aureus, E. coli strain after 24hrs, 48hrs and 72hrs, found to exhibit significant antimicrobial activity.
8. **Stability study**: For in vitro evaluation of herbal antibacterial, antifungal cream was placed at different temperatures i.e., at 8, 25 and 40˚C and 40˚C at 75% RH (relative humidity) in stability chambers for 28 days. Any change in color, liquefaction, phase separation, conductivity and pH was observed and recorded.

7. RESULT AND DISCUSSION

RESULTS

PHYTOCHEMICAL EVALUATION

Table 7.1: Chemical constituents of Madras Pea Pumpkin.

PHYTOCHEMICALS	STATUS
Carbohydrates	++
Protien	+
Alkaloids	++
Tannins	++
Flavonoids	++
Terpenoids	-
Steroids	+
Saponin	++
Reducing sugar	++
Phenolic compounds	++

EVALUATION OF CHEMICAL CONSTITUTION

Table 7.2: Evaluation test for Madras Pea Pumpkin.

Chemical constituents	Test	Status
Carbohydrate	Molisch's test	+
Protien	Biuret test	+
Saponin	Foam test	+
Alkaloids	Mayer's test	+
Tannins	Ferric chloride test	+
Steroids	Liebermann's test	+
Flavonoids	Alkaline reagent test	+
Terpenoids	Salkowski reaction	_
Reducing sugar	Benedict's test	+

EVALUATION PARAMETER OF FORMULATION.

Table 7.3: Evaluation Test of Madras Pea Pumpkin.

EVALUATION TEST	STATUS
Colour	Light green colour
Odour	Characteristics
Spreadability	3.40gm. cm/s
Irritancy test	Non-Irritancy
Stability study	Under Different Temperature
PH	7.5
Viscosity	3512 ± 0.17
Washability	Washable
Ethanol solubility test	Sparingly soluble
Type of emulsion under dye test	Oil-Water type (O/W)

EVALUATION OF ANTIFUNGAL ACTIVITY

MICROBIAL TESTING

The results of antifungal activity revealed that the formulation containing ethanolic extract of ***Madras Pea Pumpkin*** exhibited significant antifungal activity.

Both the standard sample and test sample were compared on the antifungal testing. The result showed good antifungal activity of formulated cream. It was found that F1 Formulation (containing 2.5 ml extract of madras pea pumpkin) has better antifungal action against *c. albicans* in comparison with F2 and F3 Formulation (containing less than 2ml extract of madras pea pumpkin).

ZONE OF INHIBITION (mm)

Standard (Miconazole)	
F1 Formulation	
F2 Formulation	
F3 Formulation	

DISCUSSION

- The study concluded that the formulation and evaluation of ***Madras pea pumpkin*** antifungal cream are demonstrates that plant-based cream can serve as effective and safer alternative to synthetic products.
- The ethanolic extraction method was found to effectively isolate the bioactive constituents responsible for Anti-fungal and Anti-microbial

properties.

- Formulation of antifungal cream was done and further evaluated by various evaluation parameters such as
  - Physical properties
  - PH
  - Spreadability
  - Washability
  - Non-irritancy tests
  - Viscosity
  - Stability studies
  - Microbial studies

The parameters are involved and gives the good results.

- The present work was the formulation and evaluation of antifungal cream
- The cream was non grassy in nature and easily removable after application.
- The formulation was nonirritant, not harm to the skin and less side effects.
- The presence of phytoconstituents in Madras Pea Pumpkin contributes to its potential in treating common skin problems like acne, inflammation, and fungal infections. Overall, the study supports the use of Madras Pea Pumpkin extract in herbal cream formulation highlights its promising role in the development of natural skin care products.

8. CONCLUSION

From the overall results, it was concluded that the topical formulation containing ethanolic extract of ***Madras Pea Pumpkin*** possess antifungal activity and it can be used as an herbal product in the treatment of fungal infection. The preliminary phytochemical screening showed the presence of alkaloids, steroids and flavonoids in the extract of ***Madras Pea Pumpkin*** and it was present in its leaves and other chemical constituents like which might be in part responsible for antifungal effect against ***candida albican***. It was found that F1 Formulation has better antifungal action against ***candida albican*** in comparison with F2 and F3 Formulation. Further, F1 formulation has showed almost equal antifungal action as standard formulation containing 2.5ml of Miconazole.

9. REFERENCES

1. Anaissie, E., R. Hachem, C. K-Tin-U, L. C. Stephens, and G. P. Bodey. 1993. Experimental hematogenous candidiasis caused by Candida krusei and Candida albicans: species differences in pathogenicity. Infect. Immun. 61: 1268-1271.
2. Anaissie, E. J., and G. P. Bodey. 1989. Nosocomial fungal infections old problems and new challenges. Infect. Dis. Clin. North Am. 3: 867-882.
3. Arthur, M., P. Reynolds, and P. Courvalin. 1996. Glycopeptide resistance in enterococci. Trends Microbiol. 4: 401-407.
4. Ather, M. A., and H. I. Winner. 1971. Development of resistance by Can did a species to polyene antibiotics in vitro. J. Med. Microbiol. 4: 505-517.
5. Beck-SagueÂ, C. M., W. R. Jarvis, and National Nosocomial Infections Surveillance System. 1993. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. J. Infect. Dis., 167: 1247-1251.
6. Beggs, W. H. 1983. Comparison of miconazole- and ketoconazole-induced release of K1 from Candida species. J. Antimicrobial. Chemother, 11: 381- 383.
7. Beggs, W. H., F. A. Andrews, and G. A. Sarosi. 1981. Combined action of amphotericin B and 5-¯uorocytosine on pathogenic yeasts susceptible to either drug alone. Chemotherapy, 27: 247-251.
8. Beggs, W. H., A. Sarosi, and M. I. Walker. 1976. Synergistic action of amphotericin B and rifampin against Candida species. J. Infect. Dis., 133: 206-209.
9. Belanger, P., C. Nast, R. Fratti, H. Sanati, and M. Ghannoum. 1997. Voriconazole (UK-109, 496) inhibits the growth and alters the morphology of fluconazole-susceptible and-resistant Candida species. Antimicrob. Agents Chemother, 41: 1840-1842.
10. Billot-Klein, D., L. Gutmann, D. Bryant, D. Bell, J. van Heijenoort, J. Grewal, and D. M. Shlaes. 1996. Peptidoglycan synthesis and structure in Staphylococcus haemolyticus expressing increasing levels of resistance to glycopeptide antibiotics. J. Bacteriol, 178: 4696-4703.
11. Bolard, J., and J. Milhaud. 1996. Interaction of the anti-Candida amphotericin B (and other polyene antibiotics) with lipids, p. 253-274. In R. Prasad and M. Ghannoum (ed.), Lipids of pathogenic fungi. CRC Press, Inc., Boca Raton, Fla.
12. Bozzola, J. J., R. Mehta, L. Nisbet, and J. Valenta. 1984. The effect of aculeacin A and papulacandin B on morphology and cell wall ultrastructure in Candida albicans. Can. J. Microbiol. 30: 857-863.
13. Capek, A., and A. Simek. 1971. Antimicrobial agents. XII. Relationship between biochemical resistance and microbial degradation of antimycotics. Folia Microbiol. 16: 472-475.
14. Capek, A., A. Simek, L. Bruna, A. Svab, and Z. Budesinsk. 1974. Antimcrobial agents. XXI. Dependence of antifungal activity on the structure of the side chain in the pyrimidine group. Folia Microbiol. 19: 169-171.
15. Cassone, A., R. Mason, and D. Kerridge. 1981. Lysis of growing yeast-form cells of Candida albicans by echinocandin: a cytological study. Sabouraudia, 19: 97-110.
16. Cassone, A., R. E. Mason, and D. Kerridge. 1979. Lysis of growing yeast form cells of Candida albicans following cessation of growth and their possible relationship to the development of polyene resistance. J. Gen. Microbiol. 110: 339-349.
17. Cheng, J. T., R. T. Witty, R. R. Robinson, and W. E. Yarger. 1982. Amphotericin B nephrotoxicity: increased renal resistance and tubule permeability. Kidney Int., 22: 626-633.
18. Clark, F. S., T. Parkinson, C. A. Hitchcock, and N. A. R. Gow. 1996. Correlation between rhodamine

123 accumulation and azole sensitivity in Candida species: possible role for drug efflux in drug resistance. Antimicrob. Agents Chemother, 40: 429-425.
19. Cohen, M. L. 1992. Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 257: 1050-1055.
20. Cohen, S. P., H. Hachler, and S. B. Levy. 1993. Genetic and functional analysis of the multiple antibiotic resistance (mar) locus in Escherichia coli. J. Bacteriol. 175: 1484-1492.
21. Defever, K., W. Whelan, A. Rogers, E. Beneke, J. Veselenak, and D. Soll. 1982. Candida albicans resistance to 5-¯uorocytosine: frequency of partially resistant strains among clinical isolates. Antimicrob. Agents Chemother. 22: 810-815.
22. de Kruijff, B., and R. A. Demel. 1974. Polyene antibiotic-sterol interactions in membranes of Acholeplasma laidlawaii cells and lecithin liposomes. III. Molecular structure of the polyene antibiotic-cholesterol complexes. Bio chim. Biophys. Acta 339: 57-70.
23. Diasio, R., J. Bennett, and C. Myers. 1978. Mode of action of 5-¯uorocy tosine. Biochem. Pharmacol. 27: 703-707.
24. Dick, J. D., W. G. Merz, and R. Saral. 1980. Incidence of polyene-resistant yeasts recovered from clinical specimens. Antimicrob. Agents Chemother. 18: 158-163.
25. Dowson, C., T. J. Coffey, and B. G. Spratt. 1994. Origin and molecular epidemiology of penicillin-binding-protein-mediated resistance to b-lactam antibiotics. Trends Microbiol. 2: 361-366.
26. Edwards, D. I. 1993. Nitroimidazole drugsÐaction and resistance mechanisms. II. Mechanisms of resistance. J. Antimicrob. Chemother. 31: 201- 210.
27. Espinel-Ingroff, A. 1997. Clinical relevance of antifungal resistance. Anti microb. Resist. 11: 929-944.
28. Fontana, R., M. Aldegheri, M. Ligozzi, H. Lopez, A. Sucari, and G. Satta. 1994. Overproduction of a low-affinity penicillin-binding protein and high level ampicillin resistance in Enterococcus faecium. Antimicrob. Agents Chemother. 38: 1980-1983.
29. Fothergill, W. A., D. A. Sutton, and M. G. Rinaldi. 1996. An in vitro head-to-head comparison of Schering 5692, amphotericin B, fluconazole and itraconazole against a spectrum of ®lamentous fungi, abstr. F89, p. 115. In Program and Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemother. American Society for Microbiology, Washington, D.C.
30. Fratti, R. A., P. H. Belanger, H. Sanati, A. S. Ibrahim, and M. A. Ghannoum. 1996. The effect of the new triazole, UK-109,496 on the interactions between Candida albicans and endothelial cells in vitro, abstr. F-76, p. 155. In Abstracts of the 96th General Meeting of the American Society for Microbiology 1996. American Society for Microbiology, Washington, D.C.
31. Fryberg, M. 1974. Sterol biosynthesis in antibiotic-resistant yeast: nystatin. Arch. Biochem. Biophys. 160: 83-89.
32. Gale, E. F., A. M. Johnson, D. Kerridge, and T. Y. Koh. 1975. Factors affecting the changes in amphotericin B sensitivity of Candida albicans during growth. J. Gen. Microbiol, 87: 20-36.
33. Gallis, H. A., R. H. Drew, and W. W. Pickard. 1990. Amphotericin B: 30 years of clinical experience. Rev. Infect. Dis., 12: 308-329.
34. Geber, A., C. A. Hitchcock, J. E. Swartz, F. S. Pullen, K. E. Marsden, K. J. Kwon-Chung, and J. Bennett. 1995. Deletion of the Candida glabrata ERG3 and ERG11 genes: effect on cell viability, cell growth, sterol composition, and antifungal susceptibility. Antimicrob. Agents Chemother. 39: 2708-2717.
35. Georgopapadakou, N. H., and T. J. Walsh. 1994. Human mycoses: drugs and targets for emerging pathogens. Science, 264: 371-373.
36. Georgopapadakou, N. H., and T. J. Walsh. 1996. Antifungal agents: chemotherapeutic targets and immunologic strategies. Antimicrob. Agents Chemother. 40: 279-291.
37. Ghannoum, M., M. Witt, A. Espinel-Ingroff, J. Ritchie, R. Larsen, and J. E. Edwards, Jr. 1994. Correlation of in vitro susceptibility testing of Crypto coccus neoformans with in vivo outcome: a comparison of two microdilution methods, abstr. F-103, p. 606. In Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Micro biology, Washington, D.C. 37a.Ghannoum, M. Unpublished data.
38. Ghannoum, M., I. Okagbule-Wonodi, N. Bhat, and H. Sanati. 1999. Anti fungal activity of voriconazole (UK-109,496), fluconazole and amphotericin Bagainst hematogenous Candida krusei infection in neutropenic guinea pig model. J. Chemother. 11: 34-39.
39. Ghannoum, M., B. J. Spellberg, A. Ibrahim, J. A. Ritchie, B. Currie, E. Spitzer, J. E. Edwards, Jr., and A. Casadevall. 1994. Sterol composition of Cryptococcus neoformans in the presence and absence of fluconazole. Antimicrob. Agents Chemother. 38: 2029-2033.
40. Gottesman, M. M., and I. Pastan. 1993. Biochemistry of multidrug resis tance mediated by the multidrug transporter. Annu. Rev. Biochem. 62: 385- 427.
41. Gottleib, D. H., H. E. Carter, J. H. Sloneker, and A. Ammann. 1958. Protection of fungi against polyene antibiotics by sterols. Science, 128: 361- 365.
42. Graybill, J. R., E. Montalbo, W. R. Kirkpatrick, M. F. Luther, S. G. Revankar, and T. F. Patterson. 1998. Fluconazole versus Candida albicans: a complex relationship. Antimicrob. Agents Chemother. 42: 2938-2942.
43. Graybill, J. R., E. Montalbo, W. R. Kirkpatrick, S. Revankar, M. Rinaldi, and T. Patterson. 1997. Candida albicans: less predictable than we may think, abstr. O49. In 13th International Conference

for Human and Animal Mycology.
44. Hackbarth, C. J., T. Kocagoz, S. Kocagoz, and H. F. Chambers. 1995. Point mutations in Staphylococcus aureus PBP 2 gene affect penicillin-binding kinetics and are associated with resistance. Antimicrob. Agents Chemother. 39: 103-106.
45. Hamilton-Miller, J. M. T. 1972. Physiological properties of mutagen in duced variants of Candida albicans resistant to polyene antibiotics. J. Med. Microbiol. 5: 425-440.
46. Hamilton-Miller, J. M. T. 1973. Chemistry and biology of the polyene macrolide antibiotics. Bacteriol. Rev., 37: 166-196.
47. Hata, K., J. Ueno, H. Miki, et al. 1995. ER-30346, a novel antifungal triazole. III. In vitro activity and the mode of action, abstr. F92. In Program and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
48. Hector, R. F. 1993. Compounds active against cell walls of medically important fungi. Clin. Microbiol. Rev., 6: 1-21.
49. Higgins, C. F. 1992. ABC transporters: from microorganisms to man. Annu. Rev. Cell Biol. 8: 67-113.
50. Hill, E. L., G. A. Hunter, and M. N. Ellis. 1991. In vitro and in vivo characterization of herpes simplex virus clinical isolates recovered from patients infected with human immunodeficiency virus. Antimicrobic Agents Chemother. 35: 2322-2328.
51. Hitchcock, C., K. Dickinson, S. B. Brown, E. G. Evans, and D. J. Adams. 1990. Interaction of azole antifungal antibiotics with cytochrome P450 dependent 14 a-sterol demethylase purified from Candida albicans. J. Bio chem. 266: 475-480.
52. Hitchcock, C., and P. T. Whittle. 1993. Chemistry and mode of action of fluconazole, p. 183-197.
53. In J. W. Rippon and R. A. Fromtling (ed.), Cuta neous antifungal agents: selected compounds in clinical practice and development. Marcel Dekker, Inc., New York, N.Y.
54. Hitchcock, C. A., K. A. Barrett-Bee, and N. J. Russell. 1987. The lipid composition and permeability to azole of an azole- and polyene-resistant mutant of Candida albicans. J. Med. Vet. Mycol. 25: 29-37.
55. Hitchcock, C. A., G. W. Pye, G. P. Oliver, and P. F. Troke. 1995. UK 109,496: a novel, wide spectrum triazole derivative for the treatment of fungal infections: antifungal activity and selectivity in vitro, abstr. 2739. In Program and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Wash ington, D.C.