Research Article

A Comparative Analysis of Clove Oil and Ketoconazole Against *Malassezia furfur*: A Synergistic Herbal and Synthetic Antifungal Approach to Dandruff Management

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ABSTRACT

Dandruff, most often due to Malassezia furfur, is an ongoing dermatological issue, usually treated with synthetic antifungals such as ketoconazole. But in the face of rising resistance and calls for safer formulations, herbal remedies are being investigated. This study compares the antifungal activity of *Syzygium aromaticum* (clove oil) with ketoconazole against Malassezia furfur in vitro. Clove oil was obtained through steam distillation, and its minimum inhibitory concentration (MIC) and zone of inhibition (ZOI) were conducted by using standard agar diffusion techniques. The results indicated that clove oil at 350 µg/mL resulted in a ZOI of 1.8 cm, which was greater than ketoconazole at the same dose (1.2 cm). These results indicate the strong antifungal activity of clove oil due to its bioactive compound eugenol. Although ketoconazole continues to be a popular antifungal drug, clove oil is a promising natural efficacy and may be used as a complementary or alternative therapy in anti-dandruff products. More clinical trials are required to confirm its long-term safety and therapeutic value. The investigation justifies the synergistic strategy of combining the herbal and synthetic drugs for the successful management of dandruff.

Keywords: Clove oil, Ketoconazole, *Malassezia furfur*, Dandruff, Antifungal, Eugenol, Herbal remedy.

INTRODUCTION

Dandruff, known as "Pityriasis simplex," is a widespread and often embarrassing condition [1] that impacts nearly 5% of people worldwide. It commonly arises after puberty [2], particularly between the ages of 20 and 30, and is more prevalent in males than females. This condition is marked by scalp scaling [3] and is often linked to seborrhea, the precursor to seborrheic dermatitis. The yeast *Pityrosporum ovale* [4] is responsible for causing dandruff. It thrives on skin lipids and proteins, enhancing lipase activity [5], which produces pro-inflammatory free fatty acids (FFAs) that lead to dermal inflammation [6] and tissue damage. The increased lipase activity suggests that *Pityrosporum ovale* not only triggers hypersensitivity but also releases toxic compounds that contribute to the onset of fungal infections.

Figure 1: Dandruff picture

Causes of Dandruff: [7]

1. Hormonal imbalance.
2. Excessive sweating.
3. Allergic reactions.
4. Increased stress levels.
5. Poor hygiene practices.
6. Unhealthy nutrition.
7. Insufficient sleep.
8. Genetic predispositions.
9. Inadequate shampooing and rinsing.
10. Wearing tight hats.
11. Overuse of hair styling products (gel, mousse, hair dye, curling irons, etc.).
12. Cold weather conditions.
13. Dry environments.
14. Excessive heat exposure.

Signs and Symptoms of Dandruff [8]**:**

The primary indication of dandruff, also known as seborrheic dermatitis of the scalp, is the presence of white flakes on the scalp and in the hair. These flakes are particularly noticeable on dark clothing when they fall onto the shoulders. Adults with seborrheic dermatitis of the scalp may experience red, flaky, greasy patches.

- White flakes are noticeable on the scalp and in the hair.
- Flakes may appear greasy.
- The scalp may feel tight and itchy.
- Tingling sensations can occur on the scalp.
- Soreness may develop on the scalp.

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• Red, flaky, greasy patches of skin are common in adults with seborrheic dermatitis.

• A crusty scaling rash appears on the scalp in infants with seborrheic dermatitis, also known as cradle cap.

Anti-Dandruff:

These are the agents that apply to the hair shaft and scalp to remove grease, dirt, and debris from the surface without adversely affecting the overall health of the hair and skin.

Dandruff Treatment:

Currently, there is no definitive cure for dandruff, which often results in chronic conditions. However, it can be effectively managed through various approaches.

For mild cases, daily or twice-daily cleansing with a standard shampoo is typically sufficient. It's advisable to begin with a gentle formulation, as harsher shampoos can exacerbate irritation and dryness, potentially worsening flaking.

If conventional shampoos fail to alleviate symptoms, transitioning to an anti-dandruff shampoo is recommended, with significant improvement often observable within a few weeks. Finding the optimal shampoo may require experimentation, and incorporating a rotation of different formulations can enhance scalp health.

Once an effective anti-dandruff shampoo is identified, continuous use is essential to prevent the recurrence of symptoms. For optimal results, it is beneficial to lather and rinse twice: the first cleanse removes debris, while the second allows for the active ingredients to medicate the scalp.

Dandruff is primarily attributed to an overabundance of lipophilic yeasts of the genus Malassezia, which are commensal organisms on the scalp. Managing yeast populations through targeted treatments appears to be a pragmatic strategy for treatment.

Various studies employing different clinical methodologies have demonstrated that antifungal-based antidandruff shampoos initiate a consistent therapeutic response. Several antifungal agents, including imidazole derivatives such as ketoconazole and compounds like selenium sulfide and zinc pyrithione (ZnPTO), have exhibited efficacy in mitigating dandruff.

The principal objectives of antidandruff formulations are to eliminate scaling, reduce the adherence of Malassezia species to corneocytes, and inhibit yeast proliferation. In addition to synthetic agents, numerous herbal extracts—including pepper, basil, neem, rosemary, clove, and tea tree oils—have been documented for their anti-Malassezia properties and possess potential efficacy in combating dandruff.

Clove [9,10]**:**

Cloves are the dried flower buds of a tree called *Syzygium aromaticum*, which belongs to the Myrtaceae family. Originally from Indonesia, cloves are now used as a spice in many cuisines around the world. The name "clove" comes from the Latin word for "nail," because the dried buds look a bit like small, irregular nails.

These aromatic buds have a rich history; the Chinese were using cloves as far back as the 3rd century BC, and they were known in other regions, including the Mediterranean and Europe, by the 8th century. Today, Zanzibar is the largest producer of cloves globally.

In terms of composition, cloves contain carbohydrates, proteins, oils, and fibers, along with various minerals like calcium and iron, and vitamins such as C and A. They pack a calorific value of 430.

When clove buds, stems, and leaves are steamed, they release beneficial essential oils. The oil from clove buds contains important compounds like eugenol, which has several health benefits. The oil extracted from stems and leaves has slightly different properties but is also useful.

Cloves have various medicinal uses: [11]

Nausea and Indigestion: Clove powder or tea may help with problems like nausea, bloating, and upset stomach.

Cholera: Boiling about 4 grams of cloves in 3 liters of water can help ease severe symptoms of cholera.

Asthma: A decoction made from boiling 6 cloves in a small amount of water can serve as an expectorant when mixed with honey.

Toothache: Cloves can reduce pain from toothaches and fight infections due to their antiseptic properties. Applying clove oil directly to a cavity may also help.

Earaches: Sautéing a clove in sesame oil and placing a few drops into the ear can relieve earaches.

Muscle Cramps: Applying clove oil as a poultice can help relieve muscle cramps.

Headaches: Mixing a paste of clove and salt in milk is a traditional home remedy for headaches.

Cloves are also effective for treating styes, which are painful bumps around the eyelashes. Rubbing a clove stub in water and applying it to the stye can provide relief.

In addition to their medicinal uses, cloves are commonly used in cooking, especially in curry powders, where they are mixed with other spices like chilies and turmeric. Clove oil is a popular ingredient in perfumes, soaps, and even dental products due to its pleasant scent and flavor.

KETOCONAZOLE

Ketoconazole (Nizoral) is a synthetic antifungal medication used to treat skin and fungal infections. It works by blocking the production of fungal cell membranes, inhibiting fungal growth. Approved by the FDA in 1981, it is available in scored white tablets, each containing 200 mg of ketoconazole for oral use.

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Posology and Administration: [12]

Wash the affected skin or scalp thoroughly with a 2% ketoconazole solution. Leave it on for 3 to 5 minutes to ensure maximum effectiveness before rinsing it off.

Treatment:

- Pityriasis Versicolor: Once daily for 5 days.

- Seborrheic Dermatitis and Pityriasis Capitis: Twice weekly for 2 to 4 weeks.

Prophylaxis:

-Pityriasis Versicolor: Once daily for 3 days before summer.

- Seborrheic Dermatitis and Pityriasis Capitis: Once every 1 or 2 weeks.

Overdose:

In case of accidental ingestion, provide supportive care. Do not induce vomiting or perform gastric lavage.

Side Effects:

Report the following to your healthcare professional:

- Dark yellow or brown urine, loss of appetite

- Redness, blistering, or peeling skin, skin rash

- Itching, stomach pain, yellowing of eyes or skin

Common side effects that may not need medical attention include:

- Breast tenderness, sexual difficulties (impotence in men)

- Drowsiness, dizziness, light sensitivity

- Nausea, vomiting

Uses:

Ketoconazole treats:

- Dandruff

- Reddish-brown patches on the face or chest

- Small brown or white patches on the trunk (Pityriasis Versicolor)

Malassezia furfur [14,15,16]

Scientific Classification

- Kingdom: Fungi
- Division: Basidiomycota
- Subdivision: Ustilaginomycotina
- Class: Exobasidiomycetes
- Order: Malasseziales
- Genus: Malassezia

Malassezia, formerly referred to as Pityrosporum, is a genus of related fungi that are naturally present on the skin surfaces of various animals, including humans. Certain species within this genus can, on occasion, lead to opportunistic infections that result in hypopigmentation on the trunk and other areas of the human body.

Nomenclature:

The genus Malassezia was first identified by the French scientist Louis-Charles Malassez in the late 19th century. In 1904, Raymond Sabouraud identified an organism that causes dandruff and named it *"Pityrosporum malassez"* in tribute to Malassez. However, this designation was made at the species level rather than the genus level. Following the determination that these organisms were indeed the same, the name "Malassezia" was subsequently recognized as having priority.entury, it was reclassified into two species:

•*Pityrosporum (Malassezia) ovale* is a lipid-dependent fungus that is found exclusively on humans. This species was later divided into two classifications: P. ovale and P. orbiculare. However, current sources consider these terms to refer to a single species, with M. furfur being the preferred name.

On the other hand, Pityrosporum (Malassezia) pachydermatis is lipophilic but not lipid-dependent and can be found on the skin of most animals.

In the mid-1990s, scientists at the Pasteur Institute in Paris, France, identified additional species of this fungus.

Currently, there are 10 recognized species:

- *M. furfur*
- *M. pachydermatis*
- *M. globosa*
- *M. restricta*
- *M. slooffiae*
- *M. sympodialis*
- *M. nana*
- *M. yamatoensis*
- *M. dermatis*
- *M. obtusa*

Numbers:

The number of specimens of M. globosa on a human head can be up to ten million.

Treatment of symptomatic scalp infections:

Scalp infections that show symptoms are commonly treated with shampoos containing selenium sulfide or ketoconazole. Other treatment options include ciclopirox olamine, coal tar, zinc pyrithione (ZPT), miconazole, and tea tree oil shampoos. Hydrogen peroxide can also be used occasionally to relieve itching, but it should be diluted with water. However, caution is advised as its oxidative properties can cause scarring when it reacts with catalase.

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MATERIALS AND METHODS [17,18,23,24]

Microbial source

Malassezia furfur MTCC 1374 culture from IMTECH, Chandigarh, India. Subcultures were made with modified Sabouraud's agar medium every 30 days.

Malassezia furfur

The ketoconazole solution was procured from CAS 65277-42-1 MEDCROP TECHNOLOGIESINDIA LIMITED from SECUNDERBAD, used in the study were (names not disclosed to avoid any commercial implication).

The natural ingredient clove, is procured from a grocery shop.

Procedure for Steam Distillation [19,20,21]

1. Begin by clamping a 100-mL heating mantle (do NOT fill it with sand) to a ring stand above a magnetic stirrer.

2. Secure a 100-mL round bottom flask to the ring stand; the flask should be placed in the heating mantle.

3. Weigh out 5 to 6 grams of ground spice and transfer it into the flask using weighing paper as a funnel.

4. Fill the flask halfway with distilled water and add a 1-inch magnetic stir bar.

5. Complete the distillation setup as illustrated in the provided diagram. Position a 50-mL round-bottom flask at the end of the connecting tube or vacuum adapter to collect the distillate.

6. The thermometer is not necessary, as the temperature will remain close to the boiling point of water.

7. Ensure that both the round-bottom flask and the condenser are securely clamped. Additionally, use a rubber band to hold the vacuum adapter in place at the end of the condenser.

8. Begin stirring and heating the mixture, setting the varistat to 60. If foaming occurs, lower the heating voltage as needed.

Steam Distillation:

1. Turn on the cooling water and ensure it flows through the condenser. A slight turn of the valve is enough, as the flow will be minimal. Avoid overheating the mixture to prevent bumping.

2. Insulate the top of the 100-mL round-bottom flask and the 3-way connector with glass wool to prevent steam condensation. Adjust heating to achieve a distillate drip rate of one drop every 2-5 seconds. Add water in small amounts via the separator.

3. Collect the distillate in a 50-mL round funnel to match the collection rate of the bottom flask. Keep the ice in the cooling beaker replenished as needed. Use a Pasteur pipette to manage water levels before adding more ice. The vapor should be cloudy initially; once it becomes clear, remove heat from the flask.

Isolation of Oil:

1. Extract the distillate with 30 mL of dichloromethane (3 x 10 mL portions). Dry the organic layer over sodium sulfate (Na2SO4), filter, and divide into two portions: one for gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) analysis in a short vial, and the other for biological testing in a 20-mL vial.

2. Concentrate the 20-mL portion with a gentle stream of nitrogen gas, then dissolve it in acetone.

Clove Distillation and MIC Testing:

1. Determine the Minimum Inhibitory Concentration (MIC) by adding clove oil concentrations (50 mg/mL to 10 mg/mL) to 100 mL of Sabouraud's medium. Inoculate each plate with an organism at a density of 10.

2. Incubate at 37°C for 48 hours. Use a medium without clove oil as a control. Identify the lowest concentration of clove oil that inhibits the organism's growth compared to the control.

Zone Of Inhibition (Zoi):

Diffusion-dependent antimicrobial activity of the clove oil was studied by the zone of inhibition method. The organism was uniformly inoculated on the surface of the Sabouraud's medium. The well was made in the center of the medium, and the known concentration of the oil and ketoconazole was loaded in the well. The plate was incubated at 37·c for 2 days. The zone of inhibition was measure.

RESULTS AND DISCUSSION

Dandruff may be caused by several different factors, but the exact underlying cause of dandruff is unknown. Dandruff is the visible desquamation of the scalp is the mildest manifestation of seborrheic dermatitis. Dandruff can be seasonal, in which it is most severe during winter, mildest during summer.

The results of this study showed that clove oil inhibited the growth of Malassezia furfur at a 350µl concentration when compared to ketoconazole solution.

The zone of inhibition (ZOI) of clove oil was found to be 20mm in diameter, and for ketoconazole. In the case of control, the death of *Malassezia furfur* cells could not be seen.

Table 1: Effect of clove oil and ketoconazole solution on *Malassezia furfur*

SNO	Sample	MIC	ZOI
1	Clove	50µg/ml	0.7cm
		200µg/ml	1.2cm
		250µg/ml	1.2cm
		350µg/ml	1.8cm
2	Ketoconazole	200µg/ml	0.7cm
		250µg/ml	0.8cm
		300µg/ml	1.1cm
		350µg/ml	1.2cm

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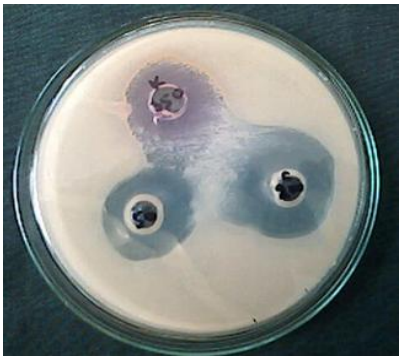
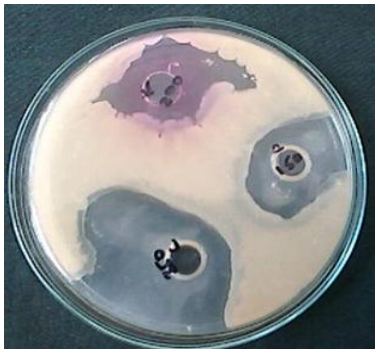
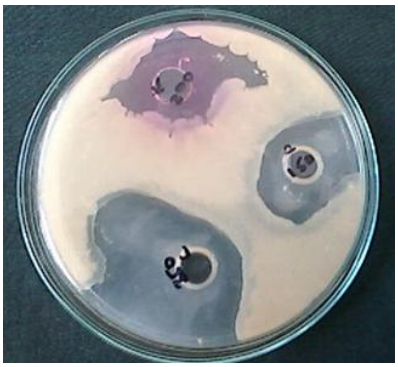
CLOVE- 200µg/ml- 1.2 cm
KETOCONAZOLE -300µg/ml-1.1cm



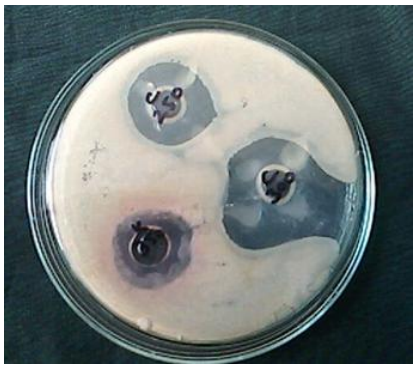
CLOVE -50µg/ml-0.7cm
KETOCONOZOLE-250µg/ml-0.8cm



CLOVE-350µg/ml-1.8cm
KETOCONAZOLE-200µg/ml-0.7cm



CLOVE-350µg/ml-1.8cm
KETOCONAZOLE-200µg/ml-0.7cm



CLOVE-250µg/ml-1.2cm
KETOCONAZOLE-350µg/ml-1.2cm

Figure 2: Effect of clove oil and ketoconazole solution on *Malassezia furfur*

CONCLUSION

These preliminary studies showed[25] that ketaconazole-based shampoos (OTC products) are used more by consumers for common dandruff problems.

Herbal ingredients like clove oil, recorded to have high anti-dandruff activity, thus minimum inhibitory concentration (MIC) are much higher than the synthetic ingredient.

This clove oil can be exploited for its anti-dandruff activity individually or in combination with anti-dandruff shampoos. But for regular usage, even shampoos with herbal anti-dandruff ingredients may suffice purpose.

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