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

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Research Article

Formulation and Evaluation of Antifungal Cream using Fenugreek oil, Neem oil, Coconut oil and Clove oil

Aditya Adwait[1]*, Kumar Anand [2], Mittal Chavi [3]

[1-2] Student, B. Pharma, Dev Bhoomi Institute of Pharmacy and Research, Dehradun
[3] Assistant Professor, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun

ARTICLE INFO

Received: 07 June 2024
Accepted: 11 June 2024
Published: 25 June 2024

Keywords:
Antifungal ointment, Aspergillus niger, Agar plate diffusion method, Synergistic effect, Alternative therapy, Antifungal properties.

DOI:
10.5281/zenodo.12532938

ABSTRACT

This research delves into creating and assessing an antifungal ointment incorporating fenugreek oil, neem oil, coconut oil, and clove oil to gauge its effectiveness against Aspergillus niger. These natural elements were chosen for their recognized antifungal properties, suggesting promise for alternative therapies. To accomplish this, varying proportions of oils were prepared using an appropriate solvent system. The resultant blend underwent rigorous testing against the standard Aspergillus niger strain via the agar plate diffusion method. Zones of inhibition surrounding the agar plates containing the formulated blend were measured, and the data were subjected to statistical analysis. The antifungal efficacy of the prepared blend demonstrated significant inhibition of Aspergillus niger growth, evident through varying inhibition zone sizes. Notably, the combination of fenugreek oil, neem oil, coconut oil, and clove oil exhibited the highest antifungal activity. Comparative analysis with a positive control representing conventional antifungal treatment demonstrated comparable or superior efficacy in reducing Aspergillus niger growth, suggesting the formulated blend's potential as an effective natural antifungal agent. The collective effects of these components highlight substantial antifungal properties, indicating their potential for treating Aspergillus niger infections as an alternative therapy. The study suggests that the synergistic action of these four components may offer an attractive option in addressing such infections. While the results are promising, the study acknowledges the need for further research to elucidate the mechanisms of action and evaluate the formula's effectiveness and safety through extensive in vivo studies and clinical trials. This research contributes to the growing understanding of potential antifungal treatments and offers prospects for innovative approaches to fungal infection management.

***Corresponding Author:** Aditya Adwait
Address: *Student, B. Pharma, Dev Bhoomi Institute of Pharmacy and Research, Dehradun*
Email : adwaitaditya01@gmail.com
Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

INTRODUCTION

1.1.Cream:

Skin creams are a form of topical preparation that can be applied to the skin for a variety of purposes. They are composed of semi-solid emulsions that can be either oil-in-water or water-in- oil varieties, with varying consistency depending on the oil-to-water ratio. These creams can be used for cosmetic objectives such as washing, beautifying, improving looks, and protecting, as well as for medicinal purposes such as delivering medications to the underlying layers of the skin or mucous membranes. They are intended to be applied topically in order to deliver medications to the affected region in a focused and localised manner.[1] Skin creams are considered pharmaceutical products and are made using pharmaceutical processes. There are both medicated and unmedicated creams that are highly successful in treating various skin disorders or dermatoses. They can be ayurvedic, herbal, or allopathic, and can comprise one or more drugs that are dissolved or spread out in a suitable base. Creams can be classed as o/w or w/o emulsion based on their phases. Traditionally, the name "cream" has been applied to semi-solid formulations that can be either water- in-oil (e.g., cold cream) or oil- in-water (e.g., disappearing cream).[2]

1.2.Fungi:-

"An ecosystem's nutrient cycling is influenced by fungi, which are usually multicellular eukaryotic organisms that are heterotrophs."[3]

Fig 1: Fungus (Candida albicans, dermatophytes and Aspergillus)

Characteristics of fungi:

Some fungus are single-celled and are known as yeast, whilst others are multicellular and may alternate between single-celled and multicellular forms depending on their life cycle stage. Fungi cells, like plant and animal cells, have a nucleus and organelles, Chitin, a hard material found in the exoskeletons of insects and arthropods such as crustaceans, is also found in fungal cell walls. "Fungi, unlike plant cell walls, do not contain cellulose." Multicellular fungi are made up of branching filaments called hyphae that have a tubular shape and are split into cell like compartments by walls called septa. These cells can have many nuclei, and organelles, including nuclei, can travel between them. Mycelium is a fungus network made up of hyphae."[4]

Types of Fungi:

1. Chytridiomycota
2. Zygomycota
3. Glomeromycota
4. Ascomycota

Chytridiomycota:

Chytrids are the organisms that are most commonly found in Chytridiomycota. They reproduce asexually by creating spores that travel via flagella, little tail- like appendages. Chytrids are known to induce fungal diseases in frogs by burrowing beneath their skin.[5]

Zygomycota:

These fungi, which are mostly found on land, can cause problems by growing on human tissues. Rhizopusstolonifer, a form of bread mould, is one example.[5]

Glomeromycota:

These fungus are often found in soil and feed on plant sugar. In exchange, they dissolve minerals in the soil to deliver nutrients to the plants. Glomeromycota fungus reproduce asexually as well.[5]

Ascomycota:

Ascomycota fungi are pathogens of both plants and animals, including humans, and cause infections such as Athlete's Foot, Ringworm, and Ergotism, which can cause vomiting, convulsions, hallucinations, and even death.[5] Fungal infections are classified into two types: superficial and profound infections. Superficial fungal infections, such as tinea versicolor and dermatophytes, attack the keratin layer of the skin, hair, and nails, causing disorders such as tinea pedis and ringworm. Candidiasis is a superficial fungal infection produced by yeast- like fungi that can cause oral thrush, vulvovaginitis, and nail infections. Deep fungal infections, on the other hand, impact internal organs such as the lungs, heart, and brain and can result in dangerous illnesses such as pneumonia, endocarditis, and meningitis. Aspergillosis, histoplasmosis, and blastomycosis are examples of deep fungal diseases. These infections are usually more difficult to cure and can be fatal, especially in people with compromised immune systems.

Fungal infections produced by dermatophytes are the most common types of infections that affect the skin, hair, and nails. These infections can develop chronic and are frequently referred to as "ringworm" or tinea infections. The formation of circular lesions or rings on the skin is a common ringworm sign. Tinea is a collective term for multiple infectious skin illnesses caused by several types of fungi. Different words are used to describe tinea infections that affect various body areas, such as the body, feet, nails, scalp, and groyne, depending on the type and location of the infection.[6]

Antifungal Cream:

An antifungal cream, which is applied topically and eliminates or inhibits the growth of fungus, is a safe and effective therapy option for fungal infections of the skin, hair, and nails. Fungal infections, which can range from superficial and localised to more serious systemic disorders. These infections can be caused by a variety of fungus, some of which are dangerous and can cause sickness even in people who have a sound immune system. According to the article, topical therapy of fungal infections offers various advantages, including tailored administration to the site of infection, decreased risk of systemic side effects, higher treatment efficacy, and greater patient compliance. To date, several topical antifungal agents have been utilised to treat dermatological skin infections, and they are accessible in traditional dosage forms such as creams, gels, lotions, and sprays. Antifungal creams are a type of medication that is used directly to the skin to treat and manage fungal infections.[7]

Plant Profile:

Fenugreek oil

Fig. 2:Fenugreek Oil

Scientific Name:

TrigonellaFoenum-graecum

Family:

Fabaceae

Subfamily:

Faboideae

Description:

Fenugreek is a fragrant plant native to the eastern Mediterranean region and extensively grown in India. Its seeds and leaves are commonly used. In Indian cuisine, fenugreek adds flavor and it's also used in traditional medicine in Indo-Pakistani regions and other countries. Historically, fenugreek had various uses, and it's rich in phytochemicals and antioxidants, serving both as a spice and a medicinal herb. It has been used for ages to treat wounds, arthritis, and digestive issues, among other things. Fenugreek oil and leaves offer health benefits such as anti-diabetic, anti-cancer, anti-inflammatory, and antioxidant effects. Various compounds in fenugreek contribute to its medicinal properties, including flavonoids, alkaloids, and saponins.[7]

1. **Antimicrobial Action:**

Fenugreek fights microbes, including fungi like Candida albicans and Aspergillus species.

2. **Bioactive Chemicals:**

Compounds like trigonelline and flavonoids in fenugreek possess antifungal properties.

3. **Disruption of Fungal Cell Membranes:**

Fenugreek extracts disrupt fungal cell membranes, leading to their destruction, particularly effective against Candida species.

4. **Anti-inflammatory Properties:**

Fenugreek helps reduce inflammation associated with fungal infections, aiding in symptom relief and healing.[8]

Neem oil:

Fig. 3: Neem Oil

Scientific Name:

AzadirachtaIndica

Family:

Meliaceae

Subfamily:

Meloideae and tribMelieae

Description:

Azadirachta Indica, commonly called neem, grows in many tropical countries and is highly valued. It originates from Burma and India, and some species, along with those brought from West Africa, are grown as medicinal plants in Nigeria. Neem is found throughout the year in our region and is important in traditional medicine, often known as "village medicine." Different parts of the neem tree, such as the bark, seeds, branches, flowers, leaves, and roots, have medicinal properties. Neem seeds, especially, contain a high oil content of around 47%, with various fatty acids like oleic acid, linoleic acid, palmitic acid, stearic acid, and arachidic acid. These seeds have been found to have properties that can reduce fever, fight malaria, and kill microbes.[9] Almost every part of the neem tree has multiple uses, leading international organizations to encourage its cultivation in Africa and Asia. With around 140 different chemicals found in various parts of the neem tree, it provides a wide range of compounds that have different effects on the body. For example, the first compounds isolated from fresh

neem leaves include polyphenolic flavonoids such as ß-sitosterol flavonoid and quercetin flavonoid, which can potentially fight against microbes.[1

Coconut Oil:

Fig. 4: Coconut oil

Botanical Name:

Cocos nucifera

Family:

Arecaceae

Subfamily:

Arecoideae

Description:

Coconut oil is clear and mainly made of medium-chain saturated fatty acids. Researchers have studied coconut oil's properties, especially its ability to fight bacteria. Its high levels of medium-chain fatty acids like lauric acid and monolaurin make it effective against many harmful microbes. People use coconut oil in their diets or as an alternative treatment for infections. Its antimicrobial and antiviral effects are particularly emphasized, and scientists are working to understand how it works against bacteria, fungi, and viruses.[11] Coconut oil, also called "minyakkelpa" in Indonesia and Malaysia, is made from mature coconuts and is known for its delicious taste. Its high fat and saturated fatty acid content make it resistant to spoiling, making it great for cooking. Coconut trees are grown in over 80 countries worldwide, with about 55 million tons of coconuts produced each year. Besides cooking, coconut is used in hygiene products, food, and various industries.[12]

Clove Oil :

Fig. 5: Clove Oil

Scientific Name:

Syzygiumaromaticum

Family:

Myrtaceae

Subfamily:

Myrtoideae

Description:

Clove oil has strong properties that fight against germs, especially fungi. Studies show it can stop the growth and movement of various parasites like Candida, Aspergillus, and dermatophytes. Clove oil, which contains eugenol, a powerful compound, reduces the amount of ergosterol in parasite cell walls, which is important for their survival. Even in small amounts, clove oil and eugenol can stop the formation of Candida albicans spores.[13] Researchers used tests like flow cytometry and ergosterol synthesis inhibition to understand how clove oil works on yeast and mold. They found that in high enough amounts, clove oil and eugenol can seriously damage the cell walls of molds, killing them.[13] Studies also show that eugenol, the active ingredient in clove oil, is effective against tough parasites, even ones resistant to common drugs like fluconazole. This

suggests that clove oil and eugenol could be helpful in treating infections caused by drug-resistant germs. Their ability to fight a wide range of germs makes them potentially useful in medicine for treating infections.[13]

MATERIALS AND METHODS:

1. The oil of Fenugreek was purchased from Fameily Drugs Pvt. Ltd. With batch number 074
2. The oil of Neem was purchased from Vyas Pharmaceuticals with batch number 00183
3. The oil of clove was purchased from Fameily Drugs Pvt. Ltd. With batch number 088
4. The oil of Coconut was purchased from Parachute with Lot no. HSJLI-31

The ingredients that are used in my formulation:

Table 1: Ingredients and their Properties

INGREDIENTS	PROPERTIES
Fenugreek oil	Anti-microbial agent
Neem oil	Anti-microbial agent
Clove oil	Anti-microbial agent
Coconut oil	Anti-microbial agent
Petroleum jelly	Soothing Agent
Hard Paraffin	Lubricant
Cetyl Alcohol	Emollient
Glycerol Monosterate	Emulsifier
Sodium Benzoate	Stabilizer
Butylated Hydroxytoluene	Preservative
Distilled Water	Base

The anti-fungal cream was prepared as following:

Table 2: Formulation Table of anti-fungal cream

INGERDIENTS	F1 (in gram)	F2 (in gram)	F3 (in gram)
Fenugreek oil	5	5	5
Neem oil	2	2	2
Clove oil	3	3	3
Coconut oil	5	5	5
Petroleum jelly	8	6	7
Hard Paraffin	2	4	5
Cetyl Alcohol	4	2	3
Glycerol Monosterate	2	4	5
Sodium Benzoate	2	2	3
Butylated Hydroxytoluene	2	4	3
Distilled Water	65	63	59

Glassware's used in the preparation process:

Table 3: List of Glasswares

Sr. No	EQUIPMENTS
1	Glass rod
2	Beaker
3	Petri dish

4	Measuring Cylinder

Preparation of Antifungal Cream:-

The preparation of an antifungal cream involves combining various ingredients to form a stable and effective formulation. Here is the preparation method:[14]

Fig. 6: Anti-fungal cream

Procedure:

1. Oil Phase Preparation:

- Fenugreek oil, Neem oil, Clove oil, Coconut oil along with Petroleum Jelly (PJ), Hard Paraffin (HP), Cetyl Alcohol (CA), and Glycerol Monostearate (GM) were combined together in a heat-resistant container.
- The mixture was gently heated until all solids are melted and the oils are well combined by continuous stirring.

2. Water Phase Preparation:

- Sodium benzoate (SB) was dissolved in distilled water (DW) and stirred until fully dissolved.

3. Emulsification:

- Oil phase was slowly poured dropwise into the Water phase while stirring continuously.
- High-shear mixer was used to emulsify the mixture thoroughly. Continuous mixing was done until a smooth, homogeneous cream is formed.
- Butylated Hydroxytoluene (BHT) was then added to the formulation.

4. Cooling Phase:

- Emulsified mixture was allowed to cool to around 40-45°C with gentle stirring.

5. Final Mixing:

- Cream was thoroughly mixed to ensure uniformity and consistency.

6. Packaging and Storage:

- Antifungal cream was transferred into clean container and stored in a cool, dry place.

Evaluation of Anti-fungal cream:

Physical Properties:

Physical properties encompass the measurable attributes of a substance without altering its chemical composition. These characteristics include color, odor, density, melting point, and solubility, offering insights into how a substance behaves under specific conditions. Essential for material identification, physical properties help determine appearance, structure, and behavior. They enable scientists and researchers to comprehend the nature of substances, aiding in classifications and applications across various fields, from chemistry to materials science. Understanding physical properties is fundamental for assessing the suitability and performance of substances in diverse contexts, contributing to scientific analysis and practical applications.[14]

Table 4: Observation of physical properties

Properties	Observation
Colour	White

Oduor	Characteristics
Appearance	Semi solid

Stability testing:

Stability testing involves subjecting a product or substance to various conditions to assess its chemical, physical, and microbiological stability over time. This process helps determine the product's shelf life, ensuring it remains effective and safe for use. Factors like temperature, humidity, and light exposure are manipulated to simulate real-world storage conditions. Periodic analysis during the testing period assesses changes in attributes like color, consistency, and potency. The results guide manufacturers in setting appropriate storage recommendations, expiration dates, and packaging requirements, ensuring consumers receive products that meet quality standards throughout their intended lifespan.[15]

Table 5: Stability testing

Test	After 1 Month
Physical Appearance	Semi solid
Texture	Smooth and Creamy
Colour	White
Oduor	Characteristics
pH value	6.0
Degradation of product	No

Determination of pH:

Determining the pH of an antifungal cream is vital for stability, efficacy, and skin compatibility. It affects active ingredient stability and skin tolerance. By monitoring pH, manufacturers optimize the cream's effectiveness and quality, ensuring safety and efficacy standards are met.[14]

Fig.7: pH test

Table 6: Determination of pH

Test	Observation
pH value at 278C628C	6.0

Irritancy test :

The irritancy test evaluates the potential of substances like antifungal creams to cause skin irritation. Applied in controlled patches, it monitors reactions like redness or swelling, ensuring product safety and user comfort. Vital for quality control, it fosters confidence in skincare and pharmaceuticals by ensuring tolerability and effectiveness. The cream was applied directly on the skin and leave for a certain period of time and the effect was studied. The cream was found to be non-irritant on the skin.[14]

Table 7: irritancy test

Test	Observation
Irritancy Test	Cream was found to be non-irritant

Spreadability test :

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

The time thatis taken upby two slides for the separation indicates the ability to spread by the cream. A shorter time suggests better spreadability, meaning the cream can spread up easily to cover a superior area.[15]

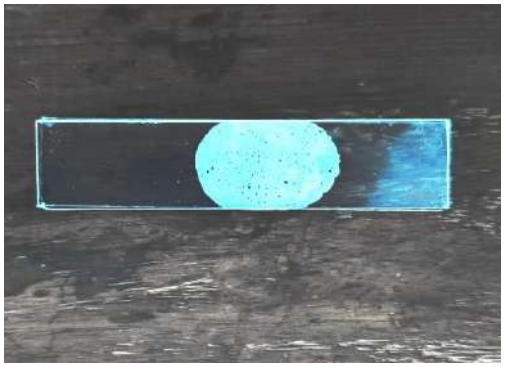

Fig. 8: Spreadability test using glass slides

Procedure:

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

Spreadability = M × L / T

Where,

M = Mean weight of cream applied (in grams)

L = Length of path spread by cream (in centimeters)

T = Time taken for the plates to separate (in seconds)

Table 8: stability test results

Formulation	Time (sec)	Spreadability (gcm/sec)
Anti-fungal cream	9	7

Viscosity test:

Viscosity testing is a crucial evaluation method that measures the resistance of a substance, such as an antifungal cream, to flow. It determines the thickness or fluidity of the cream, impacting its ease of application and adherence to the skin. During viscosity testing, a viscometer, often a Brookfield Viscometer, is employed to quantify the force required to move the cream through a defined space. This measurement helps characterize the rheological properties of the cream, influencing factors like its spreadability and stability. The viscosity test aids in optimizing the formulation, ensuring the cream maintains the desired consistency for efficient application while preventing issues like dripping or uneven coverage. Ultimately, this test contributes to the overall quality control of the antifungal cream, ensuring it possesses the ideal viscosity for optimal therapeutic efficacy and user experience.[15]

Phase separation test:

A phase separation test assesses the stability of substances like antifungal creams over time, checking for any separation into distinct phases, like oil and water, during storage. Conducted in closed containers under specific conditions, it predicts shelf life and usability, informing manufacturers about potential formulation or storage issues. The cream was prepared and stored in a close container at room temperature away from light and was undergone phase separation checks for a duration of 24 hours for a period of 30 days. The purpose of this check was was to observe if there were any changes in the phase separation of the cream over time.[15]

Table 9: Observation of phase separation

Formulation	Observation
Anti-fungal cream	No Phase separation

Anti-fungal and antimicrobial activity testing methods:

Laboratory methods like diffusion, dilution, and TLC-bioautography are commonly used to test the effectiveness of new or existing drugs against fungi and bacteria. These tests help researchers find potential medications and understand how well they work. While there are other methods like ATP bioluminescence and flow cytofluorometric, they're not as popular because they require special equipment and extra tests to make sure the results are reliable.

Agar-disk diffusion method:

The agar-disk diffusion method tests potential medications for bacterial activity before clinical trials. It involves placing drug samples on paper discs and measuring the cleared area around them after incubation. This area shows how well the drug diffuses through the agar and affects the bacteria. Factors like medium, temperature, and pH are crucial.[14]

Media used for growth of fungus:

Sabouraud Dextrose Agar:

Sabouraud agar, developed by French dermatologist Raymond J. A. Sabouraud in the late 1800s, cultivates dermatophytes, inhibits bacterial growth, and facilitates fungal identification. It uses high glucose content for vigorous fermentation, resulting in acid production that suppresses bacterial growth. Despite simplicity, its composition is complex, relying on peptones for fungal growth.

Procedure for preparing sabouraud Dextrose Agar:

Per liter of medium:

- Peptone, 10 g
- Glucose, 40 g
- Agar, 15 g

Procedure:

1. Preparation of Agar Solution:

- Appropriate amounts of peptone, glucose, and agar was weighed for the formulation. The formulation contains 10 grams of peptone, 40 grams of glucose, and 15 grams of agar per liter of water.
- In a clean flask or beaker, add the calculated amounts of peptone, glucose, and agar.
- Appropriate volume of distilled waterwas added to the beaker.
- Mixture was stirred until all components are completely dissolved. We can use a stirring rod or a magnetic stirrer for this purpose.

2. Sterilization:

- The agar solution was autoclaved at 121°C for 15 minutes to sterilize it.
- Proper safety precautions were taken when handling hot liquids and operating the autoclave.

3. Pouring Agar Plates:

- Agar plates were prepared and the agar solution was put to cool down to around 45-50°C after autoclaving but before solidification.
- The sterile agar solution was poured into sterile Petri dishes to a depth of about 5-7 mm.
- The agar was then allowed to solidify by leaving the plates undisturbed on a level surface.

4. Storage:

- Agar solution was stored in a cool, dry place away from direct sunlight.

5. Usage:

- Once the agar plates were cooled and solidified, they were ready to be used for culturing microorganisms.
- Inoculate the plates with the desired sample using sterile techniques and incubate them at the appropriate temperature (usually around 25-30°C) for the growth of fungi.
- Sterile condition were maintained throughout the process to prevent contamination ofthe agar medium.

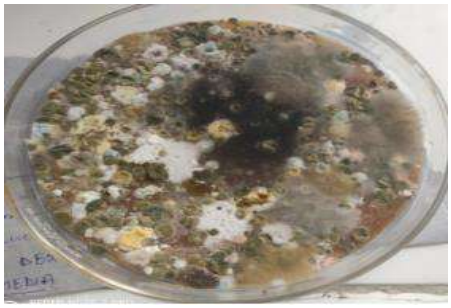

Fig. 9: Media with fungus growth

Fig. 10: Media without fungus growth

RESULT AND DISCUSSION:

For Anti-fungal cream, three different formulations were made by taking different ratios of excipirnts to get the best results and after formulation the AF cream undergo different types of evaluation Parameters as shown in table 9. And after Evaluation Parameters the cream was tested for its antifungal efficacy as shoun in figure 11 and 12 Formulation F1 exhibited a white color with a semi-solid appearance and characteristic odor. After 1 month of stability testing, F1 remained stable, maintaining its physical properties and texture. The pH value of Formulation F1 was recorded at 6.0, indicating a neutral pH level. It showed no irritant effects on the skin, passing the irritancy test successfully. F1 demonstrated good spreadability and no phase separation, highlighting its stability over time. Formulation F2 displayed similar physical properties to F1, with a white color, semi-solid appearance, and characteristic odor. It also remained stable after 1 month of testing, maintaining its physical properties. However, the pH value of Formulation F2 was slightly higher at 6.7 compared to F1. Like F1, F2 showed no irritant effects on the skin and exhibited comparable spreadability. No phase separation was observed in Formulation F2. In contrast, Formulation F3 appeared off-white with a semi-solid appearance and characteristic odor. While it initially maintained stability, F3 started to degrade slightly after 1 month, with phase separation observed. The pH value of Formulation F3 was recorded at 5.9, indicating a slightly acidic pH level. However, similar to F1 and F2, F3 showed no irritant effects on the skin and exhibited comparable spreadability. Phase separation was observed in Formulation F3, indicating instability over time. Overall, Formulation F1 demonstrated superior stability, neutral pH, absence of irritancy, and no phase separation compared to F2 and F3. These results highlight F1 as the most promising formulation among the three, suggesting its potential for further development and application in antifungal cream formulations.

Table 10: Evaluation Parameter of Anti-fungal cream:

Formulation	Physical Property	Stability Testing	pH Test	Irritancy test	Spread ability	Phase separation
F1	White colour with Semi solid appearance and characteristics odour	Stable after 1 month	6.0	Non irritant	passed	No Phase Separation
F2	White colour with Semi solid	Stable after 1 month	6.7	Non irritant	passed	No Phase Separation

	appearance and characteristics odour					
F3	Off-White colour with Semi solid appearance and characteristics odour	Started slightly degrading with phase separation	5.9	Non irritant	passed	Phase separation was seen

The Anti-fungal cream was then tested for its anti-fungal efficacy on Aspergillus niger with the F1 Formulation and also inhibited Aspergillus niger to a great extent as shown in figure 12.

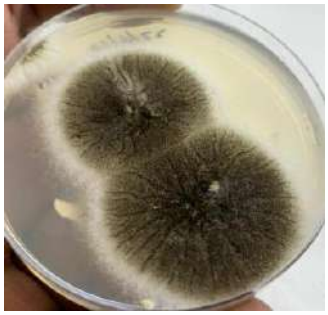

Fig. 11: A.niger growth

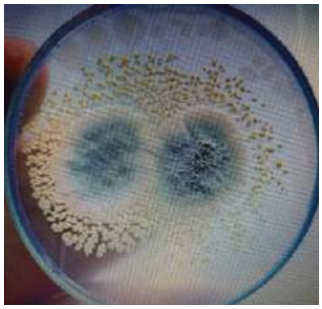

Fig. 12: A.niger inhibition by AF cream

Table. 11: Determination of Extent of growth of Aspergillusniger

Organisms	**Extent of growth**	
Aspergillus Niger	**Test**	**Control**
	No growth	Growth

Table. 12: Determination of percentage reduction of Aspergillus niger

Organisms	**Percentage reduction of organisms**	
	Anti-fungal cream	**Control**
Aspergillus niger	85%	Growth

CONCLUSION:

The study aimed to create and evaluate a cream designed to combat fungal infections. This cream was formulated using a combination of fenugreek, neem, clove, and coconut oils, all known for their beneficial properties. The process involved carefully blending these oils to produce the cream, which was then subjected to various tests to assess its effectiveness against fungal growth. Firstly, the cream was tested using an agar diffusion assay to determine its ability to inhibit the growth of fungi. The cream was applied to agar plates inoculated with different fungal strains, and after an incubation period, the size of the cleared area around the cream discs was measured. This provided insight into how well the cream diffused through the agar medium and its impact on fungal sensitivity. Subsequently, a minimum inhibitory concentration (MIC) assay was conducted to identify the lowest concentration of the cream required to inhibit fungal growth. The cream was diluted in a series of concentrations and added to fungal cultures, with the MIC defined as the lowest concentration at which no visible fungal growth occurred. The results indicated that the cream exhibited significant antifungal activity across

various fungal strains, with low MIC values suggesting potent efficacy. Furthermore, a checkerboard assay was employed to evaluate any synergistic effects between the cream and conventional antifungal medications such as fluconazole and amphotericin B. The results revealed a synergistic interaction, indicating that the cream could enhance the effectiveness of standard antifungal therapies when used in combination. Additionally, the cream was assessed for its ability to inhibit biofilm formation, a common characteristic of fungal infections that can contribute to treatment resistance. The cream demonstrated a notable reduction in biofilm formation, suggesting its potential to prevent the development of chronic fungal infections. In conclusion, the cream formulated with fenugreek, neem, clove, and coconut oils exhibited strong antifungal properties across various tests, making it a promising natural alternative or complementary therapy for fungal infections. Further research and clinical trials are warranted to validate its safety, efficacy, and mechanism of action in clinical settings.

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HOW TO CITE: Aditya Adwait , Kumar Anand , Mittal Chavi , Formulation and Evaluation of Antifungal Cream using Fenugreek oil, Neem oil, Coconut oil and Clove oil, Int. J. of Pharm. Sci., 2024, Vol 2, Issue 6, 1165-1178. https://doi.org/10.5281/zenodo.12532938