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Formulation and Evaluation of Antifungal Cream Using Ocimum Sanctum and Azadiracta Indica Leave Extract

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ABSTRACT

In recent years, it has been found that fungal infections in humans have risen. Along with its growth rate, the kind of fungal species also increased considerably. An effective formulation is necessary to treat these fungal infections in a simple manner. The main aim of my research project was to develop a formulation of a topical antifungal Cream. The formulation and development of tropical antifungal formulation have seen great results in the treatment of fungal infections. Over the conventional dosage forms, these topical drug delivery formulations have more benefits along with their extraordinary advantages. This cream formulation consists of natural as well as synthetic ingredients like; Azadirachta Indica, Ocimum sanctum Linn, Liquid paraffin, Stearic acid, Bees wax, Stearyl alcohol Glycerin Tween-80 Methyl parabens Sorbitol solution Potassium hydroxide. ingredients which have antifungal and antibacterial properties.

Keywords: Fungal infection, Topical Antifungal. Azadirachta Indica, Ocimum sanctum Linn, Liquid paraffin, Stearic acid, Bees wax, Stearyl alcohol Glycerin Tween-80 Methyl parabens Sorbitol solution Potassium hydroxide.

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INTRODUCTION

Classification of Fungal Infections:

Mycoses is a fungal infection of animals, including humans. Mycoses are classified according to the tissue levels initially colonized. The clinical nomenclatures used for the mycoses are based on the

1. Classification Based on Site
2. Classification Based on Route of Acquisition
3. Classification Based on Virulence

CLASSIFICATION BASED ON SITE

Mycoses are classified as superficial, cutaneous, subcutaneous, or systemic (deep) infections depending on the type and degree of tissue involvement and the host response to the pathogen.

Superficial mycoses

Superficial mycoses are limited to the outermost layers of the skin and hair. An example of such a fungal infection is Tinea versicolor, a fungus infection that commonly affects the skin of young people, especially the chest, back, and upper arms and legs. Tinea versicolor is caused by a fungus that lives in the skin of some adults. It does not usually affect the face. This fungus produces spots that are either lighter than the skin or a reddish brown. This fungus exists in two forms, one of them causing visible spots. Factors that can cause the fungus to become more visible include high humidity, as well as immune or hormone abnormalities. However, almost all people with this very common condition are healthy.

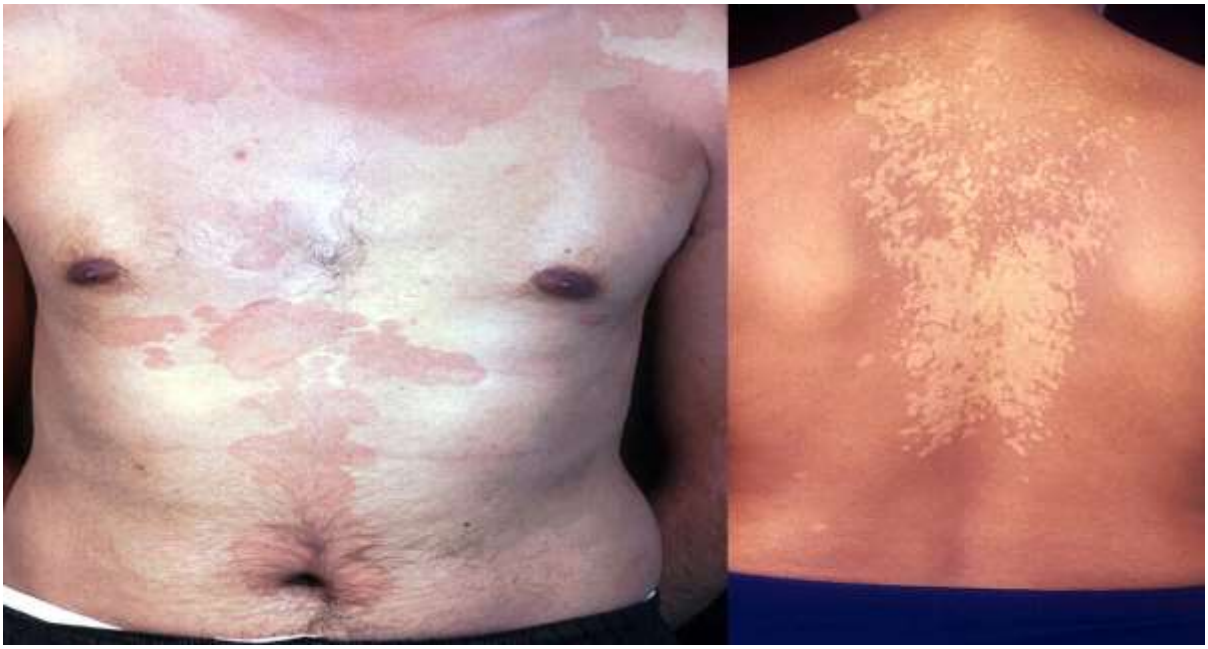


Figure 1: Superficial mycoses

Cutaneous mycoses

Cutaneous mycoses extend deeper into the epidermis, and also include invasive hair and nail diseases. These diseases are restricted to the keratinized layers of the skin, hair, and nails. The organisms that cause these diseases are called dermatophytes, the resulting diseases are often called ringworm, dermatophytosis or tinea.

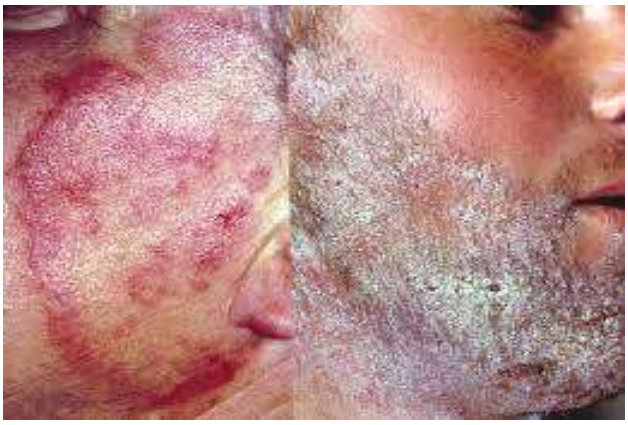


Figure 2: Cutaneous mycoses

Subcutaneous mycoses

Subcutaneous mycoses involve the dermis, subcutaneous tissues, muscle and fascia. These infections are chronic and can be initiated by piercing trauma to the skin which allows the fungi to enter. These infections are difficult to treat and may require surgical interventions such as debridement.

Figure 3: Subcutaneous mycoses

Classification Based on Route of Acquisition Infecting fungi may be either exogenous or endogenous. Routes of entry for exogenous fungi include airborne, cutaneous or percutaneous. Endogenous infection. involves colonization by a member of the normal flora or reactivation of a previous infection.

CLASSIFICATION BASED ON VIRULENCE

Primary pathogens can establish infections in normal hosts. Opportunistic pathogens cause disease in individuals with compromised host defense mechanisms.

I. **Systemic mycoses** due to primary pathogens Systemic mycoses due to primary pathogens originate primarily in the lungs and may spread to many organ systems. Organisms that cause systemic mycoses are inherently virulent. In general, primary pathogens that cause systemic mycoses are dimorphic.

II. **Systemic mycoses** due to opportunistic pathogens Systemic mycoses due to opportunistic pathogens are infections of patients with immune deficiencies who would otherwise not be infected. Examples of no compromised conditions include AIDS, alteration of normal flora by antibiotics, immunosuppressive therapy, and metastatic cancer. Examples of opportunistic mycoses include Candidiasis, Cryptococcosis and Asper gillosis.

Opportunistic Mucosal disease Candidiasis (due to C albicans and other Candida spp.) is the most common opportunistic fungal infection. Candida albicans is the most common cause of candidiasis. Candidiasis may be classified as superficial or deep. Superficial candidiasis may involve the epidermal and mucosal surfaces, including those of the oral cavity, pharynx, esophagus, intestines, urinary bladder, and vagina. The alimentary tract and intravascular catheters are the major portals of entry for deep (or visceral) candidiasis. The kidneys, liver, spleen, brain, eyes, heart, and other tissues are the major organ sites involved in deep or visceral candidiasis. The principal risk factors predisposing to deeply invasive candidiasis are protracted courses of broad-spectrum antibiotics, cytotoxic chemotherapy, corticosteroids, and vascular catheters.

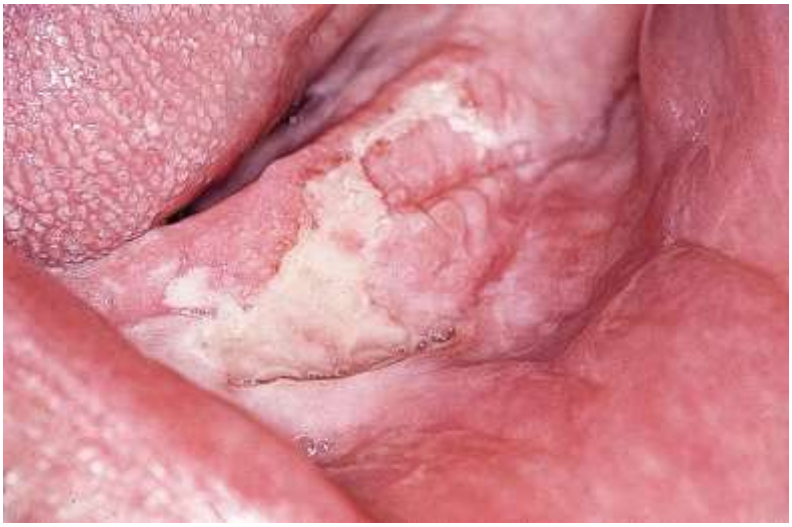


Figure 4: candidiasis

Aspergillosis

Invasive aspergillosis most frequently involves the lungs and paranasal sinuses. This fungus may disseminate from the lungs to involve the brain, kidneys, liver, heart, and bones. The main portal of entry for aspergillosis is the respiratory tract, however, injuries to the skin may also introduce

the organism into susceptible hosts. Quantitative and functional defects in circulating neutrophils are key risk factors for development of invasive aspergillosis. For example, neutropenia due to cytotoxic chemotherapy and systemic corticosteroids are common predisposing factors for invasive aspergillosis.

Opportunistic Mucosal disease Candidiasis Candidiasis (due to C albicans and other Candida spp.) is the most common opportunistic fungal infection. Candida albicans is the most common cause of candidiasis. Candidiasis may be classified as superficial or deep.

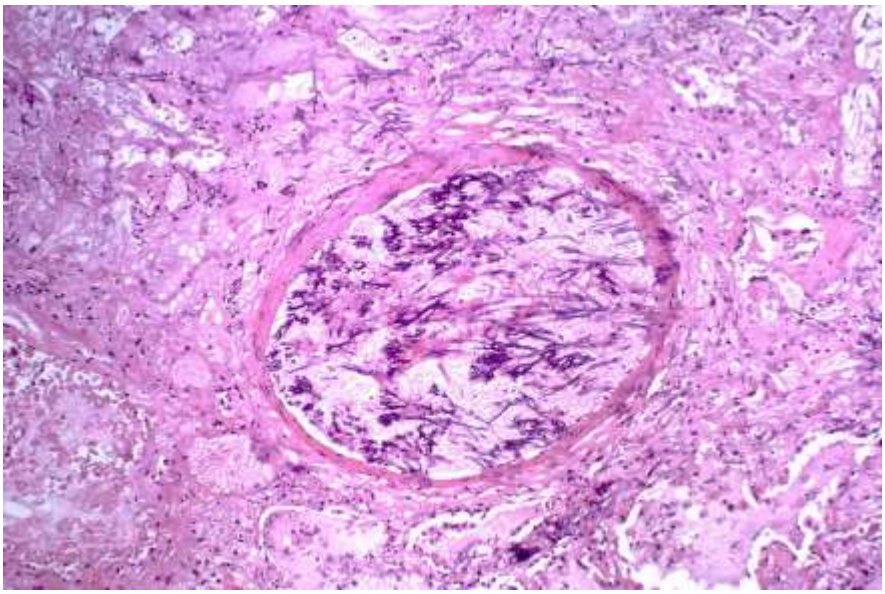


Figure 5: Candidiasis

Zygomycotic is the broadest term to refer to infections caused by bread mold fungi of the Zygomycota phylum. However, because Zygomycota has been identified as polyphyletic, and is not included in modern fungal classification systems, the diseases that zygomycotic can refer to are better called by their specific names: mucormycosis (after Mucorales), phycomycotic (after Phycomycetes) and basidiobolomycosis (after Basidiobolus). Zygomycosis due to Rhizopus, Rhizomic, Absidia, Mucor species, or other members of the class of Zygomycetes, also causes invasive sinopulmonary infections. An especially life-threatening form of zygomycotic (also known as mucormycosis), is known as the rhino cerebral syndrome, which occurs in diabetics with ketoacidosis. In addition to diabetic ketoacidosis, neutropenia and corticosteroids are other major risk factors for zygomycotic. Aspergillus sp. and the Zygomycetes have a strong propensity for invading blood vessels.

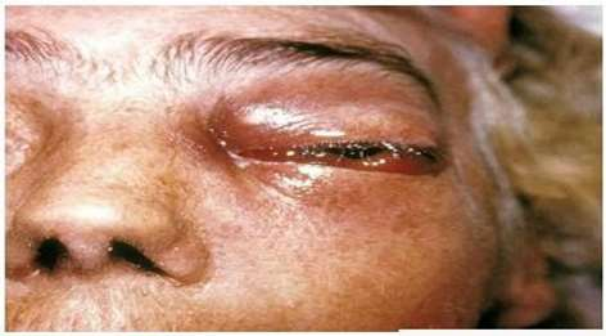


Figure 6

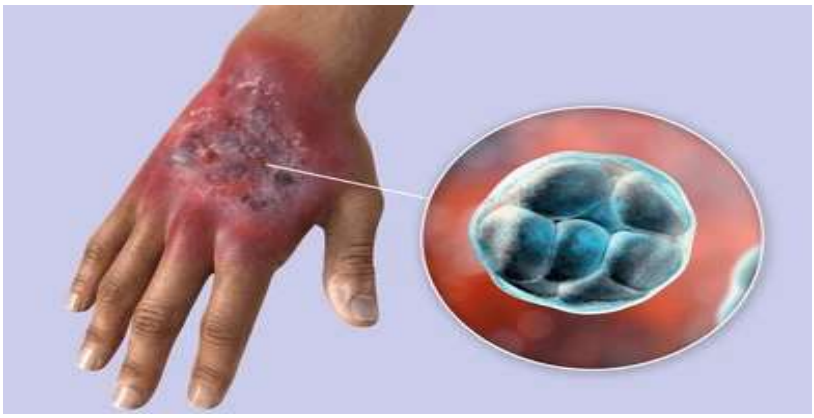


Figure 7

Mucormycosis is a very rare infection. It is caused by exposure to mucor mound which is commonly found in soil, plants, manure, and decaying fruits and vegetables. It is ubiquitous and found in soil and air and even in the nose and mucus of healthy people. It affects the sinuses, the brain and the lungs and can be life-threatening in diabetic or severely immune compromised individuals, such as cancer patients or people with HIV/AIDS.

Doctors believe mucormycosis, which has an overall mortality rate of 50%, may be being triggered by the use of steroids, a life-saving treatment for severe and critically ill Covid-19patients.

Steroids reduce inflammation in the lungs for Covid-19 and appear to help stop some of the damage that can happen when the body's immune system goes into overdrive to fight off coronavirus. But they also reduce immunity and push up blood sugar levels in both diabetics and non-diabetic Covid-19 patients. It’s thought that this drop in immunity could be triggering these cases of mucormycosis.

Cryptococcosis

Cryptococcosis is most typically an opportunistic fungal infection that most frequently causes pneumonia and/or meningitis. Defective cellular immunity, especially which associated with the acquired immune deficiency syndrome, is the most common risk factor for developing Cryptococcosis.

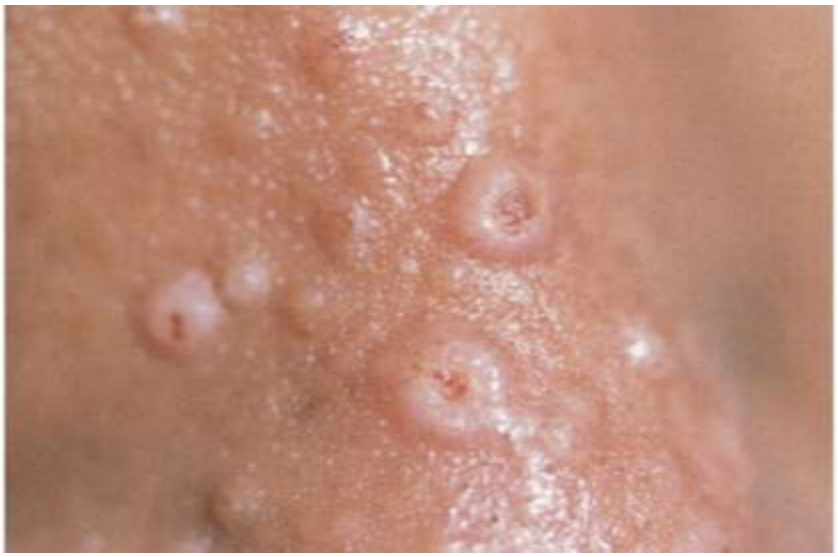


Figure 8: Cryptococcosis

Hyalohyphomycotic is an opportunistic fungal infection caused by any of a variety of normally saprophytic fungi with hyaline hyphal elements. For example, Fusarium spp. infects neutropenic patients to cause pneumonia, fungemia, and disseminated infection with cutaneous lesions.

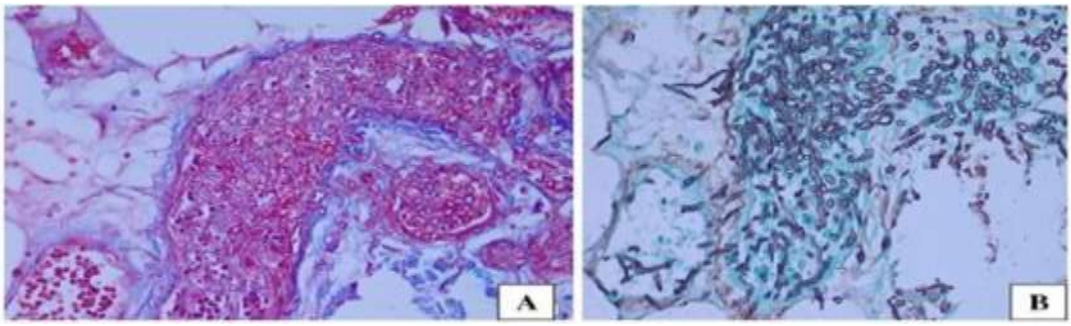


Figure 9:

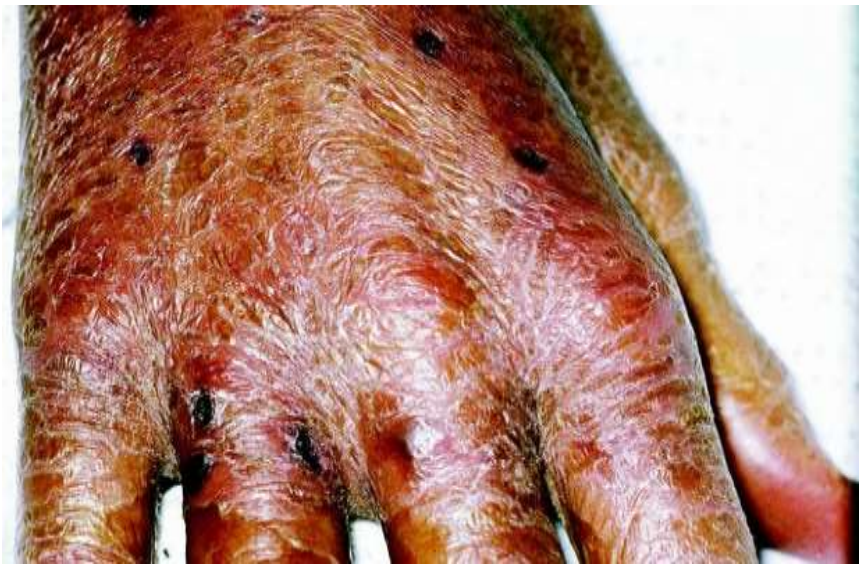


Figure 10:

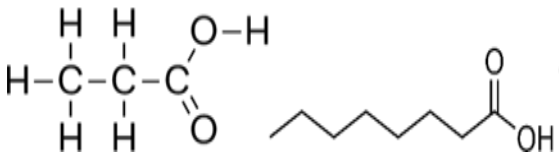
Classification of Antifungal Agents

Antifungal agents are mainly classified into following types

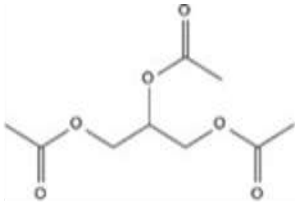
1. Synthetic agents
   - Acids and derivatives
   - Phenolic derivatives
   - Halogen containing compounds
   - Thiocarbamate derivatives
   - Pyrimidine derivatives
   - Acridine derivatives
   - Azole derivatives (imidazole derivatives and triazole derivatives)
   - Allylamine derivatives
2. Antibiotics
   - Polyenes
   - Nonpolyenes

Synthetic agents

Acids and derivatives: Fatty acids, benzoic acid, salicylic acid and triacetin (glyceryl triacetate). Fatty acids like propionic acid, caprylic acid, and undecylenic acid are this type of antifungal agents.



Caprylic acid

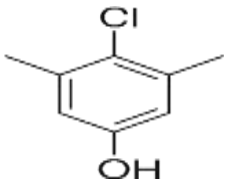


Propionic acid

Phenolic derivatives

Parachlorometaxylenol is an example of phenol derivative antifungal agent Mode of action: Denaturation of protein via the reaction of the acidic phenolic group with basic centers in the protein molecule located on cell wall of fungal cell.

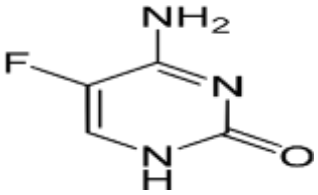
Uses: It is used topically in the treatment of tinea infection such as athlete's Foot 4-Chloro-3, 5-dimethylphenol.



Parachlorometaxylenol

Flucytosine, a pyrimidine derivative, is useful in the case of infection due to candida albicans and Cryptococcus neoformans. Another pyrimidine analogue hexitidine is also used for aphthous ulceration. E.g.: Flucytosine (Ancbon)5- Fluorocytosine or 5-FC: Flucytosine, also known as 5-fluorocytosine, is an antifungal medication. It is specifically used, together with amphotericin B, for serious Candida infections and cryptococcosis. It may be used by itself or with other antifungals for chromomycosis. Flucytosine is used by mouth and by injection into a vein.

Uses: 5-FC has a narrow spectrum. It is used orally for treatment of serious systemic infections caused by pathogenic yeasts such as Candida albicans.

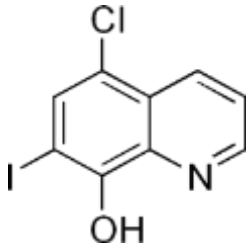


Flucytosine

Halogen containing compounds

Clioquinol is an example of this kind of antifungal agent. It is neurotoxic in large doses. It is a member of family of drugs called hydroxyquinolines which inhibit certain enzymes related to DNA replication. The drugs have been found to have activity against both viral and protozoal infections.

Mode of action: Competes with co-enzymes for metal binding sites on enzymes.



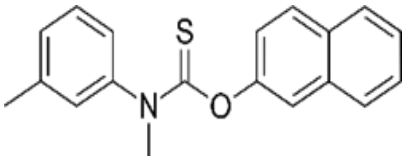
Clioquinol

Thiocarbamate derivatives clioquinol:

The thiocarbamate group of fungicides structurally resembles the rubber accelerator disulfiram (Antabuse, tetraethyl thiuram disulfide, a common sensitizer present in both the European and the North American standard patch test series (Adams and Fischer, 1990).

E.g. Tolnaftate

Uses: Treatment of superficial tinea infections of the skin in the form of 1% cream, powder, aerosol, gel, and solution.



O-2-Naphthyl-N-methyl-m-tolylthiocarbamate.

Pyrimidine derivatives: Pyrimidines are also known to exhibit antifungal properties.

Side effects: Miconazole is generally well tolerated. Oral gel can cause dry mouth, nausea and an unpleasant taste in about 1–10% of people. Anaphylactic reactions are rare. The drug prolongs the QT interval.

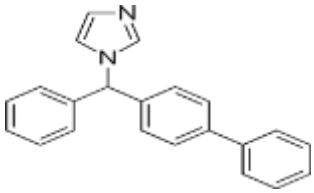
14α-sterol demethylase, resulting in a reduced production of ergosterol. In addition to its antifungal actions, miconazole, Miconazole similarly to ketoconazole, is known to act as an antagonist of the glucocorticoid receptor. The most common side effect is a burning sensation at the application site. Other reactions, such as itching, eczema or skin dryness, are rare.

Mechanism of action

Bifonazole has a dual mode of action. It inhibits fungal ergosterol biosynthesis at two points, via transformation of 24-methylendihydrolanosterol to desmethyl-sterol, together with inhibition of HMG-CoA. This enables fungicidal properties against dermatophytes and distinguishes bifonazole from other antifungal drugs.

Pharmacokinetics

Six hours after application, bifonazole concentrations range from 1000 µg/cm³ in the stratum corneum to 5 µg/cm³ in the papillary dermis.



Bifonazol

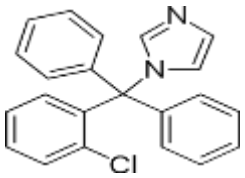
Azole derivatives

The azole antifungal agents have five-membered organic rings that contain either two or three nitrogen molecules (the imidazole's and the triazoles respectively).

Clotrimazole

Uses: Clotrimazole is used for the treatment of topical infections like tinea, mucocutaneous candidiasis, and vaginal candidiasis. It is not used orally for treatment of systemic infections as it causes severe GIT disturbances.

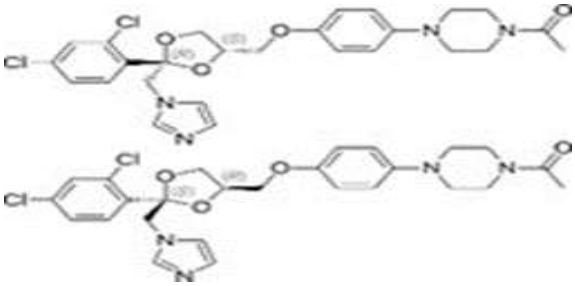
Side effects: Side effects of the oral formulation include itching, nausea, and vomiting. Less than 10% of patients using the oral formulation may have abnormal liver function tests. Side effects include rash, hives, blisters, burning, itching, peeling, redness, swelling, pain or other signs of skin irritation.



Clotrimazole

Ketoconazole

Uses – It is topically in treatment of many fungal infections and orally it is effective in many mucocutaneous and systemic mycoses, or to treat severe cutaneous dermatophytid infections, which do not respond to topical therapy or oral griseofulvin.



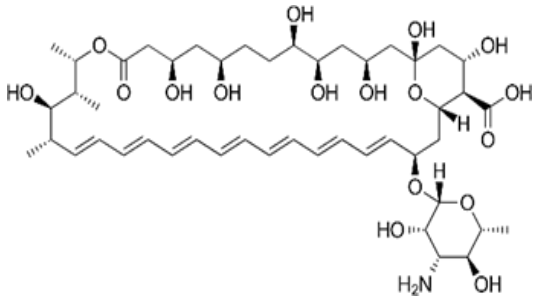
Ketoconazole

Adverse effects

Gastrointestinal: Vomiting, diarrhea, nausea, constipation, abdominal pain, upper abdominal pain, dry mouth, dysgeusia, dyspepsia, flatulence, tongue discoloration may occur.

Amphotericin B

As the name implies, amphotericin is an amphoteric substance containing a primary amino group in the sugar moiety and a carboxyl group attached to the macrolide group. Intravenously, it is indicated for the treatment of serious potentially life-threatening fungal infections & leishmaniasis, also it is used topically for treatment candida albicans. A high prevalence of adverse reactions limits the usefulness of amphotericin B. some forms of nephrotoxicity in nearly 80 % of the patients is the most important adverse reaction.



Amphotericin B

PLANT PROFILE

Figure Neem tree

Literature is enriched with several reports which prove plants as a potential source of bioactive secondary metabolites. Azadirachta indica, commonly known as neem and neem tree, belongs to the family Meliaceae (Figure 1a). In the genus Azadirachta, it is one of two species and easily available all over the world like India, Nepal, Maldives, Sri Lanka, Bangladesh etc. A. indica is easily grown in semi-tropical and tropical regions and shows many activities like antifungal, antiviral, antibacterial, contraceptive, anti-proliferative, antioxidant etc. Its constituents are applied in alternative Ayurveda, Unani, Homeopathy and modern medicinal system. e.g. for the treatment of infections, cancer diseases etc.

Fungal infections are common among all people of all age groups and development of new natural and safe therapeutic antifungal topical preparation is the plan of our study. The aim of the present study was to investigate the antifungal activity of methanolic and ethanolic extract of Ocimum sanctum and Azadirachta indica and to formulate a natural, safe antifungal cream containing the combination of both extract and to evaluate its physicochemical properties.

Ocimum Sanctum

Ocimum Sanctum also helps to prevent cancers caused by toxic compounds by reducing DNA damage and inducing apoptosis in precancerous and cancerous cells, thereby reducing the growth of experimental tumors and enhancing survival Furthermore, tulsi not only protects against the damage caused by toxic compounds, but also enables the body to more effectively transform and eliminate them by enhancing the activity of liver detoxification enzymes such as the cytochrome P450 enzymes, which deactivates toxic chemicals and enables them to be safely excreted. Infection protection

Modern research has revealed that tulsi has anti-bacterial, anti-viral and anti-fungal activity] that includes activity against many pathogens responsible for human infections. Tulsi has also been shown to boost defenses against infective threats by enhancing immune responses in non-stresses and stressed animals and healthy humans. While no human trials have been published, there is experimental evidence that tulsi may help in the treatment of various human bacterial infections including urinary tract infections, skin and wound infections, typhoid fever, cholera, tuberculosis, gonorrhea, acne, herpes simplex, leishmaniasis, various pneumonias and fungal infections, as well as mosquito-borne diseases such as dengue, malaria and filariasis.

MATERIALS AND METHOD

Materials

Excipients

"Smriti College of Pharmaceutical Education, Indore "provided all excipients including Liquid paraffin, Stearic acid, Bees wax, Stearyl alcohol, Glycerin, Tween-80, Methyl parabens, Sorbitol solution, Potassium hydroxide. All reagents and chemicals that were used in the study were of analytical quality.

Fungal species

Candida albicans was obtained from the Department of Microbiology," Smriti College of Pharmaceutical Education, Indore," Madhya Pradesh, India

Nutrient media

Nutrient agar (Hi-media) and Nutrient broth (Hi- Media) was obtained from the "Smriti College of Pharmaceutical Education, Indore" Madhya Pradesh.

Collection of plant material

Leaves of Ocimum sanctum and A. indica were collected from "Smriti College of Pharmaceutical Education, Indore, Madhya Pradesh, India. Authentication of the plant was done, and Voucher specimens were deposited at the Pharmacognosy Department, of "Smriti College of Pharmaceutical Education, Indore, Madhya Pradesh.

Extract preparation

Preparation of ethanolic extract of plant A. indica Fresh A. indica A. Juss (neem) leaves were shade- dried for few days at room temperature and powdered with a grinder. The dried powder of A. indica leaves was mixed with 70% ethyl alcohol and kept at room temperature for 36 hr. The slurry was stirred intermittently for 2 hr and left overnight using mechanical stirrer. The mixture was then filtered, and the filtrate was concentrated using water bath at 50°C and finally dried to form the extract which is kept for phytochemical screening.

Preparation of methanolic extract of plant

Ocimum sanctum

The collected leaves were thoroughly washed and dried in hot air oven at 20 ºC after washing. 50 g of the powder was macerated in 150 ml of methanol for 20 mins. The prepared suspension was stirred using a magnetic stirrer for 3 hr. Then, the methanolic suspension of powder leaves of Ocimum sanctum was kept in standing for 2 days and filtered. The filtrate was kept on water bath at 40 ºC for evaporation to form the extract which is kept for phytochemical screening.

Methods

Formulation development

Formulation of the herbal antifungal cream The formulation trails were done as per formula given in Table 1. The formulation containing

Ocimum sanctum and A. indica were 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, bees 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 phases were heated to 75 ºC 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 Ocimum sanctum and A. indica were incorporated in the emulsion. Gradually the temperature

decreased with continuous stirring and emulsion was formed which was then stored in the airtight wide-mouth container.

Table 1: Formula for preparation of antifungal cream

	Ingredients	F1 Formulation	F2 Formulation	Uses
1	Azadirachta Indica	2.5 gm	2 gm	A.P.I.
2	Ocimum sanctum	2.5 gm	2 gm	A.P.I.
3	Liquid paraffin	2.5 ml	2.5 ml	Softening agent
4	Stearic acid	1.5 gm	1.5 gm	Cleansing Property
5	Bees wax	2.5 gm	2.5 gm	Hydrating agent
6	Stearyl alcohol	5 gm	5 gm	Emollient
7	Glycerin	10 ml	10 ml	Humectant
8	Tween-80	5ml	5ml	Solubilizer
9	Methyl parabens	0.8 gm	0.68gm	Preservative
10	Sorbitol solution	4 gm	4 gm	Moisturizing
11	Potassium hydroxide	3 gm	3 gm	pH Adjuster
12	Distilled water	17 ml	17ml	Diluent

Evaluation of extract (Phytochemical screening)

1. Test for alkaloids (Dragendroffs test) 0.1 ml of extract solution + 1 drop of Dragendroffs reagent. Inference: Orange ppt. shows presence of alkaloid.
2. Test for glycosides (Legal's test) Extract dissolved in pyridine + sodium nitro prusside solution and solution were made alkaline. Inference: Red or pink colour shows presence of glycoside.
3. Test for flavonoids (Shinoda test) Extract + pinch of magnesium + Conc. HCl. Inference: pink color shows presence of flavonoids.

Evaluation of formulation

pH measurement

The determination of pH was done by using digital pH meter. Dissolved one gram cream in 100 ml of distilled water of each formulation (cream i.e. 1% of aqueous solution) and stored for two hrs. The pH measurement of each formulation was done three times and average was calculated.

Homogeneity: All formulation produces uniform distribution on skin, and this was confirmed by visual appearance and by touch.

Consistency: Consistency was estimated by visual detection.

Washability

The ease and extent washing of formulation with water were checked manually after formulations were applied on the skin.

Spreadability

Two sets of the glass slides were taken. The cream was placed over one of the slides and other slide was placed on the top of the cream, such that the cream was sandwiched between the two slides in area occupied by a distance of 6.0 cm along the slide. 100 gm weight was placed upon the upper slide so that cream between the two slides was pressed uniformly to form thin layer. Position of two slides were fixed to a stand without slightest disturbance and in such a way that only the upper slide to slip off freely by the force of weight tied to it. Carefully, 20 gm of weight was tied to the upper slide. The time taken of upper slide to travel the distance of 6.0 cm and separated away from the lower slide under the influence of the weight was noted. This experiment was repeated three times and the mean time taken was calculated. Spreadability was calculated by using the formula:

$$S = M \square L / T$$

Where, S = spreadability; M = weight tied to the upper slide, L = length moved on the glass slide, T = time (in sec) taken to separate the slide.

Antifungal testing fungal species

The test organisms (Candida albicans) were further subcultured at 37 ºC for 24 hrs. The fungus cultures were maintained in their appropriated agar slant at 4 ºC throughout the study and used as stock cultures. Nutrient Media Used Nutrient agar and Nutrient broth (Hi-Media).

Preparation of plates

Two plates were sterilized in hot air oven at 160 ºC for 2 hrs. Out of these, two Petridis were used for preparation of plates using nutrient agar as a media and other four being used for preparation of plate using nutrient agar as a media for well diffusion assay. The antibacterial activity was evaluated by the zone of inhibition (in mm).

RESULTS AND DISCUSSION

Evaluation of extract (Phytochemical screening)

Phytochemical screening of methanolic extract showed presence of alkaloid, glycoside and flavanoid.

Table 2: Phytochemical screening of Ocimum sanctum and A. indica extract:

S. No.	Name of test	Result of extract	
1	Alkaloid	+++	+++
2	Flavonoid	+++	+++
3	Glycoside	+++	+++

Evaluation of Formulation:

The formulated cream was evaluated using various physicochemical parameters. The pH of the formulation ranged from 6.40 to 6.56, which lie in the normal pH range of the human skin.

Spreadability values showed that formulation spread with an ease and the type of smear is non greasy. In stability studies, creams showed no changes in viscosity, pH, spreadability and consistency.

Table 3: Physical evaluation of cream

S. No.	TEST	F1	F2
1	pH	6.57	6.49
2	Colour	Creamy Green	Creamy Green
3	Consistency	Semi-solid	Semi-solid
4	Homogeneity	Homogenous	Homogenous
5	Washability	Good	Good
6	Spreadability	20.18	19.07

Antifungal testing

The results of antifungal activity revealed that the formulation containing methanolic extract of Ocimum sanctum and ethanolic extract of Azadirachta indica leaves 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 (Table 4). It was found that F1 formulation (containing 2.5 gm extract of Ocimum sanctum and Azadirachta indica) (Figure 2a, 2b) has better antifungal action against C. albicans in comparison with F2 formulation (containing 2.0 gm extract of and A. indica)

Table 4: Antifungal testing

Zone of inhibition (mm)	Standard (Griseofulvin)	F1 Formulation	F2 Formulation
	9.7 ± 0.6	9.4 ± 0.4	8.8 ± 0.4

Figure 2a F1 Formulation Azadirachta indica

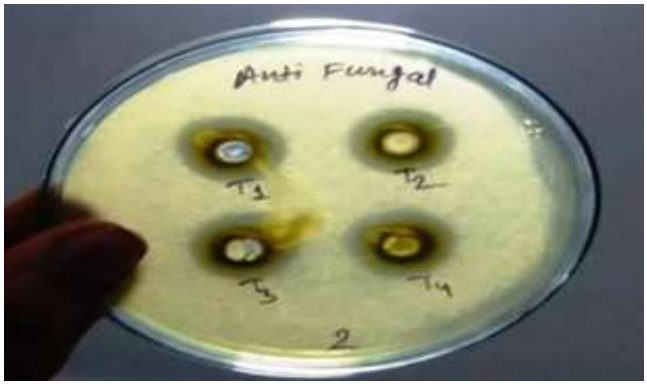


Figure 2b. F2 Formulation Ocimum sanctum

CONCLUSION:

From the overall results, it was concluded that the topical formulation containing ethanolic extract of Azadirachta indica and methanolic extract Ocimum sanctum possess significant 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 alkaloid, glycoside and flavonoid in the extract of Azadirachta indica and Ocimum sanctum and it was present in its leaves and other chemical constituents like which might be in part responsible for antifungal effect against Candida albicans. It was found that F1 formulation has better antifungal action against Candida albicans in comparison with F2 formulation. Further, F1 formulation has showed equivalent antifungal action as standard formulation containing 2.5 gm of Griseofulvin.

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