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Formulation And Evaluation Of Antifungal Cream Using Fenugreek Seed, Neem Oil, Coconut Oil And Clove Oil

Aditya Adwait[1]*, Mittal Chavi[2]*

1Student, B. Pharma, Dev Bhoomi Institute of Pharmacy and Research, Dehradun

2Associate Professor, School of Pharmacy and Research, Dev Bhoomi Uttarakhand University, Dehradun

ABSTRACT:-

This study focuses on the formulation and evaluation of an antifungal cream incorporating fenugreek seed extract, neem oil, coconut oil, and clove oil, with the aim of assessing its efficacy against Aspergillus Niger. These natural ingredients were chosen for their known antifungal properties, making them promising candidates for an alternative treatment approach. To achieve this, extracts were prepared at different ratios, employing an appropriate solvent system. The resulting mixture underwent rigorous testing against a standard Aspergillus Niger strain, utilizing the agar plate diffusion method. The zones of inhibition around the agar plates containing the formulated mixture were measured, and the information achieve was exposed for statistical investigation. The antifungal activity of the prepared mixture revealed significant inhibition of Aspergillus Niger growth, as evidenced by distinct zones of inhibition. Importantly, the size of these inhibition zones varied based on the specific ratios of fenugreek seed extract, neem oil, coconut oil, and clove oil. Notably, a unique combination of these components demonstrated the highest antifungal activity. Comparative analysis against a positive control, representing conventional antifungal treatment, indicated comparable or even superior effectiveness in reducing Aspergillus Niger growth. This suggests that the formulated mixture could serve as a potent natural antifungal agent. The combined effect of fenugreek seed extract, neem oil, coconut oil, and clove oil showcased remarkable antifungal properties, opening avenues for their potential application as an alternative treatment for Aspergillus Niger fungal infections. The study proposes that the synergistic action of these four components could offer a compelling option for managing such infections. While the results are promising, the study acknowledges the need for further investigations to delve into the underlying mechanisms of action. Additionally, assessing the effectiveness and safety of this formulation in vivo through extended research and clinical trials is imperative. The reading is funding in the rising body of knowledge on likely anti-fungal agents also presents a potential avenue for innovative fungal infection treatments.

Keyword: Antifungal activity, Aspergillus Niger, agar disc diffusion method, zones of inhibition, growth inhibition, alternative treatment, in vivo evaluation.

INTRODUCTION:-

Skin creams are a topical product that can be applied to the skin for a variety of purposes. They consist of semi-solid emulsions, which can be either O/W (oil in the water) or W/O (water in the oil) varieties, varying in uniformity according to oil in the water proportion. These creams can be used on behalf of cosmetic purposes, like washing, refining, enhancing beautification, and defending the look, and for medical purposes, such as delivering drugs to stratums underneath the layers of skin or lubricated tissues. They are planned on behalf of topical application, so that the drugs are delivered to the affected area in a targeted and localized manner.

Skin creams are treated as pharmaceutical products and are manufactured using pharmaceutical processes. There are both medicated and non-medicated creams that are very successful in treating several skin infections. These might be Ayurvedic medications, herbal medicaments or allopathic medications and may comprise one or more than one medications dissolved or distributed in the proper base. Skin Creams can be categorized as oil in the water emulsion or water in the oil emulsion created on these stages. 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 localized 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 place of contamination, reduces side effect and risk, higher treatment efficacy, and greater patient compliance. To date, several topical antifungal agents have been utilized 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. 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.

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Antifungal creams are a type of medication that is used directly to the skin to treat and manage fungal infections. Fenugreek (Trigonella foenum-graecum) is a traditional medicinal herb that was often used as a traditional medicine and cooking for millennia. It offers several health benefits, including antifungal properties. The antifungal effect of fenugreek is related to active components such as alkaloids, flavonoids, saponins and steroids. Neem oil extracted from seeds of neem has strong antifungal action.

It effectively prevents the growth and development of the fungus due to some high concentration of bioactive substances such as azadirachtin, nimbin and gedunin. It fights many fungal diseases including Candida, Aspergillus and Trichophyton. Neem oil's ability to destroy fungal hyphae and prevent their spread makes it an effective remedy against skin infections, nail fungus and plant diseases. In addition, its anti-inflammatory properties help reduce swelling and pain.

Neem oil is a natural and strong antifungal conditioner that can be applied directly to affected areas or used

as a protective spray on plants. Coconut oil has become popular as a natural remedy due to its purported antifungal properties. Its unique skin care ingredient, rich in fatty acids of medium-chains like Lauric acid, has shown antifungal activity against various types of fungi. Lauric acid damages the cell membranes of fungi, limiting their development and reproduction. In addition, the natural moisturizing properties of coconut oil help soothe and nourish the affected area. Its broad antifungal activity has shown promise in the treatment of ringworm, athlete's foot and ringworm. Although more research is needed, coconut oil appears to be a good alternative or adjunctive treatment for treating yeast infections.

Clove oil known basically by its strong anti-fungal, anti-inflammation and anti-oxidative property. Clove oil has been extensively studied against many pathogenic microorganisms and shows effective antimicrobial and antibacterial activity in various objective studies. Eugenol shows antimicrobial activity in contrast to fungi and yeasts like human pathogenous molds. Aspergillus Niger was effectively inhibited by the clove oil. The well-known antifungal action of clove oil is based on its hydrophobic nature, which allows it to enter the cell membrane of microbes, which damages the membrane structure and makes them more permeable, leading to the loss of cytoplasmic components, which leads to destruction of microbial cells

INGREDIENTS:

1. Fenugreek Seed (Trigonella foenum-graecum):

Fig. 1:Fenugreek Seed

Scientific name – Trigonella Foenum-graecum
Family - Fabaceae
Subfamily - Faboideae

Description:-

Fenugreek is almanac aromatic plant native in eastern Mediterranean region and widely cultivated in India. Plant parts like seeds and leaf are usually utilized from fenugreek. Consumed as a flavor in most of the Indian foods and also as an ingredient in conventional medicine in Indo-Pakistan Headland and former countries. In ancient times, fenugreek was found to serve many purposes. Fenugreek is wealthy in many phytochemicals as well as antioxidants, this herb for long has been used as spices for food and also as a medicinal agent. It is use to treat wounds, abscesses, arthritis, pneumonia, ulcers and indigestion. Thus, fenugreek has been used as a food and seasoning for numerous ages in several portions of the world. Researchers have reported the pharmacological use of several fenugreek plant components. Fenugreek seeds shows numerous health effects like anti-diabetic effect, anti-cancer effect, anti-inflammatory effect and anti-oxidative effect. Its leaves have antibacterial, antifungal, antidiabetic and antioxidative properties. Fenugreek seed is a traditional plant with medicinal benefits that comprises lively substances like flavonoid, alkaloid, saponin, steroid etc.

1. Antimicrobial action: Fenugreek has antimicrobial properties, including antifungal activity. It prevent

the development and activity of numerous fungal species, as well as Candida albicans, Aspergillus species and dermatophytes.

2. Fenugreek contains various bioactive chemicals that contribute to its antifungal properties. For example, trigonelline, an alkaloid found in fenugreek, exhibits antifungal action in contrast to numerous species of molds. Fenugreek flavonoids such as vitexin and isovitexin have antifungal properties.

3. Fungal cell membrane disruption: Fenugreek extracts have been shown to disrupt the cell membrane of fungal infections. This disruption can cause leakage of cell contents, loss of fungal integrity, and fungal death. This method is very effective against Candida species that cause yeast infections in humans.

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5. Anti-inflammatory properties: Inflammation is important in fungal infections and fenugreek has anti-inflammatory properties. Fenugreek reduces inflammation and helps relieve symptoms of fungal infections and facilitates healing.

2. Neem (Azadirachta Indica):

Fig. 2: Neem Oil

Scientific name - Azadirachta Indica
Family - Meliaceae
Subfamily - Meloideae and tribMelieae

Description:-

Azadirachta Indica is found in maximum tropical nations and is an ever popular tree. 1 or 2 species of Azadirachta, innate to Burma as well as India, neutralized from West Africa are widely cultivated as a medicinal plant in Nigeria. Azadirachta Indica (neem) is a plant that is widespread on our continent in all seasons. The neem tree is still considered "village medicine". Parts of the Neem tree, like the bark, seeds, branch, flower, leaves and roots, all have individual therapeutic probable for the mankind. Neem seeds contains maximum oil of all the parts of the tree. It covers about 47% of oil,that consist of oleic acid, linoleic acid, palmitic acid, stearic acid and arachidic acid as well. The kernels acts like antipyretic,
anti-malarial, and antimicrobial agents. Azadirachta Indica (neem) is a large Indian tree, used in traditional system of medicines in Asia for eras like insecticide, fungicide and contraceptive.

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Almost every part of this tree has multiple uses and many international organizations have recommended it's planting in Africa and Asia. From different parts of the neem tree there has been 140 known chemicals isolated. The neem yields a huge quantity of physiologically lively materials that is basically adjustable and chemically varied. The first compound that is a polyphenolic flavonoid was secluded from fresh leaves of neem were ß-sitosterol flavonoid and quercetin flavonoid, which may show antimicrobial activity.

3. Coconut Oil:

Fig. 3: Coconut oil

Botanical name – Cocosnucifera
Family - Arecaceae
Subfamily - Arecoideae

Description:-

The purest form of coconut oil is VCO (Virgin coconut oil) with a colorless appearance. It comprises largely of saturated fatty acids of a medium-chain. Scientists have studied the biological properties of VCO for decades, especially its antibacterial potential. The high concentration of MCFA (medium-chain fatty acids), such as lauric acid in addition to monolaurin that is its monoglyceride form which increases the antifungal action of VCO and in contrast to wide-ranging pathogenous microbes. Therefore, the oil is taken up as a day-to-day dietary increment or as alternate therapy for microbiological contaminations. We are emphasizing its antimicrobial, and antiviral activities, trying on the way to elucidate the basic processes by which the VCO prevents the development of the bacteria, fungi and virus. Coconut oil that is also an appetizing oil obtained from mature coconut fruits. It is also known as minyakkelpa in Indonesia and Malaysia. Due to its great content of lipids as well as saturated fatty acids, the oil is resistant to change in the oxidation, making it an excellent choice for cooking. Coconut trees are planted in more than 80 countries, and global coconut production is expected to grow to about 55 million tons per year. In addition to culinary applications, coconut is widely used in hygiene products, food and several industrial sectors.

4. Clove Oil :

Fig. 4: Clove Oil

Botanical name – Syzygium aromaticum
Family – Myrtaceae
Subfamily - Myrtiodeae

Description:-

Clove oil has antimicrobial properties, including antifungal properties. It has been shown to inhibit the growth and movement of parasites including Candida, Aspergillus and dermatophytes. Clove oil and eugenol also significantly reduce ergosterol amount, the special element of the cell membrane of a parasite. Candida albicans spore tube formation was entirely or near about totally inhibited by the oil application and eugenol application below the values of MIC concentration. A flow cytometric and ergosterol synthetic restriction study was performed on yeast and filamentous fungi to elucidate its mechanism of action. Quick entry of the Propidium iodide in major parts of the mold cells when cells were treated above the MIC concentrated values, indicating a fungicidal outcome is due to the broad damage to the cell film. Studies show that eugenol and has a substantial antimicrobial action in contrast to clinically essential parasites, together with strains of fluconazole-resistant microbes, which merit clinical use in the treatment of infectious contamination.

Preparation of Antifungal Cream:-

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

INGREDIENTS	USES
FENUGREEK	ANTI – MICROBIAL AGENT
COCONUT OIL	ANTI – MICROBIAL AGENT
CLOVE OIL	ANTI – MICROBIAL AGENT
NEEM OIL	ANTI – MICROBIAL AGENT
PETROLEUM JELLY	SOOTHING AGENT
HARD PARAFFIN	LUBRICANT
CETYL ALCOHOL	EMOLLIENT
GLYCEROL MONOSTERATE	EMULSIFIER
DISTILLED WATER	BASE

Table .1

Procedure:

1. Preparation of Oil Phase:
   - Combine neem oil, coconut oil, clove oil, and the fenugreek seed extract in appropriate ratios.
   - Heat the oil phase components in a beaker at around 75°C until they are melted and well-mixed.
   - Add petroleum jelly, hard paraffin, and cetyl alcohol to the oil mixture.
2. Preparation of Aqueous Phase:
   - In a separate beaker, combine distilled water and glycerol monostearate.
   - Heat the aqueous phase components to the same temperature (around 75°C) until they are melted.
3. Emulsion Formation:
   - Slowly add aqueous part to the oil part by enough agitation. Ensure uniform mixing.
   - Continue stirring until the temperature drops to around 40°C.
4. Addition of Oil:
   - Incorporate additional oil to the emulsion while stirring to achieve the desired consistency.
5. Cooling:
   - Allow the emulsion to cool to room temperature, allow it to solidify into a semisolid base.
6. pH Adjustment:
   - Adjust the pH of the cream to a suitable range, typically between 4.5 and 6.
7. Final Product:
   - The resulting cream should have a smooth texture and homogenous appearance.

This method provides a basic outline for preparing an antifungal cream using fenugreek seed extract, neem oil, coconut oil, and clove oil. Adjustments to ingredient ratios and specific procedures may be made based on the intended application and desired characteristics of the final product. It is important to conduct stability testing and evaluate the cream's physical properties before considering it for use in treating fungal infections.

Agar-disk diffusion method:

Sabouraud Dextrose Agar:

Sabouraud agar medium was developed in the late 19th century by the well-known French dermatologist Raymond J.A. Sabouraud. This platform was specifically designed to promote the growth of dermatophytes, which cause infections of the skin, hair and nails. Dr. Sabouraud dedicated his medical research to the study of bacteria and fungi that cause skin diseases, which led to the development of various agar-based media and methods for the cultivation of pathogenic molds and yeasts, including Aspergillus Niger, dermatophytes and Malassezia.

Procedure for preparing Sabouraud Dextrose Agar:

Medium per liter:

- Agar (15 grams)
- Glucose (40 grams)
- Peptone (12 grams)
- Distilled water (1000 ml)

- Weigh the agar (15 grams), glucose (40 grams), peptone (12 grams) respectively.
- Combine the weighed peptone, glucose, and agar in a large container.
- Add distilled water to the mixture, adjusting the total volume to 1000 ml.
- Stir the mixture thoroughly to ensure even distribution of the ingredients.
- Adjust the pH of the medium to approx. 5.6 using HCL acid. Check and adjust as need.
-Transfer the medium to suitable containers and autoclaved on 120°C for 15 to 17 mins. for fumigation.
- Allowing autoclaved medium to be cooled down at around 45 to 52° C.
- Now cooled agar medium was pour in the tubes for slants or disinfected petri-dish, ensuring even

distribution.
- Allow the poured medium to solidify, creating a surface suitable for fungal growth.
- Store the prepared Sabouraud Dextrose Agar plates or slants in a cool place until ready for use.

Evaluation of Anti-fungal cream:

1. Physical Properties:

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

2. Stability testing:

Stability testing is an essential element of the drug development process, beginning with the early phases of drug discovery and continuing until a molecule is discontinued or a commercial product is launched. Stability studies are undertaken to test the stability of a medicine and its formulation in accordance with the ICH (International Conference on Harmonization) criteria.

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.

3. Determination of pH:

In the context of an antifungal cream, the determination of pH is essential for multiple reasons. The pH level influences the stability, efficacy, and overall performance of the cream. Maintaining a specific pH range is crucial to ensure the stability of active ingredients, preventing degradation or alteration of their antifungal properties. Additionally, the pH of the cream impacts its compatibility with the skin, minimizing the risk of irritation or adverse reactions during application. By monitoring and controlling the pH, manufacturers can optimize the formulation to enhance the antifungal cream's effectiveness, skin tolerance, and overall quality, providing a product that meets both safety and efficacy standards.

4. Irritancy test :

The irritancy test is a crucial evaluation method that assesses the potential of a substance, like an antifungal cream, to cause irritation when applied to the skin. This test involves the controlled application of the cream on the skin, typically in small patches, and closely observing any adverse reactions. These reactions may include redness, swelling, or discomfort, providing insights into the cream's safety and tolerability. For antifungal creams, which are directly applied to the skin, ensuring minimal irritancy is paramount. This test helps manufacturers identify and address any potential skin reactions, ensuring the product is well-tolerated by users. It plays a pivotal role in quality control, contributing to the overall safety and effectiveness of topical treatments, thereby instilling confidence in users and promoting the responsible development of skincare and pharmaceutical products.

5. Spreadability test :

A spreadability test is a method used to assess how easily a substance, such as an antifungal cream, can be spread or applied onto the skin. In this test, the cream is placed between two surfaces, and the time it takes for these surfaces to separate under a specific load is measured. A shorter separation time indicates better spreadability, signifying that the cream can easily cover a larger area on the skin. This test is crucial for topical products as it influences user experience, making sure that cream should be effortlessly functional, distributed

and disseminated, promoting even coverage. Enhanced spreadability contributes to improved usability and patient compliance, making the antifungal cream more user-friendly and effective in its application.
Glass slides are used to test the spreadability of a cream recipe. Spreadability is measured by the interval of separation of the two slide when a specific load is applied.
The time that is taken up by two slides for the separation indicates the ability to spread by the cream. A shorter time suggests better spreadability, meaning the cream can spread up easily to cover a superior area.

6. 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.

7. Phase separation test:

A phase separation test is a critical evaluation conducted on substances like antifungal creams to assess their stability over time. This test examines whether the cream undergoes any separation into distinct phases, such as oil and water, during storage. The cream is stored in closed containers under specific conditions, and observations are made over a designated period. Phase separation can indicate instability, leading to changes in the cream's composition and potentially compromising its efficacy. This test is essential in predicting the shelf life and usability of the antifungal cream, informing manufacturers about potential issues related to formulation or storage conditions. By ensuring that the cream remains homogenous without separation, this test contributes to upholding its value and success of antifungal invention throughout its intended lifespan.

8. Susceptibility test:

A susceptibility test is a vital procedure to assess the sensitivity of microorganisms, particularly fungi in the context of antifungal creams, to specific antifungal agents. This test helps determine the effectiveness of the cream against targeted fungal strains and guides treatment decisions. Typically conducted in a laboratory setting, the susceptibility test involves exposing the fungal cultures to the antifungal cream and observing the inhibitory effects on their growth. The results provide valuable information about the cream's efficacy, helping researchers and clinicians select appropriate treatment options. This test is integral in understanding the spectrum of activity, potential resistance patterns, and overall therapeutic impact of the antifungal cream, contributing to informed clinical practices and the development of effective antifungal formulations.

CONCLUSION:

In conclusion, the investigation into the formulation and evaluation of an antifungal cream comprising fenugreek seed, neem oil, coconut oil, and clove oil unveils a promising prospect for natural remedies in combating fungal infections. The amalgamation of these botanical ingredients exhibited significant antifungal activity against Aspergillus Niger, suggesting a potential alternative or adjunctive treatment strategy. The distinctive properties of fenugreek, renowned for its multifaceted health benefits, neem oil with its potent antibacterial capabilities, coconut oil's purported antifungal properties, and clove oil's recognized antimicrobial potency contribute to the cream's overall efficacy.

The study reveals that the formulated cream, particularly when incorporating specific ratios of these natural components, can create zones of inhibition, indicating a hindrance to the growth of Aspergillus Niger. Notably, a special mixture comprising fenugreek seed extract, neem oil, and clove oil demonstrated the highest antifungal

activity, rivaling or surpassing conventional antifungal treatments in effectiveness. This not only underscores the potential of these natural ingredients as antifungal agents but also suggests their comparative or superior efficacy.

However, while these findings present an optimistic outlook, it is imperative to acknowledge the need for further investigations. Detailed study is needed for finding out of the primary mechanism of act, assess the safety profile, and evaluate the formulation's effectiveness in vivo. Understanding how these natural components interact and influence fungal pathogens can provide valuable insights for refining and optimizing the antifungal cream.

In essence, this research advocates for a holistic approach to antifungal therapy, leveraging the synergistic potential of natural ingredients. The encouraging results pave the way for future innovations in fungal infection treatments, emphasizing the importance of continued research to unlock the full therapeutic potential of this natural formulation. As we navigate the realms of alternative and complementary medicine, this study marks a significant stride towards developing safe, effective, and nature-inspired solutions for fungal infections.

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