

# Formulation and Evaluation of Antifungal Pelargonium graveolens Soap

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**Abstract:** Skin disorders are most serious public health issues because they affect both peoples and communities. Fungi grows as a yeasts, molds or a combination of both. Antifungal medicines treat fungal infections. Fungus in the soil, air and on your skin can cause yeast infections, ringworm, and nail and skin infections. Antifungal medication includes soaps, creams, ointments, gels, powders and oral medications which can treat fungal infection of our skin. The herbal medication like Pelargonium graveolens essential (Geranium) oil are used for Research project because it relieve itchy, dry, flaky skin. Antifungal soaps are formulated with the Geranium oil which helps to prevent the fungal infections. It contains ingredients that make your skin resistant to infections. It is having property of Antifungal which can reduce the growth of fungus and treat fungal infections. This formulation applying easily on our affected area of our body. Geranium oil contains Geraniol and citronellol oil in it which can be give effective results on infectious tissues. Using procedure we formulate the antifungal geranium soap and perform the physical parameter and evaluation tests. After all we can concluded that, the P. graveolens can influence the yield of oils, their chemical composition as well as their Antifungal, Antioxidant and Antibacterial activities.

**Keywords:** Pelargonium graveolens, Citronellol, Geraniol, Antifungal, Soap

## I. INTRODUCTION

We generally knows that the fungal infections due to fungus are mainly occurring.

Which is very dreadful, dangerous infection it can spread all over body by itching. Itching is an uncomfortable sensation on the skin that causes a desire to scratch.

Skin is the largest organ. It protects our body against germs. The skin, also known as the cutaneous membrane, protects the body's external surface. It also regulates body temperature and enables touch sensations like hot and cold. Along with our hair, nails, oil glands and sweat glands is a part of the integumentary system. Integumentary means outer covering of the body.

The skin is the exposed area of our body to the pollutants, sun, viruses and various fungal agents while this providing some protection.

Eczema, acne, rashes, psoriasis, allergies, ringworm, fungal infection and other infections are most frequent.

Skin disorders are most serious public health issues because they affect both peoples and communities.

Layers of skin includes 3 parts in it:

- Epidermis
- Dermis
- Hypodermis

### Epidermis

It is the top most layer of skin that can we see and feel it touch it. In this layer, new skin cells development occurs and it also contains the keratinocytes stem cells. Keratin is present in thus layer which is a protein inside the skin cells, makes up the cells, proteins sticks together to form Epidermis layer. It also contains melanocytes which are responsible for producing melanin which produce the pigment for your epidermis.

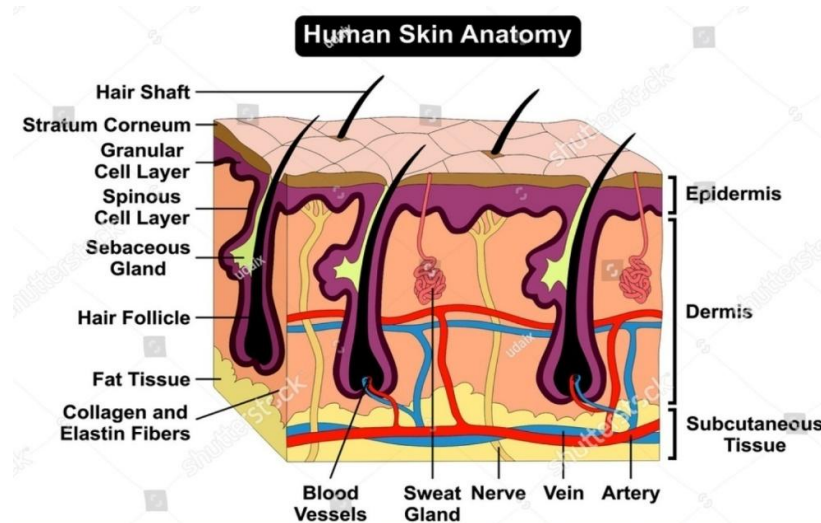


Fig.1: Human Skin Anatomy

#### Stratum Basale

**Location:** Deepest Layer of the epidermis.

**Function:** New skin cells develop in this layer, and it also contains the keratinocyte stem cells, which produce the protein keratin. Keratin helps form hair, nails and your skin's outer layer, which protect you from the harsh environment. It also contains melanocytes, which are responsible for producing melanin, which provides the pigment of your epidermis.

#### Stratum spinosum

**Location:** Between the stratum basale layer and the stratum granulosum layer.

**Function:** This layer mostly consists of keratinocytes held together by sticky proteins called desmosomes. The stratum spinosum helps make your skin flexible and strong.

#### Stratum granulosum

**Location:** Between the stratum spinosum layer and the stratum lucidum layer.

**Function:** Keratinocytes have granules within them, and in this layer they're visible under a microscope.

#### Stratum lucidum

**Location:** Between the stratum granulosum and the stratum corneum.

**Function:** The stratum lucidum is a thin, transparent layer of keratinocytes that are becoming less round and have a flatter shape.

#### Stratum corneum

**Location:** The top layer of the epidermis.

**Function:** This is the layer of the epidermis that you see. In the stratum corneum, keratinocytes become corneocytes. Corneocytes are strong, dead keratinocytes, and they protect you from harm, including abrasions, light, heat and pathogens. This layer also consists of fats that keep water from easily entering or leaving your body. The corneocytes eventually shed as new keratinocyte cells develop in the stratum basale layer and move through the other layers of skin.

**Dermis:** The dermis is a connective tissue layer sandwiched between the epidermis and subcutaneous tissue. The role of the dermis is to support and protect the skin and deeper layers, assist in thermoregulation, and aid in sensation. The dermis is divided into two layers: the papillary dermis and the reticular dermis.

**Hypodermis:** The hypodermis is also known as the subcutaneous tissue which is innermost tissue. The hypodermis protects your skeletal system, organs, muscles and tissues from harm.

It is the bottom layer of your skin, located below the epidermis (top layer) and dermis (middle layer) in your skin. The hypodermis is yellow in colour due to presence of pigment of carotene.

### **What is a fungus?**

Fungi grows as a yeasts, molds or a combination of both.

They can reproduce through very tiny spores. These spores can exist in soil or become airborne.

Naturally occurring fungi, like Candida yeast, in your body. Fungi live on your skin, inside your digestive system and vaginal area (part of the female reproductive system).

### **Who is at risk for fungal infections?**

Any person can get affected by fungal infection. Most fungi are easily treatable and they do not have any harmful effects mostly.

People who have damaged immune systems are more likely to develop serious fungal infections. These infections are called opportunistic infections. It can be life-threatening for people who have:

AIDS,

Autoimmune diseases like lupus,

Cancer,

Organ transplants, Stem cell (bone marrow) transplants.



Fig.2: Antifungal Infection (Ringworm)

### **Antifungals**

Antifungal medicines treat fungal infections. Fungus in the soil, air and on your skin can cause yeast infections, ringworm, and nail and skin infections. Breathing in fungal spores can lead to respiratory illnesses. People who have weak immune systems are more prone to fungal infections that require antifungal medicine.

Antifungals are medicines that kill or stop the growth of fungi (the plural of fungus) that cause infections. They are also called antimitotic agents.

### **Fungal infections can affect the:**

Circulatory system.

Respiratory system.

Skin and nails.

### **Antifungal medications:**

It includes soaps, creams, ointments, gels, powders and oral medications which can treat fungal infection of our skin.

During the initial stages of a fungal infection, regular washing with a medicated soap is vital. If you have dry skin or deal with other skin issues like acne, choose an antifungal soap to suit your skin type. Pick soaps with moisturizing properties in addition to medicinal ingredients if you have dry skin or eczema.

Antifungal soaps contain medicinal ingredients to treat fungal infections. Conventional antifungal medications include ketoconazole and miconazole. Both are part of a larger class of drugs called azole antifungals. These ingredients kill fungal cell membranes in order to eradicate infections.

The fungi or fungal infection in the skin, as is also known, is a condition that generates itching, redness, and scaling skin. They can appear in any area of our body, but it is especially common on the feet and hands.

Although it is mainly an aesthetic problem, it can cause more severe conditions if we do not treat it appropriately.

That is why here we recommend some anti-fungal soap options that you can add to your daily hygiene routine to heal and prevent any breakout on your skin.

#### **Anti-fungal soap with natural ingredients**

- Relieve itchy, dry, flaky skin with aloe vera's help and an organic blend of coconut, olive, and jojoba oils.
- Each one has its healing properties that hydrate and soften your skin.
- This unique tea tree blend fights stubborn fungi and bacteria, helps with fungal and bacterial infections.
- It also deodorizes and refreshes smelly feet and body odors.

## **II. INTRODUCTION TO SOAPS**

Soap is a salt of fatty acid which can be used as variety of cleansing and lubricating products and it is made by mixing of fats and oils with base of soap by combining chemical compounds in a mixer.

Soap is produced by saponification or basic hydrolysis reaction of a fat or oil.

Soaps are generally penetrating the drug substance to the inside of skin. Made of rare herbs and 100% natural ingredients, herbal soaps are found to be highly beneficial for the skin.

Soaps also contain glycerine, which is generally not used in commercial soaps. Glycerine helps in retaining the moisture in the skin thereby making these soaps for dry skin conditions. Herbal soap preparations are medicines or drugs which contain anti-bacterial & anti- fungal agents which mainly uses parts of plants such as like leaves, stem, roots & fruits for treatment for an injury or disease or to achieve good health.

#### **Hydrolysis Reaction**

Fat or oil + NaOH gives Glycerol + Sodium salts of fatty acid

Soap preparation is a medicine or drugs it contain Antibacterial & antifungal agents which e mainly uses of part of plants such as like leaves, stem, roots & fruits to treatment for a injury or disease or to achieve good health. Soaps have therapeutic and healing characteristics that offer specific benefits to the skin, such as nourishment, strength, healing, and moisturizing.

#### **Advantages of Antifungal Soaps:**

- Antifungal soaps contains ingredients that make your skin resistant to infections.
- It also boost overall health of patient.
- This are tough on germs and gentle to your skin.
- This soaps kill the germ particles that produce bad body odor.
- Soaps are mainly used to reduce itching and treat fungal infections.
- Soaps are easy to preparations and easy to apply on our skin.
- Antifungal property showing soaps are greatly effective on the skin fungal infections.
- It having stability of formulation and release of antifungal properties from soap.

#### **Ideal properties of Geranium oil formulated as antifungal soap:**

- It is having property of Antifungal which can reduce the growth of fungus and treat fungal infections.
- It also having properties like antiseptic and antibacterial.
- It is the essential oil used widely in food and flavour industries, Fragrance and cosmetic industries.
- It is best skincare oils because it is good for opening spores and cleaning oily complexes.



- The most interesting health benefits of this oil is includes its ability to lower stress and reduce swelling and boost our immune system.
- It is aromatic plant important for producing oils, this oils which is highly priced for its very profound and strong odour.
- This oil also helps to treat the heavy menstrual flows and menopause problems.
- It having properties like cell growth supporter, muscle toner and wrinkle reducer.

**Taxonomical Classification of Geranium:**



Fig.3: Geranium plant (Flowers, Stem, Leaves)

- Part used: Leaves, Stem, Flowers.
- Domain: Eukaryote
- Kingdom: Plantae
- Phylum: Tracheophytes
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Geraniales
- Family: Geraniaceae
- Genus: Pelargonium
- Species: P. graveolens

**Taxonomical Classification of Neem:**



Fig.4: Neem plant leaves

- Part used: Leaves, Fruits.
- Domain: Eukaryote
- Kingdom: Plantae
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Order: Sapindales
- Family: Meliaceae
- Genus: Azadirachta
- Species: Azadirachta indica

### III. MATERIAL AND METHODS

Selection of Herbal Essential Oil:

Geranium oil was selected by its Antifungal properties so it is very effective against fungi to prevent fungal infections, so I would like to select this plant for project.

Collection of the Essential Oil from Industry:

The Geranium oil was collected from the small scale Industry of Geranium oil A/P. Hapusbag, Tal. Junnar, Dist. Pune.

Formulation of Antifungal soap:

**Important constituents of Geranium Antifungal soap:**

1. **Aqueous Material:** This forms the aqueous phase of the soap, commonly used agents are Water and Alcohol.
2. **Oils:** Olive oil, Coconut oil, Castor oil, Palm oil, Neem oil and Geranium essential oil are widely used as vehicles for soap preparation.

**Olive oil** is used as Perfect skin moisturizer. Also having Antioxidant properties, Anti-inflammatory properties. It can be used to Relieves eczema and psoriasis. It is Extra mild and gentle in soap and Helps with acne.



Fig.5: Olive oil

**Coconut oil** is used on skin conditions for treating eczema psoriasis and other skin infections. It also help to reduce stretch marks and give soothing effect on inflamed skin, reduce redness, rehydrates skin. It is Saponifying agent.



Fig.6: Coconut oil

**Castor oil** is Superfating agent which is rich in ricinoleic acid which helps in fighting the skin bacteria that cause acne. It easily penetrates the skin layers and makes the skin smooth, supple to vitalize it naturally.



Fig.7: Castor oil



**Palm oil** is used for cleansing agent and it also helps to hardening the soap.



Fig.8: Palm oil

**Neem oil** is having antibacterial properties. It is used in soap for reduce bacterial infections and treat skin conditions.



Fig.9: Neem oil

**Geranium essential oil** is the essential oil extracted through steam distillation is used in fragrance flavor and pharmaceutical industries. The geranium oil having property like antibacterial, anti fungal and antiseptic. The pure geranium oil is almost perfume and it widely used for scenting soaps due to its stability in alkaline medium.



Fig.10: Geranium essential oil

**3.Hardening agent:** Hardening agent is the agent which gives proper shape to the formulation and Chemical agents used to increase strength and hardness of the formulations are called hardening agents. In this formulation of Geranium Antifungal soap, Stearic acid is used as hardening agent which having hardening property.





Fig.11: Stearic acid

**Lye:** A lye is a metal hydroxide mainly obtained by leaching ashes. It is a strong alkali which is highly soluble in water producing caustic basic solutions. "Lye" is commonly another name of sodium hydroxide (NaOH). In a formulation of soap sodium hydroxide is used as lye.

**Humectants:** Humectants are substances that attract water from the air or from deeper in the skin. In formulation of soap, Glycerine and propylene glycol is used as humectants.

**Moisturizing agent:** Glycerin Soap Base are enriched with moisturizing and nourishing qualities of Glycerin. It acts as a skin moisturizer and soothing agent for dry skin. It can be made with pure glycerine. It is a great emollient which is used in soaps and is highly effective for smoothing the skin and reducing inflammations and itching.



Fig.12: Glycerin soap base

**Surfactant:** Surfactants in soap help reduce water surface tension and allow oil to easily wash away. In this, SLS i.e. Sodium Lauryl Sulphate is used as a surfactant.



Fig.13: SLS

**Colouring agent:** Colouring agent is used for giving colour to the formulation. In this formulation, cochineal is used to give colour to soap. Table.1: Ingredients used for formulation of Antifungal Soap

Sr. No.	Name of Ingredients	Scientific Name	Quantity (100 gm)	Uses
1	Olive oil	Oleaeuropaea L.	0.92 ml	effective against yeasts and molds
2	Coconut oil	Cocosnucifera	0.73ml	help treat ringworm infections
3	Castor oil	Ricinuscommunis	3.07ml	acts as an antifungal agent that kills the infection.
4	Palm oil	Elaeis	2.227ml	Protect against fungi.
5	Glycerine soap base	-----	60gm	Dermatitis, dry skin, psoriasis.
6	Geranium oil	Pelargonium graveolens	12.63 ml	highest antifungal potential.
7	Neem oil	Azadirachtaindica	28.4 ml	prevent or even kill fungus.
8	Stearic acid	octadecanoic acid	1 gm	helps to harden products.
9	Sodium lauryl sulphate	Sodium dodecyl sulfate	1 gm	helps to make soaps 'froth'.

**Procedure:**

1. Firstly, accurately weigh all ingredients, oils properly.
2. Take a Beaker on water bath add in beaker glycerine soap base and stir it.
3. Then create your soap base with glycerin and add Coconut oil, Olive oil, Castor oil, Palm oil accurately according to their amounts.
4. Add 1 gm of stearic acid and 1 gm of Sodium lauryl sulphate.
5. After mixture mixed properly then add Neem oil and Essential oil i.e. Geranium oil.
6. After that pour it into soap mould and tap them to set down.
7. Then keep in refrigerator, for about 2-4 hrs.
8. Prepared Soap was packed into a suitable container box, labeled and used for further studies.

**Prepared Antifungal Soap:**

The Antifungal Granium soap was prepared by using appropriate procedure. This soap helps to prevent our body against any type of fungal infections.



Fig.14: Prepared Antifungal Geranium soap using different moulds

### Evaluation tests for Antifungal Geranium Soap

Physical parameters: Clarity and color was checked by naked eyes against white background, the odor was smelled.

Evaluations: The herbal soap formulated was evaluated for the following:

1. Organoleptic evaluation:
  - i. Color : Brown
  - ii. Odor : Dark rose like fragrance
  - iii. Appearance : Good

pH:

The pH of all the prepared formulations was determined by using digital pH meter. The formulations were dissolved in 100 ml of distilled water and stored for two hours. The measurement of pH of formulation was done in previously calibrated pH meter.

Determination of percentage free alkali:

About 5 grams of sample was taken in a conical flask and added to it into 50ml of neutralized alcohol. It was boiled under reflux on water bath for 30minutes, cooled and 1ml of Phenolphthalein solution was added it was then titrated immediately with 0.1N HCL.

Accelerated stability testing:

Accelerated stability testing of prepared PHF was at room temperature, studied for one week at  $50^{\circ}\text{C} \pm 1^{\circ}\text{C}$  for 3 months. The PHF were kept both at room and elevated temperature and observed on 0th 15th, 20th, 30th, 40th, 50th, 60th, 70th, 80th and 90<sup>th</sup> day for the following parameters.

Foam Retention:

25 ml of the 1% soap solution was taken in to a 100 ml graduated measuring cylinder. The cylinder was covered with hand and shaken 10 times. The volume of foam at 1 minute intervals for 4 minutes was recorded.

Foam Height:

0.5 grams of sample of soap was taken dispersed in 25 ml distilled water. then, transferred it in to 100ml measuring cylinder; volume was make up to 50 ml with water. 25 strokes were given and stand till aqueous volume measured up to 50 ml and measured the foam height, above the aqueous volume.

Alcohol Insoluble Matter:

5gm of sample was taken in a conical flask. Added it to 50 ml of warm ethanol and shaken vigorously to dissolve the solution was filtered through a tarred filter paper with 20 ml warm ethanol and dried it at  $105^{\circ}\text{C}$  for one hour. The weight of dried paper was taken.

**Formula:**

$$\text{Percentage alcohol insoluble matter} = \frac{\text{Weight of the residue} \times 100}{\text{Weight of sample}}$$

## IV. ANTIFUNGAL ACTIVITY STUDIES

### Determination of Minimum Inhibitory Concentration (MIC) of the oil and the aromatic constituents:

This was determined following the tube dilution method reported earlier. Different volumes of oil were mixed with sterile, cooled molten SDA supplemented with 0.75%(w/v) of sodium taurocholate to give a concentration of 1.95-1000 by two fold serial dilution method. The statistical analysis of the MIC values from three experiments was carried out by using students t-test.

### Comparison of activity of the oil and its constituents with standard antifungal drugs:

The antibiogram pattern of the pathogens was studied by disc diffusion method on SDA plates against Clotrimazole, Fluconazole, Itraconazole, Griseofulvin, Amphotericin-B and Nystatin. Sterile filter paper discs containing MIC levels of oil and aromatic constituents were also used for comparison.

## V. RESULT AND DISCUSSION

Following evaluation parameters were performed to ensure superiority of prepared Soap.

### Physical parameters:

Clarity and color was checked by naked eyes against white background, the odor was smelled.

Organoleptic evaluation:

Table 2: Physical parameters

Sr. No	Parameters	Observations
1	Colour	Brown
2	Odour	Dark rose like fragrance
3	Texture	Hard soap

### pH:

The pH of the soap was found to be in range of 5.5 to 10 in which 7-10 are mostly found. Our skin having the ideal range is between 5.5 and 6.5. Therefore it is mostly according to our skin pH. The herbal formulation of soap was shown pH i.e. pH 7(Which is Neutral).

### Determination of percentage free alkali:

Titration of the soap solution with 0.1 N HCL Was done. The % free alkali was found to be 0.26.

### Foam Retention:

Foam Retention was performed by using soap solution is about 2 cm.

### Accelerated stability testing:

Accelerated stability testing of prepared PHF was at room temperature , studied for one week at 50 °C ± 1°C for 3 month.

### Foam Height:

Foam Height was performed by using soap solution is about 2.5 cm.

**Alcohol Insoluble Matter:** % Alcohol Insoluble Matter was calculated by using formula as below:

Percentage alcohol insoluble matter =

**Weight of the residue×100 /Weight of sample =1.29×100/5.00 =25.8**

Table 3: Physical evaluation tests

Sr. No.	Chemical parameters	Herbal soap	Standard
1.	PH	7.0	6.5-7.5
2.	% free alkali	0.26	0.25
3.	Foam height(cm)	2.5cm	2.5-3.0
4.	Foam Retention(min)	2cm	0.5-2.5
5.	Alcohol insoluble matter	25.8	18-28
6.	High temperature stability	Soap melts above 45°C	45°C



PHYTOCHEMICAL ANALYSIS OF GERANIUM PLANT

Table 4: Phytochemical analysis

Phytochemical	Concentration (mg/100g)
Alkaloid	1.20 ± 0.10
Saponin	1.43 ± 0.06
Flavonoid	4.266 ± 0.10
Tannin	3.670 ± 1.15
Phenol	0.030 ± 0.02
Glycoside	0.20 ± 0.06
Oxalate	1.002 ± 0.03

Table.4: shows the different phytochemicals and their various concentrations. It was observed that the flavonoid content was highest at a value 4.266±0.10 followed by tannin at 3.67±1.15, while the value of saponin 1.43±0.06, alkaloid 1.20±0.10, oxalate 0.10±0.03, glycoside, 0.203±0.06 with phenol having the least value of 0.03 ± 0.02 mg/100g

Determination of MIC values of rose-scented geranium oil (neat) and six of its major constituents:

Table 5: Determination of MIC values

Organisms	MIC*							
	A	B	C	D	E	F	G	H
Candida albicans MTCC 183	7.8 ± 0.33	3.9 ± 0.13	3.9 ± 0.13	7.8 ± 0.16	7.8 ± 0.12	15.62 ± 0.09	61.5 ± 0.14	1.9 ± 0.03
Cyptococcusneoformans MTCC 1437	31.25 ± 0.46	3.9 ± 0.13	15.62 ± 0.14	7.8 ± 0.19	7.8 ± 0.12	15.62 ± 0.13	125 ± 0.22	1.9 ± 0.05

MIC value of neat oil is represented in µl/ml and aromatic constituents in µg/ml.

- A-Geranium neat oil;
- B-Citronellol;
- C-Linalool;
- D-Geraniol;
- E - Isomenthone;
- F - Geranyl formate;
- G -Citronellyl formate;
- H - cock tail mixture of the six constituents at 1:1 ratio.

Comparison of activity of the oil and its constituents with standard antifungal drugs:

Table 6: Comparison of activity of the oil and its constituents with standard antifungal drugs

Compounds	Potency disc	Zone sizes in mm			
		C.albicans		C.neoformans	
		SDA	SDSA	SDA	SDSA
Antifungal agents					
Clotrimazole	25µg	-	-	-	-
Itraconazole	30µg	14	14	19	19
Griseofulvin	35µg	-	-	-	-
Amphotericin-B	100U	-	-	-	-
Nystatin	100U	18	18	10	9
Fluconazole	10µg	22	22	-	-
Oils and its compounds					

Geranium oil	22	20	22	22
Citronellal	22	24	24	24
Linalool	22	24	12	12
Geranial	28	28	31	27
Isomethone	20	22	17	17
Geranyl formate	11	11	13	11
Citronellyl formate	14	11	8	12
Cock tail mix. Of six compound at 1:1 ratio	>30	>30	>32	>32

## VI. CONCLUSION

After all we can concluded that, the *P. graveolens* can influence the yield of oils, their chemical composition as well as their Antifungal, Antioxidant and Antibacterial activities. Its very challenging for searching of new, natural antifungal sources, without negative impact on the environment. The biological properties obtained by Pelargonium essential oil in this study use in numerous health problems and medical conditions. However further studies must be performed to confirm the safety of the Antifungal Soap of Pelargonium graveolens on prevention of fungal infections.

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