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PREPARATION AND EVALUATION OF HERBAL ANTIFUNGAL CREAM BASED ON THE SYNERGISTIC ACTION OF *PIPER BETEL* LEAVES AND *AEGLE MARMELOS* LEAVES

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ABSTRACT

Fungal infections of the skin are a common health problem worldwide and can cause discomfort, irritation, and inflammation. Although several synthetic antifungal drugs are available, many of them may cause side effects and resistance after prolonged use. Therefore, the use of herbal medicines with natural antifungal properties has gained increasing attention. The present study focuses on the development of a topical antifungal cream formulated using extracts of *Piper Betel* leaves and *Aegle marmelos* leaves, which are well known for their medicinal properties. *Piper Betel* leaves are widely recognized for their antimicrobial, antifungal, and anti-inflammatory activities due to the presence of bioactive compounds such as phenols and essential oils. Similarly, *Aegle marmelos* leaves possess antimicrobial, antioxidant, and healing properties that contribute to the treatment of skin infections. The combination of these two herbal extracts may enhance antifungal effectiveness and improve skin healing. The formulation of the antifungal cream involved the extraction of plant materials, selection of suitable excipients, and preparation of a stable topical cream. Various physicochemical parameters such as pH, viscosity, spreadability, and stability were evaluated to ensure the quality and effectiveness of the formulation. The prepared herbal antifungal cream showed promising antifungal activity and acceptable physical properties. The combination of *Piper Betel* and *Aegle marmelos* leaf extracts may provide a natural, safe, and effective alternative for the treatment of fungal skin infections.

However, further studies and clinical evaluations are required to confirm its safety and therapeutic efficacy.

KEYWORDS: *Piper betle*, *Aegle marmelos*, Herbal cream, Antifungal activity, Candida albicans, Topical formulation.

1. INTRODUCTION

Fungal infections are one of the deadliest infections accounting in excess of 1.5 million deaths annually worldwide. Mycosis, is a skin disease caused by a fungus and a type of microorganisms. They lead to skin irritations like rashes or bumps. Fungal infections can be caused by like, athletes' foot, ringworm, yeast infections and jock itch. Fungal infections are contagious and spread from one person to another person.[1] Infections caused by fungi are prevalent in the natural world. Fungal infections in humans arise when a fungus colonizes a specific region of the body and becomes challenging for the immune system to combat. Various types of fungi exist, including both beneficial and detrimental ones. Harmful fungi can penetrate the body, making it challenging to eliminate them completely. Moreover, they can persist in the environment and potentially re-infect individuals who are recovering from an infection.[2]

Betel vine (*Piper betle L*.) is a plant belonging to the family *Piperaceae* and the genus Piper. The leaves of the plant contain many bioactive compounds and are used in traditional medicine. Research has shown that the plant has many medicinal properties, such as anticancer, antibacterial, antifungal, antioxidant, anti-diabetic, anti-allergic, anti-malarial, wound healing, gastroprotective, and oral hygiene. The phytochemical screening of the leaves of the betel plant has shown the presence of alkaloids, tannins, glycosides, reducing sugars, and saponins and phenolic and flavonoid compounds in the plant's leaves, which are soluble in water, ethanol, ethyl acetate, acetone.[3]

Bael(*Aegle Marmelos*) shows significant antifungal potential due to bioactive compounds present in different parts of the plant. Acetone extracts of Bael fruit contain coumarin derivatives such as marmesiline, marmelonine, and hydroxysmyrindiol that contribute to antifungal activity. Ethyl alcohol extracts of Bael leaves have also demonstrated strong inhibition against dermatophytic fungi in vitro. Although the specific active compounds were not identified, the findings suggest potential for treating dermatophytosis. Additionally, petroleum ether and methanol extracts of Bael seeds yielded 1-methyl-2-(3′-methyl-but-2′-

enyloxy)-anthraquinone. This compound showed strong antifungal effects against pathogenic fungi like Aspergillus spp. and Candida albicans. Overall, these studies highlight Bael as a promising natural source of antifungal agents.[4]

Fig. 1.1: *Aegle Marmelos* leaves and *Piper Betel* leaves.

2. MATERIALS AND METHODS

Material

The raw materials including *Piper Betel* leaves and *Aegle marmelos* leaves were collected from the local market and authenticated in the Department of Pharmacognosy at the respective institute. The plant materials were washed thoroughly to remove dirt and impurities, shade dried at room temperature, and powdered separately using a grinder. The powders were stored in airtight containers until further use. Additional materials used for the formulation included stearic acid, cetyl alcohol, beeswax, liquid paraffin, glycerin, triethanolamine, methyl paraben, propyl paraben, distilled water, and rose water. All chemicals and reagents used were of analytical grade and obtained from standard suppliers. Nutrient agar, Sabouraud dextrose agar, and fungal cultures such as Candida albicans and Aspergillus niger were used for antifungal evaluation.[3,4]

METHODOLOGY

Sample Collection and Pre-treatment

Fresh leaves of *Piper betle* and *Aegle marmelos* (Beal Leaf) were collected and authenticated, then washed with distilled water. The leaves were shade dried, coarsely powdered and stored in airtight containers for further use.

2.1 Evaluation tests for *Piper Betel* extract[5]

2.1.1. Test for Alkaloid

Dragendroff's Test:-To 1 ml of the extract, a few drops of concentrated hydrochloric acid were added, followed by Dragendroff's reagent. The appearance of a reddish brown colour confirms the presence of alkaloids.

2.1.2. Test for Tannins

Ferric chloride test:- 1 ml of the extract was treated with an equal volume of freshly prepared 10% ferric chloride solution. Formation of a greenish-black colour confirmed the presence of tannins.

2.1.3. Test for saponins

Foam Test:- 1 ml of the extract was mixed with 5 ml of distilled water in a test tube and shaken vigorously. Formation of foam confirmed the presence of saponins.

2.1.4. Test for Terpenoids

In 5 ml of the extract, 2 ml of chloroform and 3 ml of concentrated sulphuric acid added along the side of the test tube to form a separate layer. The appearance of a reddish-brown precipitate at the interface confirms the presence of terpenoids.

Fig. 2.1: Phytochemical Test for *Piper Betel* Leaf Extract/

2.2 Evaluation tests for *Aegle marmelos* (Bael) Leaf extract[4]

2.2.1. Test for Alkaloid

Dragendroff's Test:- To 1 ml of the extract, hydrochloric acid was added followed by 6 drops of Dragendorff's reagent. Formation of a brownish-red precipitate indicates the presence of alkaloids.

2.2.2. Test for Glycosides

Keller-Kiliani test:- To 2 mL of the filtrate, 1 mL of glacial acetic acid containing a few drops of ferric chloride was added. Concentrated sulphuric acid was then added carefully along the side of the test tube. Formation of a brown ring at the interface or a greenish-blue colour indicates the presence of glycosides**.**

2.2.3. Test for saponins

Foam Test:- To 2 mL of the extract, 5 mL of distilled water was added and shaken vigorously for 2 minutes. Stable foam confirms the presence of saponins.

2.2.4. Test for Tannins

Ferric chloride test:- To 2 mL of the extract, 10 mL of distilled water was added and boiled.

The mixture was filtered, and a few drops of ferric chloride were added to the filtrate. Formation of a greenish-black precipitate indicates the presence of tannins.

2.2.5. Test for Flavonoids

Shinoda Test:- To the extract, add 5 ml of 95% ethanol or t-butyl alcohol, followed by a few drops of concentrated hydrochloric acid and 0.5 g of magnesium turnings. Development of orange, pink, red, or purple colour indicates the presence of flavonoids.

Fig. 2.2.: Phytochemical Test for *Aegle Marmelos* Leaf Extract.

2.3. Extraction of *Piper Betel* Leaves and *Aegle Marmelos* Leaves

Piper Betel

Cold maceration is a simple and widely used extraction technique for isolating bioactive

elements from plant in the absence of heat. This method is particularly suitable for thermolabile constituents that may degrade at higher temperatures. In the present study, leaves of P.betel were subjected to cold maceration using a suitable solvent to ensure efficient extraction of phytoconstituents such as phenolics, flavonoids, and essential oils. The finely powdered plant material was soaked in the solvent for a specified duration with intermittent shaking to facilitate maximum diffusion of active components into the solvent. This method preserves the integrity of sensitive compounds and provides a crude extract suitable for further pharmacological evaluation.[5]

Aegle Marmelos

Soxhlet extraction is a continuous hot extraction method widely employed for the efficient isolation of phytochemicals from plant materials. This technique allows repeated washing of the sample with fresh solvent, ensuring maximum extraction efficiency. In this study, dried leaves of A.marmelos were subjected to Soxhlet extraction using an appropriate organic solvent. The process involves heating the solvent to reflux, followed by condensation and percolation through the plant material in a cyclic manner. This method is particularly effective for extracting compounds such as alkaloids, tannins, and glycosides. Soxhlet extraction enhances yield and ensures thorough extraction, making it suitable for obtaining concentrated plant extracts for further analysis.[8]

Fig.2.3 Cold Maceration of *Piper Betel.*

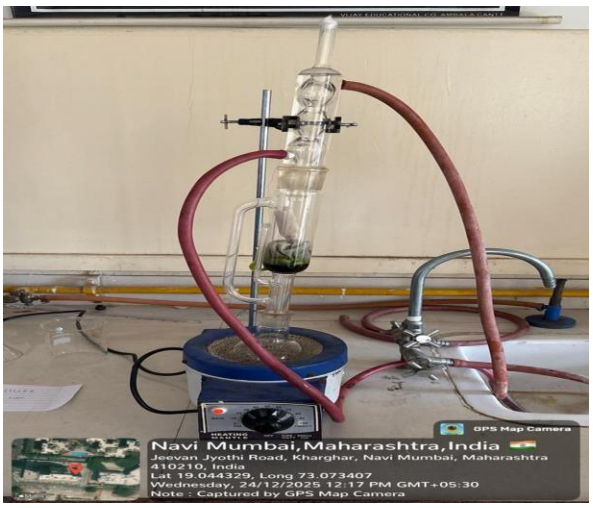


Fig.2.4 Soxhlet Extraction of *Aegle Marmelos.*

2.4 Procedure for *Piper Betel* and Aegle Marmelos Combined Cream Preparation

Table 2.1.: Materials Required.[5,6]

Active Ingredients	Base Ingredients	Eqipments Required
1. *Piper Betel* 2. *Aegle Marmelos*	1. Stearic Acid	1. Beaker (250ml /500 mL)
	2. Beeswax	2. Magnetic Stirrer
	3. Cetyl Alcohol	3. Water Bath
	4. Liquid Paraffin	4. Weighing Balance
	5. Glycerin	5. Glass rod/Stirrer
	6. Triethanolamine	6. Spatula
	7. Methyl paraben	7. Pipettes
		8. Containers for storage

Procedure of Cream Preparation[5,6]

1. Collection of Materials

Procurement of all required ingredients and equipment.

2. Weighing of Ingredients

Active Ingredients

- *Piper Betel* extract
- *Aegle marmelos* extract

3. Preparation of Oil Phase

Stearic acid, beeswax, cetyl alcohol, and liquid paraffin were melted together using a water bath.

4. Preparation of Aqueous Phase

Glycerin, triethanolamine, methyl paraben, and herbal extracts were mixed thoroughly.

5. Heating of Both Phases

Both the oil phase and aqueous phase were heated separately to the same temperature.

6. Combining of Phases

The aqueous phase was gradually added to the oil phase with continuous stirring using a magnetic stirrer to obtain a W/O (water-in-oil) emulsion.

7. Formation of Smooth Cream

Continuous stirring was carried out until a smooth and uniform cream was formed.

8. Cooling of Cream

The prepared cream was cooled at **37**°C (room temperature)

9. Transfer into Containers

The final prepared cream was stored into airtight containers.

Table 2.2 Formulation of Antifungal Cream(3 Batches)

Ingredients	Batch A	Batch B	Batch C	Uses
Piper Betel	0.5 g	0.5 g	1.0 g	Anti-fungal agent
Aegle Marmelos	0.5 g	1.0 g	0.5 g	Anti-fungal agent
Stearic acid	0.95 g	0.95 g	0.95 g	Emulsifier, thickener
Beeswax	1.0 g	1.0 g	1.0 g	Emollient
Cetyl alcohol	0.38 g	0.38 g	0.38 g	Emollient, emulsion stabilizer
Liquid paraffin	5 ml	5 ml	5 ml	Occlusive moisturizer
Glycerin	0.47 ml	0.47 ml	0.47 ml	Humectant
Triethanolamine	0.15 ml	0.15 ml	0.15 ml	pH adjuster, emulsifier
Methyl paraben Propyl Paraben	0.01 g	0.01 g	0.01 g	Preservative
Distilled water	1.8 ml	1.8ml	1.8 ml	Solvent/base

2.5 Evaluation tests for antifungal cream

2.5.1 Physical examination and Organoleptic Properties

This is the first step in the evaluation to make sure everything is the same.

Colour: Should be the same (probably a light greenish because of the herbal extracts). Smell: It should smell like the extracts.

Texture: It must be smooth and not have any visible grittiness or phase separation (when oil and water split).[1,2]

2.5.2 Finding the Ph

Because this is an antifungal cream, the pH needs to be safe for human skin so it doesn't cause irritation.

For that, add some of the cream with distilled water and use a digital pH meter.

Target: The pH should be between 5.0 and 6.5, which is like the skin's natural "acid mantle."

2.5.3 Ability to spread

The cream's spread ability tells you how easy it is to put on the affected area. Better spread ability makes sure that the herbal actives cover the fungal infection evenly.

Testing: The "Wooden Block and Glass Slide" method is the most common way to do this. It is shown as the amount of time (in seconds) it takes for two slides to come apart when a certain load is on them.[1]

Formula: S = (MxL) / T Where,

M: weight applied L: Length of slide T: Time taken

2.5.4 Washability Test

This test figures out what kind of cream dealing with, is the cream an Oil-in-Water or a Water-in-Oil emulsion and how the cream is going to behave when the patient tries to wash the cream off.

Procedure: Add a bit of the cream on the back of hand. Then wash it with tap water.

Inference: If the cream comes off easily with water and no soap then the cream is a Waterwashable cream, which is also known as an Oil-in-Water cream. This kind of cream is really good, for treating fungus because it does not stop air from getting to the area, which is avoided because do not want the infected area to be suffocated by the cream.

2.5.5 The Dye Solubility Test Procedure

Use Sudan III for this. It is a dye that can dissolve in oil.

To do the test need to mix a bit of the *Piper betle* and *Bael leaf* cream with a few drops of the dye, on a glass slide.

Then look at it under a microscope. We put a coverslip over the mixture. See what the dye does to the cream.

2.5.6 Anti-fungal Assay of Cream Materials Required

- Formulated antifungal cream
- Fungal culture (e.g., Candida albicans)
- Sabouraud Dextrose Agar (SDA)
- Nutrient broth
- Sterile Petri plates
- Sterile cork borer / wells
- Micropipette

- Sterile saline or DMSO (for dilution)
- Incubator (25–37°C)

Procedure

1. Preparation of Sample

Accurately weigh a known quantity of cream. Dissolve/disperse in a suitable solvent (e.g. Ethanol) to prepare a stock solution. Perform serial dilutions to obtain different concentrations.[6,7]

2. MIC Determination (Broth Dilution Method)

Prepare a series of test tubes containing sterile broth of 1 ml each.

↓

Add serial concentraton in to each test tube of 100,50,25,12.5 and control.

↓

Inoculate each tube with a standardized Candida Albicans suspension. Incubate at 25–37°C for 24–48 hours in an Incubator.

↓

Observe turbidity in each test tube Clear solution → No growth; Turbid → Growth present.

↓

The lowest concentration showing no visible growth is recorded as the MIC

Fig.2.5 Broth dilution of *Piper Betel* extract.

Fig.2.6 Broth dilution of *Aegle Marmelos* extract.

3. Zone of Inhibition (Agar Well Diffusion Method)

Prepare SDA agar plates and allow them to solidify. Inoculate the surface uniformly with fungal culture. Use a sterile cork borer to make wells in the agar. Add different concentrations of cream into each well. Incubate plates at 25–30°C for 24–48 hours. Measure the diameter of the clear zone (mm) around each well.[7]

Fig 2.7 ZOI exhibited by *Aegle marmelos* leaf extract against the tested bacterial strains using the agar well diffusion method.

Fig 2.8 ZOI exhibited by batches of herbal cream against candida albicans using the agar well diffusion method.

Fig 2.9 ZOI exhibited by *Piper Betel* leaf extract against the tested bacterial strains using the agar well.

In the present study, Antifungal activity of cream was done in a batches of three such as A,B,& C and is done in comparision with each other by observing the clear zone around the wells and selecting the one with highest zone of inhibition. It was tested against Gram positive Candida Albicans that shows optimistic effect for antifungal activity. The potency or activity is accessed by observing zone of Inhibition values as it is different for different concentrations such as first with 1:1, then by 1:2[*Aegle Maemelos* dominant] & 2:1[*Piper Betel* dominant]. After performing zone of inhibition it was observed that Batch C [2:1] shows highest clear zone around wells and the concentration is selected for final cream preparation. Also the final formulated cream was tested against standard drug clotrimazole and zone of inhibition was observed.

2.6 Franz Diffusion Study

In the Franz diffusion method, a fresh onion bulb is selected and its thin inner membrane is carefully peeled using forceps. The membrane is washed with distilled water and kept hydrated before use. It is mounted between the donor and receptor compartments of the Franz diffusion cell without folds or air bubbles. The receptor compartment is filled with phosphate buffer solution and maintained at 37±0.5°C with continuous stirring. A measured amount of formulation is applied to the donor compartment. Samples are withdrawn from the receptor compartment at predetermined intervals, replaced with fresh buffer, and analyzed using UV spectrophotometry to determine drug diffusion.[10]

The permeation profile of the antifungal herbal cream revealed gradual diffusion of active constituents through the membrane over a period of 60 minutes. Initial high absorbance values indicate rapid release of phytoconstituents from the cream base. The sustained diffusion pattern observed between 20–40 minutes may be due to controlled release of active compounds from the formulation matrix.

Fig. 2.10: Franz Diffusion Instrumentation.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Phytochemical screening of the ethanolic extracts of *Piper betle* and *Aegle marmelos* leaves revealed the presence of various bioactive constituents such as:

Alkaloids, Flavonoids, Tannins, Phenolic compounds, Glycosides, Saponins

These phytoconstituents are mainly responsible for the antifungal and antimicrobial activities of the herbal formulation. Phenolic compounds such as eugenol and chavicol present in Piper

betle contribute significantly to antifungal action. Flavonoids and tannins present in Aegle marmelos also possess antimicrobial and antioxidant properties.

3.2 Evaluation Tests of Cream

3.2.1 Physical Examination

The formulated herbal cream was evaluated for physical parameters such as colour, odour, consistency, texture, and homogeneity. The cream showed smooth texture, pleasant odour, good consistency, and homogenous appearance without any phase separation. Smoothness was confirmed by rubbing the cream between the fingers.

Table 3.1: Physical Properties of herbal cream.

Formulation	Appearance	Odour	Texture
Piper Betel and *Aegle marmelos* Antifungal Herbal Cream	Smooth, greenishbrown semisolid cream with uniform consistency	Characteristic herbal odour with mild aromatic smell	Soft, smooth, nongritty, and easily spreadable texture

3.2.2 Determination of pH

The pH of the formulated cream was found to be 6.5, which is suitable for topical application and compatible with normal skin pH. The formulation did not show any signs of irritation or redness on application.

3.2.3 Determination of Spreadability

The spreadability of the formulated cream was found to be 1.3 g/cm/sec, indicating good spreading property and easy application on the skin surface.

3.2.4. Dye Test

The dye test indicated that the cream was of water-in-oil (W/O) type, as the globules appeared colourless and the ground appeared to be coloured.

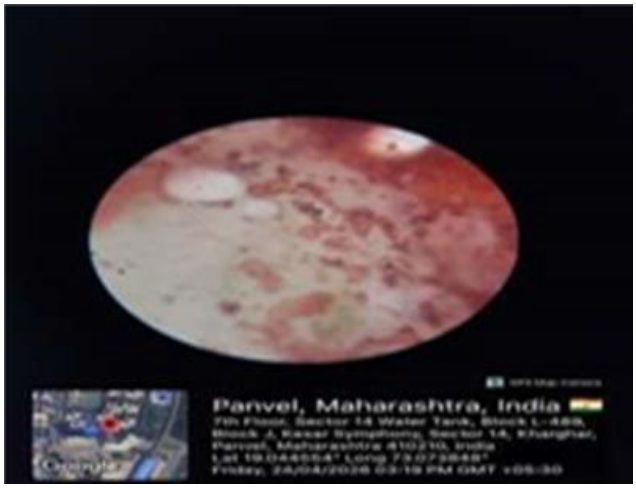


Fig. 3.1: Dye Test.

Table 3.2: MIC[Minimum Inhibitory Concentration] Observation Table.

Concentration (µg/ml)	**Growth or Turbidity(+/-)**	
	Piper Betel	*Aegle Marmelos*
100	_	_
50	_	_
25	_	+
12.5	+	_
control	+	+

Table 3.3: ZOI [Zone of Inhibition]Observation table.

Concentration	**Zone of Inhibition (mm)**
50:50	30 mm
1:2	25 mm
2:1	40 mm

Table 3.4: ZOI of Formulated Cream against Standard.

Inhouse formulated cream – 30 mm
Standard – 25 mm
Control – 0 mm

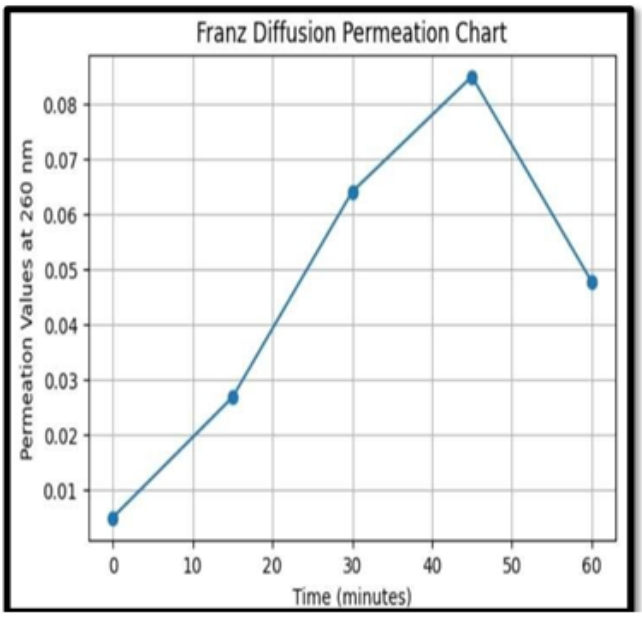
4.5. Franz Diffusion Study

The permeation graph at 260 nm showed an initial low drug release at 0 minutes with a value of 0.0050. A gradual increase in permeation was observed at 15 minutes (0.0268), indicating the beginning of diffusion through the membrane. The permeation significantly increased at 30 minutes (0.0641), showing enhanced drug release. Maximum permeation was observed at 45 minutes with a peak value of 0.0850, indicating optimal diffusion during this period. However, a notable decrease was seen at 60 minutes (0.0478), which may be due to reduced concentration gradient, saturation of the membrane, or depletion of the drug from the

formulation. Overall, the graph demonstrates a sustained permeation profile followed by a decline after reaching peak release.

Table 3.5

Time (min)	Cream(at 280nm)	% Drug Release
0 min	0.0050	5.88%
15min	0.0268	21.53%
30min	0.0641	65.41%
45min	0.0850	88.99%
60min	0.0478	46.25%



The study confirms that the cream formulation provides satisfactory permeation and sustained release characteristics suitable for topical antifungal therapy.

4. CONCLUSION

The present study successfully formulated and evaluated an antifungal herbal cream containing *Piper betle* and *Aegle marmelos* leaf extracts. The formulation exhibited good physicochemical properties including smooth texture, homogeneity, satisfactory spreadability, and suitable pH of 6.5 for topical application.

Phytochemical analysis confirmed the presence of bioactive compounds such as flavonoids, tannins, alkaloids, eugenol, and chavicol which are responsible for antifungal activity. The antifungal evaluation demonstrated significant inhibition against Candida albicans, indicating strong therapeutic potential of the herbal formulation.

The combination of *Piper betle* and *Aegle marmelos* extracts showed better antifungal activity compared to individual extracts due to synergistic interaction of phytoconstituents. The Franz diffusion study further confirmed sustained release and satisfactory permeation of active constituents from the cream base.

Therefore, the formulated herbal cream can be considered a promising, safe, economical, and effective alternative to synthetic antifungal creams for the treatment of superficial fungal infections. Further clinical studies may help establish its therapeutic efficacy and commercial application in topical antifungal therapy.

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